



(51) International Patent Classification:
C12P 7/18 (2006.01)

(21) International Application Number:
PCT/EP2015/056117

(22) International Filing Date:
23 March 2015 (23.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
14305409.6 21 March 2014 (21.03.2014) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))
- with information concerning incorporation by reference of missing parts and/or elements (Rule 20.6)

(54) Title: USE OF DJ-1 DEGLYCASE ACTIVITY

(57) Abstract: The present invention relates generally to the modification of molecules, in particular of proteins and nucleic acids, called glycation, which results in formation of advanced glycation endproducts and protein or nucleic acid crosslinks. The present invention provides products, and more particularly enzymes, that are useful for preventing or reversing protein and nucleic acid glycation. The invention in particular relates to an *in vitro* method for preventing and/or reversing glycation of a molecule of interest, said method comprising the step of bringing together the molecule of interest and a DJ-1 protein and/or one of its homologs.



USE OF DJ-1 DEGLYCASE ACTIVITY

INTRODUCTION

5 The present invention relates generally to the modification of molecules, in particular of proteins and nucleic acids, called glycation, which results in formation of advanced glycation endproducts and protein or nucleic acid crosslinks. The present invention provides products, and more particularly enzymes, that are useful for preventing or reversing protein and nucleic acid glycation.

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Advanced glycation end products, known as AGEs, are stable forms resulting from protein or nucleic acid glycation by electrophiles. AGEs comprise protein and nucleic acid adducts resulting from the reaction of proteins and nucleic acids with electrophiles, and derivatives of these adducts, and include also protein and nucleic acid crosslinks. An adduct is a product of a direct addition of two or more distinct molecules, resulting in a single reaction product containing all atoms of all components. The reaction product is considered a distinct molecular species.

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AGEs are produced *in vivo* and *in vitro* as a result of a series of chemical reactions. Glycation is the most general IUPAC term for addition of a sugar (or any electrophile compound, including dicarbonyles such as glyoxals, or lipid-derived electrophiles such as 4-hydroxynonenal) to another biomolecule. It is not to be misunderstood for glycosylation, with glycation being a nonenzymatic process and glycosylation an enzymatic process forming a glycosidic bond. Glycosylation occurs at defined sites on the target molecule and is required in order for the molecule to function. On the contrary, glycation generally impairs the proper functioning of molecules.

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In vivo, glycation occurs in all prokaryotic and eukaryotic cells (bacteria, yeasts, plants, animals...). The main glycating agents comprise reducing sugars, such as glucose, fructose, and galactose, sugar phosphates produced in glycolytic and pentose phosphate pathways, dicarbonyles compounds such as methylglyoxal and glyoxals (several thousand-fold more active than glucose) formed as byproducts of different metabolic pathways, and all other electrophile compounds (most glycating agents are endogenously formed, but also come from exogenous contaminations

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(cigarette smoke, alcohol drinking, chemical pollution, food...). Alpha-oxoaldehydes such as glyoxal, methylglyoxal and 3-deoxyglucosone are particularly noxious, and are mainly formed by degradation of glycolytic intermediates, threonine (for methylglyoxal), glycated proteins and peroxidated lipids. They are potent glycating agents of protein and nucleotides, leading to the formation of advanced glycation endproducts (AGEs)(Thornalley et al., *Drug Metabol Drug Interact*; 2008; 23(1-2):125-150).

In the literature, the term glycation has not always been used in a precise manner: it may refer to the formation of a Schiff base and/or Amadori product to an amino group in protein, to the addition of dicarbonyl compounds, such as methylglyoxal, to arginine, lysine; or to the formation of AGEs, including both adducts and crosslinks or to any covalent modification resulting in the target molecule from the initial covalent addition of the glycating agent, and harbouring atoms (the covalent modification) of the glycating agent.

Yet, it has also been shown that adducts could be formed by reaction of alpha-dicarbonyl compounds with thiol groups on amino acids, peptides, and proteins, thus supporting the fact that glycation can occur on both amino and thiol groups and principally affects cysteines, lysines and arginines of proteins (Zeng et al., *Chem Res Toxicol.*,18(8):1232-41, 2005 and Zeng et al., *Chem Res Toxicol.*,19(12):1668-76, 2006). Glycation by electrophiles, including glyoxals, also affects nucleotides and nucleic acids. Electrophiles mainly react with the amino groups of guanine residues, but also with those of adenine and cytosine residues.

In the context of the invention, glycation is thus considered as the nonenzymatic covalent addition of reducing sugars, in particular glucose, fructose, and galactose, of aldehydes and ketones derived from those sugars or oxidation reactions, or alpha-oxoaldehydes (and other alpha-dicarbonyl compounds) such as glyoxal, methylglyoxal and 3-deoxyglucosone, to nucleophilic groups (amino, imino and thiol groups) on side chains of lysyl, arginyl, and cysteinyl residues of proteins (as well as the N-terminal amino group of proteins) to lysines, arginines and cysteines under the free form (i.e. not incorporated in proteins), and to amino groups of guanine, adenine and cytidine of polynucleotides and under the free form.

When glycation of a protein residue consists in an addition of one of a carbonyl compound to a lysine amino group, the resulting product begins by formation of an aminocarbinal (-NH-CHOH-), which is rapidly dehydrated into a Schiff base (-N=CH-) (within minutes to hours) and undergoes further rearrangement into an Amadori product (after around 48 h). Amadori products are further rearranged and oxidized to form AGEs such as carboxymethyl lysine (CML) and carboxyethyl lysine (CEL). In the case of dicarbonyl electrophiles, the second carbonyl reacts with another protein lysine or arginine, leading to the formation of protein crosslinks (MOLD (methylglyoxal lysine dimers), GOLD (glyoxal lysine dimers), MODIC (methylglyoxal derived imidazolium crosslinks), GODIC (glyoxal derived imidazolium crosslinks)).

Regarding arginine residues, glycation by carbonyl compounds also begins by formation of an aminocarbinal, and occurs as described above for lysine residues. However, in the case of glycation by dicarbonyl compounds, the second carbonyl group further reacts with the arginine imino group, leading (in the case of glycation by methylglyoxal) to dihydroxyimidazolidine, hydroimidazolone (MG-H1), argpyrimidine and further complex AGEs (Rabbani and Thornalley, Amino acids 42, 1133).

When glycation of a protein residue consists in addition of one of these carbonyl compounds to the thiol group of a cysteine, the resulting product is commonly referred to an hemithioacetal (Cys-S-CHOH-). In the case of dicarbonyls, the second carbonyl frequently reacts with another protein thiol group or amino group, leading to formation of protein crosslinks. All crosslinks discussed above may also be formed on nucleic acids.

Briefly, the formation of a Schiff base involves the condensation of a carbonyl group, for example from a reducing sugar such as glucose, with a free amino group, typically the epsilon amino group of lysine residues within proteins. The resulting compound (after dehydration of the aminocarbinal (Lys-NH-CHOH-) initially formed) is called an aldimine (Lys-N=CH-). Aldimine spontaneously rearranges to form the more stable 1-amino-1-deoxy-2-ketose (ketoamine) ($\text{Lys-NH-CH}_2\text{-CO-}$ from Lys-N=CH-CHOH-), which is also known as the Amadori product. When the initial sugar is glucose, the Amadori product is commonly known as fructoselysine (FL). The extent of glycation of a protein depends on the rate of formation of the hemithioacetals,

aminocarbonols, Schiff bases, Amadori products and their rate of reversal or conversion to other products.

From this stage, the formation of advanced glycation end-products (AGEs) further involves modifications of early glycation products (including Amadori products). Such modifications involve oxidations (often catalyzed by transition metal catalysis) and non-oxidative reactions including dehydrations and complex rearrangements.

AGEs that are produced in the human body have been implicated in chronic diseases such as Parkinson disease, Alzheimer's disease and other neurodegenerative diseases, post diabetic diseases, cardiovascular diseases (including atherosclerosis), renal diseases, autoimmune diseases, ageing related diseases, diabetes-related diseases and cancer.

AGEs have also been associated with non-pathological modifications of the human body, and AGEs accumulation in different tissues is a main cause of aging. Skin is the largest organ of the human body, and the role of advanced glycation enproducts in skin aging is clearly recognized. At the cutaneous level, glycation is associated with the aging process and affects structural proteins such as collagen, elastin, glycoproteins, and glycosaminoglycans. It has been shown that in healthy integument, the accumulation of final glycation products increases with age and is amplified by ultraviolet exposure. In elderly subjects, the accumulation of AGEs modifies the mechanical properties of human skin through loss of elasticity and increased stiffening.

AGEs are also found in food, where they can impair nutritional value of food through protein crosslinking, and their levels are increased by high temperature cooking.

AGEs formation can also occur during the process of protein bioproduction, such as during recombinant protein production in mammalian cell culture for instance (Quan et al., *Anal Biochem.*;373(2):179-91, 2008). Indeed, during the cell culture process required for example for monoclonal antibody production, proteins are incubated with a large excess of reducing sugar per mole of protein product. While this high concentration of reducing sugar provides energy to the cell, as well as a means for modulating enzymatic glycosylation, it also promotes protein glycation. The glycation

of proteins is known to increase their aggregation and may compromise their pharmaceutical activity.

Glycation also occurs in bacterial cultures (Pepper et al. Appl. and Env. Microbiology, 5 76, 7925), and particularly affects overexpressed proteins. Moreover, glycation of a protein may occur during subsequent formulation, packaging, long term storage, or clinical administration steps, where sugars are commonly used as excipients in liquid or lyophilized formulations. For example, it has been reported that most commercial interferons IFN- β are glycated and that these advanced glycation endproducts 10 contribute to their immunogenicity, which results in hypersensitivities reactions. (Bozhinov et al. J. Allergy Clin. Immunol. 129, 855)

Moreover, it has been shown that glycation of the DNA, which mainly occurs on guanine residues, is associated with increased mutation frequency, DNA strand 15 breaks and cytotoxicity (Thornalley et al., *Drug Metabol Drug Interact*; 2008; 23(1-2):125-150).

Despite the need for means of inhibiting the formation of AGEs, little compounds are known that can actually reverse the glycation of a protein, and no compounds has 20 been demonstrated to be capable of reversing glycation of polynucleotides.

Some enzymes, such as aldo-keto reductases convert carbonyles into less harmful alcohols, and the glyoxylase system (composed of glyoxalase I and II) catalyze the detoxification of alpha-oxoaldehydes, such as glyoxal and methylglyoxal. Glyoxalase I 25 catalyzes the isomerization of the hemithioacetal, formed spontaneously from the alpha-oxoaldehyde and glutathione (GSH), to S-2-hydroxyacylglutathione (lactoyl glutathione), whose thioester bond is degraded by glyoxalase 2, which results in the release of glutathione and an acid-alcohol (lactate from methylglyoxal, and glycolate from glyoxal).

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Although glyoxalases help to alleviate glycation, they cannot prevent it *stricto sensu*. Indeed, although they decrease the levels of intracellular alpha-oxoaldehydes, the latter remain at sufficiently high intracellular concentrations (1-5 μM) to exert harmful effects. Moreover, glyoxalases cannot reverse glycation.

35 Amadoriases (also called fructosyl amine oxidase or FAOXs) are a class of enzymes

(initially found in *Aspergillus*) that harbor deglycase activity. These enzymes have been found in fungi, yeast, and bacteria. (Zhanglin et al., *Applied Microbiology and Biotechnology*, 86(6):1613-1619, 2010). They catalyze the deglycation of Amadori products (Takahashi et al., 1997). However, naturally occurring amadoriases can only
5 react with glycated free-amino acids (i.e. not incorporated in proteins), and their function is to provide metabolic supply to organisms that harbor them. Some bacteria also use the cooperative action of a kinase (a fructosamine-6 kinase) and a deglycase: fructosamines are first phosphorylated by a fructosamine-6 kinase to fructosamine-6-phosphates, which (after a “reverse Amadori rearrangement”) are
10 converted by the deglycase to glucose-6 phosphate and a free amino acid. However, such deglycases (like amadoriases) are used to provide metabolic supply (glycated aminoacids converted by them into aminoacids) to bacteria, and they have never been reported to deglycate proteins (van Schaftingen et al. 2012 *Amino Acids* 42, 1143-1150).

15 Eukaryotic (including human) fructosamine-3-kinases (FN3K), and related proteins, by phosphorylating lysine-ketoamines (Amadori products) of glycated proteins, moderately speed up their separation from lysine amino groups, thereby releasing the repaired protein and a dicarbonyl such as 3-deoxyglucosone. In the context of
20 the invention, “moderately speed up” means that FN3K catalyses fructosamine deglycation with a half-time of 8 hours (versus 24 hours for uncatalyzed deglycation), whereas deglycases described in the present invention display k_{cat} in the s^{-1} range.

25 While somehow interesting, amadoriases, bacterial deglycases and fructosamine-3-kinases appear quite limited and may not allow to deglycate proteins efficiently. Most of them don’t act on proteins but on glycated free amino acids, and those which act on protein only act on glycated lysines with a quite low activity.

30 There is thus still a need for improved products and methods for preventing or reversing glycation on molecules of interest, which could be useful for example in the production of said molecules, and in particular the bioproduction of proteins.

DESCRIPTION

35 The inventors have found that the enzymes of the invention display protein and

nucleic acid deglycase activities, which has never been disclosed. Indeed, although an alleged glyoxalase activity had been proposed for the DJ-1 protein and for bacterial Hsp31 (Lee et al., *Hum Mol Genet.*, 21(14):3215-25, 2012, Subedi et al., *Mol. Microbiol.* 81, 926-936), it was never suggested nor demonstrated that neither
5 the DJ-1 protein, nor its bacterial homologs Hsp31, YhbO and YajL, actually harbor a deglycase activity.

The enzymes of the invention are early protein deglycases which can notably use substrates such as aminocarbonols formed by reaction of arginines and lysines
10 residues with methylglyoxal and glyoxal, and hemithioacetals formed by reaction of cysteines residues with methylglyoxal or glyoxal (as demonstrated in the experimental part herein). Moreover, the inventors have found that the enzymes of the invention can also use as substrates the aminocarbonols formed by reaction of nucleotides aminogroups with glyoxals.

15 Interestingly, the enzymes of the invention catalyse deglycation at the second time-level, which means that they are about 40,000-fold faster than fructosamine-3-kinases (FN3K), and also impressively faster than FN3K-related proteins. The enzymes of the invention can act at the hemithioacetal step (for glycated cysteines), or the
20 aminocarbonol step (for glycated lysines, arginines and nucleotides), and release the repaired amino acid, protein or nucleotide/nucleic acid and an acid-alcohol (lactate from methylglyoxal and glycolate from glyoxal).

The enzymes of the invention can thus act as instant deglycases, on the first
25 glycation intermediates (hemithioacetals and aminocarbonols), and thus prevent formation of late intermediates, including AGEs. Indeed, there is a precursor-product relationship between early glycation products (including hemithioacetals and aminocarbonols which can be deglycated by deglycases of the invention) and late intermediates (i.e. late glycation products), including AGEs.

30 Moreover, the inventors have shown that the enzymes of the invention, by the way of their deglycase activity, can restore the activity of glycated proteins and enzymes such as bovine serum albumin, glyceraldehyde-3-phosphate dehydrogenase, fructose biphosphate aldolase and aspartate transaminase, i.e. proteins with cysteine or
35 lysine and/or arginine residue in their active site.

The enzymes of the invention can therefore be used to either to prevent or to reverse glycation on free lysine, arginine and lysine, and on lysyl-, arginyl- and cysteinyl residues of proteins, as well as on amino groups of free nucleic acids (particularly guanine residues, which are most prone to glycation (Voulgaridou et al. Mutation Res. 711, 13-27), and polynucleotides which has never been shown for any enzyme.

As such, they will usefully be used in the bioproduction of molecules, wherein they can reverse glycation on glycated proteins or polynucleotides, or even prevent the formation of newly glycated proteins or polynucleotides.

Advantageously, the enzymes of the invention can be used to prevent or reverse the effects of glycation, such as for example aging of the skin due to glycation or cosmetic effects of UV on glycation in the skin.

A first object of the invention is an *in vitro* method for preventing or reversing glycation of a molecule of interest, said method comprising the step of bringing together the molecule of interest and a DJ-1 protein and/or one of its homologs.

According to the invention, the molecule of interest may be any organic compound comprising an amino group or a thiol group. Preferably, the molecule of interest is an amino acid, a nucleic acid, or molecules formed thereof, such as for a protein or a polynucleotide for instance. More preferably, the molecule of interest is a protein or a polynucleotide.

By “**glycation**”, it is herein referred to the non-enzymatic covalent reaction between amino- or thiol- groups of amino acids (mainly arginine, lysine and cysteine), proteins (mainly on these amino acid residues), amino-lipids (mainly phosphatidylethanolamine), nucleotides (mainly guanine but also adenine and cytosine) and nucleic acids (mainly on these nucleotide residues) and, either carbonyle groups, principally those of reducing aldoses and ketoses (glucose, fructose, ribose, erythrose and their phosphorylated derivatives) and those of dicarbonyle compounds (mainly methylglyoxal, glyoxal and 3-deoxyglucosone), or a

double bonds (conjugated with a carbonyle group) of compounds such as 4-hydroxynonenal.

Preferably, the terms “glycation” refer to glycation by sugars, in particular glucose, fructose, and galactose, aldehydes derived from those sugars or oxidation reactions, or alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone. More preferably, the terms “glycation” refer to glycation by alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone. Yet preferably, the terms “glycation” refer to glycation by methylglyoxal.

Preferably the terms “glycation” refer to glycation of amino groups or thiol groups on a protein by sugars, in particular glucose, fructose, and galactose, aldehydes derived from those sugars or oxidation reactions, or alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

More preferably the terms “glycation” refer to glycation of amino groups or thiol groups on a protein by alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

Preferably the terms “glycation of proteins” refer to glycation of lysine, arginine and cysteine residues on a protein by sugars, in particular glucose, fructose, and galactose, compounds derived from those sugars or oxidation reactions, or alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

More preferably the terms “glycation of proteins” refer to glycation of lysine, arginine and cysteine residues on a protein by alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

Preferably, the method of the invention is for preventing or reversing glycation on lysine, arginine and cysteine residues of said protein of interest.

Preferably the terms “glycation of polynucleotide” refer to glycation of guanine, adenine, cytidine residues on a polynucleotide by sugars, in particular glucose, fructose, and galactose, compounds derived from those sugars or oxidation reactions, or alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

More preferably the terms “glycation of polynucleotide” refer to glycation of guanine, adenine, cytidine residues on a nucleotide or a polynucleotide by alpha-oxoaldehydes, in particular glyoxal, methylglyoxal and 3-deoxyglucosone.

Since glycation may occur on cysteines, lysines and arginines, it can affect all proteins. The numerous proteins that use one of these amino acids in their active site are particularly sensitive to glycation, which triggers dramatic loss of their activity. All other proteins, however, are sensitive to glycation because glycation at sites remote of active sites indirectly affects their activity, and their physico-chemical or mechanical properties, such as solubility, elasticity and ability to interact with other components of the cell. Many important human proteins are affected by glycation, including serum albumin (decreased drug binding...), haemoglobin (abnormal oxygen binding...), collagen (decreased physical properties, decreased resistance to repair mechanism...), respiratory chain components (resulting in production of reactive oxygen species...), lens proteins, including cristallins (post diabetic blindness...).

In the context of the invention, the terms “protein of interest” refer to any protein, polypeptide or peptide for which the prevention or reverse of glycation is contemplated. Preferably, the protein of interest is chosen from the group consisting in serum albumin, haemoglobin, collagen, cristallin proteins.

Similarly, because glycation may occur on any nucleic acid molecule, any nucleotide or polynucleotide is susceptible to be affected.

In the context of the invention, the terms “polynucleotide of interest” refer to any polynucleotide for which the prevention or reverse of glycation is contemplated. Preferably, the nucleotide of interest is chosen from the group consisting in deoxyribonucleotides or nucleotides precursors which are incorporated into DNA or RNA as XTP, and also from any DNA or RNA, since they all appear to belong (as glycated molecules) to the set of DJ-1 substrates

By hemithioacetal” it is herein referred to an adduct formed of an amino-acid, a peptide or a protein, and a sugar, in particular glucose, fructose, and galactose, an aldehydes derived from those sugars, or an alpha-oxoaldehyde, in particular glyoxal, methylglyoxal and 3-deoxyglucosone, on a thiol group of said amino-acid, peptide or protein.

For example, glycation of cysteine, arginine and lysine residues by methylglyoxal lead to the formation of a hemithioacetal (cysteine derivative), and aminocarbinal (arginine or lysine derivative).

In an embodiment, the method of the invention is for preventing glycation of a molecule of interest.

According to the invention, the terms “**preventing glycation**” refer to prevention of the formation of an aminocarbinal or of a hemithioacetal and consequently, of all the glycation products subsequently formed, such as Schiff bases, Amadori products, AGEs and crosslinks.

In an embodiment, the method of the invention is for reversing glycation of a molecule of interest.

According to the invention, the terms “reversing glycation” refers to the formation of a deglycated protein or nucleic acid from a thiol-aldehyde adduct, a thiohemiacetal or a thioester (in the case of glycation of a thiol group of a protein by a carbonyl group) or from an aminocarbinal or an amide (in the case of glycation of an amino group or a protein or nucleic acid by a carbonyl group).

By “**DJ-1**”, it is herein referred to the DJ-1 protein also known as Park7 (Parkinson disease 7), its functional variants and fragments. Preferably, “DJ-1” refers to the human protein DJ-1. In humans, DJ-1 is encoded by the PARK7 gene (ENTREZ reference: 11315), and has the sequence of NCBI reference NP_001116849, herein corresponding to the sequence SEQ ID No.1.

Preferably, by the terms “**DJ-1**”, it is herein referred to the human DJ-1 protein of sequence SEQ ID No.1, its functional variants and fragments.

According to the invention, the terms “**protein**”, “peptide” and “polypeptide” are used interchangeably and refer to a polymer of amino acid monomers having no specific length, wherein the amino acid monomers are linked by peptide bonds. Also covered by this definition, are polypeptides having undergone post-translational modifications such as those with covalently attached glycosyl groups, acetyl groups, phosphate groups, lipid groups, and the like.

According to the invention, the terms “**polynucleotide**”, refer to a polymer of nucleic acid monomers having no specific length, wherein the nucleic acid monomers are covalently bonded in a chain.

By “**functional variant of a specific peptide**”, it is herein referred to a peptide whose peptide sequence differs from the amino acid sequence of said specific peptide, but that generally retains the biological activity of said specific peptide.

By “**functional variant of DJ-1**”, it is herein referred to polypeptides whose peptide sequence differs from the amino acid sequence of DJ-1, i.e. from the sequence SEQ ID No.1, but that generally retains the biological activity of DJ-1.

The terms “activity”, “function”, “biological activity”, and “biological function” are synonymous for the purpose of the present invention and have to be understood as it is commonly known in the art.

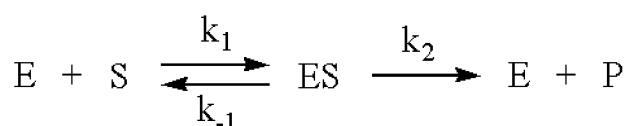
In the context of the invention, the biological activity of DJ-1 is a deglycase activity. In the sens of the invention, a deglycase activity corresponds to the cleavage of the covalent bond formed between a glycating agent and a molecule. Preferably, the extent of the deglycase activity of a specific enzyme is evaluated by the Michaelis-Menten ratio k_{cat}/K_m , also called catalytic efficiency, wherein:

k_{cat} is a constant that describes the turnover rate of an enzyme-substrate complex to product and enzyme. It is also the rate of catalyst with a particular substrate at saturating substrate concentration

K_m is the Michaelis constant that describes the amount of substrate needed for the enzyme to obtain half of its maximum rate of reaction;

The ratio k_{cat}/K_m can easily be obtained using Michaelis-Menten kinetics established and known to one skilled in the art (Leonor Michaelis, Maud Menten (1913), “Die Kinetik der Invertinwirkung” [The kinetics of invertin action], BIOCHEM. Z. 49:333-369).

For instance, the enzymatic parameters K_m and K_{cat} are determined experimentally in the presence of various concentrations of the substrate. A simplified representation of Michaelis-Menten kinetics is



wherein

E=enzyme, S=substrate, ES=enzyme-substrate complex, P=product, k_1 , k_{-1} , k_2 =rate constants.

The use of deglycase enzymes on glycated molecules therefore produces a repaired molecule (which recovers its initial structure, i.e. the structure before glycation) and
5 the glycating agent or a derivative of the glycating agent.

According to the invention, the terms “repaired molecule” refer to a molecule which has been deglycated. Said repaired molecule has the same chemical formula as the one before the glycation event.

The inventors have found that the enzymes of the invention harbor a very specific
10 activity, which has never been disclosed. The enzymes of the invention can use the aminocarbinals formed by reaction of protein arginines and lysines amino groups and of nucleotides amino groups with glyoxals, and the hemithioacetals formed by reaction of protein cysteines with glyoxals.

Preferably, in the context of the invention, the biological activity of DJ-1 is an early
15 protein deglycase activity. By “early protein deglycase activity” it is herein referred to a deglycase activity on early products of glycation, such as aminocarbinals and hemithioacetal compounds, as well as on amide and thioester intermediates of glycation/deglycation.

According to the invention, a variant of DJ-1 retains DJ-1 biological activity when it
20 has an efficient deglycase activity of at least 70, 80, 85, 90, 95, 99 % of the biological activity of DJ-1.

Preferred methods for measuring the deglycase activity are described in the experimental section, and summarized hereafter...

A preferred method for measuring deglycase activity on glycated proteins consists in
25 following deglycation of glycated Nacetylcysteine.

Thus, in a first step, Nacetylcysteine is glycated by 5 min incubation with methylglyoxal in sodium phosphate buffer 50 mM, pH 7.0. This step results in the formation of a hemithioacetal that absorbs light at 288 nm.

Then in a second step, glycated Nacetylcysteine is incubated with the enzyme to be tested. The hemithioacetal concentration is calculated by measuring absorbance at 288 nm. Deglycation is directly correlated to hemithioacetal decrease.

5 A preferred method for measuring deglycase activity on glycated nucleotides consists in following deglycation of glycated GTP or dGTP.

Thus, in a first step, GTP or dGTP is glycated for 2 hours at 37°C with 5 mM methylglyoxal in sodium phosphate buffer 50 mM pH 7.0: glycation results in the appearance of a second peak eluting slightly later than the GTP or GDP single peak, in reverse phase-HPLC chromatography (C18 HPLC column equilibrated and eluted
10 with 100 mM potassium phosphate pH 5.5).

Then in a second step, glycated GTP or dGTP is incubated with the enzyme to be tested. The glycated GTP or glycated dGTP concentration is calculated by measuring the surface of the second peak (see Fig. 8E, 8F). Deglycation is directly correlated to the decrease of the second peak.

15 Preferably, a functional variant of DJ-1 has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity with DJ-1 and retains DJ-1 biological activity.

In the context of the invention, identity between amino acid or nucleic acid
20 sequences can be determined by comparing a position in each of the sequences which may be aligned for the purposes of comparison. When a position in the compared sequences is occupied by the same amino acid or nucleotide, then the sequences are identical at that position. A degree of identity between amino acid sequences is a function of the number of identical amino acid sequences that are
25 shared between these sequences. A degree of sequence identity between nucleic acids is a function of the number of identical nucleotides at positions shared by these sequences.

To determine the percentage of identity between two amino acid sequences or two nucleic acid sequences, the sequences are aligned for optimal comparison. For
30 example, gaps can be introduced in the sequence of a first amino acid sequence or a first nucleic acid sequence for optimal alignment with the second amino acid sequence or second nucleic acid sequence. The amino acid residues or nucleotides at

corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, the molecules are identical at that position.

- 5 The percentage of identity between the two sequences is a function of the number of identical positions shared by the sequences. Hence % identity = number of identical positions / total number of overlapping positions X 100.

In this comparison, the sequences can be of the same length or may be of different lengths.

- 10 Optimal alignment of sequences may be conducted by a global homology alignment (i.e. an alignment of all amino acids or nucleotides of each sequence to be compared), such as by the global homology alignment algorithm of Needleman and Wunsch (1972), by computerized implementations of this algorithm or by visual inspection. The best alignment (i.e., resulting in the highest percentage of identity
15 between the compared sequences) generated by the various methods is selected.

- In other words, the percentage of sequence identity is calculated by comparing two optimally aligned sequences, determining the number of positions at which the identical amino acid or nucleotide occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of
20 positions and multiplying the result by 100 to yield the percentage of sequence identity.

- By “**functional fragments of a specific peptide**” that has a biological activity of interest, it is herein referred to peptides which peptide sequence is a part of the peptide sequence of the reference peptide, and that can be of any length, provided
25 the biological activity of peptide of reference is retained by said fragment.

- Thus, by “**functional fragments of DJ-1**”, it is herein referred to peptides whose peptide sequence is a part of the peptide sequence of DJ-1, and that can be of any length, provided the biological activity of DJ-1 is retained by said fragment. According to the invention, a fragment of DJ-1 retains DJ-1 biological activity when it
30 has at least 70,80, 85, 90, 95, 99 % of the biological activity of DJ-1.

According to the invention, by “**DJ-1 homologs**”, it is herein referred to proteins having a very similar primary, secondary, and tertiary structure, than the one of DJ-1, and retaining DJ-1 activity. Preferably, a protein is considered as retaining DJ-1 biological activity when it has at least 70, 80, 85, 90, 95, 99 % of the biological activity of DJ-1.

In the context of the invention, **DJ-1 homologs are chosen from the list consisting in** YajL protein, YhbO protein, Hsp31 protein.

Preferably, by the terms “YajL protein”, it is herein referred to the YajL protein of sequence SEQ ID No.2, its functional variants and fragments.

10 In the context of the invention, the biological activity of YajL is a deglycase activity. Preferably, in the context of the invention, the biological activity of YajL is a deglycase activity. Preferably, a variant of YajL retains of YajL biological activity when it has at least 70, 80, 85, 90, 95, 99 % of the biological activity of YajL.

15 Preferably, by the terms “YhbO protein”, it is herein referred to the YhbO protein of sequence SEQ ID No.3 its functional variants and fragments.

In the context of the invention, the biological activity of YhbO is a deglycase activity. Preferably, in the context of the invention, the biological activity of YhbO is a deglycase activity. Preferably, a variant of YhbO retains of YhbO biological activity when it has at least 80, 85, 90, 95, 99 % of the biological activity of YhbO.

20 Preferably, by the terms “Hsp31 protein”, it is herein referred to the Hsp31 protein of sequence SEQ ID No.4 its functional variants and fragments. In the context of the invention, the terms “Hsp31 protein” and “HchA protein” are equivalent.

In the context of the invention, the biological activity of Hsp31 is a deglycase activity. Preferably, in the context of the invention, the biological activity of Hsp31 is a deglycase activity. Preferably, a variant of Hsp31 retains of Hsp31 biological activity when it has at least 70, 80, 85, 90, 95, 99 % of the biological activity of Hsp31.

25 In the method of the invention, the molecule of interest and a DJ-1 protein and/or one of its homologs are provided. Alternatively, the molecule of interest and/or DJ-1 protein and/or one of its homologs may easily be synthesized in a production system by the person skilled in the art, using conventional techniques well known in the art.

By “**production system**”, it is herein referred to system that enables the synthesis of the molecule of interest. According to the invention, the production system may be cellular or acellular. In an embodiment, the production system is an expression system that enables the production of recombinant proteins. In another
5 embodiment, the production system is a system that enables the synthesis

For instance, when the molecule of interest is a polynucleotide, it may easily be synthesized using common chemical methods such as Alexander Todd’s H-phosphonate synthesis, Har Gobind Khorana’s phosphodiester synthesis, Letsinger and Reese Phosphotriester synthesis. Yet, preferably, the polynucleotides are synthesized
10 by solid-phase synthesis using phosphoramidite method and phosphoramidite building blocks derived from protected 2'-deoxynucleosides (dA, dC, dG, and T), ribonucleosides (A, C, G, and U), or chemically modified nucleosides. Those techniques and methods have been detailed in Brown, D. M. “*A brief history of oligonucleotide synthesis. Methods in Molecular Biology*” (Totowa, NJ, United States)
15 (1993), 20 (Protocols for Oligonucleotides and Analogs), 1-17, and in Reese (2005). “*Oligo- and poly-nucleotides: 50 years of chemical synthesis*”. Organic & Biomolecular Chemistry 3 (21): 3851.

According to the invention, DJ-1 protein and/or one of its homologs, as well as protein of interest, can easily be produced for example using synthesis by
20 recombinant DNA techniques.

Synthesis by recombinant DNA techniques, are well known from the person skilled in the art, and are further thoroughly detailed in Sambrook et al. (CSHL Press, 2001) and Ausubel et al. (John Wiley & Sons, 1988). The synthetic peptides of interest, i.e. the protein of interest and a DJ-1 protein and/or one of its homologs, may be
25 obtained by transforming a microorganism using an expression vector including a promoter and operator together with the said nucleotide sequence and causing such transformed microorganism to express the polypeptide. A non-human animal may also be used to produce the polypeptide using the said nucleotide sequence and the general techniques set forth in U.S. 4,276,282.

30 When peptides are produced by recombinant DNA techniques, they are produced in the form of recombinant peptides. Generally, for producing a recombinant peptide, a nucleic acid polymer having a nucleotide sequence encoding the reference peptide is obtained, and it is then introduced into a preferred expression vector. Preferably,

the nucleic acid polymer is a cDNA having a nucleotide sequence complementary to that of the nucleotide sequence of the mRNA coding for the reference peptide. The recombinant vector is then introduced in an expression system, usually a host cell, so as to produce said recombinant peptide.

- 5 A nucleic acid polymer having a nucleotide sequence encoding the reference peptide is preferably a cDNA having a nucleotide sequence complementary to that of the nucleotide sequence of the mRNA coding for the reference peptide. Said nucleotide sequence encoding the reference peptide may further comprise one or more sequences encoding a tag allowing purification of a protein, such as histidine (His)
10 tag, or glutathione S-transferase.

By “**vector**”, it is herein referred to a plasmid or a virus useful for performing procedures of molecular biology and genetic recombination. A vector may have the following features: an origin of replication, a selectable marker gene, and a cloning site for the insertion of a gene. A vector may be engineered to contain regulatory
15 sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector. A nucleic acid of interest can be inserted into a vector able to replicate, that is to say a vector comprising an origin of replication, in order to amplify said nucleic acid, or to express the protein encoded by said nucleic acid. These vectors are better known as “cloning vectors”
20 (to amplify a nucleic acid) or “expression vectors” (to express a protein), and are publicly available. Such vectors include, without limitation, plasmid vectors, cosmids, YACS, viral vectors (adenovirus, retrovirus, EBV episome), and phage vectors. According to the invention, an expression vector is a vector able to autonomously replicate in a host cell or able to be incorporated into the chromosome
25 of a host cell and that is designed for protein expression in cells. An expression vector may comprises an origin of replication, a selectable marker, and a cloning site for the insertion of a gene, a promoter, a translation initiation sequence such as a ribosomal binding site and start codon, a termination codon, and a transcription termination sequence. Methods for inserting a nucleic acid polymer in vectors are
30 known to those skilled in the art. Generally, a nucleic acid polymer is inserted into one or more restriction endonuclease site(s) using appropriate techniques known in the art, e.g. via ligation. It is additionally known to those skilled in the art that, depending on the nucleotide sequences present in the vector, said vector can

replicate in different host cells, and/or the protein encoded by said nucleic acid can be expressed in different host cells.

By “**expression system**”, it is herein referred to a cell, micro-organism or culture thereof, for the expression of recombinant proteins, such as herein described for example.

By “**host cell**” it is herein referred to a prokaryotic or a eukaryotic cell in which the recombinant vector of the invention can be introduced, such as to amplify the nucleic acid as described above, and/or to express the protein encoded by said nucleic acid, for example any one of a bacterium, a yeast, an animal cell, or an insect cell.

Examples of prokaryotic cells include, without limitation, bacteria such as Gram-negative bacteria of the genus *Escherichia* (e.g. *E. coli* RR1, LE392, X1776, W3110, DH5 alpha, JM109, KC8, K12), *Serratia*, *Pseudomonas*, *Erwinia*, *Methylobacterium*, *Rhodobacter*, *Salmonella* and *Zymomonas*, and Gram positive bacteria of the genus *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Arthrobacter*, and *Streptomyces*.

Examples of eukaryotic cells include, without limitation, cells isolated from fungi, plants, and animals. Such cells notably include yeasts of the genus *Saccharomyces*, cells of the fungi *Aspergillus*, *Neurospora*, *Fusarium* and *Trichoderma*, animal cells such as HEK293 cells, NIH3T3, Jurkat, MEF, Vero, HeLa, CHO, W138, BHK, COS-7, MDCK, C127, Saos, PC12, HKG, and insect cells Sf9, Sf21, Hi Five™ or of *Bombyx mori*. The use of insect cells is particularly described in the manual “Baculovirus Expression Vectors: A Laboratory Manual”, David R. O'Reilly et al. Oxford University Press, USA (1993). The expression vector may be introduced into a host cell according to any known method, depending on the type of such a host. Examples of such methods include, without limitation, transformation, electroporation, lipofection, calcium phosphate transfection, transfection using DEAE dextran, microinjection, and biolistics.

Introduction of the recombinant vector may be performed in order to obtain transient protein expression or permanent protein expression. Permanent protein expression is obtained for instance when the nucleic acid polymer having a nucleotide sequence encoding the reference peptide or the vector containing said nucleotide is stably integrated in the host cell. Techniques for obtaining stable

integration of a nucleotide sequence in a host cell have been used in high scale protein expression, and are routine techniques for the person skilled in the art, who may refer to Tingfeng (*Pharmaceuticals*; 6(5): 579-603; 2013). Briefly, after transfection with expression vectors containing expression cassettes for the recombinant protein and for selection marker genes, the cells are selected and gene-
5 amplified with the selection drug. Selection drugs such as methotrexate (MTX) and methionine sulfoximine (MSX) have been widely used in protein expression in the pharmaceutical industry. The host cell comprising the expression vector is cultured, and the recombinant peptide is then expressed and accumulates in the culture
10 medium. After completion of the culture, the recombinant peptide may be isolated and purified from the culture of the transformant according to any common method of isolating and purifying a polypeptide known in the art.

In the sense of the invention, the step of bringing together the molecule of interest and the DJ-1 protein and/or one of its homologs may be performed according to any
15 technique known in the art. For instance, the molecule of interest and the DJ-1 protein and/or one of its homologs may be first produced and purified, and then contacted.

In another example, either one of the molecule of interest and the DJ-1 protein and/or one of its homologs may be first produced and purified, and added to a
20 production system synthesizing the other molecule. For instance, a purified protein of interest may be added to an expression system expressing the DJ-1 protein. Conversely, a purified DJ-1 protein and/or one of its homologs may be added to an expression system expressing the protein of interest. Preferably, in that case, the expression system is an acellular expression system. In another example, a purified
25 polynucleotide of interest may be added to an expression system expressing the DJ-1 protein. Conversely, a purified DJ-1 protein and/or one of its homologs may be added to a production system synthesizing the molecule of interest.

In another example, DJ-1 protein and/or one of its homologs and the molecule of interest may be synthesized in the same production system. For instance, a protein
30 of interest and DJ-1 may be expressed from replication-compatible plasmids in the same cell, for example the protein of interest produced from a high copy number plasmid with a pUC replication origin, and DJ-1 produced from a low copy number plasmid (20 per cell) with pACYC replication origin.

Thus, in an embodiment of the invention, the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together directly by contacting the molecule of interest and a DJ-1 protein and/or one of its homologs.

5 In another embodiment of the invention, the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together by adding the molecule of interest to an production system synthesizing a DJ-1 protein and/or one of its homologs.

10 In yet another embodiment of the invention, the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together by adding a DJ-1 protein and/or one of its homologs to an production system synthesizing the protein of interest.

In another embodiment of the invention, the molecule of interest and a DJ-1 protein and/or one of its homologs are synthesized in the same production system, in particular they are expressed in the same expression system.

15 The purified proteins may be formulated so as to increase their interaction or stabilize their activity, or prevent their degradation. Suitable buffers commonly used include, but are not limited to, phosphate buffered saline (PBS), Tris buffer. mild surfactants such as Triton X-100 and antioxidants such as dithiothreitol may be added.

20 An object of the invention is thus the use of DJ-1 and/or one of its homologs for preventing or reversing glycation of a molecule of interest.

The person skilled in the art would perfectly understand that the activity of DJ-1 may be obtained indirectly by stimulating *in vivo* DJ-1 expression.

25 The invention thus further pertains to the use of DJ-1 inducers, preferably isothiocyanates, such as sulforaphane for example, or plant extracts, such as those from *Butea frondosa* or *Butea monosperma* for instance (Sujith et al. Asian J. of Pharmaceut. And Clin. Res. 4, 93).

30 The inventors have already shown that sulforaphane induces 3-fold the expression of Hsp31 and YhbO in bacterial cells (Figure 10B-C), and it is likely that it induces similarly DJ-1 in eukaryotic cells. Moreover, it has been reported that sulforaphane induces Nrf2 (Kerns et al. PNAS 104, 14460, Xue et al. Diabetes, 57, 2809)) and also induces glyoxalase 1 (Xue et al. Biochem J. 443, 213) and other stress genes which

belong to the Nrf2 regulon, to which DJ-1 also belongs (the oxidative and electrophile stress Nrf2 regulon).

An increase in the expression of Nrf2 leads to increased levels of DJ-1 and oxidative stress resistance genes (Ziaei, Schmedt, Chen, Jurkunas, 2013, Invest Ophthalmol Vis Sci. 54, 6724-34), and a decrease in Nrf2 in diabetes mellitus is correlated with decreased DJ-1 (Cheng, Chapple, Diabetes 62 4088). These results, our results with sulforaphane, and the novel status of DJ-1 as an electrophile stress resistance protein strongly suggest that the DJ-1 gene is a target of the Nrf2 transcriptional regulator which induces its expression. (Previous results suggesting that DJ-1 regulates Nrf2 levels, nuclear localization and activity (Clements et al., 2006, ref. 16) have been contradicted by Gan, Johnson and Johnson 2010, Eur. J. Neurosci., 31, 967).

The inventors have further discovered that the enzymes of the invention can be advantageously used for preventing or treating the cosmetic effects of glycation on the skin. To facilitate such use, the enzymes of the invention can be formulated into appropriate compositions.

Another object of the invention is thus the non-therapeutic use of a DJ-1 protein and/or one of its homologs or of a composition comprising a DJ-1 protein and/or one of its homologs, for preventing and/or treating the cosmetic effects of glycation on the skin.

According to the invention, the terms **“non-therapeutic use”** refer to a use that does not allow and/or is not intended for the prevention or for the treatment of a pathological state. A “non-therapeutic use” according to the invention can for example be the enhancement of physiological aspects or traits in the subject. Preferably, the non-therapeutic use of the invention is a cosmetic use. According to the invention, a **“cosmetic use”** is a use performed in view of the enhancement of the appearance of the human body.

By **“preventing the cosmetic effects of glycation on the skin”** it is herein referred to taking measures for reducing the risk of cosmetic effects of glycation on the skin.

By **“treating the cosmetic effects of glycation on the skin”** it is herein referred to reducing existing cosmetic effects of glycation on the skin.

Preferably, cosmetic effects due to the glycation of the skin are the loss of skin elasticity and increased stiffening of the skin.

Skin's elasticity and stiffness are both biomechanical properties of the human skin which can easily be measured by methods well known from the person skilled in the art. Methods for measuring skin elasticity have for example been described in
5 Edwards et al. (*Clin Dermatol.*;13(4):375-80, 1995), Garra BS. (*Ultrasound Q.*; 23(4):255-68, 2007). Methods for measuring skin stiffness have for example been described in Coutts et al. (*Skin Res Technol.*; 19(1):e37-44; 2013).

More preferably, the cosmetic effects of glycation on the skin are chosen from aging
10 of the skin due to glycation and cosmetic effects of UV on the skin.

By “**aging of the skin due to glycation**” it is herein referred to the effects of glycation on the physical properties or appearance of the skin.

By “**cosmetic effects of UV on the skin**” it is herein referred to the effects of UV on the physical properties or appearance of the skin. Preferably, in the context of the
15 invention, the cosmetic effects of UV on the skin are cosmetic effects due to the glycation of proteins.

It is within the ambit of a person skilled in the art to determine the appropriate amount of enzymes to be used in the invention according to usual criteria such as the age, sex, health, and surface of skin on which the enzymes are to be administered.

DJ-1 and/or one of its homologs can be formulated into compositions appropriate for the intended use. For example, the composition of the invention can be formulated for enteric or for topical administration.
20

In a particular embodiment, the composition of the invention is formulated for oral administration. For example, for a more pleasurable and convenient administration,
25 the composition can advantageously be formulated as an edible product, such as food or beverage.

Preferably, the composition of the invention, formulated for oral administration, is an edible product. According to the invention, the terms “**edible product**” refer to products and compositions in any physical form which are intended to be consumed
30 by human beings or lower animals in whole or part via the oral cavity.

In another embodiment, the composition of the invention is formulated for topic administration, more particularly for dermatological administration. Topic administration can be obtained by formulating the composition of the invention into forms suitable for that use. For instance, the composition of the invention can be formulated into cosmetic compositions such as gels, creams or lotions. Examples of composition for topic administration may for instance comprise DJ-1, DJ-1-related peptides or DJ-1 derived peptides, whose penetration through the skin may be increased by known transdermal delivery systems such as absorption enhancers or hyaluronic acid-DJ-1 conjugates or hyaluronic acid-DJ-1-related peptides conjugates. Such composition may also for example comprise small molecular weight DJ-1 inducers that would fight skin glycation by increasing DJ-1 levels in keratinocytes and fibroblasts.

In order to facilitate the skin penetration of the enzyme of interest, the composition of the invention may be formulated into liposomes formulations, for instance.

Also, in order to increase DJ-1 penetration through the skin, the composition of the invention may further comprise fatty acid(s) or any adsorption enhancers such as ethanol, linolenic acid, polyethylene glycol, and limonene.

Preferably, the composition of the invention, formulated for topic administration, is a topical cosmetic composition.

By “**topical cosmetic composition**”, it is herein referred to a solid, liquid or semi-solid composition, particularly intended for topic administration. Topical cosmetic compositions according to the invention can be in solid, liquid or semi-solid forms. Topical cosmetic compositions in solid forms can comprise for example powders, aerosols and plasters. Topical cosmetic compositions in liquid forms comprise for example lotions, liniments, solutions, emulsions and suspensions. Cosmetic compositions in liquid forms can comprise for instance ointments, creams, paste and gels.

It is well known to the skilled person that absorption of an active compound by the skin may be enhanced by excipients, preferably chosen according to the contemplated way of administration.

As it is intended for topical application, the composition of the invention may further comprise a cosmetically acceptable carrier or excipient, especially a carrier or

excipient suitable for topical administration, that is to say a carrier or excipient compatible with the skin.

By “**cosmetically acceptable excipient**” it is herein referred to excipients suitable with a cosmetical use, preferably excipients compatible with the skin. The cosmetical excipient can for example be chosen among excipients conventionally used in cosmetics, in particular topical cosmetics, such as pigments, dyes, polymers, surfactants, rheological agents, fragrances, electrolytes, pH modifiers, preservatives and mixtures thereof.

Another object of the invention is a kit comprising at least one reagent for determining the deglycase activity of DJ-1 and/or one of its homologs.

According to the invention, reagents for determining the deglycase activity of DJ-1 and/or one of its homologs comprise at least Nacetylcysteine (NacCys-S-CHOH-CO-CH₃, hemithioacetal)), preferably glycated Nacetylcysteine. Advantageously, the kit of the invention further comprises dithio-bis-nitrobenzoate (DTNB). Yet advantageously, the kit further comprises S-(N-hydroxy-N-methylcarbamoyl) glutathione.

Nacetylcysteine hemithioacetal is a substrate of DJ-1, which is degraded into Nacetylcysteine (NacCysSH) and lactate by the deglycase. DTNB enables detection of NacCysSH and lactate formation.

S-(N-hydroxy-N-methylcarbamoyl)glutathione is an inhibitor of glyoxalase which might (glyoxalase 1) interfere with the assay.

According to another embodiment, reagents for determining the deglycase activity of DJ-1 and/or one of its homologs comprises at least glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Advantageously, the kit of the invention further comprise dithio-bis-nitrobenzoate (DTNB). Yet advantageously, the kit further comprises NADP, arsenate and oxidized glutathione.

GAPDH produces NADPH (from glyceraldehyde-3-phosphate, NADP and arsenate), which reduces oxidized glutathione in the presence of glutathione reductase thereby producing reduced glutathione which gives a yellow color with DTNB

The level or/and activity of DJ-1 in red blood cells (which contain easily detectable DJ-1 amounts (Xu et al. Blood cells, Molecules and diseases 45, 227-232)) of patients

with glycation-related diseases could be determined by using several methods. DJ-1 activity could be determined on erythrocyte lysates

In an embodiment, the kit of the invention essentially consists of reagents for determining the deglycase activity of DJ-1 and/or one of its homologs.

- 5 By “**essentially consists of**” it is herein meant that the kit of the invention may comprise, in addition to the essential reagents described, other “non-essential” reagents, insofar as the essential characteristics of the kit of the invention remain unaffected. Preferably, the kit of the invention comprises a minimum of reagents other than reagents for determining the deglycase activity of DJ-1 and/or one of its
10 homologs.

FIGURE LEGEND

- Figure 1. A) Cell viability. Bacteria were grown until stationary phase ($OD_{600} = 3$) in LB medium containing 0.6% glucose, and left under agitation and aeration at 37°C for 48 h in the same medium. 4 µl of bacteria (diluted 100, 1000, 10.000 and 100.000-
15 fold (from left to right) were spotted on LB plates for viability determination. B) Protection by YhbO against UV radiations. Wild-type bacteria and wild-type bacteria overexpressing YhbO were streaked as a vertical line on a LB plate, submitted to UV stress (254 nm, 1J/m²) for 10 to 90 seconds from bottom to top, and incubated overnight at 37°C.
- 20 Figure 2. A) Protein aggregation in yajL and yhbO mutants. Bacteria (wt, lanes 1-3, yhbO mutant, lanes 4-6 and yajL mutant, lanes 7-9) were grown in LB medium at 37°C in exponential phase ($OD_{600} = 0.8$) and submitted for 20 min to electrophile stress in the presence of 0 (lanes 1, 4, 7), 2 (lanes 2, 5, 8) and 4 mM (lanes 3, 6, 9) methylglyoxal. Bacteria were collected by centrifugation, lysed by ultrasonic
25 disruption in Tris 30 mM pH 8, and 8,000g pellets were analyzed by SDS-PAGE and revealed by Coomassie Blue staining. B) YhbO overexpression reduces glycation of an overexpressed protein. The protein kinase YeaG was expressed in Escherichia coli containing both the pET-21a-yeaG plasmid and the YhbO expression plasmid pBAD33-yhbO. YhbO was either uninduced (filled circles) or induced by 3 mM arabinose
30 (triangles). Bacteria were grown in LB medium at 37°C for 5 hours until $OD_{600} = 4$, and bacterial protein extracts (after elimination of low molecular weight metabolites by gel permeation) containing 55% YeaG protein were analyzed for AGE (advanced

glycation end products) content by recording their fluorescence between 400-500 nm (arbitrary units, excitation at 340 nm) (empty circles represent buffer spectrum).

Figure 3. Protein glycation in deglycase mutants and protection by DJ-1 against glycation. A) Deglycase mutants display increased glycation levels. The 3,000g supernatants of bacterial lysates (10 µg) from the wt strain, the yhbO, yajL, yhbOyajL and hchayhbOyajL mutants were probed with anti-AGE antibodies. B) DJ-1 prevents protein glycation. The 3,000g supernatants of bacterial lysates (10 µg) from the wild type strain, the wild type strain transformed with the DJ-1 expression plasmid, the yajL mutant and the yajL mutant transformed with the DJ-1 expression plasmid were probed with anti-AGE antibodies.

Figure 4. Deglycation of N-acetyl-cysteine, N-acetyl-arginine and N-acetyl-lysine. A) N-acetyl-cysteine (2 mM) was incubated with 2 mM MGO at 22°C in 150 µl of 50 mM sodium phosphate pH 7.0, resulting in the formation of glycated NacCys in seconds (not shown) ; 2 µM DJ-1 were successively added to one sample at time 0 s, 300 s and 1100s, whereas no addition was made to the other sample, and the concentration of glycated NacCys was followed by absorbance at 288 nm. (Figure 2A displays the evolution of the absorbance at 288 nm after (or without) DJ-1 addition). B, C) N-acetyl-cysteine (2 mM) was incubated with 2 mM MGO at 22°C in 150 µl of 50 mM sodium phosphate pH 7.0, resulting in the formation of glycated NacCys in seconds ; 4 µM DJ-1 was added after 2 min ; concentrations of glycated NacCys (filled circles), NacCys (empty circles) and lactate (empty squares) were determined by reverse RP-HPLC (Fig. 2B displays the absorbance of NacCysglc, NacCys and lactate, 0, 15, 30, 60, 75 and 90 min after DJ-1 addition, and Fig 2C displays their concentrations before and after DJ-1 addition). D) N-acetyl-arginine (80 mM) was incubated at 22°C in 150 µl of 50 mM sodium phosphate pH 7.0 with 80 mM MGO. After 40 min, the mixture was 4-fold diluted and incubated with 4 µM DJ-1 ; concentrations of NacArg (empty circles) and NacArgglyc (filled circles) and lactate (empty squares) were measured by RP-HPLC. E) N-acetyl-lysine (80 mM) was incubated at 22°C in 150 µl of 50 mM sodium phosphate pH 7.0 with 80 mM MGO. After 40 min, the mixture was 4-fold diluted and incubated with 4 µM DJ-1 ; concentrations of NacLys (empty circles) and NacLysglyc (filled circles) and lactate (empty squares) were measured by RP-HPLC.

Figure 5. Deglycation and reactivation of thiol proteins. A) BSA (700 µM) was incubated with 100 mM MGO in 50 mM sodium phosphate pH 7.0 at 37°C for 110 min,

afterwhich it was separated from MGO by gel filtration, incubated at 22°C at a concentration of 300 µM, in the absence (empty circles) or presence (filled circles) of 3 µM DJ-1, and SH groups were titrated at different times (squares represent titrated BSA SH groups without MGO treatment). B) GAPDH (300 µM monomers) was incubated with 5 mM MGO in 50 mM sodium phosphate pH 7.0, and GAPDH activity (empty squares) and SH groups (empty triangles) were assayed at different times. At 40 min, GAPDH was separated from MGO on a Biogel P2 column, incubated (60 µM monomer) in the absence (empty symbols) or presence (filled symbols) of 2 µM DJ-1, and GAPDH activity (squares) and SH groups (triangles) were assayed. In a separate experiment, 2 µM DJ-1 was added to the initial glycation mixture, and GAPDH activity was measured (filled circles). C) GAPDH (300 µM monomers) was incubated with 5 mM GO in 50 mM sodium phosphate pH 7.0, either in the absence (empty circles) or presence (filled circles) of 3 µM DJ-1, and GAPDH activity was assayed at different times.

Figure 6. Deglycation of serum albumin. A, B) Prevention of Schiff base formation between lysines and methylglyoxal. A) 70 µM BSA was incubated for 3 hours with 6 mM MGO in 50 mM sodium phosphate pH 7.0 at 22°C, in the absence (curve 3) or presence of 3 µM DJ-1 (curve 2) and absorption spectra were recorded (spectrum of BSA without MGO treatment is displayed in curve 1) B) 70 µM BSA was incubated with 6 mM MGO in 50 mM sodium phosphate pH 7.0 at 22°C, in the absence (empty circles) or presence of 1.5 µM (filled circles) or 3 µM (filled triangles) DJ-1, and Schiff base formation between BSA lysines and MGO was determined by measuring the absorbance at 333 nm (100 on the ordinate represents OD= 0.1). MGO consumption by BSA, MGO and 1.5 µM DJ-1 (filled squares), BSA and MGO (empty squares), and 1.5 µM DJ-1 and MGO (empty triangles) was also measured. C) Deglycation of arginines : 500 µM BSA was incubated with 20 mM MGO for 120 minutes at 37°C in the absence (grey columns) or presence of 5 µM DJ-1 (black columns) and free arginines were titrated with phenanthrenequinone. D) Prevention of BSA glycation by ribose. BSA (150 µM) was incubated at 37°C for 3 days in 50 mM sodium phosphate buffer containing 1M ribose, in the absence or presence of 2 µM YhbO, and BSA glycation was analysed by fluorescence emission (arbitrary units)(excitation at 340 nm) (the spectrum of BSA incubated without ribose is also displayed).

Figure 7. Deglycation of FBP aldolase and aspartate aminotransferase, and deglycation of DJ-1: A, B) Fructose biphosphate aldolase reactivation : Fructose 1,6-biphosphate aldolase activity was measured after enzyme incubation at a

concentration of 15 μ M with 10 mM MGO (A) or 5 mM GO (B), in the absence of DJ-1 (empty circles) or in the presence of 4 μ M DJ-1 added to the initial glycation mixture (filled circles), after 10 min (filled triangles) or 20 min (filled squares). C) Aspartate aminotransferase reactivation: aspartate aminotransferase activity was measured after incubation for 30 min at 37°C at a concentration of 10 μ M with 0, 1, 2, 5 mM MGO (grey columns), after an additional 2 min reactivation by 4 μ M DJ-1 (black columns). D) Deglycation by DJ-1 of fructose 1,6-biphosphate aldolase and aspartate aminotransferase. The glycation status of aldolase (A) and aspartate aminotransferase (T) were assayed by immunoblotting with anti-AGE antibodies of commercial enzymes (A, T), of aldolase glycated by GO for 30 min, in the absence (AG) or presence (AGD) of DJ-1 (in conditions described in B), and of aspartate aminotransferase glycated for 30 min by 2 mM MGO in the absence (TM) or presence (TMD) of DJ-1 (in conditions described in C, except that DJ-1 was added in the initial glycation mixture). E) DJ-1 deglycation. Seven proteins (0.8 μ g each) (beta-casein, DnaK, BSA, DJ-1, GAPDH, fructose biphospahte aldolase and aspartate aminotransferase) were assayed for glycation by 5 mM MGO (4 hours at 37°C in 50 mM sodium phosphate buffer pH 7.0). After SDS-PAGE and transfer to nitrocellulose membranes, proteins were probed with anti-AGE antibodies.

Figure 8. Deglycation of nucleotides and DNA. Nucleotide deglycation : Following samples were analyzed by RP-HPLC (Grace Vydac C18) equilibrated in 100 mM potassium phosphate pH 5.5 : A, B, C) dGTP (500 μ M) incubated for 2 h at 37°C in 50 mM sodium phosphate pH 7.0, without (A) or with 5 mM MGO in the absence (B) or presence (C) of 5 μ M DJ-1 ; D, E, F) GTP (500 μ M) incubated for 2 h at 37°C without (D) or with 5 mM MGO (E, F), and subsequently treated for 30 min with 5 μ M DJ-1 (F) ; G, H, I) GTP (500 μ M) incubated overnight at 37°C with 5 mM MGO; 5 μ M DJ-1 was present in the glycation mixture (H), 5 μ M DJ-1 was added during 30 min, after the overnight incubation (I). DNA deglycation : following samples were analyzed by RP-HPLC on a SynChropack RP-4 column eluted with a linear gradient of 0-70% acronitrile in 50 mM triethanoamine pH 7.4 : primer FORyeaG (30 μ l, 30 μ M DNA (1080 μ M nucleotides)) incubated for 4 days at 37°C in J) 50 mM phosphate buffer (pH 7.0), K) phosphate buffer containing 5 mM MGO and 4 μ M DJ-1, and L) phosphate buffer containing 5 mM MGO.

Figure 9. The apparent glyoxalase activity of DJ-1 results from its deglycase activity. A) Kinetics of the reaction. 2 mM MGO was incubated with 1 μ M DJ-1 (empty circles),

8 μM DJ-1 (empty squares) or 8 μM DJ-1 and 15 μM BSA (filled circles), and MGO degradation was measured. B) Velocity versus DJ-1 concentration. DJ-1 at the indicated concentrations was incubated for 20 min in the presence of 2 mM MGO, and MGO degradation was measured. Results are presented as a function of DJ-1 concentration (filled circles) and of the square of DJ-1 concentration (empty circles) C) Stimulation by BSA. 2 μM DJ-1 was incubated for 10 min with 2 mM MGO (filled circles) or 2 mM GO (empty circles) in the presence of BSA at indicated concentrations, and MGO degradation was measured.

Figure 10. Expression of the deglycases and induction by sulforaphane. A) Expression of Hsp31 and YhbO as a function of growth phase. *E. coli* K12 MG1655 was grown at 37°C in LB medium from OD₆₀₀ 0.3 to 4, and Hsp31 (grey) and YhbO (black) levels were probed by immunoblotting whole bacteria after treatment with trichloroacetic acid, SDS-PAGE and transfer to nitrocellulose membrane. Bacteria entered into stationary phase at approximately OD = 1.3. B, C) Induction of Hsp31 and YhbO expression by sulforaphane. Bacteria were grown at 30°C to OD 0.3, supplemented with micromolar sulforaphane concentrations, and probed for Hsp31 (grey columns) and YhbO (black columns) levels 80 min later (at an OD of 1.1). Results displayed in C were obtained by quantification of protein bands with ImageJ and are the mean value of two experiments.

Figure 11. GDP/GMP sanitization and DNA/RNA deglycation.

A to F: Nucleotide deglycation.

The following samples were analyzed by C18 RP-HPLC: For GDP deglycation, 500 μM GDP was incubated for 2 h at 37°C in 50 mM sodium phosphate (pH 7.0), without (A) or with 5 mM MGO, in the absence (B) or presence (C) of 5 μM DJ-1; D, E) For GMP deglycation, 500 μM GMP was incubated for 2 h at 37°C without (D) or with 5 mM MGO in the presence of 5 μM DJ-1. E); F) GTP (500 μM) was incubated for 2 h without (left panel) or with 5 mM GO, in the absence (middle panel) or in the presence of 1 μM DJ-1 (right panel).

G, H and I: kinetics of nucleotide deglycation

G) Kinetics of GTP deglycation. GTP (5 mM) was incubated with 5 mM MGO for 2 h, and the kinetics of GTP deglycation in the absence of DJ-1 (empty circles) or by 1 μM (filled circles) or 10 μM (triangles) DJ-1 were determined by RP-HPLC. H) Kinetics of

GTP deglycation by 1 μ M DJ-1 (triangles), Hsp31 (diamonds), YhbO (filled circles), YajL (squares), 2 μ M YajL (diamonds), 10 μ M YhbO (crosses) or in the absence of deglycase (empty circles) were measured; GTP (500 μ M) was incubated for 2 h with 5 mM MGO, and deglycation was followed by measuring the surface area of peak 2 (as shown in Fig 2E). I) Stimulation of lactate production by DJ-1 by nucleotides. DJ-1 was incubated for 30 min with 2 mM MGO in the presence of dGTP (triangles), GTP (filled circles) or GDP (empty circles) at the indicated concentrations.

J: 16S ribosomal RNA deglycation

J) Fluorescence of glycated or deglycated 16S rRNA .16S rRNA was incubated for 48 h alone (empty circles), or with 5 mM MGO in the absence (filled squares) or presence (filled circles) of DJ-1, and the fluorescence emission was measured.

Figure 12. DNA deglycation

A: HPLC after hydrolysis by phosphodiesterase.

The following samples were incubated as described, DNA was separated from MGO using the Qiaquick nucleotide removal kit (Quiagen), treated for 1 h by pancreatic DNase I (10 units), followed by nuclease S1 (100 units), and analyzed by C18 RP-HPLC: DNA (16 μ M) was incubated for 10 h at 37°C in buffer (upper panel), buffer containing 2 mM MGO (middle panel), buffer containing 2 mM MGO and 4 μ M DJ-1 (lower panel).

B: PCR with native primer (DNA), glycated (DNA/MGO) or repaired (DNA/MGO/DJ-1).

DNA PCR was performed in the presence of the four dXTPs, plasmid DNA coding for protein YeaG, reverse primer, and forward primer (DNA), forward primer glycated (for 0h or 6 h) by MGO (DNA/MGO) or forward primer glycated by MGO in the presence of DJ-1 (DNA/MGO/DJ-1), as described in A.

EXAMPLES

MATERIALS AND METHODS

Bacterial strains and plasmids, and DJ-1, Hsp31, YhbO and YajL expression and purification.

The wild type *E. coli* strain MG1655 and BW25113 were used as parental strains (4, 7, 12). The *hcha::kan*, *yhbO::kan* and *yajL::kan* deletion mutants were from the Keio collection (33) and are described in (4, 7, 12). For construction of the *yhbOyajL* and *hchayhbOyajL* mutants, the *kan* resistance marker of the *yajL* mutant was
5 eliminated, and *yhbO::kan* and *hcha::cm* were transduced into the *yajL* strain by P1 transduction. The Hsp31 overexpressing plasmid pINTYEDU, YhbO overexpressing plasmid pET21a-yhbO, YajL overexpressing plasmid pCA24N-yajL and DJ-1 overexpressing plasmid pET-21a-DJ-1 are described in (3-4, 7, 9, 13). The low copy number YhbO complementing plasmid pBAD-yhbO was constructed as described in
10 (7). The substrate protein YeaG described in Fig 2B was expressed from plasmid pET21a-yeaG (34)

DJ-1, Hsp31, YhbO and YajL expression and purification.

Proteins were overexpressed from the expression plasmids described above. They
15 were purified from bacterial extracts by DEAE Sephacel and hydroxyapatite chromatography and stored in N2 gassed 50 mM sodium phosphate buffer pH 7.0 at -80°C.

DNA arrays experiments.

20 RNA extraction, DNA array experiments, microarray analysis and data processing were performed as described in (35).

Preparation of bacterial extracts and protein aggregates.

Bacterial lysates were prepared by ultrasonic disruption (Branson sonicator, 5 x 20 s,
25 40% duty) of bacteria resuspended at OD600 = 100, in 50 mM sodium phosphate pH 7.0, 50 mM NaCl. For protein purification, protein extracts were the supernatants of bacterial lysates centrifuged for 1 hour at 50,000 x g. For immunodetection of AGEs, protein extracts were the supernatants of bacterial lysates centrifuged for 5 min at 3,000 x g. Protein aggregates were prepared by differential centrifugation : cellular
30 debris were eliminated from bacterial lysates by centrifugation at 3,000 x g, and the

supernatant was centrifuged at 8,000 x g for 5 min in a Beckman microfuge, leading to protein-aggregates (in the pellet).

Immunoblotting procedures.

5 Hsp31, YajL and YhbO were detected by immunoblotting bacterial protein extracts with anti-Hsp31, anti-YhbO and anti-YajL antibodies prepared as described in (4, 7, 9). For immunoblotting with anti-AGE antibodies, we used protein extracts composed of the 3,000g supernatants (10 µg) of bacterial lysates, or purified proteins (0.2-1 µg). After SDS-PAGE, proteins were transferred to a nitrocellulose membrane and
10 probed with anti-AGE antibodies as indicated by the manufacturer (Cell Biolabs Inc.).

Glycation status of protein extracts.

The glycation status of protein extracts or of purified proteins was determined by immunoblotting with anti-AGE antibodies (see above, Fig 3A, 3B, 7D, 7E), by
15 measuring protein absorbance between 300 and 400 nm (Fig.6A) or by measuring protein fluorescence between 400 and 500 nm (excitation at 340 nm, Fig. 2B, 6D)).

Glycation/deglycation of N-acetyl-cysteine, N-acetyl-arginine and N-acetyl-lysine.

All experiments were performed in N₂-gassed 50 mM sodium phosphate buffer pH
20 7.0. Glycation/deglycation of NacCys by MGO/DJ-1 was followed by the hemithioacetal absorbance at 288 nm (27) and by reverse phase HPLC. Glycation/deglycation of NacArg and NacLys was followed by RP-HPLC. NacArg and NacLys were also detected by using phenanthrene quinone and p-benzoquinone, respectively (36). Lactate was analyzed by RP-HPLC and with D- and L-lactate
25 dehydrogenases. Amino acids, glycated amino acids and lactate were analyzed by reverse phase-HPLC (Shimadzu HPLC system interfaced with the LabSolution software). Samples were injected onto a Kromasil Eternity C18 column (length = 250 nm, internal diameter = 4 mm, particle size = 5 µm) at 40°C. Mobile phase for isocratic elution consisted of 25 mmol/L monobasic sodium phosphate, 0.3 mmol/L of
30 the ion-pairing agent 1-octane sulfonic acid, 4% (v/v) methanol, pH 2.7, adjusted

with 85% phosphoric acid. Flow rate was 1 ml/min. Products were monitored spectrophotometrically at 210 and 280 nm and quantified by integration of the peak absorbance area, employing a calibration curve established with various known concentrations of amino acids and lactate.

5

Glycation/deglycation of BSA, GAPDH, fructose 1,6-biphosphate aldolase and aspartate aminotransferase.

All experiments were performed in N₂-gassed 50 mM sodium phosphate buffer pH 7.0. Glycation/deglycation of BSA cysteine was followed by DTNB titration (37). BSA
10 was incubated with 100 mM MGO for 110 min at 37°C, separated by gel filtration on Bio-Gel P2, and incubated at a concentration of 300 µM, in the absence or presence of 3 µM DJ-1. Glycation/deglycation of GAPDH active-site cysteine was followed by measuring GAPDH activity (37) and by titrating thiol groups with DTNB. GAPDH (300
15 µM monomers, from rabbit muscle) was incubated with 5 mM MGO at 22°C for 40 min, separated from MGO by gel filtration on a Bio-Gel P2, and incubated in the absence or presence of 2 µM DJ-1. In experiments where DJ-1 was added to initial glycation mixtures, GAPDH activity was measured by adding 5 µl of the 100-fold diluted glycation mixture (omitting the gel permeation step) to a cuvette containing
20 150 µl of substrates. Glycation/deglycation of BSA lysines was followed by measuring absorbance at 333 nm (38) ; MGO concentrations during glycation/deglycation experiments were measured with 2,4-dinitrophenylhydrazine (15), and lactate concentrations were measured by using L- or D- lactate dehydrogenase. Glycation/deglycation of BSA arginines was measured by using phenanthrenequinone (32). Glycation/deglycation of fructose biphosphate aldolase A (from rabbit muscle,
25 EC 4.1.2.13, obtained from Sigma) and aspartate aminotransferase (from pig heart, cytoplasmic, EC 2.6.1.1, obtained from Sigma) were followed by measuring enzymatic activities (39, 40), and detecting their glycated forms with anti-AGE antibodies (Cell Biolabs Inc.).

30 **Glycation/deglycation of GTP, dGTP, dCTP, DNA and RNA.**

All experiments were performed in N₂-gassed 50 mM sodium phosphate buffer pH 7.0. Glycation/deglycation of nucleotides was followed by reverse phase HPLC on a

C18 Vydac column equilibrated in 100 mM potassium phosphate pH 5.5. Glycation/deglycation of DNA was followed by reverse phase HPLC (SynChropack RP-C4) eluted with a linear gradient of 0-70% acetonitrile in 50 mM triethanoamine pH 7.4.

5

Glyoxalase activity.

DJ-1 (YajL, YhbO or Hsp31), MGO or GO, were added to N₂-gassed 50 mM sodium phosphate buffer pH 7.0 at 22°C in a total volume of 70 µl. The disappearance of MGO or GO was measured as follows : the reaction was stopped by adding 10 µl of the reaction mixture to 120 µl of 0.1% 2,4-dinitrophenylhydrazine solution ; the solution was incubated for 15 min at 22°C, 160 µl of 10% NaOH was added, and after 15 min absorbance was measured (540 nm for MGO and 570 nm for GO). The appearance of lactate was measured by using L-or D-lactate dehydrogenase. The formation of glycolate was measured by RP-HPLC (15).

15 **RESULTS**

Electrophile stress sensitivities of the yajL and yhbO mutants.

The yhbO and yajL mutants were more sensitive to electrophile stress than their parent. They suffered from 10 to 100 fold viability losses compared to the parental strain on LB plates containing 1 mM electrophile such as formaldehyde, glyoxal or methylglyoxal (not shown). Moreover, the yajL and yhbOyajL mutants suffered from 10 to 100-fold viability losses after 2 days incubation in LB medium containing 0.6% glucose (Fig.1A) whereas no viability loss was observed without glucose (not shown). This result suggests that mutants suffer from electrophile stress resulting from glucose metabolism. Viability losses were canceled when the yajL mutant was transformed with DJ-1-expressing plasmids (not shown). Thus, YhbO, YajL, and DJ-1 play crucial roles in electrophile stress protection.

Protection by the deglycases against environmental stresses, including UV stress.

It is noteworthy that mutants deficient in DJ-1, Hsp31, YhbO or YajL are sensitive to many environmental stresses, including heat stress, oxidative stress, osmotic stress, acid stress, protein stress and UV stress (2, 5-7, 9). This may be explained because they miss chaperone (Hsp31, YajL, DJ-1) or covalent chaperone activities (YajL, DJ-1), or because they miss the deglycase activities described in our work, resulting in glycation and inactivation of enzymes and nucleic acids .

Interestingly, overexpression of deglycases help to fight environmental stresses. We have shown in previous work (7, 9) that YhbO and YajL protect cells against oxidative stress. Here, we show that overexpression of YhbO (Fig. 1B) or DJ-1 (not shown) in wild type cells protects against UV stress. Such property might be used for fighting UV damage in skin, since DJ-1 is expressed in fibroblasts and keratinocytes.

Overexpression of electrophile stress genes in the yajL and yhbO mutants.

DNA array experiments showed that genes involved in electrophile stress protection were overexpressed in yajL (35) and yhbO (unpublished results) mutants, including genes coding for glyoxalase GloA/GloB (2-fold), Hsp31 (alias YedU or HchA) (14-fold), aldoketoreductases YqhD and YqhE (7-fold), glutathione-S-transferase Gst (10-fold), multidrug transporters MdlA and MdlB (3-fold) and amadoriase YniA (4-fold) (not shown). These results suggest that YajL and YhbO play important roles in electrophile stress protection.

Protein aggregation in yajL and yhbO mutants.

Electrophile stress causes protein aggregation (30). SDS-PAGE analysis of bacterial extracts (8,000g pellets) showed an increase in protein aggregation in yhbO and yajL mutants, both before and after MGO addition to bacterial cultures, whereas the wild-type strain displayed only a small quantity of aggregated proteins (Fig. 2A).

Moreover, overexpression of YhbO (from the pBAD-yhbO plasmid (7)) in wild-type strains overexpressing the YeaG kinase (from pET-21a-yeaG) or chaperone DnaK (from pET-21a-dnaK) decreased protein aggregation (from 7% to 2% for the YeaG-containing extract, not shown), and decreased protein glycation (measured by

fluorescence of protein extracts containing overexpressed YeaG (Fig 2B) or DnaK (not shown)).

Protein glycation in deglycase mutants.

- 5 Electrophile stress results in protein glycation (30). Protein extracts from bacteria grown overnight in LB medium containing 0.6% glucose were separated by SDS-PAGE, transferred to nitrocellulose and probed with anti-AGE antibodies (Fig. 3A). The wild-type strain displayed a small quantity of glycated proteins; in contrast, we observed a moderate increase in protein glycation in the yhbO and yajL mutants, and a
10 massive increase in the yhbOyajL and hchayhbOyajL mutants.

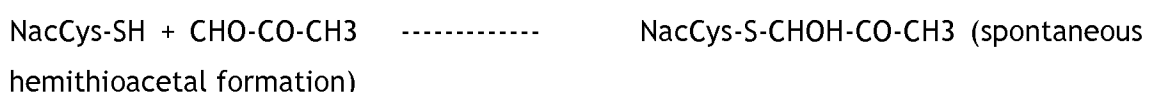
Remarkably, when DJ-1 was expressed in the wild-type strain and the yajL mutant, protein glycation decreased (Fig. 3B), showing that overexpression of the deglycases of the invention can be used to prevent or reduce protein glycation even in wild type cells.

15

DJ-1 deglycates cysteine, arginine and lysine.

- Nacetylcysteine deglycation.

- As reported previously (27), methylglyoxal reacted in a few seconds with Nacetylcysteine (2 mM) to form a hemithioacetal adduct detectable by its
20 absorbance at 288 nm (Fig. 4A) or by reverse phase HPLC (Fig. 4B). The hemithioacetal was stable for several hours (Fig. 4A), but disappeared with a half-time of 20-30 min in the presence of 5 μ M DJ-1 ($k_{cat} = 0.4 \text{ s}^{-1}$) (Fig 4A-C). Its degradation quantitatively resulted in the formation of N-acetylcysteine and lactate (Fig. 4C). Lactate analysis with L- and D-lactate dehydrogenases showed that NacCys
25 deglycation produced 67% L-lactate and 33% D-lactate (not shown). The following reactions are likely to have occurred :



NacCys-S-CHOH-CO-CH₃ ----- NacCys-S-CO-CHOH-CH₃ (H migration
catalyzed by DJ-1 (reminiscent of glyoxalase 1)

NacCys-S-CO-CHOH-CH₃ ----- NacCys-SH + COOH-CHOH-CH₃ (thioester
hydrolysis by DJ-1, reminiscent of glyoxalase 2)

5

Thus, DJ-1 degrades glycated N-acetylcysteine into N-acetylcysteine and a 67/33 mixture of L- and D- lactate. Similar results were obtained with Hsp31 and YhbO (not shown).

- N-acetylarginine deglycation.

10 80 mM NacArg was incubated with 80 mM methylglyoxal for 40 min, and the formation of 24 mM glycated NacArg was observed (i.e. 6 mM after a 4-fold dilution for analysis by reverse phase HPLC). Under similar conditions, Lo et al. reported the formation of the aminocarbinal NacArg-methylglyoxal glycosylamine, and a low amount of Nac-Nδ-(5-methyl-4-imidazolone-2yl) (27)). When the glycation mixture
15 (4-fold diluted) was incubated with 4 μM DJ-1, glycated NacArg decreased from 6 to 1 mM, leading to formation of NacArg (which increased from 14 to 19.5 mM) and lactate (which increased from 0 to 6 mM) (Fig. 4D). Thus, DJ-1 deglycates glycated N-acetylarginine into N-acetylarginine and L-lactate (L-lactate was exclusively formed by NacArg deglycation (not shown)).

20 N-acetyllysine deglycation.

80 mM NaLys was incubated with 80 mM methylglyoxal for 40 min to form 16 mM glycated NaLys (4 mM after a 4-fold dilution for RP-HPLC analysis) which likely contained the aminocarbinal NaLys-methylglyoxal glycosylamine (AcLysMGglycos) and a low amount of bis(Nacetyllysine)methylglyoxal glycosylamine
25 (AcLys)2MGglycos) (27)). When the glycation mixture (after a 4-fold dilution) was incubated with 4 μM DJ-1, glycated NaLys decreased from 4 to 0.5 mM, NaLys increased from 16 to 19.5 mM and lactate increased from 0 to 3.7 mM (Fig. 4D). Thus, DJ-1 deglycates glycated N-acetyllysine into N-acetyllysine and lactate (L-lactate was exclusively formed by NaLys deglycation ; data not shown). Following
30 reactions are likely to have occurred :

NacArg/Lys-NH₂ + CHO-CO-CH₃----- NacArg/Lys-NH-CHOH-CO-CH₃ (spontaneous aminocarbamol formation)

NacArg/Lys-NH-CHOH-CO-CH₃ ----- NacArg/Lys-NH-CO-CHOH-CH₃ (H migration catalyzed by DJ-1 (reminiscent of glyoxalase 1))

5 NacArg/Lys-NH-CO-CHOH-CH₃ -----NacArg/Lys-NH₂ + COOH-CHOH-CH₃ (amidolysis by DJ-1 (reminiscent of peptidase activity))

These results show that DJ-1 degrades aminocarbamol intermediates formed upon arginine/lysine glycation by methylglyoxal, with the quantitative release of
 10 arginine/lysine, and L-lactate. Thus, in contrast with known deglycates which only deglycate lysines (32), DJ-1 deglycates cysteines, arginines and lysines, the three major glycated amino acids.

DJ-1 deglycates protein cysteines.

15 Bovine serum albumin (BSA) contains a single exposed cysteine (Cys 34 titratable by DTNB) that is involved in oxidative stress protection (41). We reported previously that DJ-1 could repair this cysteine from sulphenylation (13). After BSA treatment with methylglyoxal (MGO) for 110 min at 37°C, the number of BSA-titrated cysteines decreased from 0.62 to 0.28 (Fig. 5A). We separated BSA from MGO by gel
 20 permeation, and incubated 300 µM BSA at 22°C, either alone or in the presence of 3 µM DJ-1. After DJ-1 addition, titratable cysteines of BSA raised from 0.28 to 0.52 with a half-time of 2 min ; they remained constant when BSA was incubated in the absence of DJ-1 (Fig. 5A). Thus, DJ-1 deglycates the exposed cysteine of BSA with a kcat of 0.3 s⁻¹.

25 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) performs covalent catalysis with its active site cysteine 150 (37). We inactivated GAPDH (300 µM monomers) with 5 mM MGO, while DTNB-titrated cysteines decreased from 1.9 to 1.1, reflecting the covalent reaction of the active-site cysteine with MGO (37) (Fig. 5B) : we then separated GAPDH from MGO by gel permeation, and incubated 60 µM GAPDH, either
 30 alone or in the presence of 2 µM DJ-1. In the absence of DJ-1, GAPDH activity decreased further to 10% of its initial activity, whereas in the presence of DJ-1, it

increased to 50% of its initial activity with a half time of 2 min, while DTNB-titrated cysteines increased from 1.1 to 1.6 with similar kinetics. Thus, DJ-1 reactivates GAPDH by deglycating its active-site cysteine. Whereas GAPDH reactivation was not complete in this experiment, when 2 μ M DJ-1 was added to the initial glycation mixture, the GAPDH activity did not decrease, suggesting that DJ-1 immediately deglycates GAPDH, thereby preventing the formation of deglycation-resistant intermediates. As shown in Fig. 5C, when added to the initial glycation mixture, DJ-1 (2 μ M) fully protected GAPDH (300 μ M) against glycation by 5 mM glyoxal.

Remarquably, if we compare the concentrations of MGO, substrate protein and DJ-1 used in these experiments with in vivo concentrations, MGO and GO concentrations are >1000-fold higher (5 mM versus 1-5 μ M for MGO and 0.2-2 μ M for GO (30)), substrate protein concentration is 9-fold lower (11 mg/ml GAPDH versus 100 mg/ml total cytoplasmic protein (30)), and DJ-1 concentrations are similar (2 μ M in our experiment versus approximately 1 μ M in skin, 10 μ M in brain and 40 μ M in liver (www.genecards.org)). Thus, DJ-1 efficiently deglycates proteins in our experiments, even though its work load in vitro is impressively heavier than in vivo.

DJ-1 prevents Schiff base formation between serum albumin lysines and MGO, but does not degrade Schiff bases.

When proteins react with methylglyoxal, they display an increase in light absorption between 300 and 400 nm, which can be attributed to Schiff base linkages between lysine side chains and MGO (the initially formed aminocarbinol Lys-NH-CHOH-CO-CH₃ dehydrates in minutes to hours, to form the Schiff base Lys-N=CH-CO-CH₃ (38)). When 70 μ M BSA was incubated with 6 mM MGO for 3 hours, its absorption spectrum displayed a shoulder between 300 and 370 nm. Remarkably, the shoulder was considerably lower when 3 μ M DJ-1 was present in the BSA/MGO mixture (Fig. 6A). In samples containing 70 μ M BSA, 6 mM MGO and 3 μ M DJ-1, the rate of Schiff base formation was reduced by 4-fold compared with that of samples without DJ-1 (Fig. 6B), while the concentration of MGO decreased by less than 15% in 60 min. This result suggests that the anti-glycation effect of DJ-1 did not result from MGO depletion, but instead from its deglycase activity; Moreover, in samples containing 6 mM MGO and 3 μ M DJ-1, the MGO concentration did not decrease significantly,

suggesting that MGO depletion correlates with deglycation (see below). Similar results were obtained with YhbO (not shown).

In contrast with its ability to prevent Schiff base formation, DJ-1 did not degrade Schiff bases : we glycated 70 μ M BSA with 6 mM MGO for 2 hours, and incubated glycated BSA (after its separation from MGO by gel permeation) in the presence of 4 μ M DJ-1. We did not observe any decrease in BSA absorbance at 333 nm over 90 minutes, suggesting that DJ-1 was unable to deglycate Schiff bases (not shown). The ability of DJ-1 to prevent Schiff base formation, but not to degrade them suggests that it degrades the aminocarbinoxyl (Schiff base precursor). Consequently, the designation of Schiff bases (which form in hours) and Amadori products (which form in days) as early glycation intermediates should be revisited, since for DJ-1, these compounds represent late intermediates, whereas hemithioacetals and aminocarbinoxyls, which form in seconds to minutes, represent early intermediates.

15 DJ-1 deglycates serum albumin arginines.

Arginine is a major target of glycation by dicarbonyl compounds (30). When 500 μ M BSA was incubated with 20 mM MGO for 2 hours, its titratable arginines (with phenanthrenequinone) decreased from 18.8 to 11.8 per BSA molecule as a consequence of arginine glycation, whereas they remained constant when 5 μ M DJ-1 was added to the glycation mixture (Fig. 6C). Thus DJ-1 efficiently deglycates BSA arginines.

YhbO protects serum albumin against glycation by ribose and glucose.

Whereas deglycates of the invention principally protect proteins against glycation by methylglyoxal and glyoxal, YhbO, and to a lesser extent the other deglycates, protect serum albumin against glycation by ribose and galactose. When BSA (150 μ M) was incubated at 37°C for 3 days in the presence of 1M ribose or for 7 days in the presence of 1M glucose, the addition of 2 μ M YhbO decreased the extent of BSA glycation, as judged by the 400-500 nm fluorescence spectrum (Fig. 6D for ribose, not shown for glucose).

DJ-1 deglycates FBP aldolase and aspartate transaminase.

The active site of rabbit muscle fructose-1,6-biphosphate aldolase (FBP) contains 3 lysines (K108, K147, and K230 which forms a covalent bond with dihydroxyacetone phosphate) and an arginine (R304 which stabilizes the substrate phosphate anion). As reported previously (39), 15 μ M FBP was rapidly inactivated by 10 mM MGO (Fig. 7A); 4 μ M DJ-1 prevented (when added to the initial glycation mixture), stopped (when added after 10 min) or partially reversed (when added after 20 min) FBP inactivation (Fig. 7A). FBP (15 μ M) was also inactivated by 5 mM GO, and 4 μ M DJ-1 prevented its inactivation (Fig. 7B) (Hsp31, YhbO and YajL also prevented the inactivation of FBP by 5 mM MGO (not shown). As shown in Fig. 7D, in which aldolase was revealed with anti-AGE antibodies, DJ-1 afforded full protection to aldolase against glycation by glyoxal.

The active site of pig heart cytoplasmic aspartate aminotransferase contains two arginines, R293 and R387 involved in aspartate binding, and a lysine, K259 which forms a transient covalent bond with pyridoxal phosphate. As reported previously (40), aspartate aminotransferase (10 μ M) was inactivated by 20-90% by 1-5 mM methylglyoxal (Fig. 7C). The addition of 2 μ M DJ-1 30 min after MGO rapidly restored (in 2 minutes) up to 90-100% of aspartate aminotransferase activity following 1-2 mM methylglyoxal stress, and up to 60% after a 5 mM methylglyoxal stress (Fig. 7C). As shown in Fig. 7D, in which aspartate aminotransferase was revealed with anti-AGE antibodies, DJ-1 afforded full protection against glycation by 2 mM methylglyoxal.

DJ-1 deglycation.

Seven proteins were incubated separately with 5 mM MGO for 4 hours at 37°C (beta-casein, DnaK, BSA, DJ-1, GAPDH, fructose biphosphate aldolase and aspartate aminotransferase) and their glycation status was checked by using anti-AGE antibodies. All proteins, except DJ-1, were glycated (Fig. 7E). The apparent virginity of DJ-1 results from its deglycase activity, with DJ-1 molecules deglycating each other (except for the active site which may undergo autodeglycation).

DJ-1 deglycates nucleotides, DNAs and RNAs.

DNA is susceptible to glycation by glyoxal and methylglyoxal, and the most reactive nucleotide is by far deoxyguanosine (dG) (estimates of glycated dG in human mononuclear leukocytes were 16 dG-MG per 106 nucleotides (versus 3 for the famous major oxidative adduct 8-oxo-dG), suggesting that glycation by methylglyoxal is an important DNA damage in vivo (26) (deoxyadenosine and deoxycytosine are glycated to a much lesser extent). DNA glycation results in mutations, deletions, DNA strand breaks and cytotoxicity, and glycation induces DNA repair systems. In fact, we observed in yajL and yhbO mutants a 4-6-fold overexpression of genes coding for translesional DNA polymerases (Pol2 and Pol4) and of proteins involved in DNA repair (MutS, UvrABCD, RecA) (34).

We first investigated nucleotide deglycation by DJ-1 and its homologs. When analyzed by reverse-phase HPLC, dGTP and GTP migrated each as a single peak (Fig. 8, A, D). 80% and 46% of dGTP and GTP (500 μ M), respectively, were glycated after 2 hours incubation with 5 mM MGO (Fig. 8, B, E) ; most glycated dGTP and GTP migrated each as peak 2 eluting 1 min after dGTP and GTP. In the presence of DJ-1 (5 μ M) in the glycation mixture, dGTP (panel C) and GTP glycation (not shown) were negligible, suggesting that DJ-1 efficiently deglycates these nucleotides. When 5 μ M DJ-1 was added to glycated GTP after 2 hours, it efficiently performed its deglycation (7F), showing that glycated GTP is deglycable by DJ-1 several hours after glycation onset. Hsp31, YhbO and YajL were also able to deglycate these nucleotides (not shown).

When GTP was incubated overnight with 5 mM MGO, glycated GTP migrated as three major peaks (peaks 2-4) (Fig. 8G). Peak 2 contained deglycable molecular species (see below), probably similar to those of panel E, whereas peaks 3 and 4 contained undeglycable molecular species (advanced glycation end products) evolved from peak 2 (our study of GTP glycation as a function of time showed a precursor-product relationship between molecular species of peak 2 and those found in peaks 3 and 4 (not shown). The presence of DJ-1 in the overnight glycation mixture decreased GTP glycation to negligible levels (Fig. 8H). In contrast, when DJ-1 was added after overnight glycation of GTP (Fig. 8I), it only deglycated molecular species displayed in peaks 2 of panels E and G, and missing in panel I, whereas a minimal fraction of species contained in peaks 3 and 4 was deglycated. Thus, when present in the glycation mixture (as it occurs in vivo) DJ-1 efficiently prevented glycation of nucleotides because it deglycates them as glycation goes along, before the formation

of undeglycable molecular species (generally designed as AGEs (advanced glycation endproducts)). In contrast, when added after glycation, DJ-1 only deglycated deglycable species.

DJ-1 and its prokaryotic homologs protected DNA and RNA against glycation by MGO.

The DNA primer FORyeaG GGTGGTTGCTCTTCACATATGAATATATTCGATCAC (30 μ M) was incubated with 5 mM MGO in the absence or presence of 1 μ M DJ-1 (for 4 days at 37°C) and analyzed DNAs by reverse phase HPLC : native DNA and DNA incubated with MGO and DJ-1, both eluted at 16.60 min (in a 25 min 0-70% acetonitrile gradient) whereas DNA incubated with MGO eluted at 25 min (Fig. 8J). In a similar experiment, DJ-1 protected Escherichia coli tRNAs against glycation by 5 mM MGO (not shown).

Thus, DJ-1 and its prokaryotic homologs are the first nucleotides/nucleic acid deglycases ever described, and constitute a novel class of nucleic acid repair enzymes.

The previously reported glyoxalase activity of DJ-1 reflects its deglycase activity.

DJ-1 and Hsp31 have been reported to function as glutathione-independent glyoxalases, displaying 1.000-fold lower activities than glutathione-dependent glyoxalases Glo1 and Glo2 (15, 20). The previously reported glyoxalase activities of DJ-1 and Hsp31 reflect their deglycase activities. Firstly, kinetics of MGO degradation (at micromolar DJ-1 concentrations) displayed a lag, which was likely required for spontaneous formation of the actual substrate, i.e. glycated DJ-1 or glycated BSA : the duration of the lag ranged from 40 min at 1 μ M DJ-1, to 5 min at 8 μ M DJ-1 and 1 min at 8 μ M DJ-1 in the presence of 15 μ M BSA (Fig. 9A). Secondly, the apparent glyoxalase activity of DJ-1 increased with the square of DJ-1 concentration, in accordance with DJ-1 being both enzyme (deglycase) and a substrate (glycated DJ-1) (Fig. 9B). Thirdly, the negligible levels of apparent glyoxalase activity of DJ-1 (1 μ M) measured during the first 10 minutes were strongly stimulated by BSA with an apparent K_a of approximately 5 μ M BSA (Fig. 9C) ; stimulation by BSA was also observed when glyoxal was used as a glycating agent (Fig. 9C). These results are consistent with the DJ-1 substrates being glycated proteins (DJ-1 or BSA in this section) instead of glyoxals.

Expression of deglycases Hsp31 and YhbO, and induction by sulforaphane.

The expression of electrophile stress genes in eukaryotes is controlled (at least in part) by transcription factor Nrf2, which is induced by either oxidative or electrophile stress, but also by less harmful compounds such as extracts of flowers *Butea frondosa* or the isothiocyanate sulforaphane (a natural compound found in vegetables such as broccolis (42)). In bacteria, the transcriptional response to electrophiles is complex (induction of the oxydative OxyR and SoxRS responses, the NemR response and the DNA damage SOS response), and appears to reflect the covalent modification of specific proteins and DNA bases rather than integration of gene regulation through a master regulator (43). We first investigated the expression of Hsp31 and YhbO as a function of the bacterial phase growth. As shown in Figure 10 A, Hsp31 displayed higher expression levels in stationary phase (as previously reported (44)) whereas YhbO was preferentially expressed in exponential phase. Since methylglyoxal and most electrophiles are bacteriostatic and/or bactericide, we tried to induce the deglycases with sulforaphane, a naturally occurring isothiocyanate derived from cruciferous vegetables, which is a potent inducer of phase 2 cytoprotective enzymes and protects cells against electrophiles, oxidative stress and inflammation (42) (sulforaphane likely acts by stabilization of Nrf2, which results in the induction of oxidative and electrophile stress resistance genes). As shown in Figure 10 B, C, sulforaphane, at micromolar concentrations which did not affect bacterial growth, induced Hsp31 and YhbO expression by 3 to 4-fold.

REFERENCES

1. Quigley, P. M., K. Korotkov, F. Baneyx, and W. G. Hol. 2003. The 1.6-A crystal structure of the class of chaperones represented by *Escherichia coli* Hsp31 reveals a putative catalytic triad. *Proc Natl Acad Sci U S A* 100:3137-42.
2. Sastry, M. S., K. Korotkov, Y. Brodsky, and F. Baneyx. 2002. Hsp31, the *Escherichia coli* yedU gene product, is a molecular chaperone whose activity is inhibited by ATP at high temperatures. *J Biol Chem* 277:46026-34.

3. Lee, S. J., S. J. Kim, I. K. Kim, J. Ko, C. S. Jeong, G. H. Kim, C. Park, S. O. Kang, P. G. Suh, H. S. Lee, and S. S. Cha. 2003. Crystal structures of human DJ-1 and *Escherichia coli* Hsp31, which share an evolutionarily conserved domain. *J Biol Chem* 278:44552-9.
- 5 4. Malki, A., T. Caldas, J. Abdallah, R. Kern, V. Eckey, S. J. Kim, S. S. Cha, H. Mori, and G. Richarme. 2005. Peptidase activity of the *Escherichia coli* Hsp31 chaperone. *J Biol Chem* 280:14420-6.
5. Cookson, M. R. 2005. The biochemistry of Parkinson's disease. *Annu Rev Biochem* 74:29-52
- 10 6. Wilson, M. A. 2011. The role of cysteine oxidation in DJ-1 function and dysfunction. *Antioxid Redox Signal* 15:111-22.
- 7 Abdallah, J., Caldas, T., Kthiri, F., Kern, R. and Richarme, G. (2007) YhbO protects cells against multiple stresses. *J. Bacteriol.* 189, 9140-9144.
8. Wilson, M. A., D. Ringe, and G. A. Petsko. 2005. The atomic resolution crystal
15 structure of the YajL (ThiJ) protein from *Escherichia coli*: a close prokaryotic
homologue of the Parkinsonism-associated protein DJ-1. *J Mol Biol* 353:678-91.
9. Kthiri, F., H. T. Le, V. Gautier, T. Caldas, A. Malki, A. Landoulsi, C. Bohn, P. Bouloc, and G. Richarme. 2010. Protein aggregation in a mutant deficient in YajL, the bacterial homolog of the Parkinsonism-associated protein DJ-1. *J Biol Chem*
20 285:10328-36.
10. Shendelman, S., A. Jonason, C. Martinat, T. Leete, and A. Abeliovich. 2004. DJ-1 is a redox-dependent molecular chaperone that inhibits alpha-synuclein aggregate formation. *PLoS Biol* 2:e362.
11. Zhou, W., M. Zhu, M. A. Wilson, G. A. Petsko, and A. L. Fink. 2006. The
25 oxidation state of DJ-1 regulates its chaperone activity toward alpha-synuclein. *J Mol Biol* 356:1036-48.
12. Le, H. T., V. Gautier, F. Kthiri, A. Malki, N. Messaoudi, M. Mihoub, A. Landoulsi, Y. J. An, S. S. Cha, and G. Richarme. 2012. YajL, prokaryotic homolog of Parkinsonism-associated protein DJ-1, functions as a covalent chaperone for thiol
30 proteome. *J Biol Chem* 287:5861-70.

13. Gautier, V., H. T. Le, A. Malki, N. Messaoudi, T. Caldas, F. Kthiri, A. Landoulsi, and G. Richarme. 2012. YajL, the prokaryotic homolog of the parkinsonism-associated protein DJ-1, protects cells against protein sulfenylation. *J Mol Biol* 421:662-70.
- 5 14. Andres-Mateos, E., C. Perier, L. Zhang, B. Blanchard-Fillion, T. M. Greco, B. Thomas, H. S. Ko, M. Sasaki, H. Ischiropoulos, S. Przedborski, T. M. Dawson, and V. L. Dawson. 2007. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. *Proc Natl Acad Sci U S A* 104:14807-12.
15. Lee, J.Y., Song, J., Kwon, K., Jang, S., Kim, C., Baek, J. and Park, C. (2012)
- 10 Human DJ-1 and its homologs are novel glyoxalases. *Hum. Mol. Genetics* 21, 3215-3225.
16. Clements, C. M., R. S. McNally, B. J. Conti, T. W. Mak, and J. P. Ting. 2006. DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. *Proc Natl Acad Sci U S A* 103:15091-6.
- 15 17. Junn, E., H. Taniguchi, B. S. Jeong, X. Zhao, H. Ichijo, and M. M. Mouradian. 2005. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. *Proc Natl Acad Sci U S A* 102:9691-6.
18. van der Brug, M. P., J. Blackinton, J. Chandran, L. Y. Hao, A. Lal, K. Mazan-Mamczarz, J. Martindale, C. Xie, R. Ahmad, K. J. Thomas, A. Beilina, J. R. Gibbs, J.
- 20 Ding, A. J. Myers, M. Zhan, H. Cai, N. M. Bonini, M. Gorospe, and M. R. Cookson. 2008. RNA binding activity of the recessive Parkinsonism protein DJ-1 supports involvement in multiple cellular pathways. *Proc Natl Acad Sci U S A* 105:10244-9.
19. Guzman, J. N., J. Sanchez-Padilla, D. Wokosin, J. Kondapalli, E. Ilijic, P. T. Schumacker, and D. J. Surmeier. 2010. Oxidant stress evoked by pacemaking in
- 25 dopaminergic neurons is attenuated by DJ-1. *Nature* 473:696-700.
20. Subedi, K.P., Choi, D., Kim, I., Min, B. and Park, C. (2011) Hsp31 of *Escherichia coli* K-12 is glyoxalase III. *Mol. Microbiol.* 81, 926-936.
21. Kern, R., Malki, A., Holmgren, A. and Richarme, G. (2003) Chaperone properties of *E. coli* thioredoxin and thioredoxin reductase. *Biochem. J.* 371, 965-
- 30 972.

22. Hestertamp T. and Bukau, B. (1996) The Escherichia coli Trigger factor. FEBS Lett. 389, 32-34.
23. Huyen, T., Elamphungporn, W., Mader, U., Liebeke, M., Lalk, M., Hecker, M., Helmann, J.D. and Antelmann, H. (2009) Genome-wide responses to carbonyl electrophiles in *Bacillus subtilis*. Mol. Microbiol. 71, 876.
24. Maillard, L.C. (1912) Action des acides aminés sur les sucres: formation des mélanoidines par voie méthodique. C.R. Acad. Sci. Ser.2 154, 66-68.
25. Rahbar, S. (1968) An abnormal hemoglobin in red cells in diabetics. Clin. Chim. Acta 22, 296-298.
- 10 26. Thornalley, P.J. (2008) Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems. Role in ageing and disease. Drug metabol. Drug Interact. 23, 125-150.
27. Lo, T.W.C., Westwood, M.E., McLellan, Selwood, T. and Thornalley, P.J. (1994) Binding and modification of proteins by methylglyoxal under physiological conditions. J. Biol. Chem. 269, 32199-3230
- 15 28. Jez, J.M., Bennett, M.J., Schlegel, B.P., Lewis, M. and Penning, T.M. (1997) Comparative anatomy of the aldo-keto reductase family. Biochem. J. 326, 625-636
29. Neuberg, C. (1913) The destruction of lactic aldehyde and methylglyoxal by animal organs. Biochem.Z. 49, 502-506.
- 20 30. Rabbani, N. and Thornalley, P.J. (2012) Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino acids 42, 1133-1142.
31. Smitherman, P.K., Townsend, A.J., Kute, T.E. and Morrow, C.S. (2004) Role of multidrug resistance protein 2 in alkylating agent detoxification. J. Pharmacol. Exp. Ther. 308, 260-268.
- 25 32. Van Schaftingen, E, Collard, F., Wiame, E. and Veiga-da Cunha, M. (2012) Enzymatic repair of Amadori products. Amino acids 42, 1143-1150.
33. Datsenko K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* using PCR products Proc. Natl. Acad. Sci. USA 97, 6640-6645.

34. Tagourt, J., Landoulsi, A. and Richarme G. (2009) Cloning, expression and purification of the stress kinase YeaG Prot. Exp. Purif. 59, 79-85.
35. Messaoudi, N., Gautier, V., Kthiri, F., Lelandais, G., Mihoub, M., Joseleau-Petit, D., Caldas, T., Bohn, C., Tolosa, L., Rao, G., Tao, K., Landoulsi, A., Boulloc, P., and Richarme, G. (2013) Global stress response in a prokaryotic model of DJ-1-associated Parkinsonism. J. Bacteriol. 195, 1167-1178.
36. Patthy, L. and Smith, E.L. (1975) Identification of functional arginine residues in ribonulcease A and lysozyme. J. Biol. Chem. 250, 565-569.
37. Morgan, P.E., Dean, R.T. and Davies, M.J. (2002) Inactivation of cellular enzymes by carbonyls and protein-bound glycation/glycoxidation products. Arch. Biochem. Biophys. 403, 259-269.
38. McLaughlin, J.A., Pethig, R. and Szent-Györgyi, A. (1980) Spectroscopic studies of the protein-methylglyoxal adduct. Proc. Natl. Acad. Sci. U.S.A. 77, 949-951.
39. Leoncini, G., Ronchi, S., Maresca, M. and Bonsignore, A. (1980) Studies of the interaction of fructose 1,6-P2 with methylglyoxal. Ital. J. Biochem. 29, 289-299.
40. Seidler, N.W. and Kowalzski, C. (2003) Methylglyoxal-induced glycation affects protein topography. Arch. Biochem. Biophys. 410, 149-154.
41. Carballal, S., Tadi, R., Kirk, M.C., Barnes, S., Freeman, B.A. and Alvarez, B. (2003) Sulfenic acid formation in human serum albumin by hydrogen peroxide and peroxinitrite. Biochemistry, 42, 9906-9914.
42. Dinkova-Kostova, A.T. and Kostov, R.V. (2012) Glucosinolates and isothiocyanates in health and disease. Trends Mol. Medicine 18, 337-347.
43. Ozyamak, E., de Almeida, C., de Moura, A., Miller, S. and Booth, I.R. (2013) Integrated stress response of Escherichia coli to methylglyoxal. Mol. Microbiol. 88, 936-950.
44. Mujacic, M. and Baneyx, F. (2006) Regulation of Escherichia coli hchA, a stress-inducible gene encoding molecular chaperone Hsp31 Mol. Microbiol. 60, 2576-1585.

45. Curtin, N.J. (2012) DNA repair dysregulation from cancer driver to therapeutic targets. *Nature Rev.* 12, 801-817.

46. Yamagishi, S. (2012) Potential clinical utility of advanced glycation product cross-link breakers in age-and diabete-associated disorders. *Rejuvenation Res.* 15,
5 564572.

CLAIMS

1. An *in vitro* method for preventing and/or reversing glycation of a molecule of interest, said method comprising the step of bringing together the molecule of interest and a DJ-1 protein and/or one of its homologs.
5
2. The *in vitro* method of claim 1, wherein the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together directly by contacting the molecule of interest and a DJ-1 protein and/or one of its homologs.
10
3. The *in vitro* method of claim 1, wherein the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together by adding the molecule of interest to an production system synthesizing a DJ-1 protein and/or one of its homologs.
15
4. The *in vitro* method of claim 1, wherein the molecule of interest and a DJ-1 protein and/or one of its homologs are brought together by adding a DJ-1 protein and/or one of its homologs to an production system synthesizing the molecule of interest.
20
5. The *in vitro* method of claim 1, wherein the molecule of interest and a DJ-1 protein and/or one of its homologs are synthesized in the same production system.
6. The *in vitro* method of any of claims 1 to 5, wherein the molecule of interest is a protein, a nucleotide or a polynucleotide.
25
7. The *in vitro* method of any of claims 1 to 6, wherein the DJ-1 homologue is chosen from the list consisting in YajL protein, YhbO protein, and Hsp31 protein.
- 30 8. The *in vitro* method of claim 7, wherein the protein of interest the YhbO protein.
9. The *in vitro* method of any of claims 1 to 8, said method being for preventing or reversing glycation on both amino and thiol group, more particularly on lysine, arginine and cysteine residues of said protein of interest.
35

10. The *in vitro* method of any of claims 1 to 9, said method being for reversing glycation of said protein of interest.
11. The *in vitro* method of any of claims 1 to 10, said method being for preventing glycation of said protein of interest.
12. Use of a DJ-1 protein and/or one of its homologs for preventing or reversing deglycation of a molecule of interest.
13. Non-therapeutic use of a DJ-1 protein and/or one of its homologs or of a composition comprising a DJ-1 protein and/or one of its homologs, for preventing and/or treating the cosmetic effects of glycation on the skin.
14. Non-therapeutic use according to claim 13, wherein said cosmetic effects of glycation on the skin are aging of the skin due to glycation.
15. Non-therapeutic use according to claim 13, wherein said cosmetic effects of glycation on the skin are cosmetic effects of UV on the skin.
16. A kit comprising at least one reagent for determining the deglycase activity of a DJ-1 protein and/or one of its homologs.
17. Non-therapeutic use of DJ-1 inducers for preventing and/or treating the cosmetic effects of glycation on the skin, wherein DJ-1 inducers are preferably chosen from the list consisting in Nrf2 activators, preferably sulforaphane, plant extracts, preferably from *Butea frondosa* or *Butea monosperma*, and compounds discovered by screening chemical libraries for DJ-1 induction.

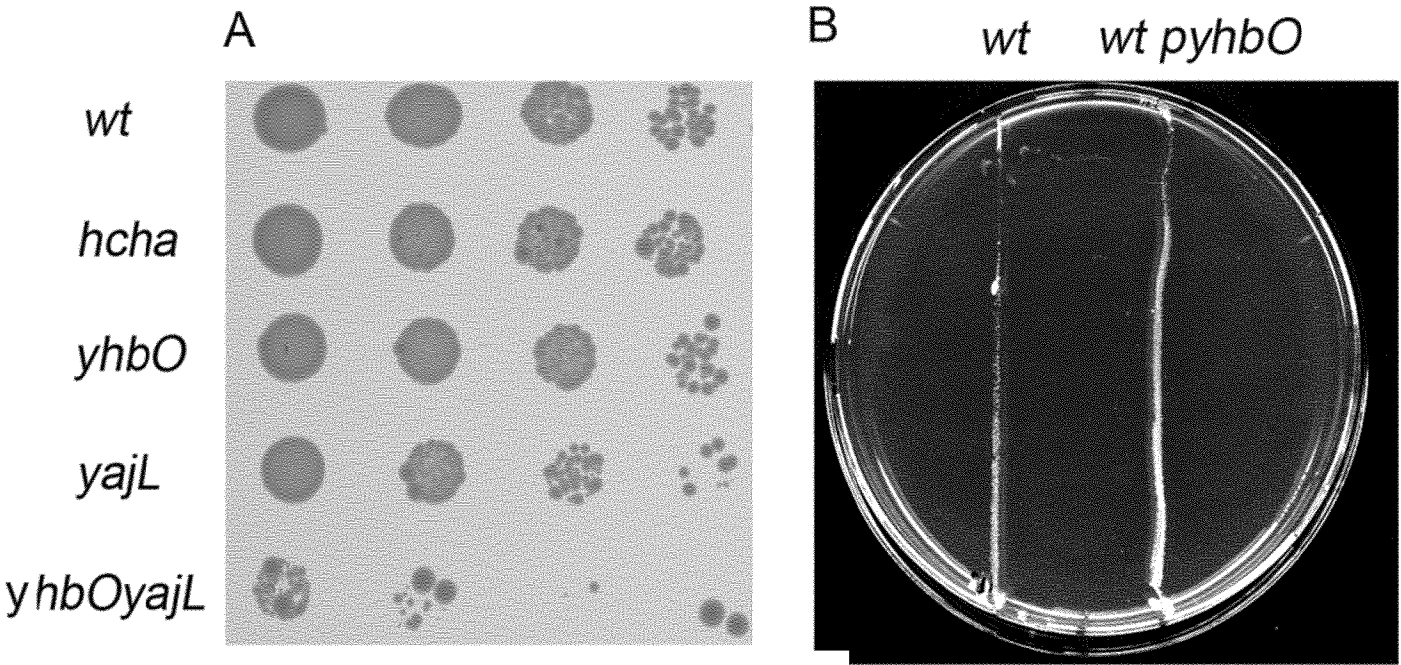


Fig. 1

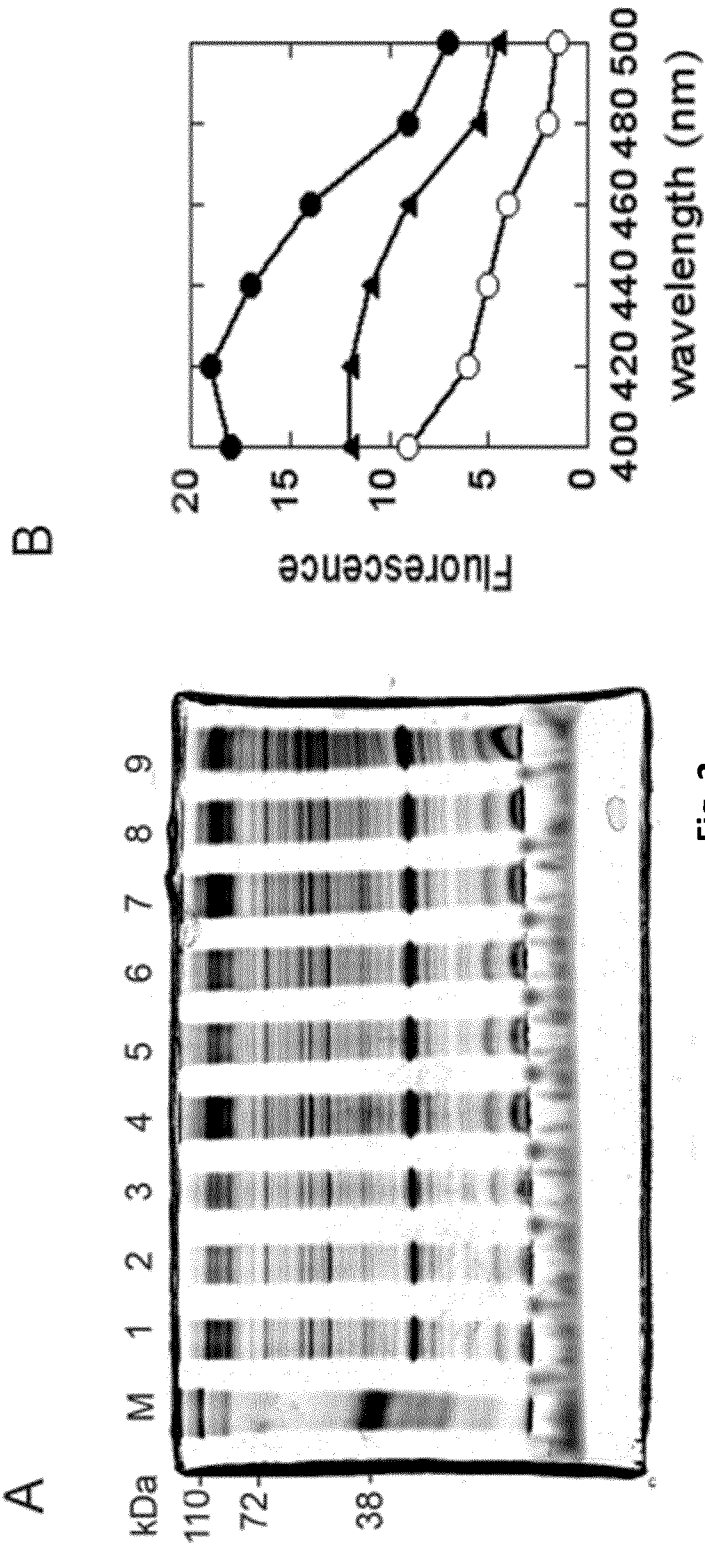


Fig. 2

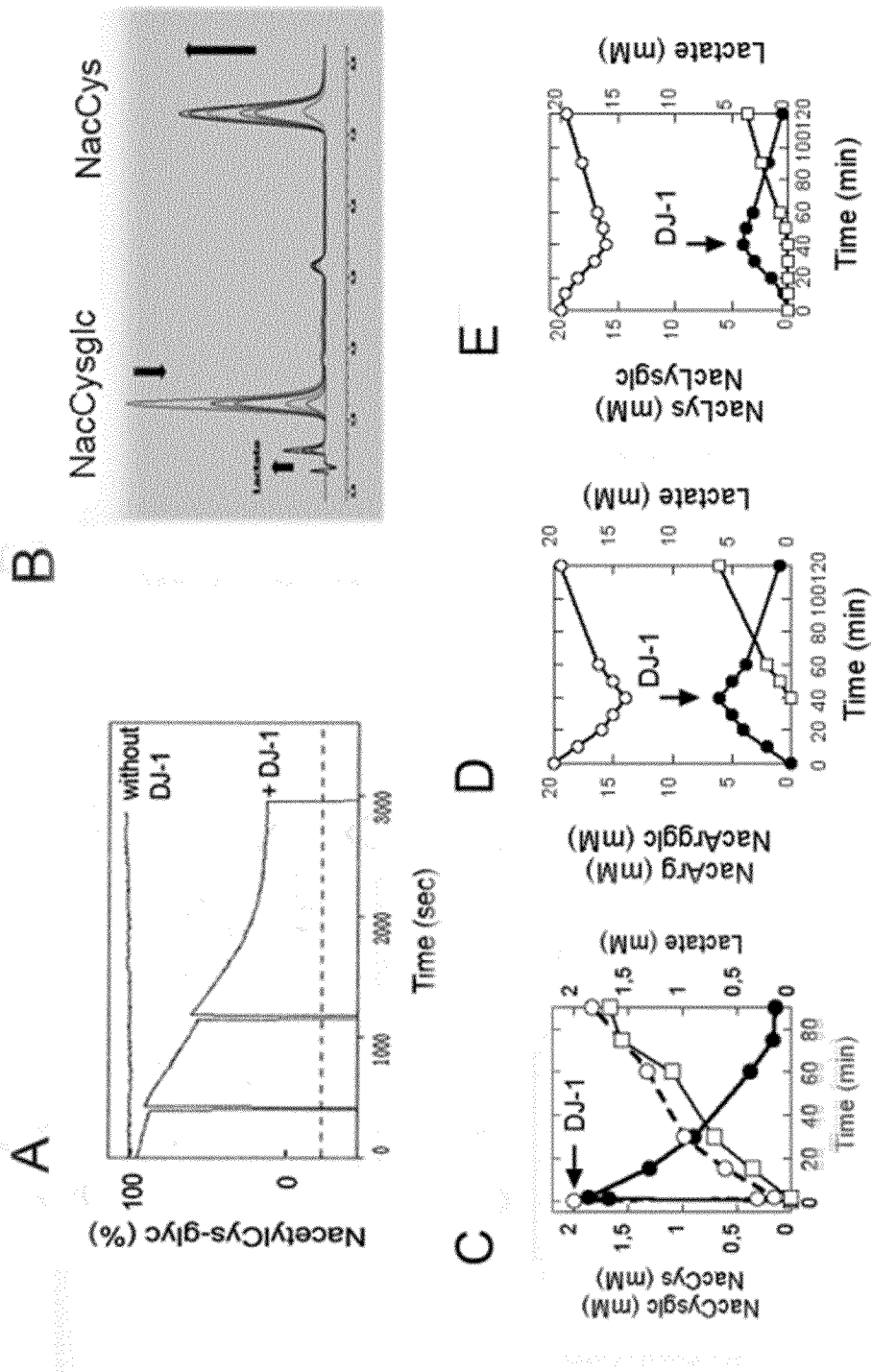


Fig. 4

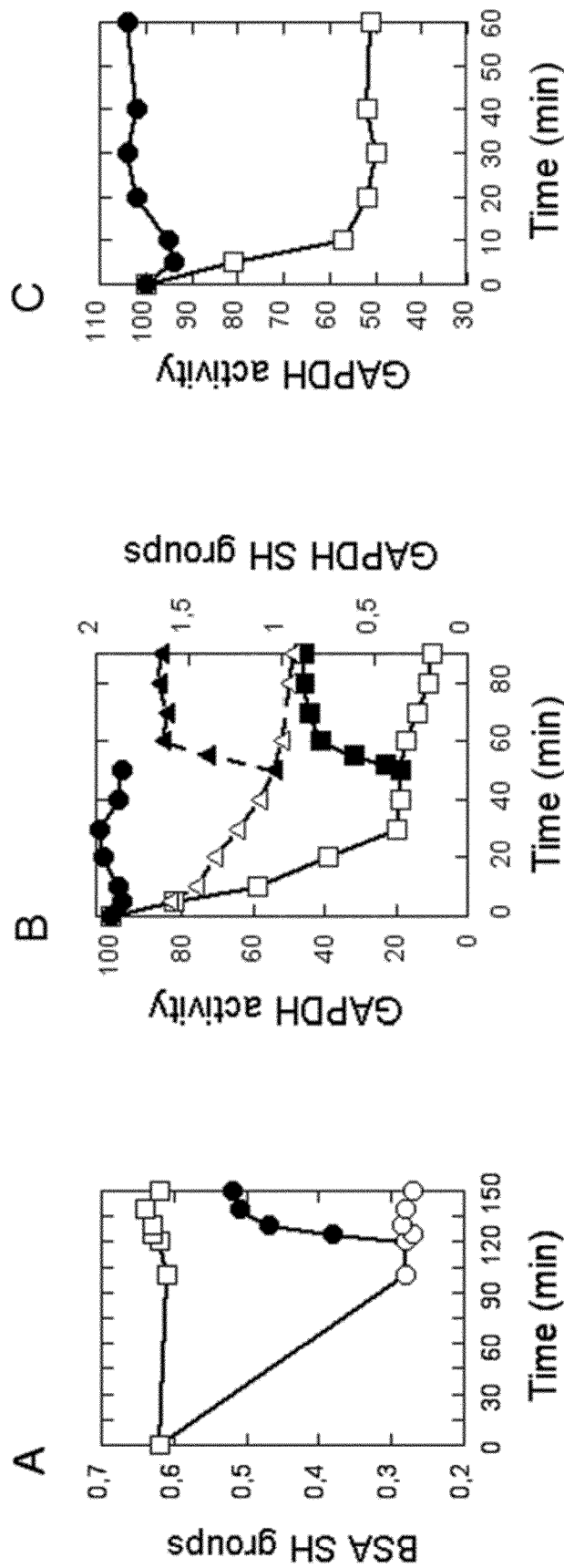


Fig. 5

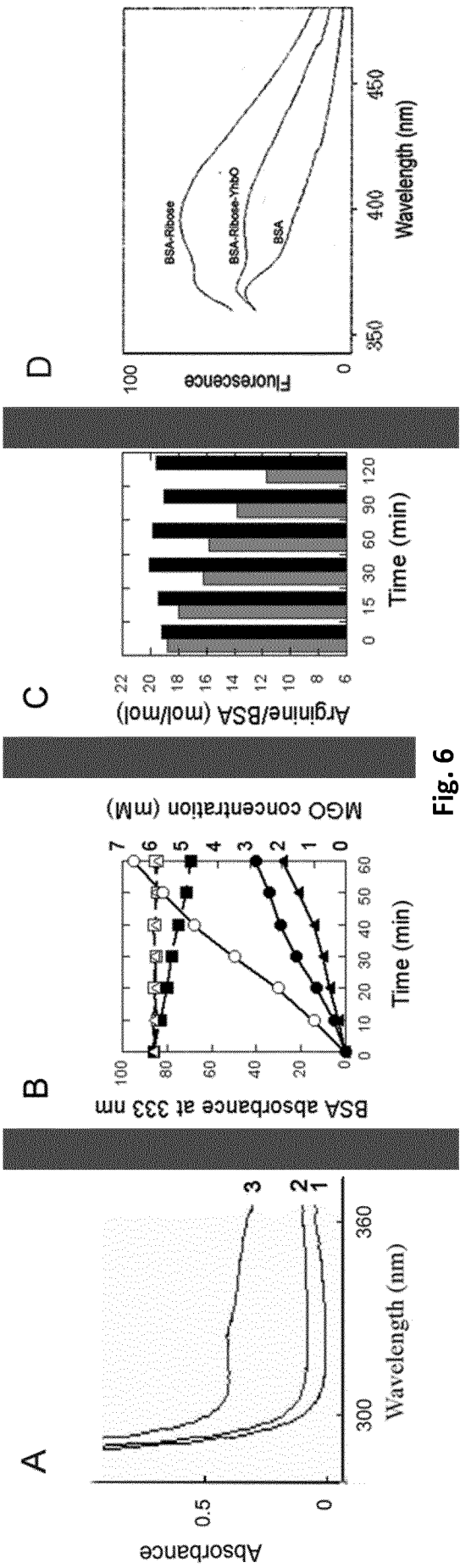
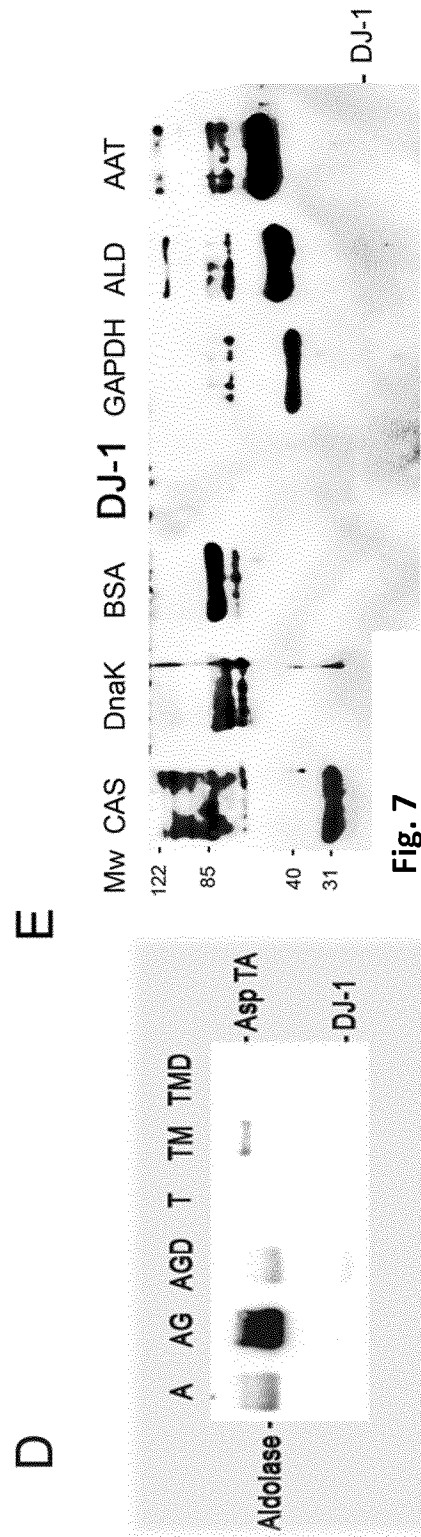
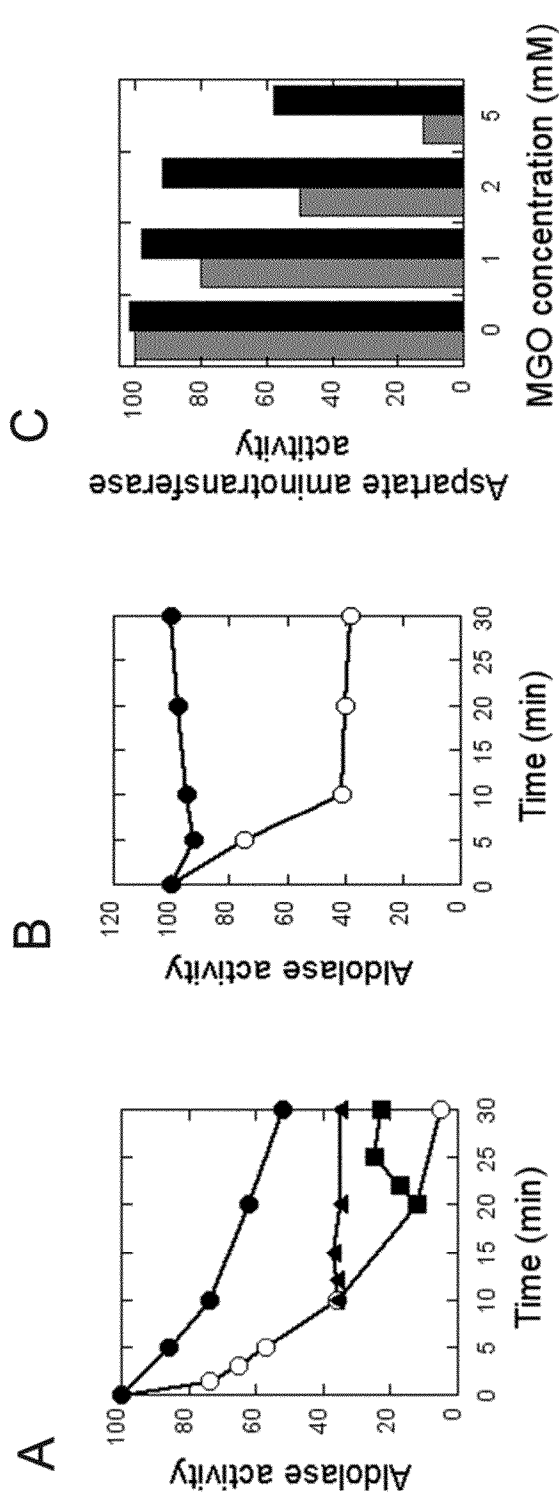


Fig. 6



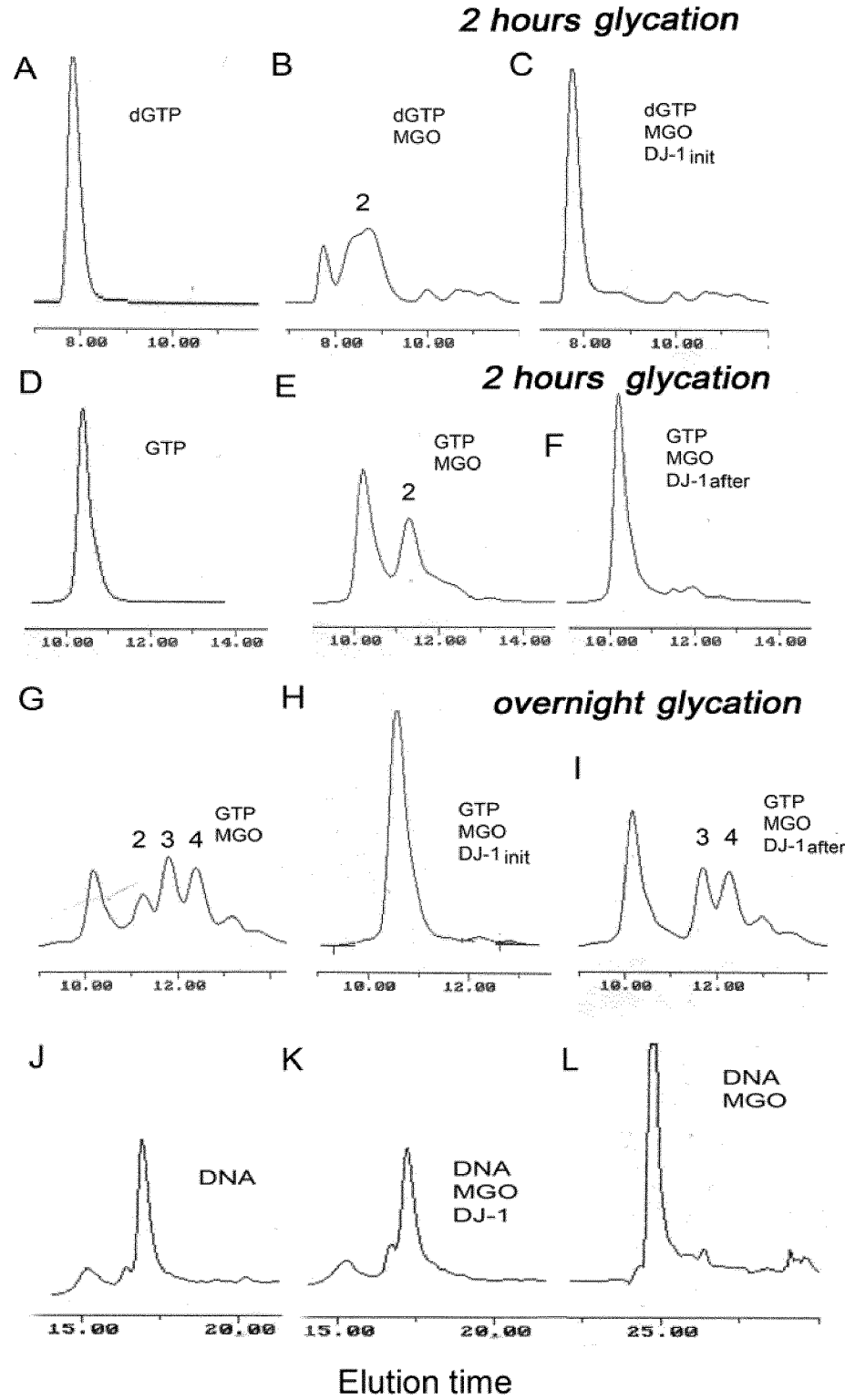


Fig. 8

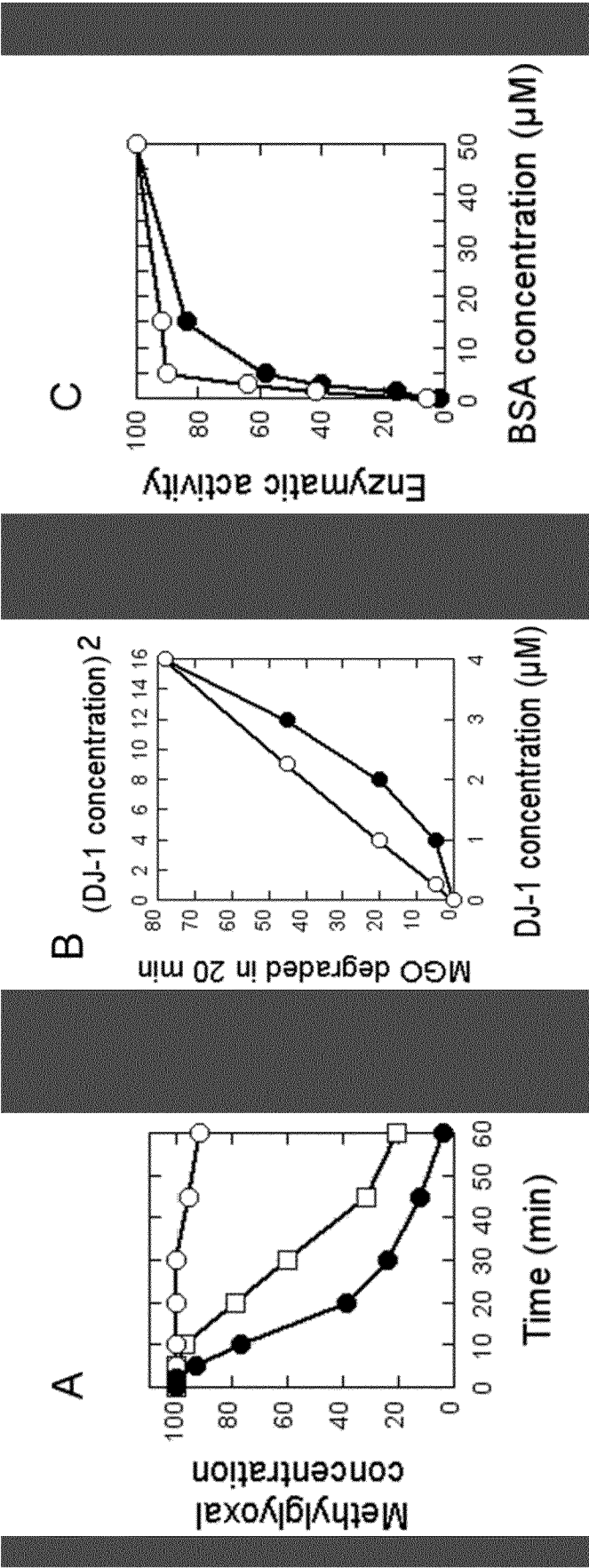


Fig. 9

Fig. 10

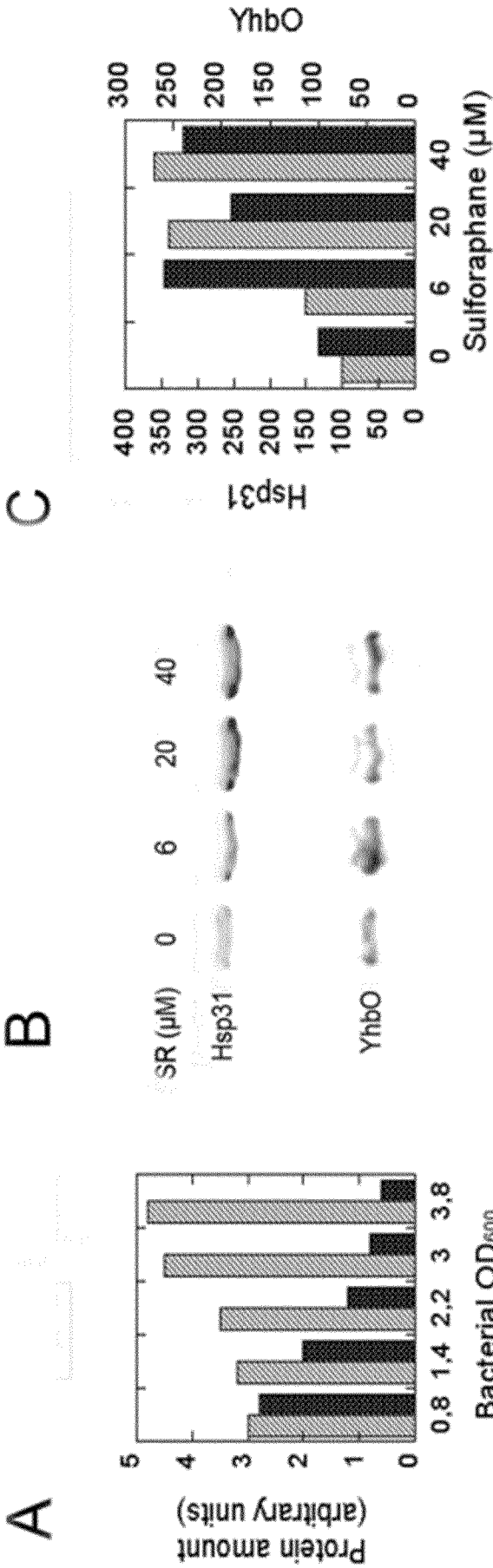


Fig. 10

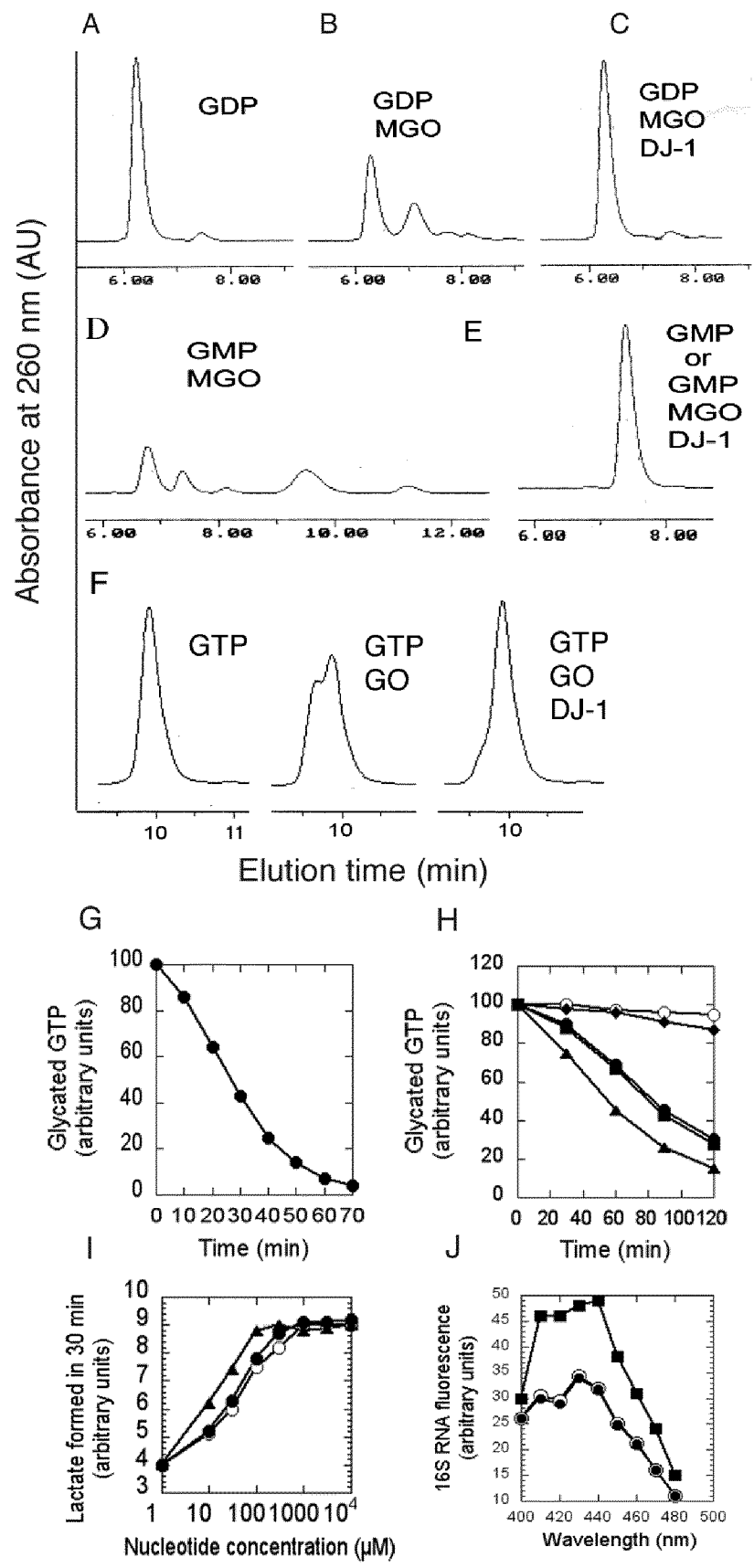
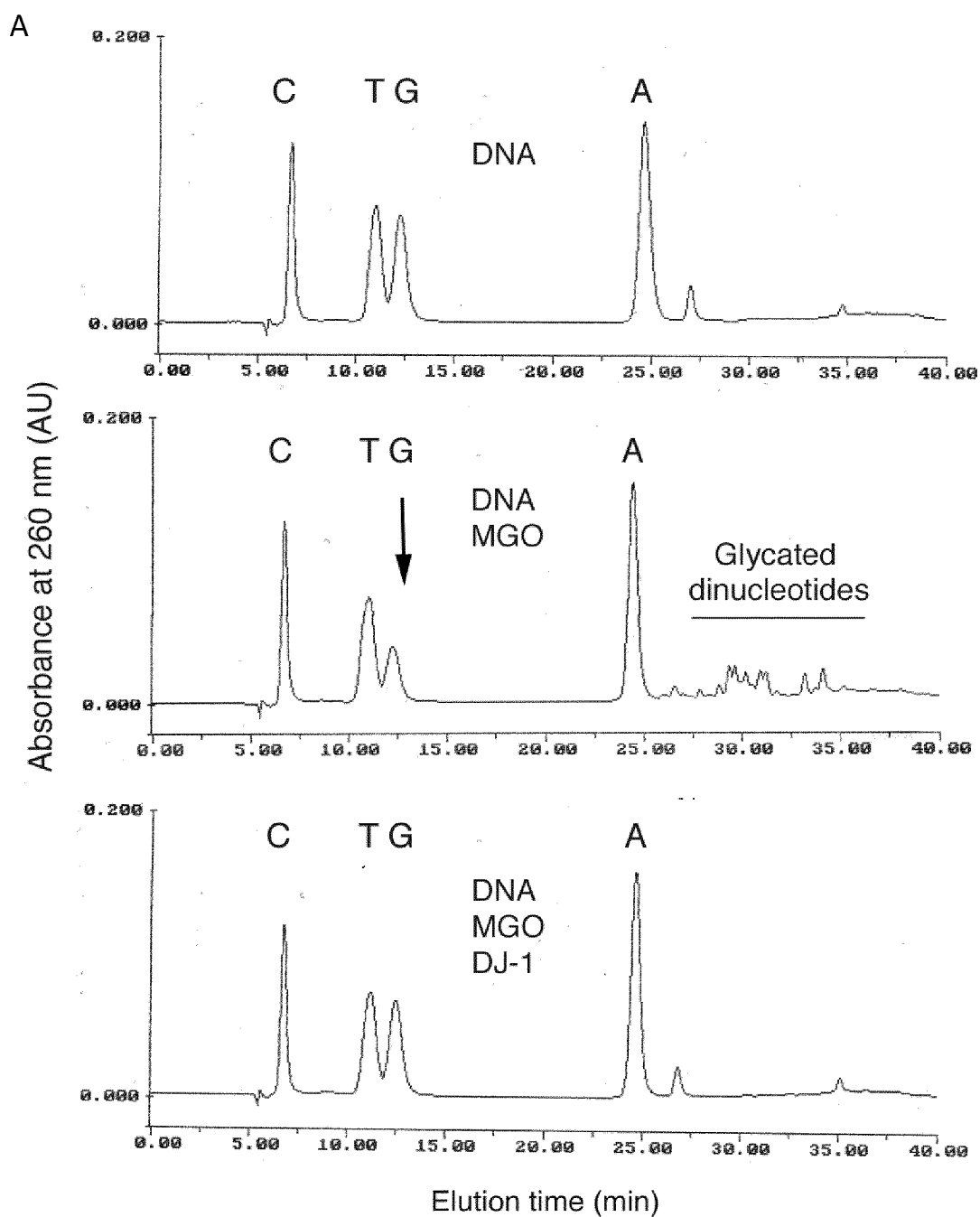
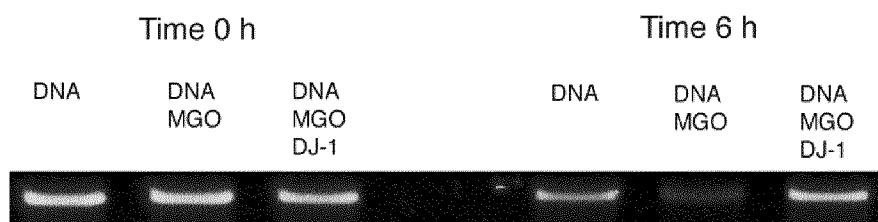


Fig. 11

**B****Fig. 12**