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(54) Titre : FUCOSIDASE ISSUE DE BACTEROIDES ET SES PROCEDES D'UTILISATION
(54) Title: FUCOSIDASE FROM BACTEROIDES AND METHODS USING THE SAME

Figure 1(a) Kinetics of BfFucH

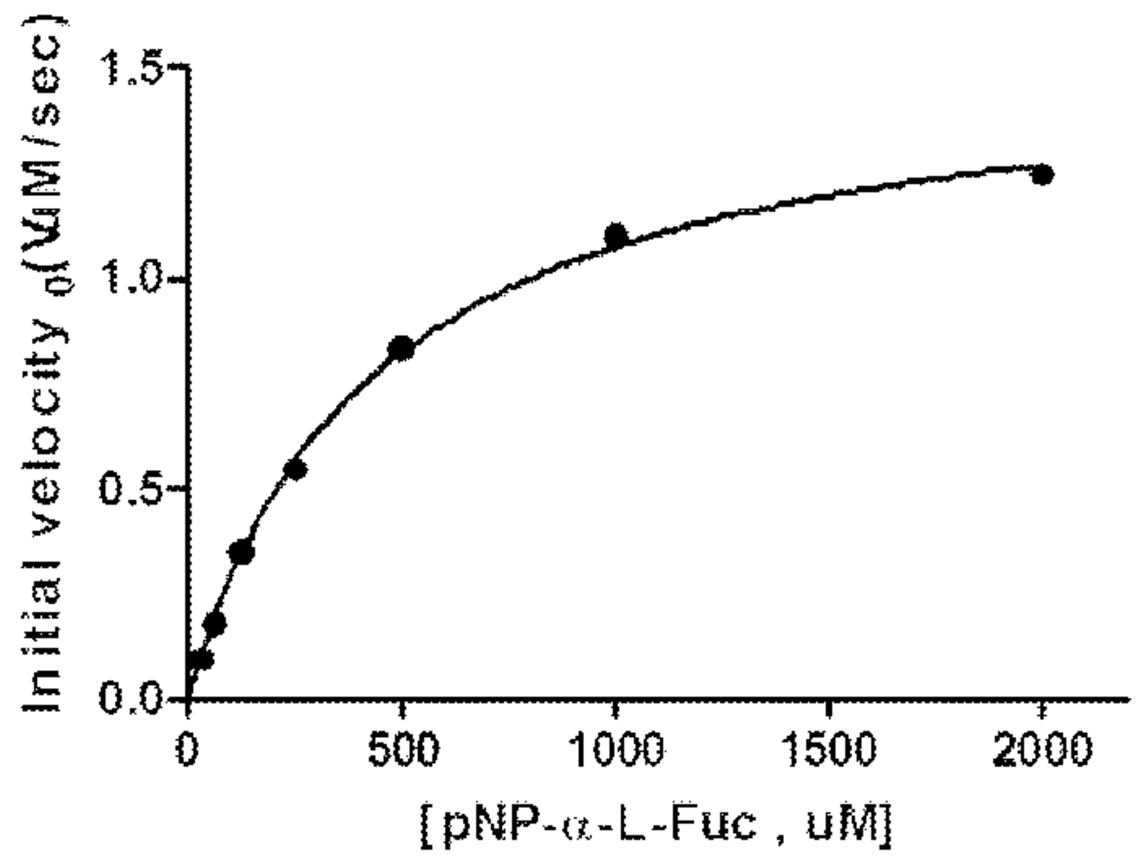

Fig. 1(b)

Parameter	Value
Kcat	183.8 s^{-1}
Km	437.0 uM
Kcat/Km	0.42 s^{-1}/uM
Vmax	1.544 uM/sec

Fig. 1(c)

Parameter	Relative activity (%)
pNP-α-D-Glc	0
pNP-α-D-Gal	0
pNP-α-D-GlcNAc	0
pNP-α-D-GalNAc	0
pNP-α-D-Man	0
pNP-α-L-Fuc	**100**
pNP-β-L-Fuc	0
pNP-α-L-Rha	0

Figure 1

(57) **Abrégé/Abstract:**
The present disclosure relates to an a-fucosidase having a-(1,2), a-(1,3), a-(1,4), and a-(1,6) fucosidase activity. The present disclosure also relates to the compositions comprising the a-fucosidase, and the methods of producing and using the a-fucosidase in cleaving a-(1,2), a-(1,3), a-(1,4), and/or a-(1,6)-linked fucoses in the glycoconjugates. Accordingly, the present invention provides the compositions and methods for the improved enzymatic hydrolysis of fucose in vitro. In particular, the present invention is useful for the efficient cleavage of core fucose in native glycoproteins without denaturation or functional deterioration of glycoproteins. The compositions and methods of the invention can facilitate the Fc glycoengineering of Fc fusion proteins or antibodies, such as therapeutic antibodies.

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(54) **Title**: FUCOSIDASE FROM BACTEROIDES AND METHODS USING THE SAME

Figure 1(a) Kinetics of BfFucH

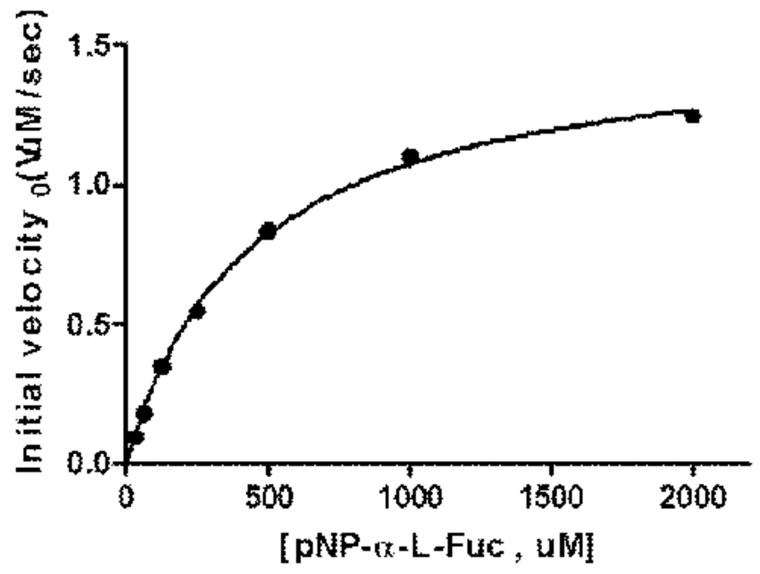

Fig. 1(b)

Parameter	Value
Kcat	183.8 s^{-1}
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Fig. 1(c)

Parameter	Relative activity (%)
pNP-α-D-Glc	0
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pNP-α-D-GlcNAc	0
pNP-α-D-GalNAc	0
pNP-α-D-Man	0
pNP-α-L-Fuc	**100**
pNP-β-L-Fuc	0
pNP-α-L-Rha	0

Figure 1

(57) **Abstract**: The present disclosure relates to an a-fucosidase having a-(1,2), a-(1,3), a-(1,4), and a-(1,6) fucosidase activity. The present disclosure also relates to the compositions comprising the α-fucosidase, and the methods of producing and using the α-fucosidase in cleaving a-(1,2), a-(1,3), a-(1,4), and/or a-(1,6)-linked fucoses in the glycoconjugates. Accordingly, the present invention provides the compositions and methods for the improved enzymatic hydrolysis of fucose in vitro. In particular, the present invention is useful for the efficient cleavage of core fucose in native glycoproteins without denaturation or functional deterioration of glycoproteins. The compositions and methods of the invention can facilitate the Fc glycoengineering of Fc fusion proteins or antibodies, such as therapeutic antibodies.

WO 2015/184008 A1

FUCOSIDASE FROM BACTEROIDES AND METHODS USING THE SAME

RELATED APPLICATIONS

[0001] This application claims the benefit of US provisional aplications United States Serial Number ("USSN") 62/003,136 filed May 27, 2014, USSN 62/003,104 filed May 27, 2014, USSN 62/003,908 filed May 28, 2014, USSN 62/020,199 filed July 2, 2014 and USSN 62/110,338 filed January 30, 2015. The contents of which is hereby incorporated in its entirety.

BACKGROUND OF THE INVENTION

[0002] Fucose is an important component of many O- or N-linked oligosaccharide structures of glycoconjugates. Fucose-containing glycans are involved in numerous biological events, including development and apoptosis, and are involved in the pathology of inflammation, cancer, and cystic fibrosis. Defucosylation of the glycoconjugates is an important process for understanding the biological effects of the glycoconjugates.

[0003] α-L-fucosidases (α-fucosidase) are exo-glycosidases, responsible for the removal of fucose residues from the non-reducing end of glycoconjugates by hydrolyzing α-(1,2), α-(1,3), α-(1,4), and α-(1,6) linkages of fucoses attached, primarily to galactose or N-acetylglucosamine.

[0004] Both human serum IgG and therapeutic antibodies are well known to be heavily fucosylated. Antibody-dependent cellular cytotoxicity (ADCC) has been found to be one of the important effector functions responsible for the clinical efficacy of therapeutic antibodies. ADCC is triggered upon the binding of lymphocyte receptors (FcεRs) to the antibody Fc region. ADCC activity is dependent on the amount of fucose attached to the innermost GlcNAc of N-linked Fc oligosaccharide via an α-(1,6) linkage.

SUMMARY OF THE INVENTION

[0005] Accordingly, the present invention provides the compositions and methods for the improved enzymatic hydrolysis of fucose *in vitro*. In particular, the present invention is useful for the efficient cleavage of core fucose in native glycoproteins without denaturation or functional deterioration of glycoproteins. The compositions and methods of the invention can facilitate the Fc glycoengineering of Fc fusion proteins or antibodies, such as therapeutic antibodies. This invention also provides the application of glycan sequencing for distinguishing fucose position on a glycoconjugate. The glycoconjugate may be a glycolipid, glycoprotein, oligosaccharide, or glycopeptide.

[0006] In one aspect, the present invention relates to an α-fucosidase comprising a polypeptide having at least 85% sequence identity to SEQ ID NO: 1. In some embodiments, the α-fucosidase comprises a polypeptide having at least 88% sequence identity to SEQ ID NO: 1.

In some embodiments, the α-fucosidase comprises a polypeptide having the sequence identity to SEQ ID NO: 1. In certain embodiments, the α-fucosidase comprises a polypeptide having the sequence identity to SEQ ID NO: 2. SEQ ID NOs: 1 and 2 share 88% sequence identity.

[0007] The fucosidase described herein can hydrolyze one or more α(1,2), α(1,3), α(1,4), and α(1,6)-linked fucoses. The fucoses may be present in N- and/or O- linked glycans in a glycoconjugate. In certain embodiments, the α-fucosidase is a recombinant *Bacteroides* α-fucosidase.

[0008] In preferred embodiments, the α-fucosidase exhibits pH optimum at 4-9.

[0009] In another aspect, the present invention relates to a composition comprises the α-fucosidase described above. The composition may further comprise at least one glycosidase. In some embodiments, the glycosidase may be an exoglycosidase. The exoglycosidase includes, but not limited to, sialidase, galactosidase, alpha-fucosidase, and bariants thereof. In some embodiments, the glycosidase may be an endoglycosidase. The endoglycosidase includes, but not limited to, Endo-beta-N-acetylglucosaminidases (NAG), EndoA, EndoF1, EndoF2, EndoF3, EndoH, EndoM, EndoS, and variants thereof.

[0010] The composition of the invention is useful for making defucosylation of a glycoconjugate *in vitro*. In particular, the composition described herein is useful for making core defucosylation of glycoproteins *in vitro*. In some embodiments, the core defucosylation is core α(1,6) defucosylation. In certain embodiments, the core defucosylation is core α(1,3) defucosylation. The defucosylation can be performed without denaturation or functional deterioration of glycoproteins.

[0011] Another aspect of the invention provides a method for making defucosylation of a glycoconjugate *in vitro*. The inventive method comprises the step of contacting the glycoconjugate with the α-fucosidase of the invention described above. The glycoconjugate comprises one or more fucoses selected from α(1,2), α(1,3), α(1,4), and α(1,6)-linked fucoses. The fucoses may be present in N- and/or O- linked glycans in a glycoconjugate.

[0012] In some embodiments, the glycoconjugate is a glycoprotein. In some embodiments, the glycoprotein comprises a core fucose. In some embodiments, the core fucose is a core α-(1,3)-linked fucose or a core α-(1,6)-linked fucose.

[0013] In some embodiments, the method further comprises contacting the glycoconjugate with at least one glycosidase. In certain embodiments, the glycosidas is an endoglycosidase. Endoglycosidase is used to trim off the variable portions of an oligosaccharide in the N-glycan. Examples of endoglycosidases used herein include, but not limited to, Endo-beta-N-acetylglucosaminidases (NAG), EndoA, EndoF1, EndoF2, EndoF3, EndoH, EndoM, EndoS, and variants thereof. Exoglycosidase

[0014] For core defucosylation, the glycoconjugate can be treated with an endoglycosidase and an α-fucosidase sequentially or simultaneously. The core defucosylation may be core α(1,3) defucosylation or α(1,6) defucosylation.

[0015] The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1. shows the biochemical properties of BfFucH.

[0017] Figure 2. (a) pH profile of BfFucH (b) temperature effects on the enzyme activity of BfFucH (c) metal ion effects on the enzyme activity of BfFucH.

[0018] Figure 3. shows the time course of of BfFucH treatment for Rituxan.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The absence of core fucose residues in the Fc glycans is known to substantially increase the ADCC activity of IgG as nonfucosylated antibodies bind to the FcgRIIIα receptor with significantly increased affinity. To improve FcgRIIIα binding and ADCC, several strategies have been developed to reduce fucosylation of IgG, including the development of production cell lines that abolish or reduce expression levels of α-(1,6) fucosyltransferase. Alternative strategies to reduce fucosylation include silencing the α-(1,6) fucosyltransferase gene using RNAi. However, core defucosylation of N-glycans has not been able to be achieved enzymatically *in vitro*, mainly because N-glycans are embedded between two Fc domains. The enzymatic defucosylation efficiency is much lower due to steric hindrance, i.e., access of α-fucosidase to fucose residues is blocked by potions of the Fc domains.

[0020] A number of α-fucosidases are known in the art. Examples include α-fucosidases from Turbo cornutus, Charonia lampas, Bacillus fulminans, Aspergillus niger, Clostridium perfringens, Bovine kidney (Glyko), Chicken liver (Tyagarajan et al., 1996, Glycobiology 6:83-93) and α-fucosidase II from Xanthomonas manihotis (Glyko, PROzyme). Some fucosidase are also commercially available (Glyko, Novato, Calif.; PROzyme, San Leandro, Calif.; Calbiochem-Novabiochem Corp., San Diego, Calif.; among others). None of these α-fucosidases are able to efficiently cleave the core fucoses from N-linked glycans without denaturing the glycoproteins first.

[0021] WO 2013/12066 disclosed the defucosylation of (Fucα1,6) GlcNAc-Rituximab by an α-fucosidase from bovine kidney. As described in WO 2013/12066, a reaction mixture of (Fuc α1, 6) GlcNAc-Rituximab was incubated with α-fucosidase from bovine kidney (commercially available from Prozyme) at 37°C for 20 days to completely remove the fucose in (Fucα1,6) GlcNAc-Rituximab. Thermal instability of immunoglobulin is known in the art (Vermeer et al., Biophys J. Jan 78: 394–404 (2000)). The Fab fragment is most sensitive to heat treatment, whereas the Fc fragment is most sensitive to decreasing pH. It is contemplated that the antibody will significantly lose the binding affinity to CD20 after prolonged thermal treatment, such as at 37°C for 20 days, as described in WO 2013/12066.

[0022] The limitation of currently known α-fucosidases has prevented effective manipulation of certain N-linked glycans. Thus, a need remains for new α-fucosidases suitable for Fc glycoengineering of of Fc fusion proteins or antibodies for development of human therapeutics.

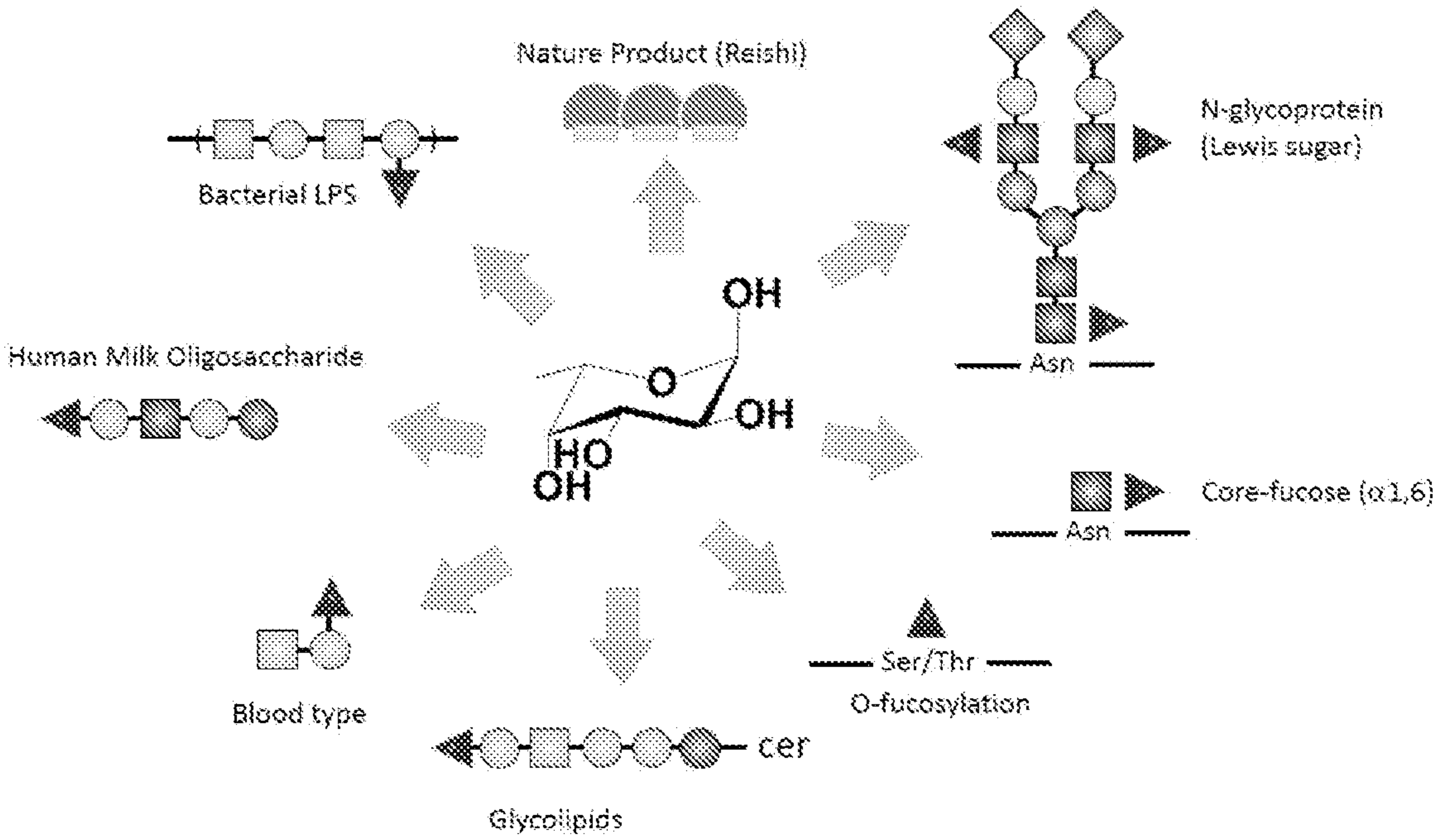
[0023] The present disclosure relates to an unexpected discovery of a bacterial α-fucosidase that is able to efficiently cleave core fucose from N-linked glycans.

[0024] The present disclosure relates to an unexpected discovery of a bacterial α-fucosidase that is able to efficiently cleave core fucoses from N-linked glycans.

[0025] In some examples, the α-fucosidase may be an α-fucosidase from *Bacteroides fragilis* (BfFucH). In some examples, the α-fucosidase may be an α-fucosidase from *Bacteroides thetaiotaomicron* (BtFucH). The α-fucosidase can be expressed from bacteria, yeast, baculovirus/insect, or mammalian cells. In some embodiments, the α-fucosidase can be a recombinant *Bacteroides* α-fucosidase. In some embodiments, the α-fucosidase can be a recombinant *Bacteroides* α-fucosidase expressed from *E. coli.*

[0026] The α-fucosidase can hydrolyze one or more α(1,2), α(1,3), α(1,4), and α(1,6)-linked fucoses. The fucose may be present in N- and/or O- linked glycans in a glycoconjugate. The fucose can be a core α-(1,3) fucose or a core α-(1,6) fucose.

[0027] Scheme 1 shows various fucose-containing glycoconjugates.



[0028] Examples of the substrates suitable for the enzyme include, but not limited to, milk oligosaccharides, cancer associated carbohydrate antigens such as Globo H, Lewis blood groups (a, b, x, y), and sialyl Lewis a (SLe^a) and x (SLe^x). Unlike the reports known in the arts, the α-fucosidase can hydrolyze sialyl Lewis a (SLe^a) and x (SLe^x) withour cleaving the terminal sialic acid. Milk oligosaccharides may bear α-(1,2), α-(1,3) and/or α-(1,4) linked fucoses.

Compositions

[0029] The present invention also relates to a compostion of the α-fucosidase described above. The α-fucosidase comprises a polypeptide having at least 85% sequence identity to SEQ ID NO: 1. In some embodiments, the α-fucosidase comprises a polypeptide having at least 88% identity to SEQ ID NO: 1, or a functional variant thereof. In some embodiments, the α-fucosidase comprises a polypeptide having an amino acid sequence of SEQ ID NO: 1. In some embodiments, the α-fucosidase comprises a polypeptide having an amino acid sequence of SEQ ID NO: 2. SEQ ID NO: 2 has 88% sequence identity to SEQ ID NO: 1.

[0030] Variant polypeptide as described herein are those for which the amino acid sequence varies from that in SEQ ID NO: 1 or 2, but exhibit the same or similar function of the enzyme comprising the polypeptide having an amino acid sequence of SEQ ID NO: 1 or 2.

TABLE 1
SEQ ID: 1 QQKYQPTEANLKARSEFQDNKFGIFLHWGLYAMLATGEWTMTNNNLNYKEYAKLAGGFYPSK FDADKWVAAIKASGAKYICFTTRHHEGFSMFDTKYSDYNIVKATPFKRDVVKELADACAKHG IKLHFYYSHIDWYREDAPQGRTGRRTGRPNPKGDWKSYYQFMNNQLTELLTNYGPIGAIWFD GWWDQDINPDFDWELPEQYALIHRLQPACLVGNNHHQTPFAGEDIQIFERDLPGENTAGLSG QSVSHLPLETCETMNGMWGYKITDQNYKSTKTLIHYLVKAAGKDANLLMNIGPQPDGELPEV AVQRLKEVGEWMSKYGETIYGTRGGLVAPHDWGVTTQKGNKLYVHILNLQDKALFLPIVDKK VKKAVVFADKTPVRFTKNKEGIVLELAKVPTDVDYVVELTID **SEQ ID: 2** QSSYQPGEENLKAREEFQDNKFGIFLHWGLYAMLATGEWTMTNNNLNYKEYAKLAGGFYPSK FDADKWVAAIKASGAKYICLTSRHHDGFSMFDTQYSDFNIVKATPFKRDIIKELAAACSKQG IKLHFYYSHLDWTREDYPWGRTGRGTGRSNPQGDWKSYYQFMNNQLTELLTNYGPVGAIWFD GWWDQDGNPGFNWELPEQYAMIHKLQPGCLIGNNHHQTPFAGEDIQIFERDLPGENTAGLSG QSVSHLPLETCETMNGMWGYKITDQNYKSTKTLIHYLVKAAGKNANLLMNIGPQPDGELPEV AVQRLKEMGEWMNQYGETIYGTRGGAVAPHDWGVTTQKGNKLYVHILNLQDKALFLPLADKK VKKAVLFKNGTPVRFTKNKEGVLLEFTEIPKDIDYVVELTID

[0031] As used herein percent (%) sequence identity with respect to a sequence is defined as the percentage of amino acid residues in a candidate polypeptide sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0032] It will be understood that the polypeptide of the α-fucosidase of the invention may be derivatized or modified to assist with their isolation or purification. Thus, in one embodiment of the invention, the polypeptide for use in the invention is derivatized or modified by addition of a ligand which is capable of binding directly and specifically to a separation means. Alternatively, the polypeptide is derivatized or modified by addition of one member of a binding pair and the separation means comprises a reagent that is derivatized or modified by addition of the other member of a binding pair. Any suitable binding pair can be used. In a preferred embodiment where the polypeptide for use in the invention is derivatized or modified by addition of one member of a binding pair, the polypeptide is preferably histidine-tagged or biotin-tagged. Typically the amino acid coding sequence of the histidine or biotin tag is included at the gene level and the proteins are expressed recombinantly in *E. coli*. The histidine or biotin tag is typically present at one end of the polypeptide, either at the N-terminus or at the C-terminus. The histidine tag typically consists of six histidine residues, although it can be longer than this, typically up to 7, 8, 9, 10 or 20 amino acids or shorter, for example 5, 4, 3, 2 or 1 amino acids. Furthermore, the histidine tag may contain one or more amino acid substitutions, preferably conservative substitutions as defined above.

Applications of the compositions

[0033] The composition of the invention can be used for making defucosylation of a glycoconjugate *in vitro*. The inventive method comprises the step of contacting the glycoconjugate with the α-fucosidase of the invention described abov. The glycoconjugate comprises one or more fucoses selected from α-(1,2), α-(1,3), α-(1,4), and α-(1,6)-linked fucoses. The fucoses may be present in N- and/or O- linked glycans in a glycoconjugate.

[0034] In some embodiments, the glycoconjugate is a glycoprotein. In some embodiments, the glycoprotein comprises a core fucose. In some embodiments, the core fucose is a core α-(1,3) linked fucose or a core α-(1,6) linked fucose.

[0035] In some embodiments, the method further comprises contacting the glycoconjugate with at least one glycosidase. In certain embodiments, the glycosidas is an endoglycosidase. Endoglycosidase is used to trim off the variable portions of an oligosaccharide in the N-glycan. Examples of endoglycosidases used herein include, but not limited to, Endo-beta-N-acetylglucosaminidases (NAG), EndoA, EndoF1, EndoF2, EndoF3, EndoH, EndoM, EndoS, and variants thereof.

[0036] For core defucosylation, the glycoconjugate can be treated with an endoglycosidase and an α-fucosidase sequentially or simultaneously. The core defucosylation may be core α(1,3) defucosylation or α(1,6) defucosylation.

[0037] The method of the invention can be useful for making Fc glycoengineering from monoclonal antibodies. Exemplary methods of engineering are described in, for example, Wong et al USSN12/959,351, the contents of which is hereby incorporated by reference. Preferrably, the monoclonal antibodies are therapeutic monoclonal antibodies. In some examples, the method for making a homogeneously glycosylated monoclonal antibody comprises the steps of (a) contacting a monoclonal antibody with an α-fucosidase and at least one endoglycosidase, thereby yielding a defucosylated antibody having a single N-acetylglucosamine (GlcNAc), and (b) adding a carbohydrate moiety to GlcNAc under suitable conditions. In certain embodiments, the glycan can be prepared by treatment with endo-GlcNACase and exemplary fucosidase, then followed by exemplary endo-S mutant and a glycan oxazoline.

[0038] In a specific example, the monoclonal antibody according to the method of the invention is Rituximab. In certain embodiments, the carbohydrate moiety according to the method the invention is slected from the group consisting of Sia_2(α2-6)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia_2(α2-6)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia_2(α2-3)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia_2(α2-3)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia_2(α2-3/α2-6)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia_2(α2-6/α2-3)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia_2(α2-3/α2-6)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia_2(α2-6/α2-3)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia(α2-6)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia(α2-3)$Gal_2GlcNAc_2Man_3GlcNAc$, Sia(α2-6)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia(α2-3)$Gal_2GlcNAc_3Man_3GlcNAc$, Sia(α2-6)$GalGlcNAc_2Man_3GlcNAc$, Sia(α2-3)$GalGlcNAc_2Man_3GlcNAc$, Sia(α2-6)$GalGlcNAc_3Man_3GlcNAc$, Sia(α2-3)$GalGlcNAc_3Man_3GlcNAc$, $Gal_2GlcNAc_2Man_3GlcNAc$ and $Gal_2GlcNAc_3Man_3GlcNAc$.

[0039] In some embodiments, the carbohydrate moiety is a sugar oxazoline.

[0040] Step (b) in the method of the invention may lead to sugar chain extension. One method for sugar chain extension is through an enzyme-catalyzed glycosylation reaction. It is well known in the art that glycosylation using a sugar oxazoline as the sugar donor among the enzyme-catalyzed glycosylation reactions is useful for synthesizing oligosaccharides because the glycosylation reaction is an addition reaction and advances without any accompanying elimination of acid, water, or the like. (Fujita, et al., *Biochim. Biophys. Acta* 2001, 1528, 9–14)

[0041] Suitable conditions in step (b) include incubation of the reaction mixture for at least 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 70 minutes, 80 minutes, 90 minutes or 100 minutes, preferably less than 60 minutes. Incubation preferably takes place at room temperature, more preferably at approximately 20°C, 25 °C, 30°C, 35 °C, 40°C or 45 °C, and most preferably at approximately 37°C.

[0042] As used herein, the terms “fucose” and “ L-fucose” are used interchangeably.

[0043] As used herein, the terms “core fucose” and “ core fucose residue” are used interchangeably and refer to a fucose in α1,3-position or α1,6-position linked to the asparagine-bound N-acetylglucosamine.

[0044] As used herein, the term "α-(1,2) Fucosidase" refers to an exoglycosidase that specifically catalyzes the hydrolysis of α-(1,2) linked L-fucose residues from oligosaccharides.

[0045] As used herein, the term " α-(1,4) Fucosidase" refers to an exoglycosidase that specifically catalyzes the hydrolysis of α-(1,4) linked L-fucose residues from oligosaccharides.

[0046] As used herein, the term “glycan” refers to a polysaccharide, oligosaccharide or monosaccharide. Glycans can be monomers or polymers of sugar residues and can be linear or branched. A glycan may include natural sugar residues (e.g., glucose, N-acetylglucosamine, N-acetyl neuraminic acid, galactose, mannose, fucose, hexose, arabinose, ribose, xylose, etc.) and/or modified sugars (e.g., 2′-fluororibose, 2′-deoxyribose, phosphomannose, 6′ sulfo N-acetylglucosamine, etc).

[0047] As used herein, the terms “N-glycan”, “N-linked glycan”, “N-linked glycosylation”, “Fc glycan” and “Fc glycosylation” are used interchangeably and refer to an N-linked oligosaccharide attached by an N-acetylglucosamine (GlcNAc) linked to the amide nitrogen of an asparagine residue in a Fc-containing polypeptide. The term “Fc-containing polypeptide” refers to a polypeptide, such as an antibody, which comprises an Fc region.

[0048] As used herein, the term “glycosylation pattern” and “glycosylation profile“ are used interchangeably and refer to the characteristic “fingerprint” of the N-glycan species that have been released from a glycoprotein or antibody, either enzymatically or chemically, and then analyzed for their carbohydrate structure, for example, using LC-HPLC, or MALDI-TOF MS, and the like. See, for example, the review in Current Analytical Chemistry, Vol. 1, No. 1 (2005), pp. 28-57; herein incorporated by reference in its entirety.

[0049] As used herein, the term “glycoengineered Fc” when used herein refers to N-glycan on the Fc region has been altered or engineered either enzymatically or chemically. The term “Fc glycoengineering” as used herein refers to the enzymatic or chemical process used to make the glycoengineered Fc.

[0050] The terms “homogeneous”, “uniform”, “uniformly” and “homogeneity” in the context of a glycosylation profile of Fc region are used interchangeably and are intended to mean a single glycosylation pattern represented by one desired N-glycan species, with no trace amount of precursor N-glycan.

[0051] As used herein, the terms " IgG", "IgG molecule", "monoclonal antibody", "immunoglobulin", and "immunoglobulin molecule" are used interchangeably. As used herein, "molecule" can also include antigen binding fragments.

[0052] As used herein, the term "glycoconjugate", as used herein, encompasses all molecules in which at least one sugar moiety is covalently linked to at least one other moiety. The term specifically encompasses all biomolecules with covalently attached sugar moieties, including for example N-linked glycoproteins, O-linked glycoproteins, glycolipids, proteoglycans, etc.

[0053] As used herein, the term "glycolipid" refers to a lipid that contains one or more covalently linked sugar moieties (i.e., glycans). The sugar moiety(ies) may be in the form of monosaccharides, disaccharides, oligosaccharides, and/or polysaccharides. The sugar moiety(ies) may comprise a single unbranched chain of sugar residues or may be comprised of one or more branched chains. In certain embodiments, sugar moieties may include sulfate and/or phosphate groups. In certain embodiments, glycoproteins contain O-linked sugar moieties; in certain embodiments, glycoproteins contain N-linked sugar moieties.

[0054] As used herein, the term "glycoprotein" refers to amino acid sequences that include one or more oligosaccharide chains (e.g., glycans) covalently attached thereto. Exemplary amino acid sequences include peptides, polypeptides and proteins. Exemplary glycoproteins include glycosylated antibodies and antibody-like molecules (e.g., Fc fusion proteins). Exemplary antibodies include monoclonal antibodies and/or fragments thereof, polyclonal antibodies and/or fragments thereof, and Fc domain containing fusion proteins (e.g., fusion proteins containing the Fc region of IgGl, or a glycosylated portion thereof).

[0055] As used herein, the term "N-glycan" refers to a polymer of sugars that has been released from a glycoconjugate but was formerly linked to the glycoconjugate via a nitrogen linkage (see definition of N-linked glycan below).

[0056] As used herein, the term "O-glycan" refers to a polymer of sugars that has been released from a glycoconjugate but was formerly linked to the glycoconjugate via an oxygen linkage (see definition of O-linked glycan below).

[0057] As used herein, a functional variant of a wild-type enzyme possesses the same enzymatic activity as the wild-type counterpart and typically shares a high amino acid sequence homology, *e.g.*, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of the wild-type counterpart. The "percent identity" of two amino acid sequences is determined using the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci.* USA 87:2264-68, 1990, modified as in Karlin and Altschul *Proc. Natl. Acad. Sci.* USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al. J. Mol. Biol.* 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res*. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. A functional variant can have various mutations, including addition, deletion, or substitution of one or more amino acid residues. Such a variant often contain mutations in regions that are not essential to the enzymatic activity of the wild-type enzyme and may contain no mutations in functional domains or contain only conservative amino acid substitutions. The skilled artisan will realize that conservative amino acid substitutions may be made in lipoic acid ligase mutants to provide functionally equivalent variants, i.e., the variants retain the functional capabilities of the particular lipoic acid ligase mutant.

[0058] As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.* Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D. Any of the enzymes involved in the deglycosylation system can be prepared via routine technology. In one example, the enzyme is isolated form a natural source. In other examples, the enzyme is prepared by routine recombinant technology. When necessary, the coding sequence of a target enzyme can be subjected to coden optimization based on the host cell used for producing the enzyme. For example, when *E. coli* cells are used as the host for producing an ezyme via recombinant technology, the gene encoding that enzyme can be modified such that it contains codons commonly used in *E. coli*. The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appending claims.

[0059] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

EXAMPLE 1: Protein Expression Constructs

[0060] The α-fucosidases were amplified by PCR from *Bacteroides fragilis* NCTC 9343 genomic DNA (ATCC 25285) and *Bacteroides Thetaiotaomicron* VPI-5482 (ATCC 29148), respectively, and cloned into pET47b+ (EMD Biosciences, San Diego, CA) with N-termial poly-histine with internal AcTEV protease cutting site. Other enzymes used in the study such as Endo F1 (GenBank: AAA24922.1), Endo F2 (GenBank: AAA24923.1), Endo F3 (GenBank: AAA24924.1), Endo H (GenBank: AAA26738.1) and PNGase F (Genbank: GenBank: J05449.1) were codon optimized for *E.coli*, and cloned into pET28a with MBP fusion in N-terminus, respectively. All sequences of the clones were first confirmed by Applied Biosystems 3730 DNA Analyzer.

[0061] Primers used for protein expression constructs in *E. coli* are listed in the table below.

SEQ ID NO	Primer[a]	Sequence (5′→3′)	Restriction enzyme site	Gene source from genome or cDNA pool
SEQ ID NO: 1	*BfFucH*-F	TTCAGGGA**GCGATCGC**TCAGCAAAAGTATCAACCGACA[b]	AsiSI	Bacteroides fragilis (BfFucH, e.g. GenBank accession no. YP_212855.1)
SEQ ID NO: 2	*BfFucH*-R	GTCATTAC**GTTTAAAC**TTAGTCAATTGTAAGTTCTACCA	PmeI	
SEQ ID NO: 3	*BtFucH*-F	TTCAGGGA**GCGATCGC**TCAGTCTTCTTACCAGCCTGGT	AsiSI	Bacteroides thetaiotaomicron (BtFucH, e.g. GenBank accession no. AAO76949.1)
SEQ ID NO: 4	*BtFucH*-R	GTCATTAC**GTTTAAAC**TTAGTCAATTGTAAGTTCTACAAC	PmeI	
SEQ ID NO: 5	*EndoF1*-F	TTCAGGGA**GCGATCGC**TGCGGTTACCGGTACCACCA	AsiSI	Elizabethkingia miricola (e.g. GenBank accession no. AAA24922.1)
SEQ ID NO: 6	*EndoF1*-R	GTCATTAC**GTTTAAAC**TTACCAGTCTTTAGAGTACGGGG	PmeI	

SEQ ID NO	Primer[a]	Sequence (5′→3′)	Restriction enzyme site	Gene source from genome or cDNA pool
SEQ ID NO: 7	*EndoF2-F*	TTTCAGGGA**GCGATCGC**TGCGGTTAACCTGTCTAACCT	AsiSI	Elizabethkingia miricola (e.g. GenBank accession no. AAA24923.1)
SEQ ID NO: 8	*EndoF2-R*	GTCATTAC**GTTTAAAC**TTACGGGTTCATGATTTTGATCAG	PmeI	
SEQ ID NO: 9	*EndoF3-F*	TTCAGGGA**GCGATCGC**TGCGACCGCGCTGGCGGGTT	AsiSI	Elizabethkingia miricola (e.g. GenBank accession no. AAA24924.1)
SEQ ID NO: 10	*EndoF3-R*	GTCATTAC**GTTTAAAC**TTAGTTTTTAACCGCGTCACGAAC	PmeI	
SEQ ID NO: 11	*EndoH-F*	TTCAGGGA**GCGATCGC**TGCGCCGGCGCCGGTTAAACA	AsiSI	Streptomyces plicatus (e.g. GenBank accession no. AAA26738.1)
SEQ ID NO: 12	*EndoH-R*	GTCATTAC**GTTTAAAC**TTACGGGGTACGAACCGCTTCAG	PmeI	
SEQ ID NO: 13	*endoS*-F	TTCAGGGA**GCGATCGC**TACCCACCATGATTCACTCAAT	AsiSI	Streptococcus pyogenes (e.g. GenBank accession no. AAK34539.1)
SEQ ID NO: 14	*endoS*-R	GTCATTAC**GTTTAAAC**TTATTTTTTTAGCAGCTGCCTTTTC	PmeI	
SEQ ID NO: 15	*PNGase F*-F	TTCAGGGA**GCGATCGC**TGCGCCGGCGGACAACACCGT	AsiSI	Chryseobacterium meningosepticum (e.g. GenBank accession no. J05449.1)
SEQ ID NO: 16	*PNGase F*-R	GTCATTAC**GTTTAAAC**TTAGTTGGTAACAACCGGCGCAGA	PmeI	

[0062] [a] a pair of primers for forward (F) and reversed (R) PCR reactions to amplify the coding sequence of each gene.

[0063] [b] Underline with bold means the site of restriction enzyme recognition.

[0064] [c] Codon optimization for *E. coli.* See, *e.g.*, Puigbò *et al.*, *Nucleic Acids Research* (2007) 35(S2):W126-W130.

Protein expression and purification

[0065] Protein expression constructs were transformed into BL21(DE3) (EMD Biosciences, San Diego, CA) for protein expression using 0.2 mM isopropyl β-D-thiogalactopyranoside (IPTG) in 16°C for 24hours. Cells were disrupted by microfluidizer and then centrifuged. Supernatants were collected and loaded onto Ni-NTA agarose column (QIAGEN GmbH, Hilden, Germany) and washed with ten folds of washing buffer (sodium phosphate buffer (pH

7.0), 300 mM sodium chloride, and 10 mM imidazole). Elution was employed by two folds of elution buffer (sodium phosphate buffer (pH 7.0), 300 mM sodium chloride, and 250 mM imidazole), followed bybuffer exchanging into reaction buffer by Amicon Ultra-15 10K (EMD Millipore Chemicals, Billerica, MA). Protein purity was examined by SDS-PAGE and quantitative protein concentration was measured by Qubit® Protein Assay Kits (Invitrogen, Carlsbad, CA). The recombinant fucosidase with his-tag followed by Ni-NTA column purification resulted in a yield of 60 mg/ L with greater than 95% purity. Protein concentration was determined according to the method of Brandford (Protein Assay; Bio-Rad, Hercules, CA, USA) with bovine serum albumin as standards. The purity and molecular mass of the enzyme was examined by SDS-PAGE.

[0066] The purified fucosidase from *Bacteroides fragilis* exhibited a molecular mass of about 50 kDa in sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) which is close to the theoretical molecular weight of 47.3 kDa.

<u>EXAMPLE 2</u>: Enzymatic Assays

Characteristics of enzymes

[0067] Unlike fucosidases from mammalian or bacterias, which have optimum pH in the acid condition (pH 4.0-6.0), BfFucH preformed well at mild condition (pH 7.0-7.5). In addition, BfFucH is not affected by certain divalent metal ions, and exogenous addition of metal ions did not influence the activity. However, Ni^{2+} can dramatically reduce the enzymatic activity by 60%. Also, Zn^{2+} and Cu^{2+} can completely abolish the enzymatic activity. The chelator EDTA showed no effect on the enzymatic activity, indicating the metal ions do not participate in the catalytic reaction. The enzyme is funationally active and stable at room temperate and at 4°C.

Enzyme activity on N-linked glycas

[0068] The fucosidases described herein can be used to determine the fucose position in N-glycan. The BfFucH hydrolysis activity on N-glycans with various fucoses attached at different positions was evaluated. Two synthetic glycopeptides, 0800F and 0823F, were prepared. Both glycopeptides have the fucoses bound to outer GlcNAc and the innermost GlcNAc at the glycosylation site, respectively.

[0069] Enzymatic assays revealed that the fucose could be released only from the outer GlcNAc in the sample 0800F, but not in the glycopeptide 0823F where the fucose is bound to the innermost GlcNAc. This result indicated that the steric hindrance of the G0 structure in N-glycan may shield and protect the fucose from fucosidase hydrolysis. In contrast, if 0823F was treated with BfFucH and endo-β-N-acetylglucosaminidase (endo M) simultaneously in a one-pot reaction, the core fucose could easily be removed. This result indicated that the α-fucosidase can be used to distinguish the position of fucose bound to a glycan.

Enzyme activity on oligosaccharides

[0070] The lipopolysaccharide (LPS) of serotypes O86, 0128, and O111 of *E. coli* strains contains various monosaccharides, e.g, Gal, GalNAc, and fucose. By the formaldehyde dehygrogenase (FDH) coupled assay, we confirmed that BfFucH can liberate L-fucose from LPS of *E.coli* O128:B12 strain in a dose-dependent manner. We also tested the enzymatic activity of the enzyme on various substrates including 2'-fucosyllactose (2'FL), 3'-fucosyllactose (3’FL), lacto-N-fucopentaose I (LNPT I), Globo H, Lewis a (Le^a), Lewis x (Le^x), Lewis b (Le^b), Lewis y (Le^y), Sialyl Lewis a (SLe^a), Sialyl Lewis x (SLe^x), and pNP (para-Nitrophenol)-α-L-fucoside. Results showed the α-fucosidase are able to hydrolyze all of the substrates.

<u>EXAMPLE 3</u>: Core defucosylation of glycoproteins

[0071] Aleuria aurantia possesses a fucose-specific lectin (AAL) that is widely used as a specific probe for fucose. AAL recognizes and binds specifically to fucose and terminal fucose residues on complex oligo saccharides and glycoconjugates. AAL can be used to determine the core defucosylation. Endoglycosidase is useful for trimming off the variable portions of an oligosaccharide in the N-glycan. After the treatment of a cocktail of endoglycosidases (Endo F1, Endo F2, Endo F3 and Endo H), the antibody (Humira or Rituxan) showed a high AAL-bloting signal, indicating the presence of core fucose in the antibody. However, after the treatment of a combination of a cocktail of endoglycosidases (Endo F1, Endo F2, Endo F3 and Endo H) and BfFucH, the antibody (Humira or Rituxan) lost the AAL-bloting signal due to the hydrolysis of core fucose. These results demonstrated the BfFucH is active for core defucosylation.

Materials and Methods

[0072] Unless otherwise noted, all compounds and reagents were purchased from Sigma-Aldrich or Merck. Anti-tumor necrosis factor-alpha (TNFα) antibody, Adalimumab (Humira®), was purchased from (North Chicago, IL). Anti-human CD20 mouse/human chimeric IgG1 rituximab (Rituxan®) was purchased from Genentech, Inc. (South San Francisco, CA)/IDEC Pharmaceutical (San Diego, CA). TNF receptor-Fc fusion protein Etanercept (Enbrel®) was purchased from Wyeth Pharmaceuticals (Hampshire, UK). Epoetin beta (Recormon®) was purchased from Hoffmann-La Roche Ltd (Basel, Switzerland). Interferon β1a (Rebif®) was purchased from EMD Serono, Inc. (Boston, MA).

[0073] Para-Nitrophenyl α- or β-monosaccharide, Lewis sugars, blood type sugars and human milk oligosaccharides were purchased from Carbosynth Limited. (Berkshire, UK). Primary antibody against IgG Fc region, Recormon®, and Rebif® were purchased from

Chemicon (EMD Millipore Chemicals, Billerica, MA). Biotinylated Aleuria Aurantia Lectin (AAL) and HRP-Conjugated Streptavidin were purchased from Vector Laboratory (Burlingame, CA). Chemiluminescence on protein blots was visualized and quantified using the ImageQuant LAS 4000 biomolecular imager system.

BfFucH Activity Analytical Methods

[0074] Enzyme activity was measured at 25°C in 50mM sodium phosphate buffer, pH 7.0, using pNP-α-L-Fuc (p-nitrophenyl-α-L-Fuc) as a substrate, as standard assay condition. One unit of α-L-fucosidase activity was defined as the formation of 1 μmol of pNP and Fuc from the pNP-α-L-Fuc per minute in 50 mM sodium phosphate buffer, pH 7.0, at 25°C. Values for Michaelis constants (Km), Turnover Number (Kcat) and Vmax were calculated for pNP-α-L-Fuc from the Michaelis-Menten equation by non-linear regression analysis by GraphPad Prism v5 software (La Jolla, CA).

Activity Measurement of optimum pH of BfFucH.

[0075] The optimum pH for fucosidase activity was determined in the standard enzyme assay mentioned above in the pH range 4.0-10.0, including sodium acetate, MES, MOPS, HEPES, Tris-HCl, CHES buffer. All reactions were performed in triplicate for statistical evaluation.

Activity Measurement of optimum divalent metal ion of BfFucH

[0076] The assay for metal requirement was performed in standard assay condition. Enzymes were mixed with metal ion (Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, or Ni^{2+}, Fe^{2+}, Cu^{2+}) in a final concentration of 5mM, in the presence and absence of EDTA. All reactions were performed in triplicate for statistical evaluation.

Activity Measurement of optimum temperature of BfFucH

[0077] The effect of temperature on the activity of enzymes were determined by incubating sufficient amount of purified fucosidase with pNP-α-L-Fuc in sodium phosphate buffer (pH 7.0). In order to keep the assay consist, all components were mixed well and preheated at assay temperature for 10 min, and the reaction was started by adding the enzyme and recorded by multimode plate readers (SpectraMax M5, Molecular Devices) in constant temperature. The temperature ranged from 4 to 80°C. All reactions were performed in triplicate for statistical evaluation.

Fucose dehydrogenase-based (FDH) assay

[0078] The fucose dehydrogenase-based assay was slightly modified from previous reports. Unlike other fucose dehydrogenases from Pseudomonas sp sold by Sigma-Aldrich, which are active only react with NADP+, the recombinant form of FDH from Mesorhizobium loti are functional with only NAD+. The NADH formed was measured by NADPH fluorescence at around 450nm when excited with 340nm by multimode plate readers (SpectraMax M5, Molecular Devices) at 25°C. By using this method, fucosyl-conjugates in various oligosaccharides such as Lewis sugar and human milk oligosaccharides (HMOs) were quantitated within 5 min.

Generation of mono-GlcNAc or GlcNAc-(Fuc α-1,6) of immunoglobulin G, Fc-fusion protein, EPO, interferon (IFNβ1a) and Influenza Hemagglutinin (HA)

[0079] All the glycoproteins were buffer exchanging by reaction buffer 50mM sodium phosphate buffer (pH 7.0). First the endoglycosidases cocktail solution, including EndoF1, EndoF2, EndoF3, EndoH and EndoS (1mg/mL), were added in order to remove all the N-glycan chain except the GlcNAc bound to Asn of glycoproteins followed by the suitable quantities of fucosidase. Incubate at 37°C for 48 hours in order to completely remove the core-fucose bound to GlcNAc of glycoproteins.

CLAIMS

We claim:

1. A composition comprising an α-fucosidase comprising a polypeptide having at least 85% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 2, and at least one glycosidase.

2. The compostion of claim 1, wherein the α-fucosidase comprises an isolated polypeptide having at least 88% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 2.

3. The compostion of claims 1-2, wherein the α-fucosidase comprises an isolated polypeptide having an amino acid sequence set forth in SEQ ID NO: 1.

4. The compostion of claims 1-2, wherein the α-fucosidase comprises an isolated polypeptide having an amino acid sequence set forth in SEQ ID NO: 2.

5. The compostion of claim 1, wherein the glycosidase is an endoglycosidase.

6. The compostion of claim 4, wherein the endoglycosidase is selected from the group consisting of Endo-beta-N-acetylglucosaminidases (NAG), EndoA, EndoF1, EndoF2, EndoF3, EndoH, EndoM, EndoS, and variants thereof.

7. The compostion of claim 1, wherein the glycosidase is an exoglycosidase.

8. The compostion of claim 1, wherein the enzyme is a recombinant *Bacteroides* α-L-fucosidase.

9. The compostion of claim 1, wherein the enzyme can hydrolyze α-(1,2), α-(1,3), α-(1,4), and α-(1,6)-linked fucoses present in N- and/or O- linked glycans in a glycoconjugate.

10. The composition of claim 1, wherein the α-fucosidase has pH optimum at 4-9.

11. A method for removing one or more fucoses in a glycoconjugate, the method comprising contacting the glycoconjugate with an α-fucosidase comprising a polypeptide having at least 85% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 2.

12. The method of claim 10, wherein the α-fucosidase comprises an isolated polypeptide having an amino acid sequence set forth in SEQ ID NO: 1.

13. The method of claim 10, wherein the α-fucosidase comprises an isolated polypeptide having an amino acid sequence set forth in SEQ ID NO: 2.

14. The method of claim 10, wherein the glycoconjugate comprises one or more fucoses selected from α-(1,2), α-(1,3), α-(1,4), and α-(1,6)-linked fucoses.

15. The method of claim 12, wherein the α-(1,2), α-(1,3), α-(1,4), and/or α-(1,6)-linked fucoses are present in N- and/or O- linked glycans in a glycoconjugate.

16. The method of claim 10, wherein the glycoconjugate is a glycolipid, glycoprotein, oligosaccharide, or glycopeptide.

17. The method of claim 14, wherein the glycoconjugate is a glycoprotein.

18. The method of claim 15, wherein the glycoprotein comprises a core fucose.

19. The method of claim 16, wherein the core fucose is a core α-(1,3)-linked fucose or a core α-(1,6)-linked fucose.

20. The method of claim 10, further comprising one or more endoglycosidases.

21. The method of claim 18, wherein the one or more endoglycosidases are selected from the group consisting of Endo-beta-N-acetylglucosaminidases (NAG), EndoA, EndoF1, EndoF2, EndoF3, EndoH, EndoM, EndoS, and variants thereof.

Figure 1(a) Kinetics of BfFucH

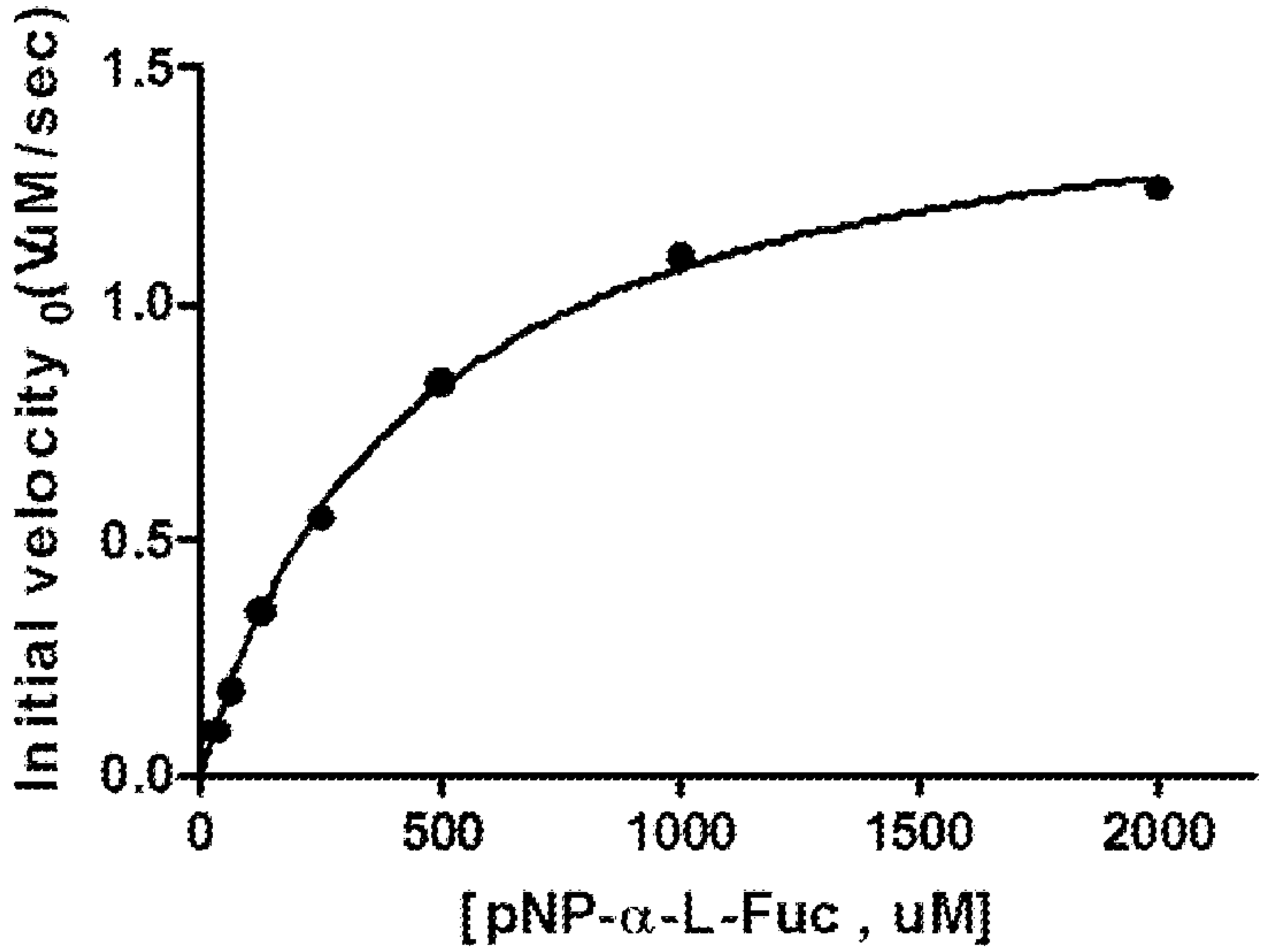


Fig. 1(b)

Parameter	Value
Kcat	183.8 s^{-1}
Km	437.0 uM
Kcat/Km	0.42 s^{-1}/uM
Vmax	1.544 uM/sec

Fig. 1(c)

Parameter	Relative activity (%)
pNP-α-D-Glc	0
pNP-α-D-Gal	0
pNP-α-D-GlcNAc	0
pNP-α-D-GalNAc	0
pNP-α-D-Man	0
pNP-α-L-Fuc	**100**
pNP-β-L-Fuc	0
pNP-α-L-Rha	0

Figure 1

FIG 2 (a) pH profile of BfFucH

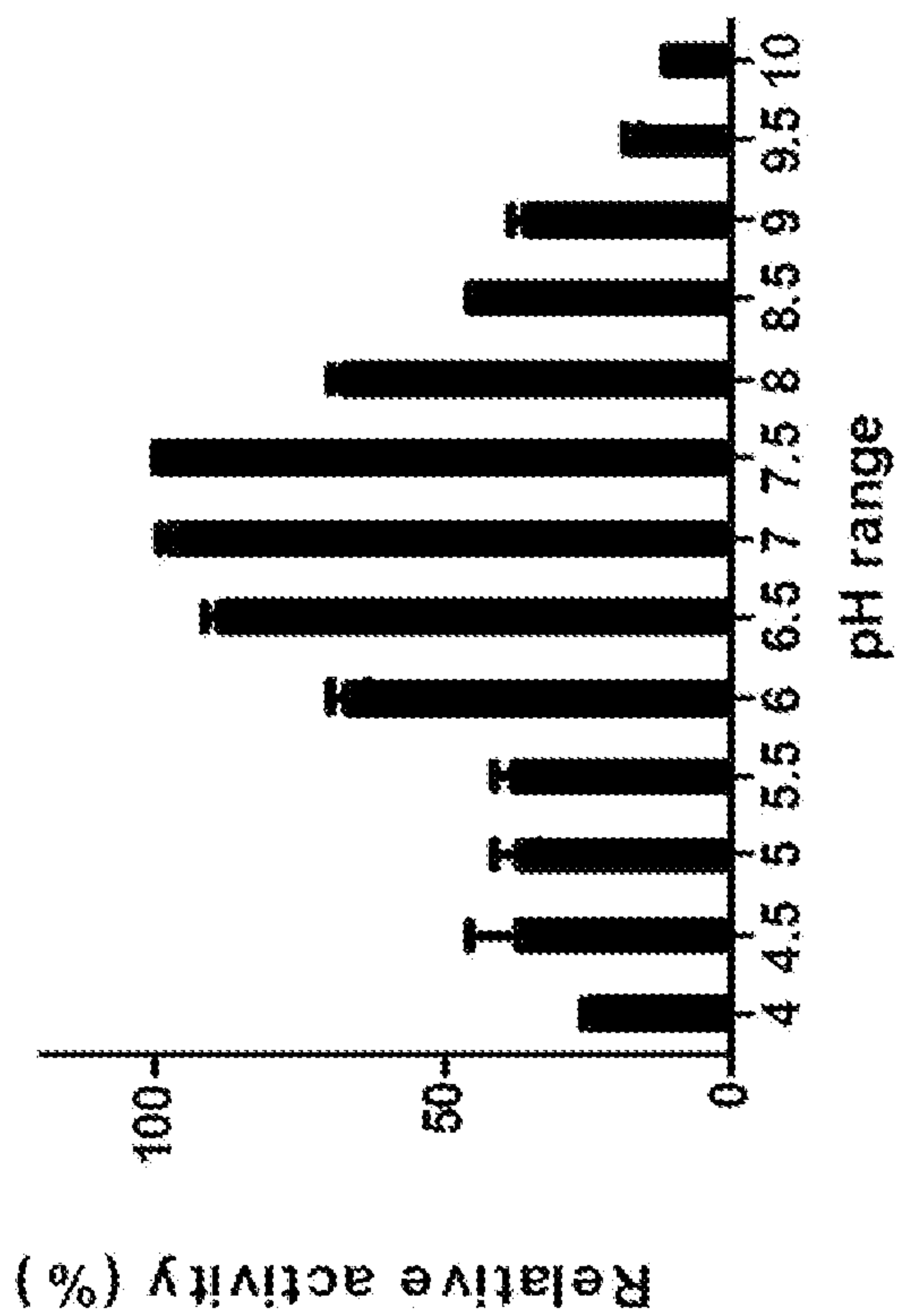


FIG 2 (b) Profile of BfFucH at different temperature

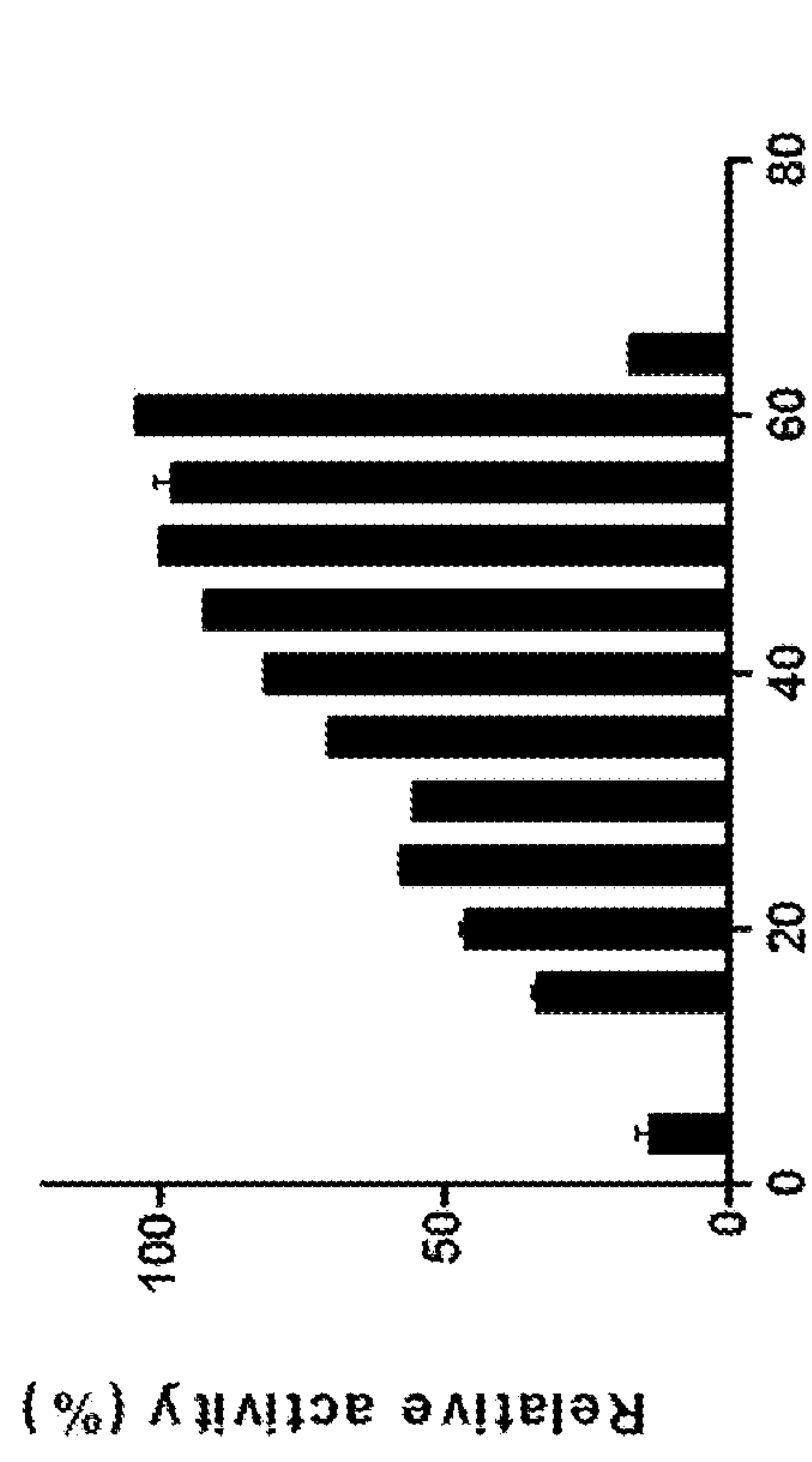
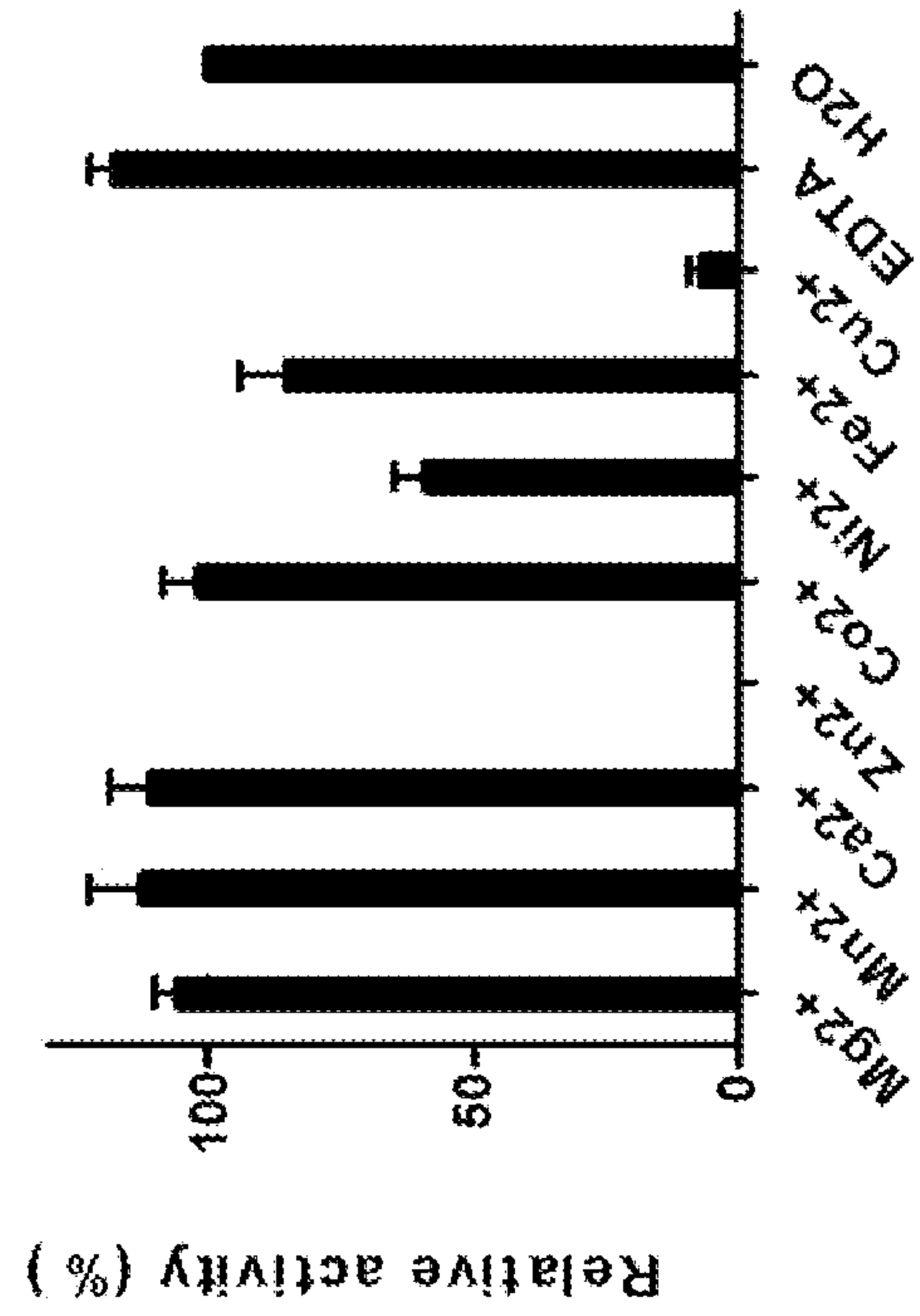
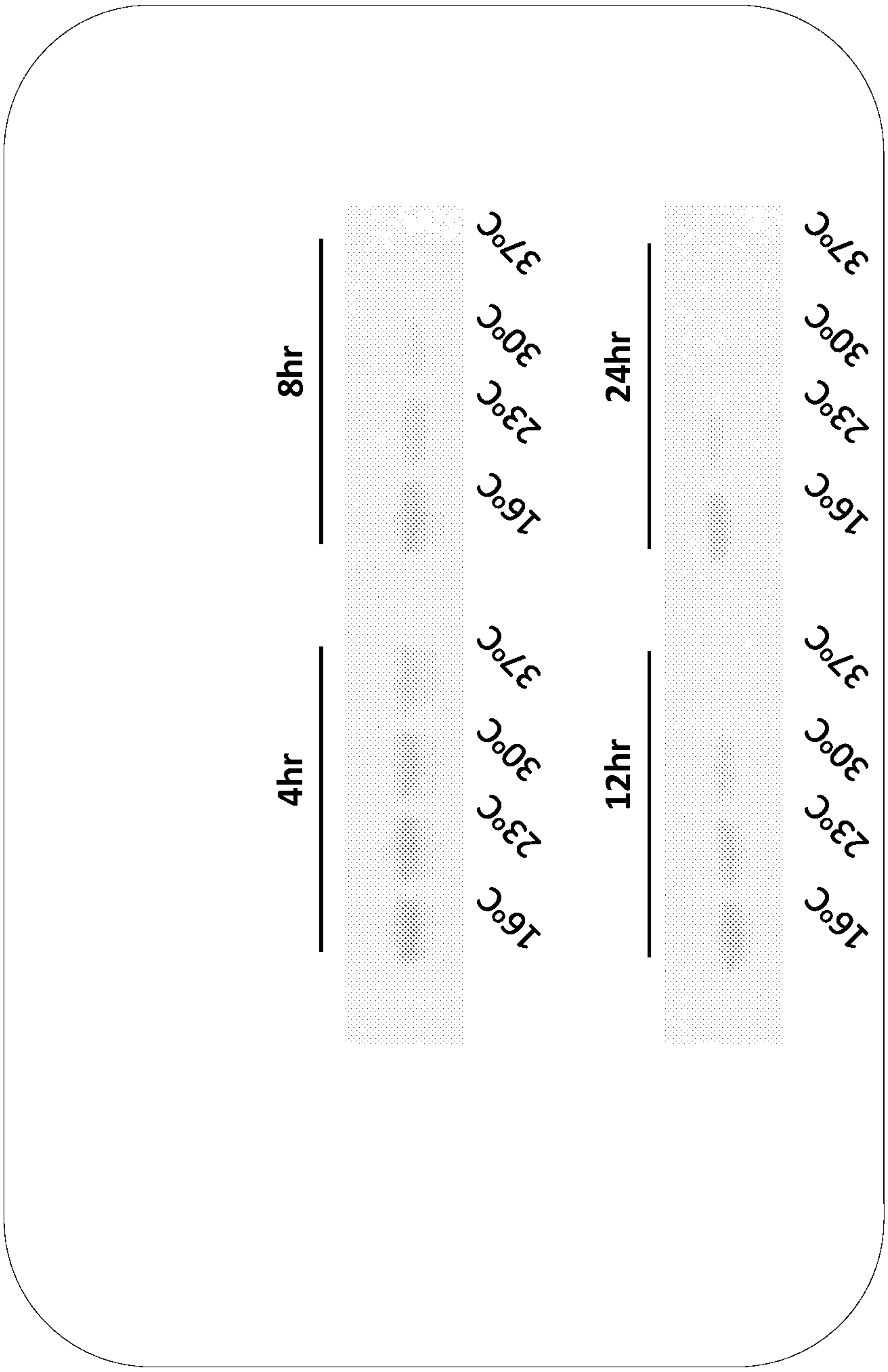


FIG 2 (c) Metal ion influence in fucosidase, BfFucH




8hr
37oC
30oC
23oC
16oC
4hr
37oC
30oC
23oC
16oC
24hr
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30oC
23oC
16oC
12hr
37oC
30oC
23oC
16oC

Figure 3

Figure 1(a) Kinetics of BfFucH

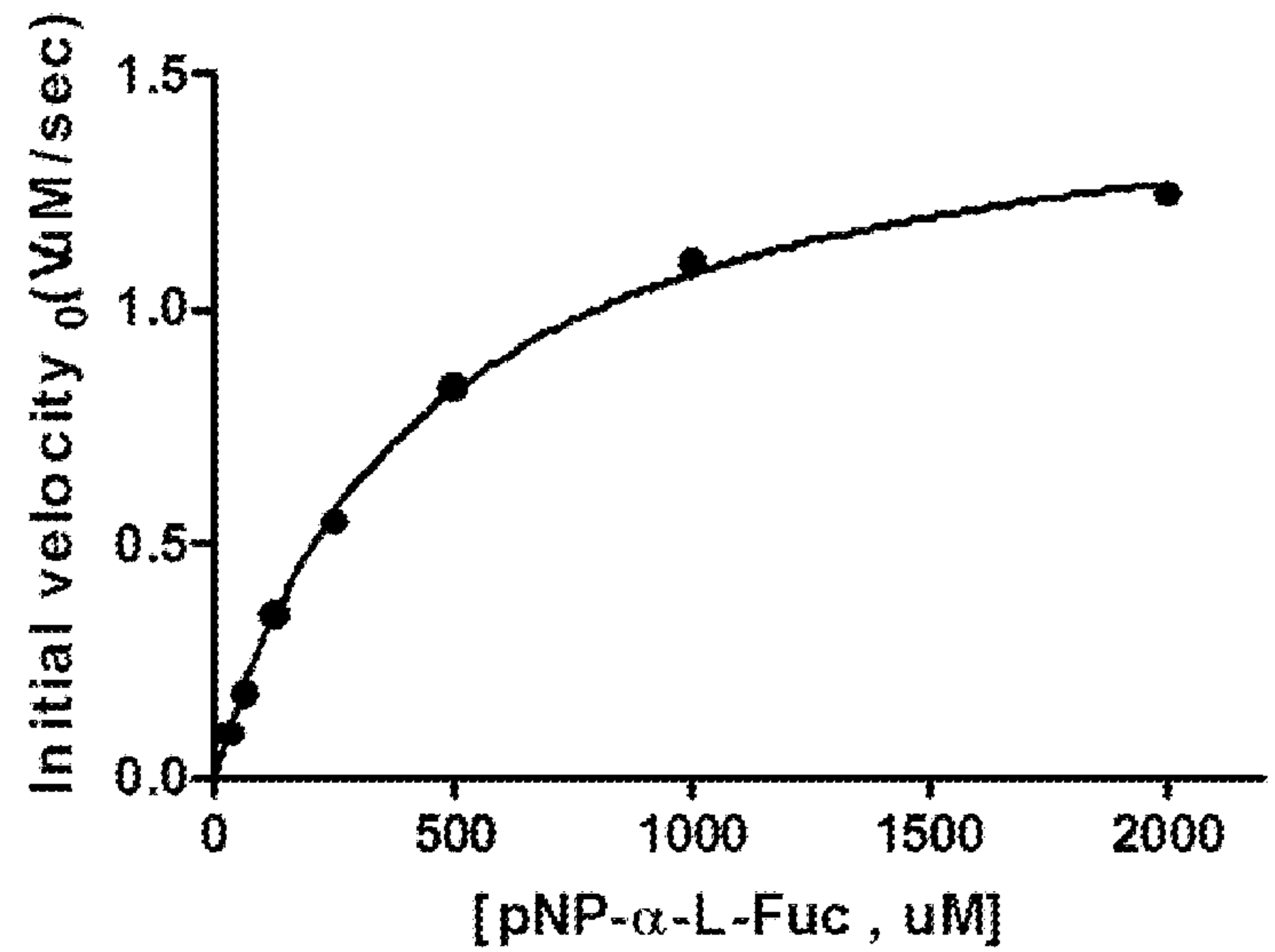

Fig. 1(b)

Parameter	Value
Kcat	183.8 s^{-1}
Km	437.0 uM
Kcat/Km	0.42 s^{-1}/uM
Vmax	1.544 uM/sec

Fig. 1(c)

Parameter	Relative activity (%)
pNP-α-D-Glc	0
pNP-α-D-Gal	0
pNP-α-D-GlcNAc	0
pNP-α-D-GalNAc	0
pNP-α-D-Man	0
pNP-α-L-Fuc	**100**
pNP-β-L-Fuc	0
pNP-α-L-Rha	0

Figure 1