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(54) PURIFICATION OF CYSTATHIONINE BETA-SYNTHASE

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Description

Cross-Reference to Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 61/615,629, filed March 26, 2012, and U.S. Application No. 13/830,494, filed March 14, 2013.

Field of the Invention

[0002] The present invention generally relates to methods for producing an enriched Cystathionine β-Synthase (CBS), particularly truncates thereof.

Background of the Invention

[0003] Cystathionine β-synthase (CBS) plays an essential role in homocysteine (Hcy) metabolism in eukaryotes (Mudd et al., 2001, in The Metabolic and Molecular Bases of Inherited Disease, 8 Ed., pp. 2007-2056, McGraw-Hill, New York). The CBS enzyme catalyzes a pyridoxal 5'-phosphate (PLP; Vitamin B_6)-dependent condensation of serine and homocysteine to form cystathionine, which is then used to produce cysteine by another PLP-dependent enzyme, cystathionine γ-lyase. In mammalian cells that possess the transsulfuration pathway, CBS occupies a key regulatory position between the remethylation of Hcy to methionine or its alternative use in the biosynthesis of cysteine. The relative flux between these two competing pathways is roughly equal and is controlled by intracellular S-adenosylmethionine (AdoMet) concentrations (Finkelstein and Martin, 1984, J. Biol. Chem. 259:9508-13). AdoMet activates the mammalian CBS enzyme by as much as 5-fold with an apparent dissociation constant of 15μM (Finkelstein et al., 1975, Biochem. Biophys. Res. Commun. 66: 81-87; Roper et al., 1992, Arch. Biochem. Biophys. 298: 514-521; Kozich et al., 1992, Hum. Mutation 1: 113-123).

[0004] The C-terminal regulatory domain of human CBS consists of ~140 amino acid residues (Kery et al., 1998, Arch. Biochem. Biophys. 355: 222-232). This region is required for tetramerization of the human enzyme and AdoMet activation (Kery *et al.,* 1998, *id*.). The C-terminal regulatory region also encompasses the previously defined "CBS domains" (Bateman, 1997, Trends Biochem. Sci. 22: 12-13). These hydrophobic sequences (CBS 1 and CBS 2), spanning amino acid residues 416-468 and 486-543 of SEQ ID NO: 1, respectively, are conserved in a wide range of otherwise unrelated proteins. Their function remains unknown, although the sharp transition of thermally induced CBS activation and the observation that mutations in this domain can constitutively activate the enzyme indicates that they play a role in the autoinhibitory function of the C-terminal region (Janosik et al., 2001, Biochemistry 40: 10625-33; Shan et al., 2001, Hum. Mol. Genet. 10: 635-643; Miles and Kraus, 2004, J. Biol. Chem. 279: 29871-4). Two well-conserved CBS domains are also present in the C-terminal region of the yeast CBS, which is of approximately the same length as the human enzyme.

[0005] In healthy normal individuals, CBS-mediated conversion of Hcy to cystathionine is the rate-limiting intermediate step of methionine (Met) metabolism to cysteine (Cys). Vitamin B_6 is an essential coenzyme for this process. In patients with certain genetic mutations in the CBS enzyme, the conversion of Hcy to cystathionine is slowed or absent, resulting in elevations in the serum concentrations of the enzymatic substrate (Hcy) and a corresponding decrease in the serum concentrations of the enzymatic product (cystathionine). The clinical condition of an elevated serum level of Hcy, and its concomitant excretion into the urine, is collectively known as homocystinuria.

[0006] Deficiency of CBS is the most common cause of inherited homocystinuria, a serious life-threatening disease that results in severely elevated homocysteine levels in plasma, tissues and urine. Estimates on the prevalence of homocystinuria vary widely. Ascertainment by newborn screening and clinical ascertainment have indicated a prevalence ranging from 1:200,000 to 1:335,000 (Mudd et al., 1995, The Metabolic and Molecular Basis of Inherited Diseases, McGraw-Hill: New York, p. 1279). The primary health problems associated with CBS-deficient homocystinuria (CBSDH) include: cardiovascular disease with a predisposition to thrombosis, resulting in a high rate of mortality in untreated and partially treated patients; connective tissue problems affecting the ocular system with progressive myopia and lens dislocation; connective tissue problems affecting the skeleton characterized by marfanoid habitus, osteoporosis, and scoliosis; and central nervous system problems, including mental retardation and seizures. Symptoms include dislocated optic lenses, skeletal disorders, mental retardation and premature arteriosclerosis and thrombosis (Mudd *et al.,* 2001, *id*.). Homozygous CBS deficiency is associated with a multitude of clinical symptoms, including mental retardation, osteoporosis, kyphoscoliosis, stroke, myocardial infarction, ectopia lentis, and pulmonary embolism. Cardiovascular complications of the disease, in particular arterial and venous thrombosis, are the principal contributors to early mortality.

[0007] The pathophysiology of CBS deficiency is undoubtedly complex, but there is a consensus that the fundamental instigator of end-organ injury is an extreme elevation of serum Hcy, a substrate of CBS that builds-up in tissues and blood due to the absence of its CBS-catalyzed condensation with L-serine to form cystathionine. The toxicity of profound elevations in blood and tissue concentrations of Hcy may ensue from the molecular reactivity and biological effects of

Hcy *per se* or from its metabolites (*e.g.* Hcy-thiolactone) that affect a number of biological processes (Jakubowski et al., 2008, FASEB J 22: 4071-6). Abnormalities in chronic platelet aggregation, changes in vascular parameters, and endothelial dysfunction have all been described in patients with homocystinuria.

[0008] Currently, three treatment options exist for the treatment of CBSDH:

1) Increase of residual activity of CBS activity using pharmacologic doses of Vitamin B_6 in Vitamin B_6-responsive patients
2) Lowering of serum Hcy by a diet with a strict restriction of the intake of Met; and
3) Detoxification by betaine-mediated conversion of Hcy into Met, thus lowering serum Hey concentration.

[0009] Each of these three therapies is aimed at lowering serum Hcy concentration. The standard treatment for individuals affected with Vitamin B_6 non-responsive CBSDH consists of a Met-restricted diet supplemented with a metabolic formula and Cys in the form of cysteine (which has become a conditionally essential amino acid in this condition). Intake of meat, dairy products, and other food high in natural protein is prohibited. Daily consumption of a poorly palatable, synthetic metabolic formula containing amino acids and micronutrients is required to prevent secondary malnutrition. Supplementation with betaine (trade name: Cystadane™, synonym: trimethylglycine) is also standard therapy, wherein betaine serves as a methyl donor for the remethylation of Hcy to Met catalyzed by betaine-homocysteine methyltransferase in the liver (Wilcken et al., 1983, N. Engl. J. Med. 309: 448-53). Dietary compliance generally has been poor, even in those medical centers where optimal care and resources are provided, and this non-compliance has major implications on the development of life-threatening complications of homocystinuria.

[0010] To enable patients with homocystinuria enjoy a far less restrictive diet (*e.g.* daily intake limited to 2 g protein per kg, which is easily attainable), and have a significantly decreased Hcy plasma level leading in the long-term to clinical improvement, a strategy for increasing enzyme activity provides potential for treatment as set forth in co-pending U.S. provisional patent application Serial No. 61/758,138 . The most effective therapeutic strategy is to increase enzyme activity, as is evident when Vitamin B_6-responsive homocystinuria patients are given pyridoxone. However, this strategy is not possible for Vitamin B_6 non-responsive patients due to the nature of the mutations. Enzyme replacement therapy (ERT) as a way to increase enzyme activity in these patients requires exogenous enzyme, which is not present in the art and thus raises a need in the art for improved reagents and methods for producing CBS in greater yields of sufficiently purified enzyme for therapeutic administration.

[0011] Kraus and colleagues have developed expression systems and fermentation conditions for generating active recombinant human CBS and variants thereof (US patents No. 5,635,375, 5,523,225 and 7,485,307. These proteins were purified by processes relevant for academic purposes, including use of protein leads on the proteins which are not considered useful for preparation of pharmaceuticals.

[0012] In order to employ methods of increasing CBS enzyme activity, an efficient method of CBS enzyme purification is required. Existing methods of purification for recombinant CBS protein rely on affinity tags to facilitate purification that does not provide the desired purity and efficiency. Therefore to more efficiently obtain the necessary levels of CBS required for therapeutic use there is a need for improved downstream purification of CBS protein produced in microbial cells.

[0013] "Kinetic characterization of recombinant human cystathionine beta-synthase purified from E. coli", by Belew M S et al.. Protein Expression and Purification, Academic Press, San Diego, CA, vol. 64, no. 2, pages 139 - 145, ISSN 1046-5928 suggests that cystathionine P synthase (CBS) catalyzes the pyridoxal-5'-phosphate-dependent condensation of 1-serine and 1-homocysteine to form 1-cystathionine in the first step of the transsulfuration pathway. A series of five expression constructs, each incorporating a 6-His tag, are described to enable the efficient purification of hCBS via immobilized metal ion affinity chromatography. Two of the constructs are described as expressing hCBS in fusion with a protein partner, while the others are described as only bear the affinity tag. The addition of an amino-terminal, 6-His tag, in the absence of a protein fusion partner and in the absence or presence of a protease-cleavable linker, is described as being sufficient for the purification of soluble hCBS and resulting in enzyme with 86-91% heme saturation and with activity similar to that reported for other hCBS expression constructs.

Summary of the Invention

[0014] This invention provides methods for producing an enriched CBS solution, wherein the CBS solution comprises a naturally occurring, chemically cleaved or genetically engineered truncated CBS protein, the method comprising: (a) providing a CBS containing solution comprising one or a plurality of impurities; (b) performing chromatographic separation of the CBS-containing solution using an immobilized metal affinity chromatography (IMAC) resin charged with zinc; and (c) performing at least one additional chromatographic separation selected from the group consisting of: hydrophobic interaction chromatography (HIC), ceramic hydroxyapatite (CHAP), and ion exchange chromatography, thereby producing the enriched cystathionine p-synthase solution. In certain embodiments the truncated CBS protein has an amino

acid sequence identified by SEQ ID NO: 3. In particular embodiments, the CBS solution is a clarified CBS solution.

[0015] In certain embodiments the truncated CBS protein is produced in a recombinant cell. In particular embodiments, the recombinant cell is a bacterial cell.

[0016] In certain embodiments, the CBS-containing solution is obtained by homogenizing recombinant bacterial cells expressing a recombinant construct comprising a nucleic acid sequence encoding CBS. In particular embodiments, the nucleic acid sequence encodes a truncated CBS protein. In particular embodiments, the truncated CBS protein has been truncated to an ending position of one of amino acid residues from 382-532 or 543-550 of SEQ ID NO: 4. In particular embodiments, the CBS nucleic acid sequence comprises SEQ ID NO: 4. In particular embodiments, the bacterial cells are *E. coli* cells. In particular embodiments, the nucleic acid sequence encoding the truncated CBS protein is optimized for expression in *E. coli* cells.

[0017] In another aspect, the invention provides a use of a method in the production of a CBS solution, wherein the method comprises: (a) providing a CBS-containing solution in the presence of at least one impurity, wherein said CBS protein is a naturally occurring truncated protein, or a chemically cleaved or genetically engineered truncate thereof; and (b) performing chromatographic separation of the CBS-containing solution using a metal affinity chromatography (IMAC) resin charged with zinc.

[0018] In certain embodiments, the use further comprises (c) performing chromatographic separation using ion exchange chromatography. In particular embodiments, the ion exchange chromatography column is a weak anion exchanger. In other embodiments the weak anion exchanger is a DEAE-Sepharose FF column.

Brief Description of Drawings

[0019] The following detailed description of the embodiments of the present invention can be best understood when read in conjunction with the following drawings.

Fig. 1 is a purification train summary from scale-up generation runs using a multi-step chromatography method including DEAE-Sepharose-FF, Zn-IMAC and HIC chromatography.

Fig. 2 is a purification summary from purification experiments using a DEAE-Sepharose-FF column and CBS purified using the "non-optimized" bacterial expression construct. Mobile phases included 10% ethylene glycol in addition to other components as set forth in the Examples.

Fig. 3 is a purification train summary from scale-up generation runs using a multi-step chromatography method including DEAE-Sepharose-FF, Zn-IMAC, ceramic hydroxyapaptite resin and HIC chromatography.

Fig. 4 is a photoimage of a SDS page gel showing the relative amounts of CBS protein and impurities for each stage of the purification step using a DEAE column.

Fig. 5 is a photoimage of a SDS page gel showing the relative amounts of CBS protein and impurities for a 3 column purification method including: a DEAE column, a Zn-IMAC column and HIC column.

Fig. 6 is a photoimage of a SDS page gel showing the relative amounts of CBS protein and impurities for a 4 column purification method including: a DEAE column, a Zn-IMAC column, a ceramic hydroxyapaptite resin and a HIC column.

Fig. 7 is chromatograms demonstrating the components of the separated mixture following purification using Zn-IMAC.

Fig. 8 is a purification summary from development runs using a Ni-IMAC column.

Fig. 9 is a summary table demonstrating the total protein following a purification method using a Ni-IMAC column.

Fig. 10 is a photoimage of a SDS page gel showing the relative amounts of CBS protein and impurities for each stage of the purification step using a Ni-IMAC column.

Fig. 11 is a purification summary from scale-up generation runs using a Cu-IMAC column.

Fig. 12 is a summary table demonstrating the total protein following a purification method using a Zn-IMAC column.

Fig. 13 is a photoimage of a SDS page gel showing the relative amounts of CBS protein and impurities for each stage of the purification step using a Zn-IMAC column.

Fig. 14 is a scheme of the purification method using multi-step chromatography purification steps.

Detailed Description of the Invention

[0020] This invention provides methods for producing an enriched CBS solution, wherein the CBS solution comprises naturally occurring, chemically cleaved or genetically engineered truncated CBS protein. In particular, the methods include the steps (a) providing a CBS-containing solution in the presence of at least one impurity; and (b) performing chromatographic separation of said CBS-containing solution using a metal affinity chromatography (IMAC) resin charged with zinc; and (c) performing at least one additional chromatographic separation selected from the group consisting of: hydrophobic interaction chromatography (HIC), ceramic hydroxyapatite (CHAP), and ion exchange chromatography. In

one embodiment, the ion exchange chromatography column is an anion exchanger, preferably a weak anion exchanger. Various types of anion exchange resins can be used, including DEAE-Sephadex, QAE-Sephadex, DEAE-Sephacel, DEAE-cellulose and DEAE-Sepharose-FF. According to one embodiment, the anion exchange resin is DEAE-Sepharose-FF.

[0021] The metal affinity chromatography (IMAC) resin having appropriate pH and conductivity allows the protein to bind to the column while selective intermediate washes are used to remove weaker binding proteins and other molecular species. In certain embodiments, varying concentrations of imidazole were used to modulate the partitioning during the chromatography. HIC is useful for removing impurities that have relatively closely related chromatographic properties that are eluted together with the target protein during the capture step. "Ceramic hydroxyapatite" or "CHAP" refers to an insoluble hydroxylated calcium phosphate of the formula ($Ca_{10}(PO_4)_6(OH)_2$), which has been sintered at high temperatures into a spherical, macroporous ceramic form. The methods of the invention also can be used with hydroxyapatite resin that is loose or packed in a column. The choice of column dimensions can be determined by the skilled artisan.

[0022] Chromatography matrices useful in the method of the invention are materials capable of binding biochemical compounds, preferably proteins, nucleic acids, and/or endotoxins, wherein the affinity of said biochemical compounds to said chromatography matrix is influenced by the ion composition of the surrounding solution (buffer). Controlling the ion composition of said solution allows to use the chromatography materials of the invention either in subtractive mode (CBS passes through said chromatography matrix, at least certain contaminants bind to said chromatography matrix) or, preferably, in adsorptive mode (CBS binds to the chromatography matrix).

[0023] In particular embodiments, the method for producing an enriched CBS solution comprises the step of homogenizing recombinant bacterial cells expressing a recombinant construct comprising a nucleic acid sequence encoding CBS. In particular embodiments, CBS is a truncated CBS protein and the nucleic acid sequence encoded said truncate has been optimized for expression *in E. coli* cells. A specific embodiment of such a nucleic acid sequence optimized for CBS expression in *E. coli* is set forth in SEQ ID NO: 4. In said methods, cells are harvested, e.g. by centrifugation, and optionally stored at -80 degree °C. Homogenization of host cells is performed by disrupting the cells host using physical, chemical or enzymatic means or by a combination thereof. Advantageously, for purification from bacterial sources homogenation is performed by disrupting the cell wall of said bacterial host by sonication. Alternatively or additionally homogenizing is performed by destabilizing the bacterial cell wall of the host by exposure to a cell wall degrading enzyme such as lysozyme.

[0024] Disclosed herein is also a clarified CBS homogenate, wherein cell debris is removed from the homogenate by either filtration or centrifugation. In certain aspects, clarifying is performed by centrifuging the homogenate at an effective rotational speed. The required centrifugation time depends *inter alia* on the volume of the homogenate, which can be determined empirically to obtain a sufficiently solid pellet. To obtain an essentially cell debris-free clarified homogenate a combination of centrifugation and filtration can be performed on the homogenate.

[0025] The term "recombinant cell" as used herein refers to suitable cells (including progeny of such cells) from any species into which has been introduced a recombinant expression construct capable of expressing a nucleic acid encoding CBS protein, preferably human CBS protein and most particularly a human CBS protein that is a naturally occurring truncated variant, or a chemically cleaved or genetically engineered truncate thereof. In specific embodiments, the truncated CBS protein encoded by said recombinant expression construct has an amino acid sequence as set forth in SEQ ID NO: 3.

[0026] The term, "bacterial cell", as used herein refers to bacteria that produces a mammalian, preferably human, CBS protein *inter alia* using recombinant genetic methods including progeny of said recombinant cell, wherein said CBS protein is a naturally occurring truncated variant, or a genetically engineered truncate thereof.

[0027] The term "recombinant expression construct" as used herein refers to a nucleic acid having a nucleotide sequence of a mammalian, preferably human, CBS protein, and sequences sufficient to direct the synthesis of CBS protein in cultures of cells into which the recombinant expression construct is introduced and the progeny thereof.

[0028] As used herein, reference to CBS protein or polypeptide preferably includes a naturally occurring, chemically cleaved or genetically engineered truncate CBS protein, or fusion proteins, or any homologue (variant, mutant) thereof, and specifically mammalian CBS and preferably human CBS. Such a CBS protein can include, but is not limited to, purified CBS protein, recombinantly produced CBS protein, soluble CBS protein, insoluble CBS protein, and isolated CBS protein associated with other proteins. In addition, a "human CBS protein" refers to a CBS protein from a human *(Homo sapiens)* preferably includes a naturally occurring truncated variant, or a chemically cleaved or genetically engineered truncate thereof., As such, a human CBS protein can include purified, partially purified, recombinant, mutated/modified and synthetic proteins. As disclosed herein and in related U.S. Patent No. 8,007,787 and 7,485,307, the CBS protein truncates are advantageously soluble CBS proteins that are produced in bacteria without the creation of insoluble inclusion bodies.

[0029] As used herein, the term "homologue" (or variant or mutant) is used to refer to a protein or peptide which differs from a naturally occurring protein or peptide (*i.e.*, the "prototype" or "wild-type" protein) by modifications to the naturally occurring protein or peptide, but which maintains the basic protein and side chain structure of the naturally occurring

form. Such changes include, but are not limited to: changes in one, few, or even several amino acid side chains; changes in one, few or several amino acids, including deletions (*e.g.*, a truncated version of the protein or peptide), insertions and/or substitutions; changes in stereochemistry of one or a few atoms; and/or minor derivatizations, including but not limited to: methylation, glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidyl inositol. A homologue can have enhanced, decreased, changed, or substantially similar properties as compared to the naturally occurring protein or peptide. A homologue can include an agonist of a protein or an antagonist of a protein.

[0030] Homologues can be the result of natural allelic variation or natural mutation. A naturally occurring allelic variant of a nucleic acid encoding a protein is a gene that occurs at essentially the same locus (or loci) in the genome as the gene which encodes such protein, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. One class of allelic variants can encode the same protein but have different nucleic acid sequences due to the degeneracy of the genetic code. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (*e.g.*, in regulatory control regions). Allelic variants are well known to those skilled in the art.

[0031] Homologues can be produced using techniques known in the art for the production of proteins including, but not limited to, direct modifications to the isolated, naturally occurring protein, direct protein synthesis, or modifications to the nucleic acid sequence encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis. CBS variants are described in U.S. Patent No. 8,007,787, in particular and preferred embodiments, the reagents and methods of the invention set forth herein preferably include a naturally occurring truncated variant, or a chemically cleaved or genetically engineered truncate of human CBS protein. Particular truncated forms of SEQ ID NO: 3 according to the present invention include N-terminal deletion variants, C-terminal deletion variants, and variants having both N-terminal and C-terminal deletions.

[0032] As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein *in vitro, ex vivo* or *in vivo.* For a protein to be useful *in vitro, ex vivo* or *in vivo*, it is preferably substantially free of contaminants, other proteins and/or chemicals that might interfere or that would interfere with its use, or that at least would be undesirable for inclusion with a CBS protein (including homologues thereof).

[0033] As used herein an enriched CBS solution is a solution subjected to one or more purification steps.

[0034] The purity of protein can be determined by calculating fold purification, *i.e.* a formula that provides a measure of how much more a purified solution is compared to a less purified solution or crude extract. Fold purification is calculated using the following formula:

$$\textit{Specific activity final fraction/Specific activity crude fraction.}$$

[0035] Another measurement to assess purity is the "specific activity" which measures the purity of an enzyme. Specific activity can be measured using the following formula:

$$\frac{Units}{mL} \times \frac{mL}{mg} = \frac{Units}{mg}$$

[0036] CBS protein compositions provided by this invention are useful for regulating biological processes and particularly, processes associated with the catalysis of the pyridoxal 5'-phosphate (PLP)-dependent condensation of serine and homocysteine to form cystathionine. In particular, compositions of the present invention are useful for producing cystathionine and cysteine in vitro or for treating a patient that will benefit from increased CBS activity (*e.g.*, a patient with homocystinuria). In certain embodiments, the invention provides said compositions of CBS protein, preferably human CBS protein, wherein said CBS protein is a naturally occurring truncated variant, or a chemically cleaved or genetically engineered truncate of human CBS protein, as pharmaceutical compositions comprising said CBS protein and a pharmaceutically acceptable carrier.

[0037] As used herein, a "pharmaceutically acceptable carrier" includes pharmaceutically acceptable excipients and/or pharmaceutically acceptable delivery vehicles, suitable for use in suitable administration of the composition *in vitro, ex vivo* or *in vivo.* Suitable *in vitro*, *in vivo* or *ex vivo* administration preferably comprises any site where it is desirable to regulate CBS activity. Suitable pharmaceutically acceptable carriers are capable of maintaining a CBS protein as provided by this invention in a form that, upon arrival of the protein at the target cell or tissue in a culture or in patient, the protein has its expected or desired biological activity. Examples of pharmaceutically acceptable carriers include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable

auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity. Compositions of the present invention can be sterilized by conventional methods and/or lyophilized.

[0038] The following examples are provided for the purpose of illustration and are not intended to limit the scope of the present invention.

Examples

Example 1: Production of Truncated CBS Protein in Bacteria

[0039] A truncated human CBS variant lacking specific portions of the non-conserved regions (r-hCβSΔC; SEQ ID No: 3) were constructed and over-expressed using the previously described *E. coli* based expression system (Kozich and Kraus, 1992, *supra).* In the modification of this system disclosed herein (*i.e.*, expressing the truncate rather than the full-length CBS protein), the CBS truncate encoded by SEQ ID NO: 3 was expressed without any fusion partner under the control of the *tac* promoter. Constructs encoding the truncated human CBS protein variant r-hCβSΔC (SEQ ID NO: 4) were generated by a modification of the previously described pHCS3 CBS expression construct (Kozich and Kraus, 1992, Hum. Mutat. 1,113-123) which contains the CBS full-length coding sequence (SEQ ID NO: 1) cloned into pKK388.1. In this construct, CBS expression was governed by the IPTG inducible *lac* promoter. To generate C-terminal deletion constructs, CBS cDNA fragments spanning the desired nucleotide residues were amplified using primers incorporating Sph I and Kpn I sites to the 5' and 3' respective ends of the PCR product. All PCR products were then cut with Sph I and Kpn I and cloned by ligation into the pHCS3 vector digested with Sph I and Kpn I. An Sph I site naturally occurs in the CBS cDNA, just upstream of the antisense primer hybridization site (base pare position 1012, according to the CBS cDNA numbering, ref. 25). PCR products thus generated were then digested with Nco I and Sph I and ligated into the pHCS3 plasmid cut with the same enzymes.

pKK CBS A414-551

[0040]

sense: CGTAGAATTCACCTTTGCCCGCATGCTGAT (SphI)(SEQ ID NO: 5)
antisense: TACGGGTACCTCAACGGAGGTGCCACCACCAGGGC (KpnI)(SEQ ID NO: 6)

[0041] Finally, the construct was transformed into *E. coli* BL21 (Stratagene). The authenticity of the construct was verified by DNA sequencing using a Thermo Sequenase Cy5.5 sequencing kit (Amersham Pharmacia Biotech) and the Visible Genetics Long-Read Tower System-V3.1 DNA sequencer according to the manufacturer's instructions.

[0042] *Bacterial Expression analysis of CBS Deletion Mutants-Growth of E. coli.* BL21 cells bearing the CBS truncation mutant construct, induction of expression and the generation of crude cell lysates were performed as described previously (Maclean et al., 2002, Hum. Mutat. 19(6), 641-55). Briefly, bacteria were grown at 37° C aerobically in 1 L NZCYMT media (Gibco/BRL, Gaithersburg, Md.) containing 75 μg/mL ampicilin and 0.001% thiamine in the presence or absence of 0.3 mM δ-aminolevulinate (δ-ALA) until they reached turbidity of 0.5 at 600 nm. IPTG was then added to 0.5 mM and the bacteria were grown further. The insoluble fraction was prepared as follows: after the centrifugation of the sonicated homogenate, pelleted cell debris were thoroughly washed with chilled Ix Tris-buffered saline, pH 8.0. The pellets were then resuspended inlml of the lysis buffer (Maclean *et al.*, *ibid.)* followed by a brief sonication in order to homogenize the insoluble fraction.

[0043] *CBS Activity Assay*- CBS activity was determined by a previously described radioisotope assay using [^{14}C] serine as the labeled substrate (Kraus, 1987, Methods Enzymol. 143,388-394). Protein concentrations were determined by the Lowry procedure (Lowry et al., 1951, J. Biol. Chem. 193, 265-275) using bovine serum albumin (BSA) as a standard. One unit of activity is defined as the amount of CBS that catalyzes the formation of 1 μmol of cystathionine in 1 h at 37°C.

[0044] *Denaturing and Native Polyacrylamide Gel Electrophoresis and Western Blotting*-Western blot analysis of crude cell lysates under both denaturing and native conditions was performed as described previously (Janosik, 2001, *supra)* with some modifications. Soluble fractions of *E. coli* lysates containing the expressed mutant protein were mixed with sample buffer and run on a 6% native PAGE without a stacking gel. The final composition of the sample buffer was: 50mM Tris-HCl, pH 8.9, 1mM DTT, 10% glycerol, 0.001% bromphenol blue. Detection of heme was performed using a previously described method that relies on heme peroxidase activity (Vargas et al., 1993, Anal. Biochem. 209(2), 323-6).

[0045] *Densitometric Scanning Analysis-Quantitative* densitometry analysis was performed using the Imagemaster ID (version 2.0) software (Pharmacia). To construct a calibration curve, 50, 75, 100,250,500 and 1000ng of purified wild type CBS protein were run on an SDS-PAGE together with crude cell lysates of the individual mutants. Following

electrophoresis, Western blot immunoanalysis was conducted using rabbit anti-CBS serum. The signals corresponding to the experimentally observed CBS mutant subunits were all within the linear range of the calibration curve constructed with purified human CBS.

Example 2: Preparation of Crude Extraction

[0046] Crude CBS protein-containing extracts was prepared for use in downstream chromatography steps. Frozen pellets (cells) obtained from fermentation of recombinant bacteria producing human truncated CBS variant (r-hCβSΔC; SEQ ID No: 3) were lysed, wherein said bacteria expressed truncated human CBS encoded by SEQ ID NO: 4. Lysis buffer for initial isolations contained 1 mM DTT, 1% Triton X-100, and Protease Inhibitor. These components were eventually removed from the buffer. The buffer used for the final isolations that produced material for scale-up runs consisted of 20mM Sodium Phosphate, 50 mM NaCl, 0.1 mM PLP (pH 7.2), with lysozyme added to a concentration of 2 mg/mL after homogenization. Following mixing with lysozyme for 1 hr at 4°C, the homogenate was sonicated until viscosity was reduced and then subjected to centrifugation at 20,000 rpm (48,000 x g) for 30 min. The supernatant was collected, aliquoted, and stored at -70°C until use. Generally, the crude extract was thawed at 37°C prior to chromatographic purification.

Example 3: DEAE-Sepharose FF Chromatography

[0047] DEAE-Sepharose FF was used in this Example of the purification methods for CBS because it possesses good capacity and flow properties and has been manufactured consistently for several years. This step employed a drip/gravity column that contained approximately 6 mL of resin. The column was equilibrated in Sodium Phosphate buffer with 50 mM NaCl, pH 7.0. Loading of the crude extract was targeted at approximately 20 mg total protein/mL resin. After loading the column, the red color of the load was concentrated near the top of the column. Following a wash with equilibration buffer, the column was washed with a buffer containing 150 mm NaCl, whereby the majority of color eluted from the column (all steps were performed at pH 7.0). Essentially all color was removed from the column with a 300mM NaCl wash. Based on these results, a column was packed that could be operated in flow mode. The conditions employed equilibration/loading at a NaCl concentration 50mM, with elution at 250mM NaCl. The final conditions required dilution of the column load with H_2O to approach the ionic strength of the equilibration/wash buffer (50mM NaCl), and elution with 137 mM NaCl (Figs. 1, 2 and 3). Samples were analyzed by SDS-PAGE to determine the relative amounts of CBS protein and impurities (Fig. 4). The following tables represent column operational parameters and data from the scale-up runs that employed them.

Table 1. Operational Parameters for DEAE Capture Step

Process Step	Column load target (total protein mg/mL)	NaCl Concentration (with 20mM Na_3PO_4 pH 7.0)	Column Volumes (mL)	Contact time Column vol./flow rate (min.)
Equilibration	N/A	50 mM	3-5	10
Load	20-25 mg/mL	Approx. 50 mM	Variable	15
Wash	N/A	50 mM	3	10
Elution	N/A	137 mM	Variable*	15
2M NaCl Strip	N/A	2 M	3	10
* Note: Eluate collection starts at approx. 0.4 AU and ends at approx. 0.55 AU. Void volume typically approx. 1 column volume.				

Table 2. Data from Scale-up Runs (n=6)

Input		Output		
Column loading (per mL Resin)				
Total Protein (mg)	Units	Recovery (%)	Fold Purif. (By S.A.)	
14.5 - 19.8	3275 - 5443	79.3 - 93.0	2.5 - 3.3	*Range*

(continued)

Input		Output		
Column loading (per mL Resin)				
Total Protein (mg)	Units	Recovery (%)	Fold Purif. (By S.A.)	
18.2	4451	86	2.8	*Average*

Example 4: IMAC Chromatography

[0048] The ability for an immobilized metal affinity column (IMAC) to separate CBS protein from impurities and other contaminants from a biological source, such as a recombinant bacterial cell homogenate, was demonstrated. Because of the desire to avoid low pH conditions (< 5, anecdotal), varying concentrations of imidazole were used to modulate partitioning during the chromatography.

[0049] Copper (Cu^{++} was tested as a candidate species of IMAC column based on its relatively strong binding characteristics. Prior to being applied to the IMAC column, the CBS solution was adjusted to 0.4M NaCl. The results indicated that capture was near complete, with an acceptable activity recovery (70-80%). Recovery of CBS was obtained using 100mM imidazole, which resulted in significant precipitation upon thawing from storage at -70° C. (Fig. 11). In addition, there was only a small increase in purity relative to the load. Thus, experiments employing Ni^{++}IMAC were conducted as the metal of choice. In these experiments, the CBS sample was run through a G-25 column to remove dithiothreitol (DTT) prior to loading the solution onto the IMAC column. Purity enhancement remained low and selectivity was similar to Cu^{++}, as evidenced by a relatively small A_{280} peak in the high imidazole strip fraction. (Figs 8, 9 and 10).

[0050] The relatively weak binding Zn^{++} was also tested. Although capture, wash and elution conditions required significantly lower imidazole concentrations, potential for purity enhancement provided positive results due to the significant size of the A_{280} peaks in the post-load wash and high imidazole strip fractions. 0.4 M NaCl and 0.01% Triton X-100 were added to the equilibration and wash buffers to minimize non-specific binding. (Figs. 1 and 3). Samples were analyzed by SDS-PAGE to determine the relative amounts of CBS protein and impurities (Fig. 13). The results of the IMAC experiments are presented in Fig. 7. The following tables represent column operational parameters and data from the scale-up runs that employed them.

Table 3. Operational Parameters for Zn-IMAC Step

Process Step	Column load target (total protein mg/mL)	Imidazole Concentration (with 20mM Na_3PO_4 pH 7.0)	Column Volumes (mL)	Contact time Column vol./flow rate (min.)
Equilibration	N/A	1 mM	3	10
Load	10	0	Variable	10
Wash	N/A	1 mM	3	10
Elution	N/A	11 mM	Variable*	10
Strip	N/A	100 mM	3	10
* Note: Eluate collection starts at approx. 0.25 AU and ends at approx. 0.16 AU. Void volume typically approx. 1.5 column volumes.				

Table 4. Data from Scale-up Runs (n=5)

Input		Output		
Column loading (per mL Resin)				
Total Protein (mg)	Units	Recovery (%)	Fold Purif. (By S.A.)	
6.5-9.3	4414-7038	71.8 - 84.6	1.3 - 1.6	*Range*
8.1	5687	80	1.4	*Average*

Example 5: HIC Chromatography

[0051] Multiple experiments were conducted to identify the parameters for HIC chromatography. Initial drip column experiments were conducted that employed a resin with a relatively strong binding ligand (phenyl) with an IMAC eluate as starting material/load. This experiment resulted in empirically complete binding at 1.3M $(NH_4)_2SO_4$. However, there was evidence of significant retention of CBS on the column even after washing with a low ionic strength buffer. Based on these results, a resin with a weaker binding ligand (butyl) was tested. Initial experiments with this resin showed no apparent capture at 0.5M $(NH_4)_2SO_4$. The non-binding flow through of this column experiment was collected and adjusted to 1.25M $(NH_4)_2SO_4$, and reloaded on to a column equilibrated to the same concentration of $(NH_4)_2SO_4$. In this case there was evidence of significant binding to the column. A 20 column volume $(NH_4)_2SO_4$ gradient elution was performed from 1.25M to 0.25M $(NH_4)_2SO_4$ with fractions collected. SDS-PAGE analysis of the fractions indicated that there was significant potential for impurity clearance on the lower end of the gradient. Experiments utilizing step gradient washes at varying concentrations of $(NH_4)_2SO_4$ determined the final operational parameters. (Figs. 1 and 3). Those parameters and the scale-up run data are summarized in the tables below.

Table 5. Operational parameters for HIC Step (n=6)

Process Step	Column load target (total protein mg/mL)	$(NH_4)_2SO_4$ Concentration (with 20mM Na_3PO_4 pH 7.0)	Column Volumes (mL)	Contact time Column vol./flow rate (min.)
Equilibration	N/A	1.4M	3	10
Load	5-10	1.4M	Variable	10
Wash	N/A	1.4M	3	10
Elution	N/A	1.1M	Variable*	10
Strip	N/A	0.05M NaCl	3	10
* Note: Eluate collection starts at approx. 0.25 AU and ends at approx. 0.15 AU. Void volume typically approx. 1.4 column volumes.				

Table 6. Data from Scale-up Runs (n=5)

Input		Output		
Column loading (per mL Resin)				
Total Protein (mg)	Units	Recovery (%)	Fold Purif. (By S.A.)	
5.1 - 7.2	5375 - 9248	77.8 - 92.7	1.0 - 1.3	*Range*
6.3	7638	85	1.2	*Average*

Example 6: CHAP Chromatography

[0052] Ceramic hydroxyapatite is a resin that has a unique, potentially mixed binding mode chemistry that was utilized in a CBS purification method. CBS displayed acidic characteristics and therefore initial investigation focused on using phosphate-modulated partitioning. The initial experiments utilized HIC eluate that was buffer exchanged into a 0.05M NaCl, 0.005M Potassium Phosphate (pH 6.8) buffer. A 5 mL ceramic hydroxyapatite (Type 1) cartridge was equilibrated in the same buffer and the conditioned HIC eluate was loaded onto the column. There was no visible breakthrough of protein (as measured by A_{280}) during the load and subsequent wash with equilibration/wash buffer. A linear gradient (5%) of 0.005M to 0.5M Potassium Phosphate was then run and fractions were collected. Based on the chromatogram, samples were analyzed by SDS-PAGE to determine the relative amounts of CBS protein and impurities. In subsequent experiments (based on analysis of the results of previous experiments), step washes with varying levels of phosphate were employed to determine optimal conditions for load, wash, and elution steps. In addition, the composition of buffer salts was transitioned from Potassium to Sodium Phosphate. (Fig. 3). The following tables represent column operational parameters and data from the scale-up runs that employed them.

Table 7. Operational Parameters for CHAP Step

Process Step	Column load target (total protein mg/mL)	Na_3PO_4 Concentration (with 50mM NaCl, pH 7.0)	Column Volumes (mL)	Contact time Column vol./ flow rate (min.)
Equilibration	N/A	10 mM	3	6
Load	10-15	10 mM	Variable	6
Wash	N/A	30 mM	3	6
Elution	N/A	90 mM	Variable*	6
Strip	N/A	150 mM	3	6
* Note: Eluate collection starts at approx. 0.20 AU and ends at approx. 0.16 AU. Void volume typically approx. 1.0 column volumes.				

Table 8. Data from Scale-up Runs (n=5)

Input		Output		
Column loading (per mL Resin)				
Total Protein (mg)	Units	Recovery (%)	Fold Purif. (By S.A.)	
9.9 - 12.2	11205 - 12297	84.6 - 92.4	1.1 - 1.2	*Range*
11.1	11751	89	1.2	*Average*

Example 7: **Integrated Process Results**

[0053] The particular multi-step method described in these Examples was evaluated at the scale of a 60mL capture column. All of the purification trains utilized starting material (crude extract) obtained from fermentations that were seeded with recombinant cells comprising a construct comprising a truncated variant of human CBS encoded by a nucleic acid having codons optimized for expression in *E. coli.* This construct resulted in starting material that was approximately 2-fold higher in specific activity, and significantly impacted the final purity achieved from the integrated purification method. The overall purification results using the multi-step method were measured by SDS-PAGE and Specific Activity (Figs. 5 and 6). The results demonstrated that the purity and specific activity met or exceeded that of the purified tagged truncated CBS. All Specific Activities of final column eluates obtained by the largest scale currently possible exceeded 1200 U/mg total protein. The following table summarizes the overall purification results from the scale-up runs.

Table 9. Overall Results from Scale-Up Runs

	Total Recovery (%)		Fold Purification	
	Range	Average	Range	Average
	Total Recovery (%)		Fold Purification	
3 Column Train (n=3)	57 - 60	58	5.7 - 6.2	5.9
4 Column Train (n=2)	47 - 52	50	4.6 - 5.4	5.0
Specific Activity of Final Column Eluate = 1206 -1509.				

[0054] Having described the invention in detail and by reference to specific embodiments thereof, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims. More specifically, although some aspects of the present invention are identified herein as particularly advantageous, it is contemplated that the present invention is not necessarily limited to these particular aspects of the invention.

Table 10: CβS Sequences

Molecule	SEQ ID NO	Sequence
Native human CβS nucleic acid sequence	**1**	atgccttctgagacccccaggcagaagtggggcccacag gctgcccccaccgctcagggccacactcggcgaaggggag cctggagaaggggtccccagaggataaggaagccaaggag cccctgtggatccggcccgatgctccgagcaggtgcacct ggcagctgggccggcctgcctccgagtccccacatcacca cactgccccggcaaaatctccaaaaatcttgccagatatt ctgaagaaaatcggggacacccctatggtcagaatcaaca agattgggaagaagttcggcctgaagtgtgagctcttggc caagtgtgagttcttcaacgcgggcgggagcgtgaaggac cgcatcagcctgcggatgattgaggatgctgagcgcgacg ggacgctgaagcccggggacacgattatcgagccgacatc cgggaacaccgggatcgggctggccctggctgcggcagtg aggggctatcgctgcatcatcgtgatgccagagaagatga gctccgagaaggtggacgtgctgcgggcactgggggctga gattgtgaggacgcccaccaatgccaggttcgactccccg gagtcacacgtgggggtggcctggcggctgaagaacgaaa tccccaattctcacatcctagaccagtaccgcaacgccag caaccccctggctcactacgacaccaccgctgatgagatc ctgcagcagtgtgatgggaagctggacatgctggtggctt cagtgggcacgggcggcaccatcacgggcattgccaggaa gctgaaggagaagtgtcctggatgcaggatcattggggtg gatcccgaagggtccatcctcgcagagccggaggagctga accagacggagcagacaacctacgaggtggaagggatcgg ctacgacttcatccccacggtgctggacaggacggtggtg gacaagtggttcaagagcaacgatgaggaggcgttcacct ttgcccgcatgctgatcgcgcaagaggggctgctgtgcgg tggcagtgctggcagcacggtggcggtggccgtgaaggct gcgcaggagctgcaggagggccagcgctgcgtggtcattc tgcccgactcagtgcggaactacatgaccaagttcctgag cgacaggtggatgctgcagaagggctttctgaaggaggag gacctcacggagaagaagccctggtggtggcacctccgtg ttcaggagctgggcctgtcagccccgctgaccgtgctccc gaccatcacctgtgggcacaccatcgagatcctccgggag aagggcttcgaccaggcgcccgtggtggatgaggcggggg taatcctgggaatggtgacgcttgggaacatgctctcgtc cctgcttgccgggaaggtgcagccgtcagaccaagttggc aaagtcatctacaagcagttcaaacagatccgcctcacgg acacgctgggcaggctctcgcacatcctggagatggacca cttcgccctggtggtgcacgagcagatccagtaccacagc accgggaagtccagtcagcggcagatggtgttcggggtgg tcaccgccattgacttgctgaacttcgtggccgcccagga gcgggaccagaagtga
Native human CβS polypeptide sequence	**2**	MPSETPQAEVGPTGCPHRSGPHSAKGSLEKGSPEDKEAKE PLWIRPDAPSRCTWQLGRPASESPHHHTAPAKSPKILPDI LKKIGDTPMVRINKIGKKFGLKCELLAKCEFFNAGGSVKD RISLRMIEDAERDGTLKPGDTIIEPTSGNTGIGLALAAAV RGYRCIIVMPEKMSSEKVDVLRALGAEIVRTPTNARFDSP ESHVGVAWRLKNEIPNSHILDQYRNASNPLAHYDTTADEI LQQCDGKLDMLVASVGTGGTITGIARKLKEKCPGCRIIGV DPEGSILAEPEELNQTEQTTYEVEGIGYDFIPTVLDRTVV DKWFKSNDEEAFTFARMLIAQEGLLCGGSAGSTVAVAVKA AQELQEGQRCVVILPDSVRNYMTKFLSDRWMLQKGFLKEE DLTEKKPWWWHLRVQELGLSAPLTVLPTITCGHTIEILRE KGFDQAPVVDEAGVILGMVTLGNMLSSLLAGKVQPSDQVG KVIYKQFKQIRLTDTLGRLSHILEMDHFALVVHEQIQYHS TGKSSQRQMVFGVVTAIDLLNFVAAQERDQK

(continued)

Molecule	SEQ ID NO	Sequence
Truncated, Human CβS polypeptide sequence	**3**	MPSETPQAEVGPTGCPHRSGPHSAKGSLEKGSPED KEAKEPLWIRPDAPSRCTWQLGRPASESPHHHTAP AKSPKILPDILKKIGDTPMVRINKIGKKFGLKCEL LAKCEFFNAGGSVKDRISLRMIEDAERDGTLKPGD TIIEPTSGNTGIGLALAAAVRGYRCIIVMPEKMSS EKVDVLRALGAEIVRTPTNARFDSPESHVGVAWRL KNEIPNSHILDQYRNASNPLAHYDTTADEILQQCD GKLDMLVASVGTGGTITGIARKLKEKCPGCRIIGV DPEGSILAEPEELNQTEQTTYEVEGIGYDFIPTVL DRTVVDKWFKSNDEEAFTFARMLIAQEGLLCGGSA GSTVAVAVKAAQELQEGQRCVVILPDSVRNYMTKF LSDRWMLQKGFLKEEDLTEKKPWWWHLR
Truncated, Optimized Human CβS nucleic acid sequence	**4**	ATGCCGTCAGAAACCCCGCAGGCAGAAGTGGGTCCGACGG GTTGCCCGCACCGTAGCGGTCCGCATTCTGCAAAAGGCAG TCTGGAAAAAGGTTCCCCGGAAGATAAAGAAGCCAAAGAA CCGCTGTGGATTCGTCCGGACGCACCGTCACGCTGTACCT GGCAGCTGGGTCGTCCGGCAAGCGAATCTCCGCATCACCA TACGGCTCCGGCGAAAAGTCCGAAAATTCTGCCGGATATC CTGAAGAAAATTGGTGACACCCCGATGGTTCGTATCAACA AAATCGGCAAAAAATTCGGTCTGAAATGCGAACTGCTGGC TAAATGTGAATTTTTCAATGCGGGCGGTTCCGTGAAAGAT CGTATCTCACTGCGCATGATTGAAGATGCTGAACGCGACG GCACCCTGAAACCGGGTGATACGATTATCGAACCGACCTC TGGCAACACGGGTATCGGTCTGGCACTGGCGGCGGCAGTC CGTGGTTATCGCTGCATTATCGTGATGCCGGAAAAAATGA GCTCTGAAAAAGTTGATGTCCTGCGTGCTCTGGGCGCGGA AATTGTTCGTACCCCGACGAATGCCCGCTTCGACAGTCCG GAATCCCATGTGGGTGTTGCATGGCGCCTGAAAAACGAAA TCCCGAATTCGCACATTCTGGATCAGTATCGTAACGCTAG CAATCCGCTGGCGCATTACGATACCACGGCCGACGAAATC CTGCAGCAATGTGATGGCAAACTGGACATGCTGGTCGCTT CTGTGGGTACCGGCGGTACCATTACGGGCATCGCGCGTAA ACTGAAAGAAAAATGCCCGGGCTGTCGCATTATCGGTGTG GATCCGGAAGGCAGTATTCTGGCGGAACCGGAAGAACTGA ACCAGACCGAACAAACCACGTATGAAGTTGAAGGCATCGG TTACGATTTTATTCCGACCGTCCTGGATCGCACGGTGGTT GACAAATGGTTCAAAAGCAATGACGAAGAAGCCTTTACCT TCGCACGTATGCTGATCGCTCAGGAAGGTCTGCTGTGCGG TGGTTCAGCAGGTTCGACGGTCGCAGTGGCAGTTAAAGCT GCGCAGGAACTGCAAGAAGGTCAACGTTGTGTCGTGATTC TGCCGGATTCTGTTCGCAACTACATGACCAAATTTCTGAG TGACCGTTGGATGCTGCAAAAAGGCTTCCTGAAAGAAGAA GATCTGACCGAGAAAAAACCGTGGTGGTGGCACCTGCGCT AA

CLAUSES:

[0055]

1. A method for purifying cystathionine β-Synthase (CBS) protein, wherein the CBS protein is a naturally occurring, chemically cleaved or genetically engineered truncated CBS protein, comprising the steps of:

(a) providing a CBS-containing solution comprising one or a plurality of impurities; and
(b) performing chromatographic separation of the CBS-containing solution using an ion exchange chromatography column and a metal affinity chromatography (IMAC) resin, wherein the impurities are removed thereby.

2. The method of clause 1, further comprising the step of performing chromatographic separation using a Hydrophobic Interaction Chromatography (HIC) column.

3. The method of clauses 1-2, further comprising the step of performing chromatographic separation using a ceramic hydroxyapaptite resin.

4. The method of clauses 1-3, wherein the ion exchange chromatography column is a weak anion exchanger.

5. The method of clause 4, wherein the weak anion exchanger is a DEAE-Sepharose FF column.

6. The method of clauses 1-5, wherein the metal affinity chromatography (IMAC) resin is charged with a divalent metal cation.

7. The method of clause 6, wherein the divalent metal cation is nickel, copper, cobalt or zinc.

8. The method of clause 7, wherein the divalent metal ion is zinc.

9. The method of clauses 1-8, further comprising eluting CBS from the metal affinity chromatography (IMAC) resin with an elution buffer comprising imidazole.

10. The method of clauses 1-9, wherein the truncated CBS protein has an amino acid sequence identified by SEQ ID NO: 3.

11. The method of clauses 1-10, wherein the CBS-containing solution is a clarified CBS solution.

12. The method of clauses 1-11, wherein the CBS is produced in a recombinant cell.

13. The method of clause 12, wherein the recombinant cell is a bacterial cell.

14. The method of clause 13, wherein the CBS-containing solution is obtained by homogenizing recombinant bacterial cells expressing a recombinant construct comprising a nucleic acid sequence encoding CBS.

15. The method of clause 14, wherein the CBS nucleic acid encodes a truncated CBS protein.

16. The method of clause 15, wherein the truncated CBS protein has been truncated to an ending position of one of amino acid residues from 382-532, 382-550 or 543-550 of SEQ ID NO:2.

17. The method of clause 16, wherein the CBS nucleic acid sequence comprises SEQ ID NO. 4.

18. The method of clauses 12-17, wherein the recombinant cells are *E. coli* cells.

19. The method of clause 16, wherein the nucleic acid sequence encoding the truncated CBS protein is optimized for expression in *E. coli* cells.

20. A substantially purified CBS solution produced by the method of clauses 1-9.

21. The substantially purified CBS solution of clause 20 formulated in a pharmaceutically acceptable carrier.

22. A method for producing an enriched CBS solution, wherein the CBS protein is a naturally occurring, chemically cleaved or genetically engineered truncate thereof, the method comprising:

(a) providing a CBS-containing solution comprising one or a plurality of impurities; and
(b) performing chromatographic separation of the CBS-containing solution using an immobilized metal affinity chromatography (IMAC) resin charged with a divalent metal ion, wherein the impurities are removed thereby.

23. The method of clause 22, wherein the divalent metal ion is nickel, copper, cobalt or zinc.

24. The method of clause 23, wherein the divalent metal ion is zinc.

25. The method of clauses 22-24, wherein the truncated CBS protein has an amino acid sequence identified by SEQ ID NO: 3.

26. The method of clauses 22-25, wherein the CBS solution is a clarified CBS solution.

27. The method of clauses 22-26, wherein the CBS is produced in a recombinant cell.

28. The method of clause 27, wherein the recombinant cell is a bacterial cell.

29. The method of clause 28, wherein the CBS solution is obtained by homogenizing recombinant bacterial cells expressing a recombinant construct comprising a nucleic acid sequence encoding CBS.

30. The method of clause 29, wherein the CBS encodes a truncated CBS protein.

31. The method of clause 30, wherein the truncated CBS protein has been truncated to an ending position of one of amino acid residues from 382-532 or 543-550 of SEQ ID NO:2.

32. The method of clause 31, wherein the CBS nucleic acid sequence comprises SEQ ID NO. 4.

33. The method of clauses 22-32, wherein the bacterial cells are *E. coli* cells.

34. The method of clauses 22-33, wherein the nucleic acid sequence encoding the truncated CBS protein is optimized for expression in *E. coli* cells.

35. An enriched CBS solution produced by the method of clauses 22-34.

SEQUENCE LISTING

[0056]

<110> The Regents of the University of Colorado
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<120> PURIFICATION OF CYSTATHIONINE BETA-SYNTHASE

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<150> US 13/830,494
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tacatgacca agttcctgag cgacaggtgg atgctgcaga agggctttct gaaggaggag 1200

gacctcacgg agaagaagcc ctggtggtgg cacctccgtg ttcaggagct gggcctgtca 1260

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cacatcctgg agatggacca cttcgccctg gtggtgcacg agcagatcca gtaccacagc 1560

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        35                  40                  45

Pro Ser Arg Cys Thr Trp Gln Leu Gly Arg Pro Ala Ser Glu Ser Pro
    50                  55                  60

His His His Thr Ala Pro Ala Lys Ser Pro Lys Ile Leu Pro Asp Ile
65                  70                  75                  80

Leu Lys Lys Ile Gly Asp Thr Pro Met Val Arg Ile Asn Lys Ile Gly
                85                  90                  95

Lys Lys Phe Gly Leu Lys Cys Glu Leu Leu Ala Lys Cys Glu Phe Phe
            100                 105                 110

Asn Ala Gly Gly Ser Val Lys Asp Arg Ile Ser Leu Arg Met Ile Glu
        115                 120                 125
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```
Asp Ala Glu Arg Asp Gly Thr Leu Lys Pro Gly Asp Thr Ile Ile Glu
    130                 135                 140

Pro Thr Ser Gly Asn Thr Gly Ile Gly Leu Ala Leu Ala Ala Ala Val
145                 150                 155                 160

Arg Gly Tyr Arg Cys Ile Ile Val Met Pro Glu Lys Met Ser Ser Glu
                165                 170                 175

Lys Val Asp Val Leu Arg Ala Leu Gly Ala Glu Ile Val Arg Thr Pro
            180                 185                 190

Thr Asn Ala Arg Phe Asp Ser Pro Glu Ser His Val Gly Val Ala Trp
        195                 200                 205

Arg Leu Lys Asn Glu Ile Pro Asn Ser His Ile Leu Asp Gln Tyr Arg
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Asn Ala Ser Asn Pro Leu Ala His Tyr Asp Thr Thr Ala Asp Glu Ile
225                 230                 235                 240

Leu Gln Gln Cys Asp Gly Lys Leu Asp Met Leu Val Ala Ser Val Gly
                245                 250                 255

Thr Gly Gly Thr Ile Thr Gly Ile Ala Arg Lys Leu Lys Glu Lys Cys
            260                 265                 270

Pro Gly Cys Arg Ile Ile Gly Val Asp Pro Glu Gly Ser Ile Leu Ala
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Glu Pro Glu Glu Leu Asn Gln Thr Glu Gln Thr Thr Tyr Glu Val Glu
    290                 295                 300

Gly Ile Gly Tyr Asp Phe Ile Pro Thr Val Leu Asp Arg Thr Val Val
305                 310                 315                 320

Asp Lys Trp Phe Lys Ser Asn Asp Glu Glu Ala Phe Thr Phe Ala Arg
                325                 330                 335

Met Leu Ile Ala Gln Glu Gly Leu Leu Cys Gly Gly Ser Ala Gly Ser
            340                 345                 350

Thr Val Ala Val Ala Val Lys Ala Ala Gln Glu Leu Gln Glu Gly Gln
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Arg Cys Val Val Ile Leu Pro Asp Ser Val Arg Asn Tyr Met Thr Lys
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Asp Leu Thr Glu Lys Lys Pro Trp Trp Trp His Leu Arg Val Gln Glu
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Leu Gly Leu Ser Ala Pro Leu Thr Val Leu Pro Thr Ile Thr Cys Gly
            420                 425                 430

His Thr Ile Glu Ile Leu Arg Glu Lys Gly Phe Asp Gln Ala Pro Val
        435                 440                 445

Val Asp Glu Ala Gly Val Ile Leu Gly Met Val Thr Leu Gly Asn Met
    450                 455                 460

Leu Ser Ser Leu Leu Ala Gly Lys Val Gln Pro Ser Asp Gln Val Gly
465                 470                 475                 480

Lys Val Ile Tyr Lys Gln Phe Lys Gln Ile Arg Leu Thr Asp Thr Leu
                485                 490                 495

Gly Arg Leu Ser His Ile Leu Glu Met Asp His Phe Ala Leu Val Val
            500                 505                 510

His Glu Gln Ile Gln Tyr His Ser Thr Gly Lys Ser Ser Gln Arg Gln
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            20                  25                  30
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Pro Ser Arg Cys Thr Trp Gln Leu Gly Arg Pro Ala Ser Glu Ser Pro
    50                  55                  60

His His His Thr Ala Pro Ala Lys Ser Pro Lys Ile Leu Pro Asp Ile
65                  70                  75                  80

Leu Lys Lys Ile Gly Asp Thr Pro Met Val Arg Ile Asn Lys Ile Gly
                85                  90                  95

Lys Lys Phe Gly Leu Lys Cys Glu Leu Leu Ala Lys Cys Glu Phe Phe
            100                 105                 110

Asn Ala Gly Gly Ser Val Lys Asp Arg Ile Ser Leu Arg Met Ile Glu
        115                 120                 125

Asp Ala Glu Arg Asp Gly Thr Leu Lys Pro Gly Asp Thr Ile Ile Glu
    130                 135                 140

Pro Thr Ser Gly Asn Thr Gly Ile Gly Leu Ala Leu Ala Ala Ala Val
145                 150                 155                 160

Arg Gly Tyr Arg Cys Ile Ile Val Met Pro Glu Lys Met Ser Ser Glu
                165                 170                 175

Lys Val Asp Val Leu Arg Ala Leu Gly Ala Glu Ile Val Arg Thr Pro
            180                 185                 190

Thr Asn Ala Arg Phe Asp Ser Pro Glu Ser His Val Gly Val Ala Trp
        195                 200                 205

Arg Leu Lys Asn Glu Ile Pro Asn Ser His Ile Leu Asp Gln Tyr Arg
    210                 215                 220

Asn Ala Ser Asn Pro Leu Ala His Tyr Asp Thr Thr Ala Asp Glu Ile
225                 230                 235                 240

Leu Gln Gln Cys Asp Gly Lys Leu Asp Met Leu Val Ala Ser Val Gly
                245                 250                 255

Thr Gly Gly Thr Ile Thr Gly Ile Ala Arg Lys Leu Lys Glu Lys Cys
            260                 265                 270

Pro Gly Cys Arg Ile Ile Gly Val Asp Pro Glu Gly Ser Ile Leu Ala
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Glu Pro Glu Glu Leu Asn Gln Thr Glu Gln Thr Thr Tyr Glu Val Glu
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Gly Ile Gly Tyr Asp Phe Ile Pro Thr Val Leu Asp Arg Thr Val Val
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Asp Lys Trp Phe Lys Ser Asn Asp Glu Glu Ala Phe Thr Phe Ala Arg
                325                 330                 335

Met Leu Ile Ala Gln Glu Gly Leu Leu Cys Gly Gly Ser Ala Gly Ser
            340                 345                 350

Thr Val Ala Val Ala Val Lys Ala Ala Gln Glu Leu Gln Glu Gly Gln
        355                 360                 365

Arg Cys Val Val Ile Leu Pro Asp Ser Val Arg Asn Tyr Met Thr Lys
    370                 375                 380

Phe Leu Ser Asp Arg Trp Met Leu Gln Lys Gly Phe Leu Lys Glu Glu
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ctgaaatgcg aactgctggc taaatgtgaa tttttcaatg cgggcggttc cgtgaaagat      360

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ctgcagcaat gtgatggcaa actggacatg ctggtcgctt ctgtgggtac cggcggtacc 780

attacgggca tcgcgcgtaa actgaaagaa aaatgcccgg gctgtcgcat tatcggtgtg 840

gatccggaag gcagtattct ggcggaaccg gaagaactga accagaccga acaaaccacg 900

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Claims

1. A method for producing an enriched cystathionine β-synthase solution, wherein the cystathionine β-synthase solution comprises a naturally occurring, chemically cleaved or genetically engineered truncated cystathionine β-synthase protein, the method comprising:

   (a) providing a cystathionine β-synthase-containing solution comprising one or a plurality of impurities;
   (b) performing chromatographic separation of the cystathionine β-synthase-containing solution using an immobilized metal affinity chromatography (IMAC) resin charged with zinc; and
   (c) performing at least one additional chromatographic separation selected from the group consisting of: hydrophobic interaction chromatography (HIC), ceramic hydroxyapatite (CHAP), and ion exchange chromatography, thereby producing the enriched cystathionine β-synthase solution.

2. The method of claim 1, wherein the truncated cystathionine β-synthase protein has an amino acid sequence identified

by SEQ ID NO: 3.

3. The method of any of claims 1 or 2, wherein the cystathionine β-synthase solution is a clarified cystathionine β-synthase solution.

4. The method of any of claims 1-3, wherein the truncated cystathionine β-synthase protein is produced in a recombinant cell.

5. The method of claim 4, wherein the recombinant cell is a bacterial cell.

6. The method of any of claims 1-5, wherein the cystathionine β-synthase-containing solution is obtained by homogenizing recombinant bacterial cells expressing a recombinant construct comprising a nucleic acid sequence encoding cystathionine β-synthase.

7. The method of claim 6, wherein the nucleic acid sequence encodes a truncated cystathionine β-synthase protein.

8. The method of claims 1-7, wherein the truncated cystathionine β-synthase protein has been truncated to an ending position of one of amino acid residues from 382-532 or 543-550 of SEQ ID NO: 2.

9. The method of claim 8, wherein the cystathionine β-synthase nucleic acid sequence comprises SEQ ID NO: 4.

10. The method of claim 9, wherein the bacterial cells are *E. coli* cells.

11. The method of any of claims 4-10, wherein the nucleic acid sequence encoding the truncated cystathionine β-synthase protein is optimized for expression in *E. coli* cells.

12. A use of a method in the production of a cystathionine β-synthase solution, wherein the method comprises:

    (a) providing a cystathionine β-synthase-containing solution in the presence of at least one impurity, wherein said cystathionine β-synthase protein is a naturally occurring truncated protein, or a chemically cleaved or genetically engineered truncate thereof; and
    (b) performing chromatographic separation of the cystathionine β-synthase-containing solution using a metal affinity chromatography (IMAC) resin charged with zinc.

13. The use of claim 12, further comprising: (c) performing chromatographic separation using ion exchange chromatography.

14. The use of claim 13, wherein the ion exchange chromatography column is a weak anion exchanger.

15. The use of claim 14, wherein the weak anion exchanger is a DEAE-Sepharose FF column.

Patentansprüche

1. Verfahren zum Herstellen einer angereicherten Cystathionin-β-Synthase-Lösung, wobei die Cystathionin-β-Synthase-Lösung ein natürlich vorkommendes, chemisch gespaltenes oder genetisch verändertes verkürztes Cystathionin-β-Synthase-Protein umfasst, wobei das Verfahren Folgendes umfasst:

    (a) Bereitstellen einer Cystathionin-β-Synthase enthaltenden Lösung, die eine oder eine Vielzahl von Verunreinigungen umfasst;
    (b) Durchführen von chromatographischer Trennung der Cystathionin-β-Synthase enthaltenden Lösung unter Verwendung eines Harzes für immobilisierte Metallaffinititätschromatographie (IMAC), das mit Zink beladen ist; und
    (c) Durchführen mindestens einer zusätzlichen chromatographischen Trennung, ausgewählt aus der Gruppe, bestehend aus: hydrophober Interaktionschromatographie (HIC), keramischem Hydroxylapatit (CHAP) und Ionenaustauschchromatographie, wodurch die angereicherte Cystathionin-β-Synthase-Lösung hergestellt wird.

2. Verfahren nach Anspruch 1, wobei das verkürzte Cystathionin-β-Synthase-Protein eine Aminosäuresequenz auf-

weist, die durch SEQ ID NO: 3 identifiziert wird.

3. Verfahren nach einem der Ansprüche 1 oder 2, wobei die Cystathionin-β-Synthase-Lösung eine geklärte Cystathionin-β-Synthase-Lösung ist.

4. Verfahren nach einem der Ansprüche 1-3, wobei das verkürzte Cystathionin-β-Synthase-Protein in einer rekombinanten Zelle hergestellt wird.

5. Verfahren nach Anspruch 4, wobei die rekombinante Zelle eine Bakterienzelle ist.

6. Verfahren nach einem der Ansprüche 1-5, wobei die Cystathionin-β-Synthase enthaltende Lösung durch Homogenisieren von rekombinanten Bakterienzellen erhalten wird, die ein rekombinantes Konstrukt exprimieren, das eine Nukleinsäuresequenz umfasst, die Cystathionin-β-Synthase codiert.

7. Verfahren nach Anspruch 6, wobei die Nukleinsäuresequenz ein verkürztes Cystathionin-β-Synthase-Protein codiert.

8. Verfahren nach den Ansprüchen 1-7, wobei das verkürzte Cystathionin-β-Synthase-Protein auf eine Endposition von einem der Aminosäurereste von 382-532 oder 543-550 von SEQ ID NO: 2 verkürzt wurde.

9. Verfahren nach Anspruch 8, wobei die Cystathionin-β-Synthase-Nukleinsäuresequenz SEQ ID NO: 4 umfasst.

10. Verfahren nach Anspruch 9, wobei die Bakterienzellen *E.* coli-Zellen sind.

11. Verfahren nach einem der Ansprüche 4-10, wobei die Nukleinsäuresequenz, die das verkürzte Cystathionin-β-Synthase-Protein codiert, für die Expression in *E.* coli-Zellen optimiert ist.

12. Verwendung eines Verfahrens bei der Herstellung einer Cystathionin-β-Synthase-Lösung, wobei das Verfahren Folgendes umfasst:

    (a) Bereitstellen einer Cystathionin-β-Synthase enthaltenden Lösung in Gegenwart mindestens einer Verunreinigung, wobei das Cystathionin-β-Synthase-Protein ein natürlich vorkommendes, verkürztes Protein oder eine chemisch gespaltene oder genetisch veränderte Verkürzung davon ist; und
    (b) Durchführen von chromatographischer Trennung der Cystathionin-β-Synthase enthaltenden Lösung unter Verwendung eines Harzes für Metallaffinitätschromatographie (IMAC), das mit Zink beladen ist.

13. Verwendung nach Anspruch 12, weiter umfassend: (c) Durchführen von chromatographischer Trennung unter Verwendung von Ionenaustauschchromatographie.

14. Verwendung nach Anspruch 13, wobei die Säule für Ionenaustauschchromatographie ein schwacher Anionenaustauscher ist.

15. Verwendung nach Anspruch 14, wobei der schwache Anionenaustauscher eine DEAE-Sepharose-FF-Säule ist.

Revendications

1. Méthode de production d'une solution de cystathionine β-synthase enrichie, dans laquelle la solution de cystathionine β-synthase comprend une protéine de cystathionine β-synthase naturelle tronquée chimiquement clivée ou génétiquement modifiée, la méthode comprenant les étapes consistant à :

    (a) fournir une solution contenant de la cystathionine β-synthase comprenant une ou une pluralité d'impuretés ;
    (b) mettre en oeuvre une séparation chromatographique de la solution contenant de la cystathionine β-synthase à l'aide d'une résine de chromatographie d'affinité pour ions métalliques (IMAC) chargée avec du zinc ; et
    (c) mettre en oeuvre au moins une séparation chromatographique additionnelle choisie dans le groupe consistant en : une chromatographie d'interaction hydrophobe (CIH), de la céramique d'hydroxyapatite (CHAP), et une chromatographie d'échange d'ions, en produisant ainsi la solution de cystathionine β-synthase enrichie.

2. Méthode selon la revendication 1, dans laquelle la protéine de cystathionine β-synthase tronquée a une séquence d'acides aminés identifiée par SEQ ID NO : 3.

3. Méthode selon l'une quelconque des revendications 1 ou 2, dans laquelle la solution de cystathionine β-synthase est une solution de cystathionine β-synthase clarifiée.

4. Méthode selon l'une quelconque des revendications 1-3, dans laquelle la protéine de cystathionine β-synthase tronquée est produite dans une cellule recombinée.

5. Méthode selon la revendication 4, dans laquelle la cellule recombinée est une cellule bactérienne.

6. Méthode selon l'une quelconque des revendications 1-5, dans laquelle la solution contenant de la cystathionine β-synthase est obtenue par l'homogénéisation de cellules bactériennes recombinées exprimant une construction recombinée comprenant une séquence d'acides nucléiques codant pour la cystathionine β-synthase.

7. Méthode selon la revendication 6, dans laquelle la séquence d'acides nucléiques code pour une protéine de cystathionine β-synthase tronquée.

8. Méthode selon les revendications 1-7, dans laquelle la protéine de cystathionine β-synthase tronquée a été tronquée jusqu'à une position terminale de l'un parmi les résidus d'acides aminés parmi 382-532 ou 543-550 de SEQ ID NO : 2.

9. Méthode selon la revendication 8, dans laquelle la séquence d'acides nucléiques de cystathionine β-synthase comprend SEQ ID NO : 4.

10. Méthode selon la revendication 9, dans laquelle les cellules bactériennes sont des cellules d'*E. coli.*

11. Méthode selon l'une quelconque des revendications 4-10, dans laquelle la séquence d'acides nucléiques codant pour la protéine de cystathionine β-synthase tronquée est optimisée pour une expression dans des cellules d'*E. coli.*

12. Utilisation d'une méthode dans la production d'une solution de cystathionine β-synthase, dans laquelle la méthode comprend les étapes consistant à :

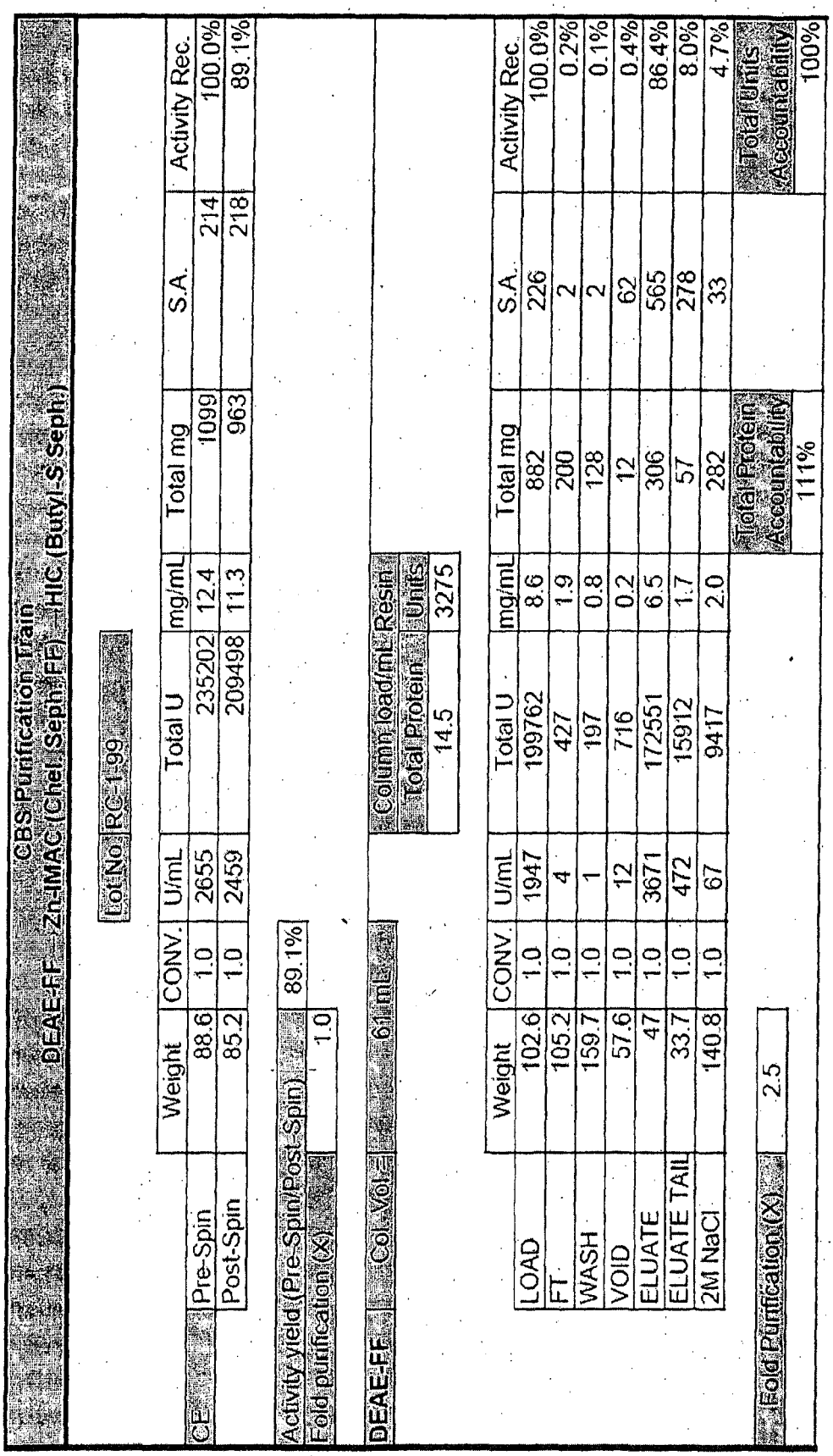
    (a) fournir une solution contenant de la cystathionine β-synthase en présence d'au moins une impureté, dans laquelle ladite solution de cystathionine β-synthase est une protéine tronquée naturelle, ou un composé chimiquement clivé ou génétiquement modifié de celle-ci ; et
    (b) mettre en oeuvre une séparation chromatographique de la solution contenant de la cystathionine β-synthase à l'aide d'une résine de chromatographie d'affinité pour ions métalliques (IMAC) chargée avec du zinc.

13. Utilisation selon la revendication 12, comprenant en outre l'étape consistant à :
    (c) mettre en oeuvre une séparation chromatographique à l'aide d'une chromatographie d'échange d'ions.

14. Utilisation selon la revendication 13, dans laquelle la colonne de chromatographie d'échange d'ions est un échangeur d'anions faibles.

15. Utilisation selon la revendication 14, dans laquelle l'échangeur d'anions faibles est une colonne de DEAE-Sépharose FF.

Figure 1A



CBS Purification Train
DEAE-FF Zn-IMAC (Chel Seph FF) HIC (Butyl-S Seph)

Lot No RC-1-99

CE		Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
	Pre-Spin	88.6	1.0	2655	235202	12.4	1099	214	100.0%
	Post-Spin	85.2	1.0	2459	209498	11.3	963	218	89.1%

Activity yield (Pre-Spin/Post-Spin) 89.1%
Fold purification (X) 1.0

DEAE-FF Col. Vol = 61 mL

Column load/mL Resin

Total Protein	Units
14.5	3275

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD	102.6	1.0	1947	199762	8.6	882	226	100.0%
FT	105.2	1.0	4	427	1.9	200	2	0.2%
WASH	159.7	1.0	1	197	0.8	128	2	0.1%
VOID	57.6	1.0	12	716	0.2	12	62	0.4%
ELUATE	47	1.0	3671	172551	6.5	306	565	86.4%
ELUATE TAIL	33.7	1.0	472	15912	1.7	57	278	8.0%
2M NaCl	140.8	1.0	67	9417	2.0	282	33	4.7%

Total Protein Accountability 111%
Total Units Accountability 100%

Fold Purification (X) 2.5

Figure 1B

CBS Purification Train DEAE-FF → Zn-IMAC (Chel. Seph. FF) → HIC (Butyl-S Seph.)									
Zn-IMAC	Col. Vol =	40 mL			Column load/mL Resin				
					Total Protein	Units			
					6.5	4414			
		Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
	LOAD	47.3	1.0	3733	176573	5.5	260	679	100.0%
	FT	156	1.0	13	1998	0.43	67	30	1.1%
	WASH/VOID	54.8	1.0	22	1186	0.07	4	309	0.7%
	ELUATE PK 1	39.4	1.0	941	37071	0.91	36	1034	21.0%
	ELUATE PK 2	50.5	1.0	2158	108961	2.10	106	1027	61.7%
	ELUATE COMB PKS	88	1.0	1681	147928	1.50	132	1121	83.8%
	100 mM STRIP	86	1.0	64	5517	0.58	50	111	3.1%
							Total Protein Accountability		Units Accountability
	Fold Purification (X)	1.5					97%		89%

Figure 1C

CBS Purification Train
DEAE-FF →Zn-IMAC (Chel. Seph. FF) →HIC (Butyl-S Seph.)

HIC	Col. Vol.=	23 mL

Column load/mL Resin	
Total Protein	Units
6.5	6745

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD	103.9	1.1	1357	155143	1.58	149	1040	100.0%
FT/WASH	85	1.1	26	2470	0.080	6	400	1.6%
VOID	30.5	1.1	34	1133	0.060	2	681	0.7%
ELUATE	41.1	1.1	2671	120762	2.50	93	1293	77.8%
STRIP	43.8	1.0	282	13591	0.87	38	357	8.8%

Fold Purification (X)	1.2

Total Protein Accountability		Units Accountability
93%		89%

Recovery Total Protein From First Column Load
10.6%

Recovery Total Units From First Column Load
60%

Total Fold Purification (X)
5.7

Figure 2

RC-1-64 DEAE FF (DEAE-IMAC-HIC Process Train)

Date	04/21/11
Column ID	RC-3-10
Exp. No.	RC-1-64
Col Vol =	30
Total protein/mL resin loaded	26 mg/mL

Fraction ID	Weight or Volume (g or mL)	CONV.	Protein (mg/mL)	Total Protein in fraction (mg)	Cystathionine from assay (nmol.mL.hr)	Dilution factor from assay	Total Units in fraction	S.A. Fraction	% Rec. Units (%)	% Rec. Total Protein (%)
CE Pre-Spin	37.6	1.0	20.9	784.3	46423	40	69820	89.0	100.0%	100.0%
Ce Post-Spin	36.3	1.0	20.7	752.9	52427	40	76124	101.1	109.0%	96.0%
DEAE Load	49.8	1.0	15.4	766.8	36740	40	73186	95.4	96.1%	101.8%
DEAE FT	49.7	1.0	3.0	151.0	3403	1	169	1.1	0.2%	20.1%
DEAE Void	31	1.0	0.3	8.8	10298	1	319	36.4	0.4%	1.2%
DEAE Eluate	24.9	1.0	5.2	130.0	63422	40	63168	485.8	83.0%	17.3%
DEAE Eluate Tail	18.9	1.0	1.9	35.16	69389	5	6557	186.5	8.6%	4.7%
DEAE Wash	89.3	1.0	1.6	143.16	5519	1	493	3.4	0.6%	19.0%
DEAE 2M NaCl	76	1.0	3.2	241.44	13935	5	5295	21.9	7.0%	32.1%

Mass Balance	
Units	99.8%

Mass Balance Units (from load)	Mass Balance Protein
103.8%	94.2%

Recovery units (from load)
86.3%

Buffers:

Equilibration	0.02 M Na3PO4, 0.05 M NaCl, 10% Ethylene Glycol, pH 7.0
Wash	0.02 M Na3PO4, 0.05 M NaCl, 10% Ethylene Glycol, pH 7.0
Elution	0.02 M Na3PO4, 0.137 M NaCl, 10% Ethylene Glycol, pH 7.0
Strip	2 M NaCl
Sanitze	1.0 M NaOH

CBS Purification Train
DEAE-FF →Zn-IMAC (Chel. Seph. FF) →HAP (Ceramic Type 1) →HIC (Butyl-S Seph.)

Lot No. RC-1-103

		Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
CE	Pre-Spin	87.1	1.0	3369	293422	13.9	1211	242	100.0%
	Post-Spin	100.4	1.0	3050	306270	11.7	1175	261	**104.4%**

Activity yield (Pre-Spin/Post-Spin)	104.4%
Fold purification (X)	**1.1**

DEAE-FF Col. Vol = 60 mL

Column load/mL Resin	
Total Protein	Units*
19.6	5105

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD*	100.4	1.0	3050	306270	11.7	1175	261	100.0%
FT	102.6	1.0	6	585	2.3	236	2	0.2%
WASH	164.6	1.0	2	410	1.0	165	2	0.1%
VOID	56.3	1.0	11	644	0.3	17	38	0.2%
ELUATE	52.6	1.0	4976	261744	7.2	379	691	**85.5%**
ELUATE TAIL	30.6	1.0	441	13488	1.3	40	339	4.4%
2M NaCl	115.4	1.0	75	8635	3.0	346	25	2.8%
						Total Protein Accountability		Total Units Accountability
						101%		**93%**

Fold Purification (X)	2.7

**Load same as Post-Spin;*

Dilution occurred during balancing of tubes for centrifugation

CBS Purification Train
DEAE-FF →Zn-IMAC (Chel. Seph. FF) →HAP (Ceramic Type 1)→HIC (Butyl-S Seph.)

Zn-IMAC	Col. Vol.=	40 mL

Column load/mL Resin	
Total Protein	Units
9.3	6337

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD	52.9	1.0	4792	253492	7.0	370	685	100.0%
FT/WASH	144.9	1.0	23	3388	0.65	94	36	1.3%
VOID	52.3	1.0	64	3355	0.16	8	401	1.3%
ELUATE COMB PKS	82.6	1.0	2457	202919	2.67	221	920	80.0%
100 mM STRIP*	89	1.0	19	1689	0.15	13	127	0.7%

Fold Purification (X)	1.3

Total Protein Accountability	Units Accountability
91%	83%

**Precipitation observed in strip sample.*

HAP	Col. Vol.=	18 mL

Column load/mL Resin	
Total Protein	Units
12.2	11205

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD	82.1	1.0	2457	201691	2.67	219	920	100.0%
FT/WASH	132.2	1.0	26	3383	0.08	11	320	1.7%
ELUATE	37.5	1.0	4552	170694	4.34	163	1049	84.6%
STRIP	34.5	1.0	348	12013	0.55	19	633	6.0%

Fold Purification (X)	1.1

Total Protein Accountability	Units Accountability
88%	92%

CBS Purification Train
DEAE-FF →Zn-IMAC (Chel. Seph. FF) →HAP (Ceramic Type 1)→HIC (Butyl-S Seph.)

HIC	Col. Vol.=	21 mL

Column load/mL Resin	
Total Protein	Units
7.2	8447

	Weight	CONV.	U/mL	Total U	mg/mL	Total mg	S.A.	Activity Rec.
LOAD	43.8	1.1	3682	177384	3.82	152	1166	100.0%
FT/WASH	105.7	1.1	1	96	0.020	2	50	0.1%
VOID	28.7	1.1	4	126	0.020	1	241	0.1%
ELUATE	44.9	1.1	3251	160544	3.26	***133***	***1206***	**90.5%**
STRIP	40.2	1.0	325	14375	0.54	22	662	8.1%

Fold Purification (X)	1.0

Total Protein Accountability		**Units Accountability**
103%		**99%**

Recovery Total Protein From First Column Load
11.3%

Recovery Total Units From First Column Load
52%

Total Fold Purification (X)
4.6

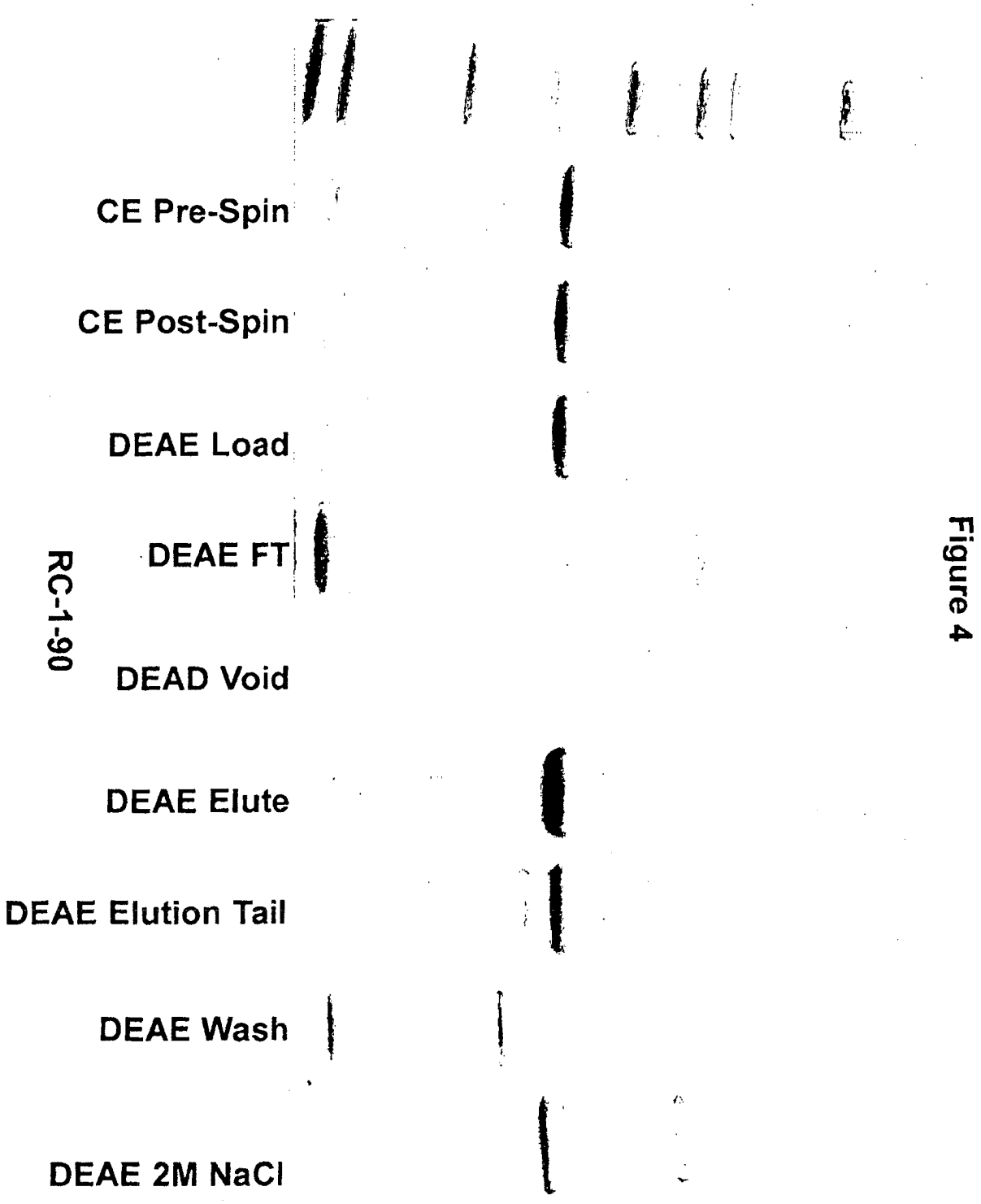

CE Pre-Spin
CE Post-Spin
DEAE Load
DEAE FT
DEAD Void
DEAE Elute
DEAE Elution Tail
DEAE Wash
DEAE 2M NaCl
RC-1-90

Figure 4

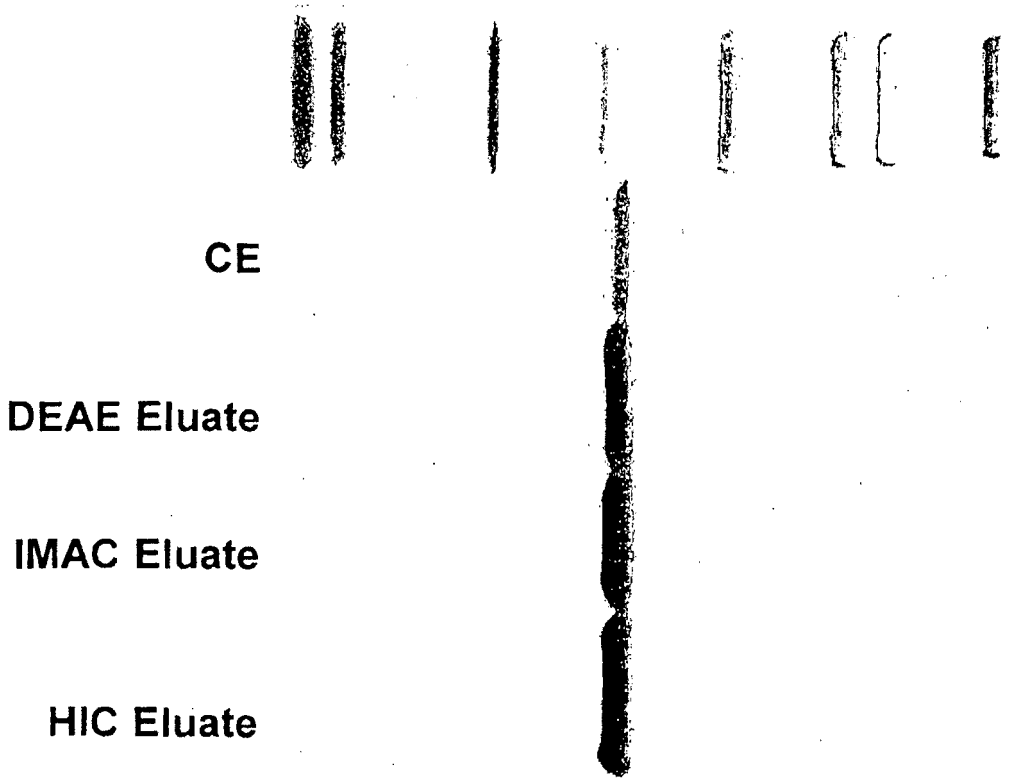


Figure 5

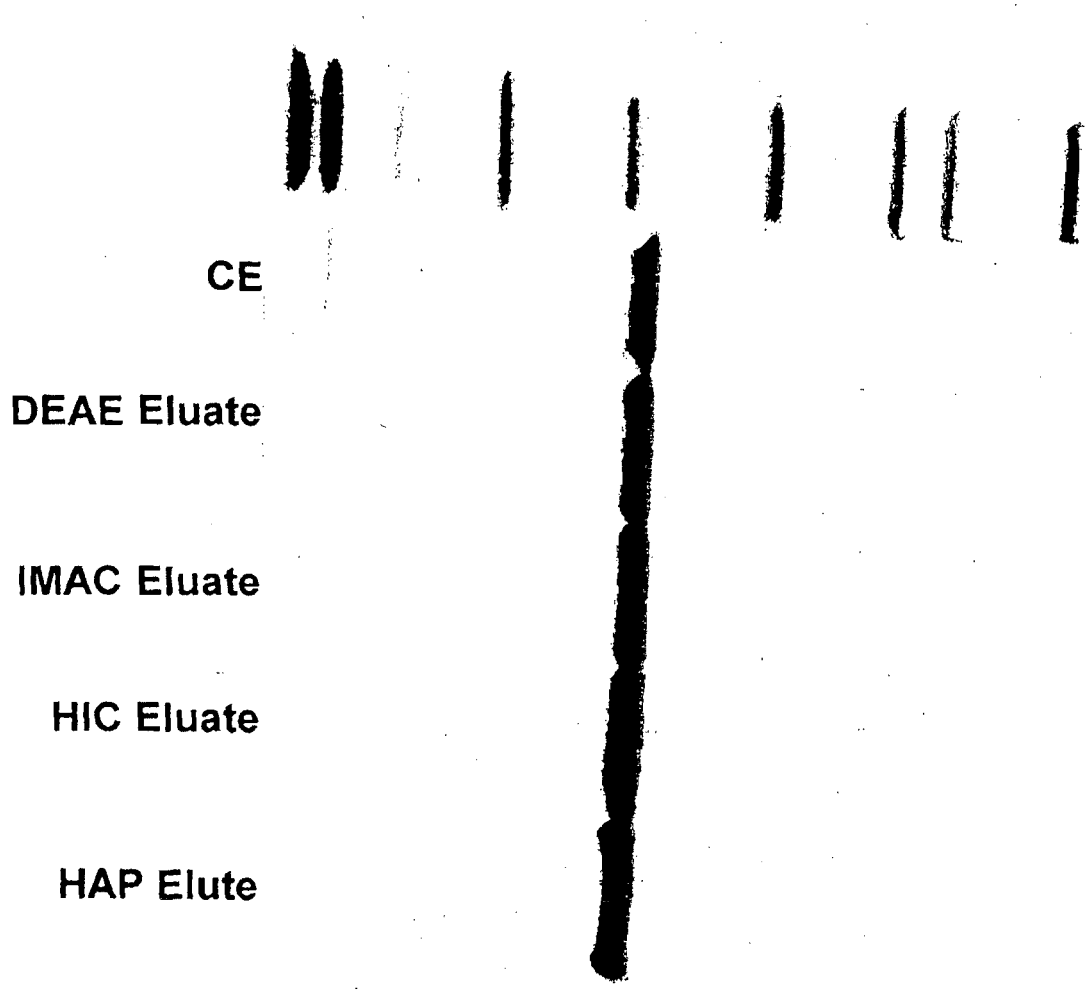


Figure 6

Figure 7A

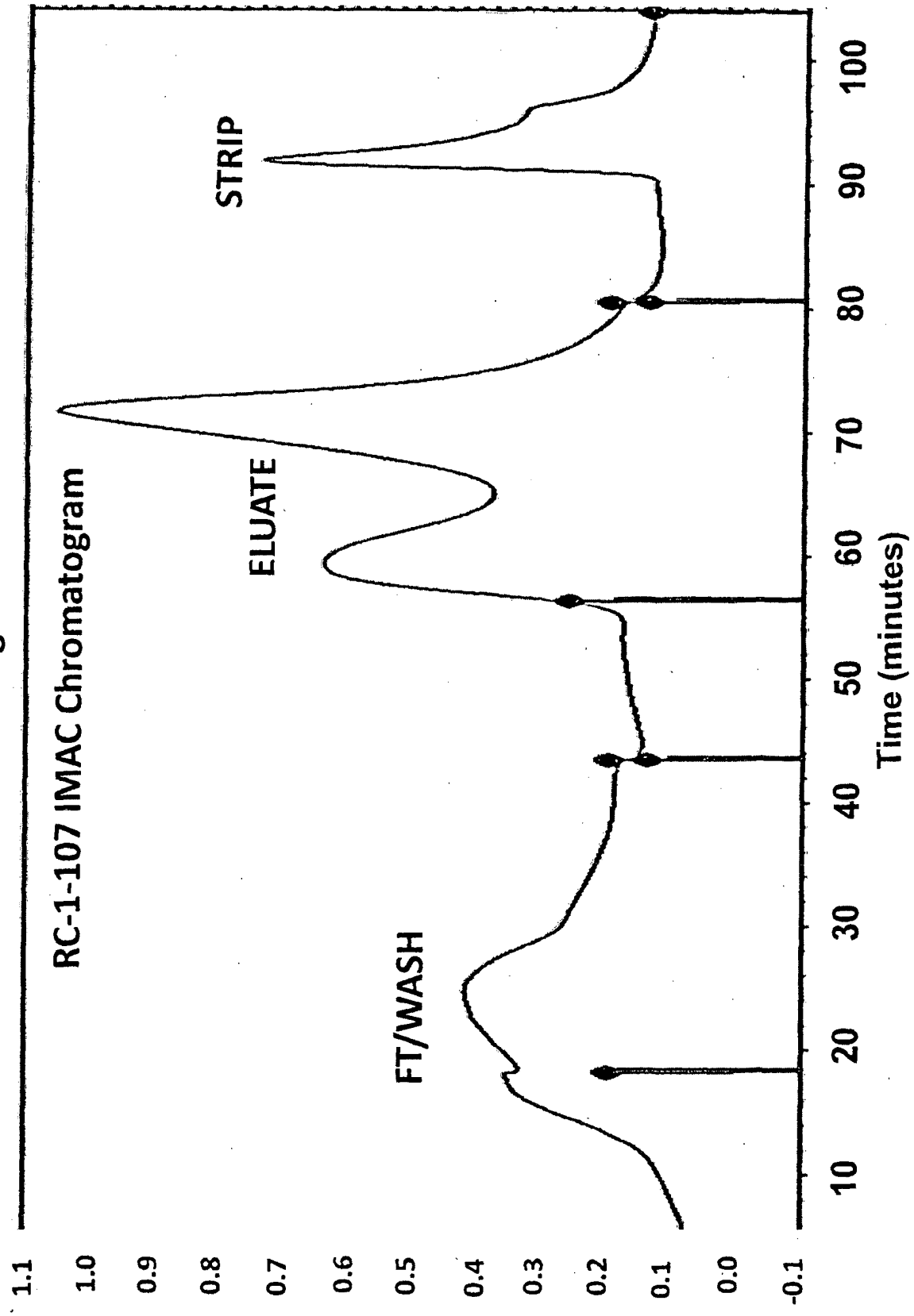

RC-1-107 IMAC Chromatogram
STRIP
ELUATE
FT/WASH
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
10
20
30
40
50
60
70
80
90
100
Time (minutes)

Figure 7B

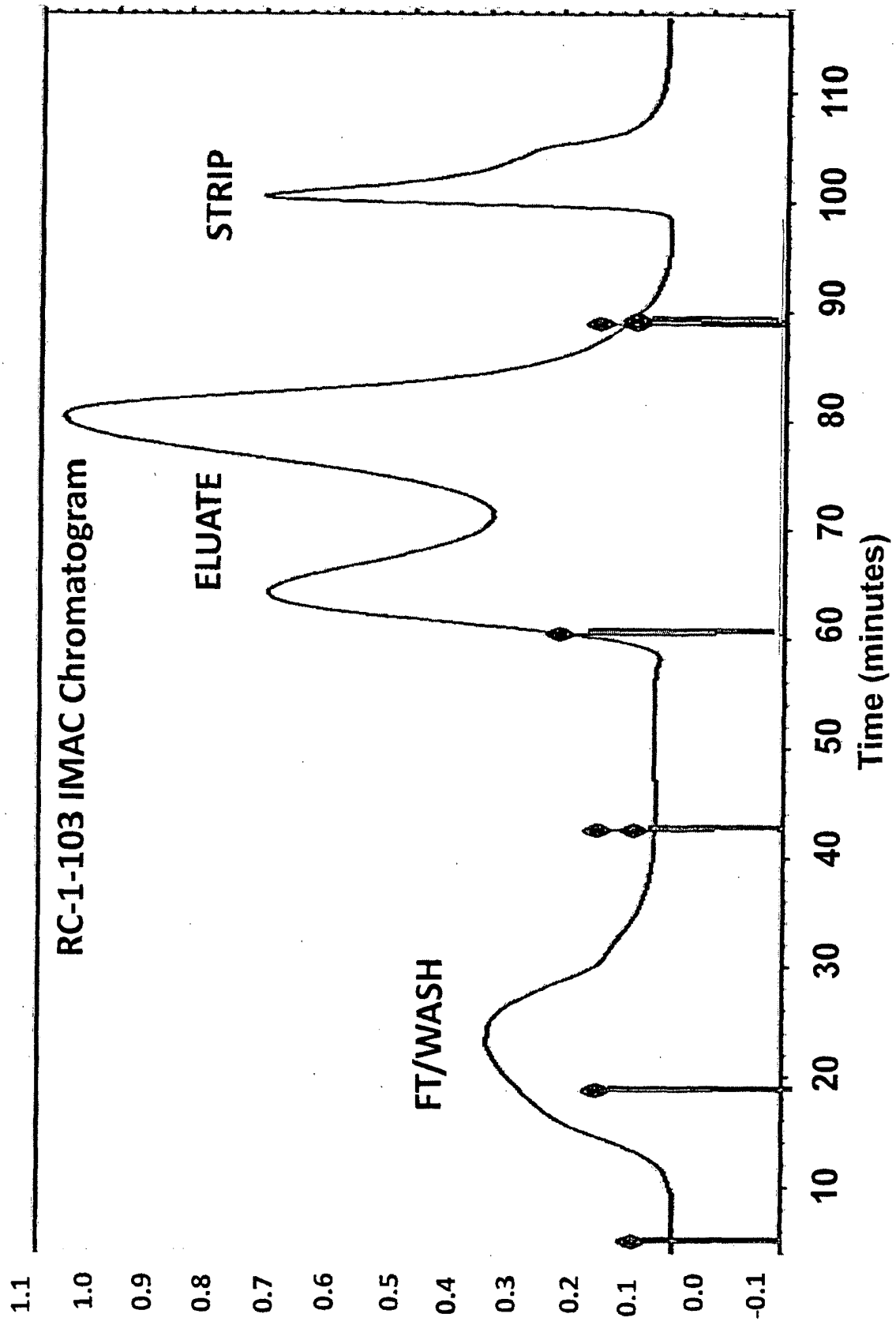

RC-1-103 IMAC Chromatogram
STRIP
ELUATE
FT/WASH
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
10
20
30
40
50
60
70
80
90
100
110
Time (minutes)

Figure 8

Date	04/06/11
Column ID	RC-3-

Exp. No.	RC-1-57 IMAC

Col Vol =	9	
Total protein/mL resin loaded	29	mg/mL

Fraction ID	Weight or Volume (g or mL)	CONV.	Protein (mg/mL)	Total Protein in fraction (mg)	Cystathionine from assay (nmol.mL.hr)	Dilution factor from assay	Total Units in fraction	S.A. Fraction	% Rec. Units (%)	% Rec. Total Protein (%)
CE	16	1.0	15.3	244.1	76039	20	24332	100	100.0%	100.0%
IMAC Load	34.7	1.0	7.6	264.7	40808	20	28321	107	116.4%	108.5%
IMAC FT	34.3	1.0	2.3	77.9	50	20	34	0	0.1%	31.9%
IMAC Equil Wash	32.6	1.0	2.8	91.6	4111	1	134	1	0.5%	37.5%
IMAC 5mM Imid.	34.3	1.0	0.6	19.2	9211	1	316	16	1.1%	7.9%
IMAC Void	6.9	1.0	0.4	3.0	3813	1	264	89	0.9%	1.2%
IMAC Eluate Front	14	1.0	0.7	9.8	77625	1	1087	110	3.8%	4.0%
IMAC Eluate	16.6	1.0	4.6	77.2	75941	20	25212	327	89.0%	31.6%
IMAC Eluate Tail	6.7	1.0	1.4	9.3	40388	5	1353	145	4.8%	3.8%
IMAC 100mM Imid.	38	1.0	0.4	16.7	29197	1	1109	66	3.9%	6.8%

Mass Balance Units (from load)	Mass Balance Protein
104.2%	115.1%

Buffers:

Charge	50 mM NiSO4
Equilibration	0.02 M Na3PO4, 0.4 M NaCl, 0.002 M Imidazole, 0.01%Triton, pH 7.0
Wash	0.02 M Na3PO4, 0.4 M NaCl, 0.005 M Imidazole, 0.01%Triton, pH 7.0
Elution	0.02 M Na3PO4, 0.4 M NaCl, 0.050 M Imidazole, 0.01%Triton, pH 7.0
Strip	100 mM Imidazole
Sanitize	1 M NaOH
Storage	0.001 M NaOH

Figure 9

Bradford Assay (Total Protein) **RC-1-57 IMAC**

Date: 4/7/2011

Standard Curve: BSA Stock: 2000 ug/mL

Well#	ug/mL BSA	BSA (uL)	Water (uL)
1	0	0	40
2	50	1	39
3	125	2.5	37.5
4	250	5	35
5	500	10	30
6	750	15	25
7	1000	20	20

Samples:

Number of samples Don't include triplicates): 10

Amount of Bradford Reagent Needed (mL): 19

For 10uL per standard, run in triplicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 1	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 17	Sample 17	Sample 17
B	Std 2	Std 2	Std 2	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 18	Sample 18	Sample 18
C	Std 3	Std 3	Std 3	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 19	Sample 19	Sample 19
D	Std 4	Std 4	Std 4	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 20	Sample 20	Sample 20
E	Std 5	Std 5	Std 5	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 21	Sample 21	Sample 21
F	Std 6	Std 6	Std 6	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14	Sample 22	Sample 22	Sample 22
G	Std 7	Std 7	Std 7	Sample 7	Sample 7	Sample 7	Sample 15	Sample 15	Sample 15	Sample 23	Sample 23	Sample 23
H				Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24

1 Remove appropriate amount of reagent and let it come to RT
2 Add 10uL Standard or sample to each well
3 Add 300uL Reagent to each well using multichannel pipettor
4 Incubate at RT 10min
5 Read on VersaMax

Sample #		Dilution	Protein (uL)	Water (uL)	Bradford (ug/mL)	ug/uL
1	CE	100	2	198	**15255.9**	15.2559
2	IMAC Load	50	4	196	**7629.2**	7.6292
3	IMAC FT	50	4	196	**2270.7**	2.2707
4	IMAC Equil Wash	25	8	192	**2810.3**	2.8103
5	AC 5mM Imidizole Wash	10	20	180	**559.3**	0.5593
6	IMAC Void	10	20	180	**432.3**	0.4323
7	IMAC Eluate Front	10	20	180	**702.7**	0.7027
8	IMAC Eluate	50	4	196	**4649.2**	4.6492
9	IMAC Eluate Tail	25	8	192	**1389**	1.389
10	IMAC 100mM Imidizole	10	20	180	**439.3**	0.4393

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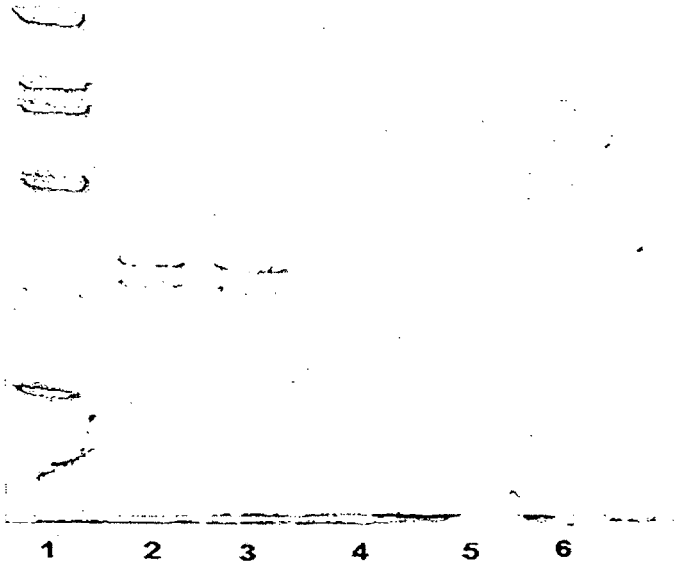
Gel 1

Figure 10A

Lane	Sample	TP Conc. (ug/ul)	Conc. for gel (ug)	Dilution (X)	Sample buffer (ul)	Protein (for 10ug/25uL)	ddH2O (ul)	
1	BIORAD Standard	--	--	1	12.5	1.00	11.50	
2	CE	15.256	10	1		0.66	11.84	
3	IMAC Load	7.6292	10	1		1.31	11.19	
4	IMAC FT	2.2707	10	1		4.40	8.10	
5	IMAC Equil Wash	2.8103	10	1		3.56	8.94	
6	IMAC 5mM Imidizole Wash	0.5593	10	1		12.50	0.00	Only 7ug loaded



Gel 1:

Figure 10B

Lane	Sample	TP Conc. (ug/ul)	Conc for gel (ug)	Dilution (X)	Sample buffer (ul)	Protein (for 10ug/25uL)	ddH2O (ul)	
1	BIORAD Standard	--	--	1	12.5	1.00	11.5	
2	IMAC Void	0.4323	10	1		12.50	0.00	Only 5.4ug loaded
3	IMAC Eluate Front	0.7027	10	1		12.50	0.00	Only 8.78ug loaded
4	IMAC Eluate	4.6492	10	1		2.15	10.35	
5	IMAC Eluate Tail	1.389	10	1		7.20	5.3	
6	IMAC 100mM Imidizole	0.4393	10	1		12.50	0.00	Only 5.49ug loaded

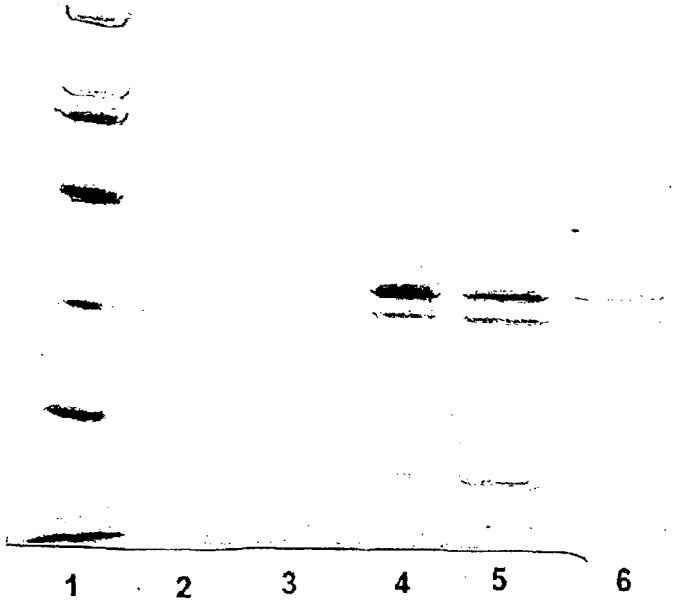


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Figure 11

CBS Crude Extract on Chelating Sepharose FF

Date	02/18/11
Column ID	RC-3-2 Chelating Seph. FF

Exp. No. RC-1-39

Col Vol.= 6.2 mL

Note: column charged to 6.2 mL, 6.5cm

Total protein/mL resin loaded 14 mg/mL

Fraction ID	Weight or Volume (g or mL)	CONV.	Protein (mg/mL)	Total Protein in fraction (mg)	Cystathionine from assay (nmol.mL.hr)	Dilution factor from assay	Total Units in fraction	S.A. Fraction	% Rec. Units (%)	% Rec. Total Protein (%)
CE	11	1.0	8.0	88.0	74067	10	8147	92.6	100.0%	100.0%
Load	11.4	1.0	8.1	92.3	82223	10	9373	101.5	115.0%	104.9%
Void	16	1.0	0.3	4.8	7	1	0	0.0	0.0%	5.5%
FT/Wash	62	1.0	1.2	74.4	5939	1	368	4.9	4.5%	84.5%
Eluate	25	1.0	2.1	52.5	56851	5	7106	135.4	87.2%	59.7%
500 mM Imid. Strip	36	1.0	0.2	7.2	1152	1	41	5.8	0.5%	8.2%

Recovery in Eluate (from load) 75.8%

Mass Balance Units (from CE)	Mass Balance Protein
92.3%	152.4%

Mass Balance Units (from load) 80.2%

Buffers:

Equilibration	0.02 Phosphate, 0.002 M imidazole, 0.4 M NaCl, pH 7.0
Wash	0.02 Phosphate, 0.002 M imidazole, 0.4 M NaCl, pH 7.0
Elution	0.02 Phosphate, 0.1 M imidazole, 0.4 M NaCl, pH 7.0
Strip	0.5 M Imidazole
Sanitize	1.0 M NaOH
Storage	0.001 M NaOH

Figure 12

Bradford Assay (Total Protein)					RC-1-63								
Date:	4/18/2011												
Standard Curve:			BSA Stock:	2000	ug/mL		Samples:						
		Well#	ug/mL BSA	BSA (uL)	Water (uL)		Number of samples (Don't include triplicates):					6	
		1	0	0	40								
		2	50	1	39		Amount of Bradford Reagent Needed (mL):					15.4	
		3	125	2.5	37.5								
		4	250	5	35								
		5	500	10	30								
		6	750	15	25								
		7	1000	20	20								
		For 10uL per standard, run in triplicate											

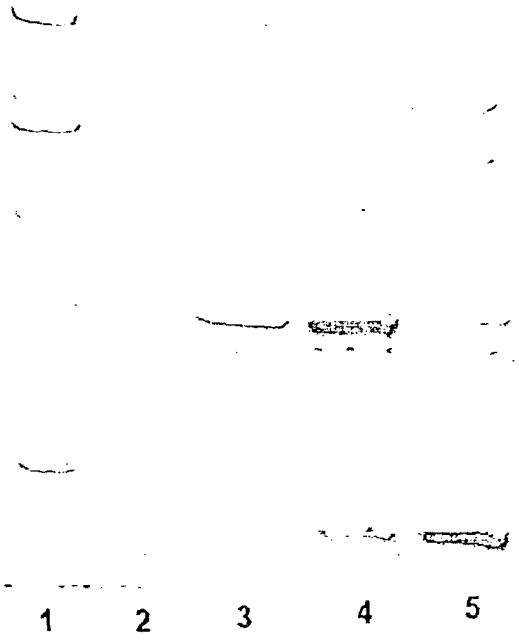
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	Std 1	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 17	Sample 17	Sample 17
B	Std 2	Std 2	Std 2	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 18	Sample 18	Sample 18
C	Std 3	Std 3	Std 3	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 19	Sample 19	Sample 19
D	Std 4	Std 4	Std 4	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 20	Sample 20	Sample 20
E	Std 5	Std 5	Std 5	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 21	Sample 21	Sample 21
F	Std 6	Std 6	Std 6	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14	Sample 22	Sample 22	Sample 22
G	Std 7	Std 7	Std 7	Sample 7	Sample 7	Sample 7	Sample 15	Sample 15	Sample 15	Sample 23	Sample 23	Sample 23
H				Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24

1. Remove appropriate amount of reagent and let it come to RT
2. Add 10uL Standard or sample to each well
3. Add 300uL Reagent to each well using multichannel pipettor
4. Incubate at RT 10min
5. Read on VersaMax

Sample #		Dilution	Protein (uL)	Water (uL)	Bradford (ug/mL)	ug/uL
1	Post Spin CE Load	100	2	198	**32187.209**	32.187209
2	Equil Wash	20	10	190	**11830.182**	11.830182
3	FT	10	20	180	**3310.385**	3.310385
4	5mM Wash	10	20	180	**252.568**	0.252568
5	8mM Wash	10	20	180	**670.278**	0.670278
6	20mM Wash	10	20	180	**524.895**	0.524895

Figure 13

Lane	Sample	TP Conc. (ug/ul)	Conc for gel (ug)	Dilution (X)	Sample buffer (ul)	Protein (for 10ug/25uL)	ddH2O (ul)	
1	BIORAD Standard	--	--	1	12.5	1.00	11.50	
2	Equil Wash	11.83	10	1		0.85	11.65	
3	5mM Wash	0.2526	10	1		12.50	0.00	3.16 ug protein loaded
4	8mM Wash	0.6703	10	1		12.50	0.00	8.38 ug protein loaded
5	20mM Wash	0.5249	10	1		12.50	0.00	6.56 ug protein loaded



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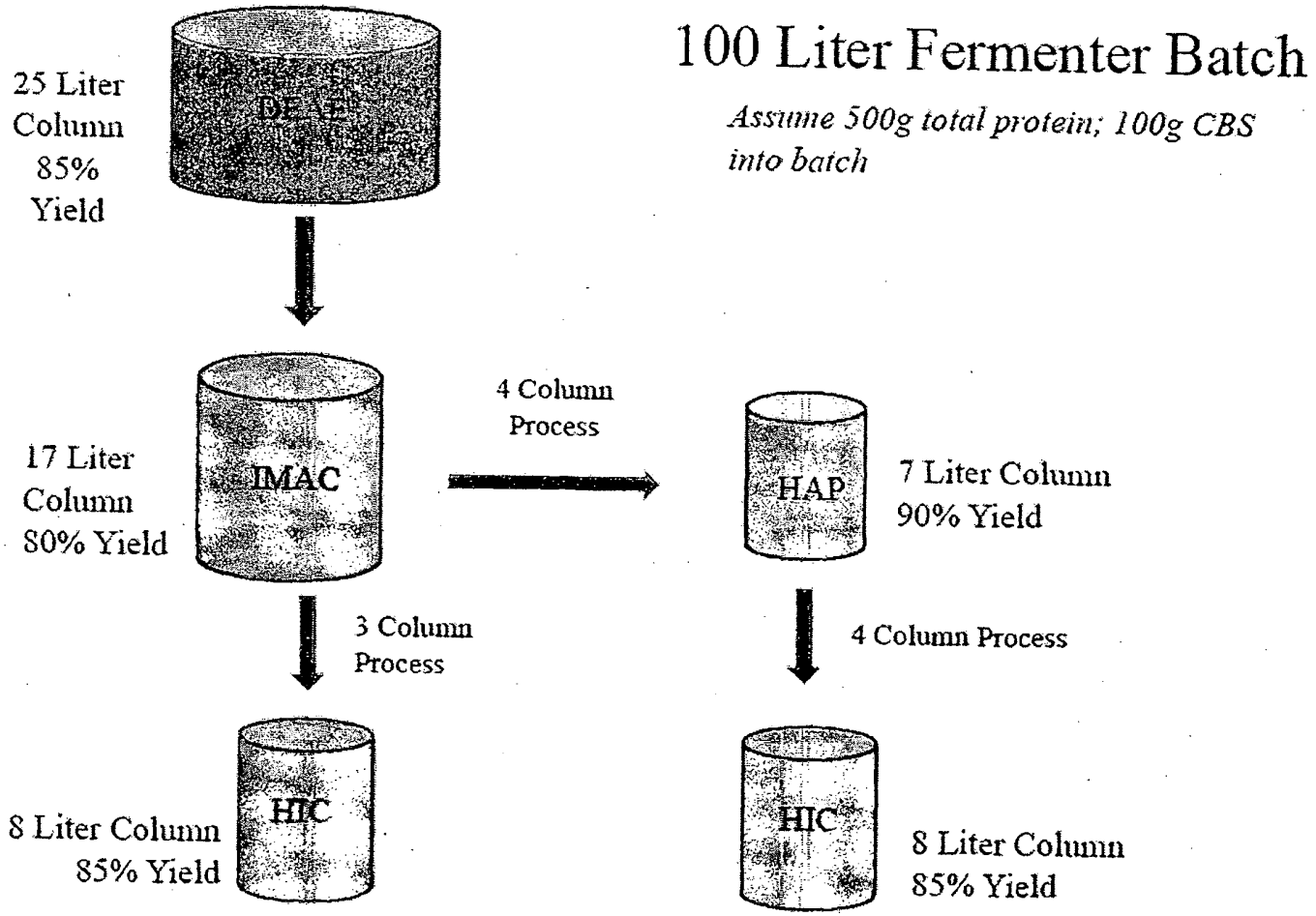
Process Flow Chart

Figure 14



REFERENCES CITED IN THE DESCRIPTION

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