2. Description of Related Art

[0006] Cystinuria is a hereditary disorder caused by mutations in the *SLC3A1* and *SLC7A9* genes encoding the kidney proximal tubule's cystine and dibasic amino acid transporter that leads to abnormal excretion of cystine (the disulfide form of the amino acid cysteine) and the formation of cystine crystals/stones in the urinary tract. There are few therapeutics available to patients suffering from the hereditary disorder cystinuria wherein a defective kidney transporter is unable to re-uptake cystine during renal filtration. Cystine, the disulfide form of the amino acid L-cysteine, is highly insoluble and in cystinuria patients reaches high concentrations in the urinary tract resulting in the formation of cystine crystals and stones. Existing therapies that reduce circulating cystine levels partially prevent urinary tract stone formation but have significant adverse effects that limit their use. Therapies are needed to reduce and prevent cystine stone formation in the kidney and bladder.

SUMMARY

[0007] The present disclosure describes the engineering of primate cystathionine-gamma-lyase ("CGL") enzymes such that both L-cystine and L-cysteine (referred to herein as "L-cyst(e)ine") can be efficiently degraded from serum, and providing the modified CGL enzymes in a formulation suitable for human therapy. To develop an enzyme displaying low K_M and high catalytic activity, k_{cat}, as compared to the native enzyme, the native enzyme was engineered by modifying selected amino acids, which modifications result in an enzyme having dramatically improved enzymatic properties. As such, modified CGL enzymes, as described herein, overcome a major deficiency in the art by providing novel enzymes that comprise human or primate polypeptide sequences having improved L-cyst(e)ine-degrading catalytic activity. As this enzyme is comprised of a human sequence, it is not likely to induce adverse immunological responses. As such, these modified enzymes may be suitable for human therapy and have low immunogenicity.

[0008] Methods are disclosed of utilizing an engineered human cystathionine-gamma-lyase (CGL) enzyme that efficiently converts cystine to cysteine-persulfide, which subsequently decays to free cysteine and H_2S, such that it is a suitable therapy for treating cystinuria patients by preventing cystine accumulation and formation of stones in the kidney and urinary tract. In addition, the engineered human CGL enzyme can convert cysteine to pyruvate, ammonia, and hydrogen sulfide, in effect reducing the amount of cysteine that can oxidize to form cystine. As cystine is a non-essential amino acid, which is normally

produced by most cells, no toxicities have been found to be induced by long-term cystine depletion in animal models. The ability of a cystine-degrading therapeutic to non-toxically ablate the total levels of circulating cystine indicate that it would be a superior therapeutic regimen for preventing cystine stone formation than existing therapeutic regimens.

[0009] Disclosed herein are modified CGL enzymes, having L-cyst(e)ine degrading activity, that are derived from primate CGL enzymes. A modified CGL enzyme may be derived from a human CGL enzyme (SEQ ID NO: 1), a *Pongo abelii* CGL enzyme (Genbank ID: NP_001124635.1; SEQ ID NO: 2), a *Macaca fascicularis* CGL enzyme (Genbank ID: AAW71993.1; SEQ ID NO: 3), a *Pan troglodytes* CGL enzyme (Genbank ID: XP_513486.2; SEQ ID NO: 4), or a *Pan paniscus* CGL enzyme (Genbank ID: XP_003830652.1; SEQ ID NO: 5). The native CGL enzyme may be modified by one or more other modifications, such as chemical modifications, substitutions, insertions, deletions, and/or truncations.

[0010] A modified CGL enzyme may be derived from a native, primate CGL enzyme by modifying by one, two, three, four or more substitutions at amino acid position(s) 51, 55, 59, 91, 163, 189, 193, 200, 234, 311, 336, 339, and/or 353 of SEQ ID NOs: 1-5. In these examples, the first methionine of each sequence corresponds to amino acid position 1, and each amino acid is numbered sequentially therefrom. The substitutions at amino acid positions 51 may be tryptophan (W), 55 may be glutamic acid (E), 59 may be threonine (T) or isoleucine (I), 91 may be methionine (M) or serine (S), 163 may be arginine (R), 189 may be serine (S), 193 may be glycine (G) or alanine (A), 200 may be proline (P) or histidine (H), 234 may be lysine (K), 311 may be glycine (G), 336 may be aspartic acid (D), 339 may be valine (V), and/or 353 may be serine (S).

[0011] A modified CGL enzyme may have an amino acid sequence according to SEQ ID NOs: 6-95. A modified CGL enzyme may be capable of degrading L-cyst(e)ine under physiological conditions. The modified CGL enzyme may have a catalytic efficiency for L-cyst(e)ine (k_{cat}/K_M) of at least or about 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10^4, 10^5, 10^6 $s^{-1}M^{-1}$ or any range derivable therein. An exemplary CGL enzyme may have a catalytic efficiency of $>10^4$ $s^{-1}M^{-1}$ for L-cystine and $>10^3$ $s^{-1}M^{-1}$ for L-cysteine.

[0012] The substitutions may be a combination of P193A, T311G, E339V, and I353S of human CGL (for example, the modified polypeptide having the amino acid sequence of SEQ ID NO: 6 or a fragment or homolog thereof). For example, an equivalent substitution of

containing such nucleic acids. A nucleic acid encoding the modified CGL enzyme can be operably linked to a promoter, including but not limited to heterologous promoters. A modified CGL enzyme may be delivered to a target cell by a vector (*e.g.*, a gene therapy vector). Such vectors may have been modified by recombinant DNA technology to enable the expression of the modified CGL-encoding nucleic acid in the target cell. These vectors may be derived from vectors of non-viral (*e.g.*, plasmids) or viral (*e.g.*, adenovirus, adeno-associated virus, retrovirus, lentivirus, herpes virus, or vaccinia virus) origin. Non-viral vectors may be complexed with agents to facilitate the entry of the DNA across the cellular membrane. Examples of such non-viral vector complexes include the formulation with polycationic agents, which facilitate the condensation of the DNA, and lipid-based delivery systems. Lipid-based delivery systems include liposome-based delivery of nucleic acids.

[0021] Host cells comprising such vectors are provided. The host cells may be bacteria (*e.g.*, *E. coli*), fungal cells (*e.g.*, yeast), insect cells, or mammalian cells.

[0022] A vector can be introduced into host cells for expressing the modified CGL enzyme. The modified CGL enzymes may be expressed in any suitable manner. The modified CGL enzymes may be expressed in a host cell such that the protein is glycosylated. Alternatively, the modified CGL enzymes may be expressed in a host cell such that the protein is aglycosylated.

[0023] Therapeutic formulations containing the modified CGL enzyme and a pharmaceutically acceptable carrier are provided. Therapeutic formulations can be administered intravenously, intradermally, intraarterially, intraperitoneally, intramuscularly, subcutaneously, by infusion, by continuous infusion, *via* a catheter, in lipid compositions (*e.g.*, liposomes).

[0024] In one aspect, the present disclosure refers to a use of a formulation as disclosed herein in the manufacture of a medicament for treating a subject having or at risk of developing cystinuria. .Methods are disclosed for treating a subject having or being at risk of developing cystinuria, the methods comprising administering to the subject a therapeutically effective amount of a formulation comprising an isolated, modified primate CGL enzyme having at least one substitution relative to a native primate CGL amino acid sequence (see SEQ ID NOs: 1-5) or a nucleic acid comprising a nucleotide sequence encoding the isolated, modified primate CGL enzyme. The isolated, modified primate CGL enzyme may have a sequence according to any one of SEQ ID NOs: 6-95. The methods may reduce the level of cysteine and/or cystine in the subject's plasma, serum, and/or urine.

[0025] The subject may be any animal, such as a mouse. For example, the subject may be a mammal, a rodent, a primate, or a human patient. The subject or patient may be maintained on a L-cyst(e)ine-restricted diet, a methionine-restricted diet or a normal diet in combination with being treated with the described compositions.

[0026] The subject may have previously been treated for cystinuria and the CGL enzyme is administered to treat or moderate the recurrence of cystinuria or ameliorate one or more conditions and/or symptoms associated with cystinuria. The methods may also comprise administering at least a second therapy to the subject, such as a second cystinuria therapy, such as modifications of diet, nutritional therapy, and shock wave therapy. The methods may reduce the volume of cystine stones or the number of cystine stones in the subject's urinary tract, kidney, and/or bladder. The methods may prevent the formation of cystine stones in the subject's urinary tract, kidney, and/or bladder. The methods may reduce the number of cystine crystals in the subject's urine. The methods may prevent the formation of cystine crystals in the subject's urine.

[0027] As such, methods are provided for preventing the formation of cystine stones in patients having cystinuria, the methods comprising administering to the subject a therapeutically effective amount of a formulation comprising an isolated, modified primate CGL enzyme having at least one substitution relative to a native primate CGL amino acid sequence (see SEQ ID NOs: 1-5) or a nucleic acid comprising a nucleotide sequence encoding the isolated, modified primate CGL enzyme. The isolated, modified primate CGL enzyme may have a sequence according to any one of SEQ ID NOs: 6-95. The methods may prevent the formation of cystine stones in the patient's kidney and/or bladder.

[0028] A composition comprising a modified CGL enzyme or a nucleic acid encoding a modified CGL enzyme is provided for use in the treatment of cystinuria in a subject. The use of an isolated or modified CGL enzyme, a nucleic acid encoding a modified CGL enzyme, or a composition comprising said modified CGL enzyme in the manufacture of a medicament for therapeutic application to a cystinuria patient is provided. The modified CGL enzyme may be any modified CGL enzyme disclosed herein.

[0029] Also provided herein are:

1. In one aspect, the present disclosure refers to an isolated, modified primate cystathionine-γ-lyase (CGL) enzyme comprising at least the following substitutions relative to a native human CGL amino acid sequence (SEQ

ID NO: 1), wherein the modified enzyme has both cystinase and cysteinase activity, said substitutions being selected from the group consisting of:

(a) alanine at position 193, glycine at position 311, valine at position 339, and serine at position 353;

(b) proline at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(c) alanine at position 193, proline at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(d) glutamic acid at position 55, threonine at position 59, methionine at position 91, lysine at position 234, aspartic acid at position 336, and valine at position 339;

(e) glutamic acid at position 55, threonine at position 59, serine at position 91, lysine at position 234, aspartic acid at position 336, and valine at position 339;

(f) serine at position 189, glycine at position 193, glycine at position 311, valine at position 339, and serine at position 353;

(g) arginine at position 163, glycine at position 311, valine at position 339, and serine at position 353;

(h) tryptophan at position 51, glutamic acid at position 55, threonine at position 59, aspartic acid at position 336, and valine at position 339;

(i) glutamic acid at position 55, alanine at position 193, glycine at position 311, aspartic acid at position 336, valine at position 339, and serine at position 353;

(j) histidine at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(k) glycine at position 311, valine at position 339, and serine at position 353;

(l) isoleucine at position 59, valine at position 339, and serine at position 353;

(m) methionine at position 91 and valine at position 339; and

(n) valine at position 339 and serine at position 353.

2. The isolated, modified CGL enzyme as disclosed herein, wherein the modified CGL enzyme is a modified *Pongo abelii* CGL enzyme.

3. The isolated, modified CGL enzyme as disclosed herein, wherein the modified *Pongo abelii* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and V353S;

(b) A200P, T311G, E339V, and V353S;

(c) P193A, A200P, T311G, E339V, and V353S;

(d) H55E, V59T, L91M, N234K, T336D, and E339V;

(e) H55E, V59T, L91S, N234K, T336D, and E339V;

(f) T189S, P193G, T311G, E339V, and V353S;

(g) T163R, T311G, E339V, and V353S;

(h) A51W, H55E, V59T, T336D, and E339V;

(i) H55E, P193A, T311G, T336D, E339V, and V353S;

(j) A200H, T311G, E339V, and V353S;

(k) T311G, E339V, and V353S;

(l) V59I, E339V, and V353S;

(m) L91M and E339V; and

(n) E339V and V353S.

4. The isolated, modified CGL enzyme as disclosed herein, wherein the modified CGL enzyme is a modified human CGL enzyme, a modified *Pan troglodytes* CGL enzyme, or a modified *Pan paniscus* CGL enzyme.

5. The isolated, modified CGL enzyme as disclosed herein, wherein the modified human CGL enzyme, the modified *Pan troglodytes* CGL enzyme, or the modified *Pan paniscus* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and I353S;

(b) A200P, T311G, E339V, and I353S;

(c) P193A, A200P, T311G, E339V, and I353S;

(d) H55E, E59T, L91M, N234K, T336D, and E339V;

(e) H55E, E59T, L91S, N234K, T336D, and E339V;

(f) T189S, P193G, T311G, E339V, and I353S;

(g) T163R, T311G, E339V, and I353S;

(h) A51W, H55E, E59T, T336D, and E339V;

(i) H55E, P193A, T311G, T336D, E339V, and I353S;

(j) A200H, T311G, E339V, and I353S;

(k) T311G, E339V, and I353S;

(l) E59I, E339V, and I353S;

(m) L91M and E339V; and

(n) E339V and I353S.

6. The isolated, modified CGL enzyme as disclosed herein, wherein the modified CGL enzyme is a modified *Macaca fascicularis* CGL enzyme.

7. The isolated, modified CGL enzyme as disclosed herein, wherein the modified *Macaca fascicularis* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and I353S;
(b) A200P, T311G, E339V, and I353S;
(c) P193A, A200P, T311G, E339V, and I353S;
(d) H55E, E59T, L91M, N234K, T336D, and E339V;
(e) H55E, E59T, L91S, N234K, T336D, and E339V;
(f) T189S, P193G, T311G, E339V, and I353S;
(g) V163R, T311G, E339V, and I353S;
(h) A51W, H55E, E59T, T336D, and E339V;
(i) H55E, P193A, T311G, T336D, E339V, and I353S;
(j) A200H, T311G, E339V, and I353S;
(k) T311G, E339V, and I353S;
(l) E59I, E339V, and I353S;
(m) L91M and E339V; and
(n) E339V and I353S.

8. The isolated, modified CGL enzyme as disclosed herein, further comprising a heterologous peptide segment or a polysaccharide.

9. The isolated, modified CGL enzyme as disclosed herein, wherein the heterologous peptide segment is an XTEN peptide, an IgG Fc, an albumin, or an albumin binding peptide.

10. The isolated, modified CGL enzyme as disclosed herein, wherein the polysaccharide comprises polysialic acid polymers.

11. The isolated, modified CGL enzyme of any one as disclosed herein, wherein the enzyme is coupled to polyethylene glycol (PEG).

12. The isolated, modified CGL enzyme as disclosed herein, wherein the enzyme is coupled to the PEG via one or more lysine residues.

13. In another aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the enzyme as disclosed herein.

14. The nucleic acid as disclosed herein, wherein the nucleic acid is codon optimized for expression in bacteria, fungus, insects, or mammals.

15. The nucleic acid as disclosed herein, wherein the bacteria are *E. coli*.

16. The nucleic acid as disclosed herein, wherein the nucleic acid comprises a sequence according to one of SEQ ID NOs: 81-95.

17. In yet another aspect, the present disclosure refers to an expression vector comprising the nucleic acid as disclosed herein.

18. In another aspect, the present disclosure refers to a host cell comprising the nucleic acid as disclosed herein.

19. The host cell as disclosed herein, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, or a mammalian cell.

20. In another aspect, the present disclosure refers to a therapeutic formulation comprising an enzyme as disclosed herein, or the nucleic acid as disclosed herein, in a pharmaceutically acceptable carrier.

21. A method of treating a subject having or at risk of developing cystinuria comprising administering to the subject a therapeutically effective amount of a formulation of aspect 20.

22. The method as disclosed herein, wherein the subject is maintained on a L-cystine and/or L-cysteine restricted diet.

23. The method as disclosed herein, wherein the subject is maintained on a methionine-restricted diet.

24. The method as disclosed herein, wherein the subject is maintained on a normal diet.

25. The method as disclosed herein, wherein the subject is a human patient.

26. The method as disclosed herein, wherein the formulation is administered intravenously, intraarterially, intraperitoneally, intramuscularly, intravascularly, subcutaneously, by injection, by infusion, by continuous infusion, or via a catheter.

27. The method as disclosed herein, wherein the subject has previously been treated for cystinuria and the enzyme is administered to prevent the recurrence of cystinuria.

28. The method as disclosed herein, further comprising administering at least a second cystinuria therapy to the subject.

29. The method as disclosed herein, wherein the second cystinuria therapy is a surgical therapy or a shock wave therapy.

30. The method as disclosed herein, wherein the second cystinuria therapy comprises an agent or drug selected from the group consisting of: bicarbonate, tris-hydroxymethylene-aminomethane (tromethamine-E), acetylcysteine, D-penicillamine, α-mercaptopropionylglycine, and captopril.

31. The method as disclosed herein, wherein the method reduces the level of cysteine and/or cystine in the subject's plasma and/or serum.

32. The method as disclosed herein, wherein the method reduces the level of cysteine and/or cystine in the subject's urine.

33. The method as disclosed herein, wherein the method prevents the formation of cystine stones in the subject's urinary tract.

34. The method as disclosed herein, wherein the method prevents the formation of cystine stones in the subject's kidneys.

35. The method as disclosed herein, wherein the method reduces the number of cystine stones in the subject's kidneys.

36. Use of an isolated or modified CGL enzyme as disclosed herein or a composition comprising said modified CGL enzyme for the manufacture of a medicament for therapeutic application to a cystinuria patient.

[0030] As used herein the terms "encode" or "encoding," with reference to a nucleic acid, are used to make the disclosure readily understandable by the skilled artisan; however, these terms may be used interchangeably with "comprise" or "comprising," respectively.

WHAT IS CLAIMED IS:

1. An isolated, modified primate cystathionine-γ-lyase (CGL) enzyme comprising at least the following substitutions relative to a native human CGL amino acid sequence (SEQ ID NO: 1), wherein the modified enzyme has both cystinase and cysteinase activity, said substitutions being selected from the group consisting of:

(a) alanine at position 193, glycine at position 311, valine at position 339, and serine at position 353;

(b) proline at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(c) alanine at position 193, proline at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(d) glutamic acid at position 55, threonine at position 59, methionine at position 91, lysine at position 234, aspartic acid at position 336, and valine at position 339;

(e) glutamic acid at position 55, threonine at position 59, serine at position 91, lysine at position 234, aspartic acid at position 336, and valine at position 339;

(f) serine at position 189, glycine at position 193, glycine at position 311, valine at position 339, and serine at position 353;

(g) arginine at position 163, glycine at position 311, valine at position 339, and serine at position 353;

(h) tryptophan at position 51, glutamic acid at position 55, threonine at position 59, aspartic acid at position 336, and valine at position 339;

(i) glutamic acid at position 55, alanine at position 193, glycine at position 311, aspartic acid at position 336, valine at position 339, and serine at position 353;

(j) histidine at position 200, glycine at position 311, valine at position 339, and serine at position 353;

(k) glycine at position 311, valine at position 339, and serine at position 353;

(l) isoleucine at position 59, valine at position 339, and serine at position 353;

(m) methionine at position 91 and valine at position 339; and

(n) valine at position 339 and serine at position 353.

2. The isolated, modified CGL enzyme of claim 1, wherein the modified CGL enzyme is a modified *Pongo abelii* CGL enzyme.

3. The isolated, modified CGL enzyme of claim 2, wherein the modified *Pongo abelii* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and V353S;

(b) A200P, T311G, E339V, and V353S;

(c) P193A, A200P, T311G, E339V, and V353S;

(d) H55E, V59T, L91M, N234K, T336D, and E339V;

(e) H55E, V59T, L91S, N234K, T336D, and E339V;

(f) T189S, P193G, T311G, E339V, and V353S;

(g) T163R, T311G, E339V, and V353S;

(h) A51W, H55E, V59T, T336D, and E339V;

(i) H55E, P193A, T311G, T336D, E339V, and V353S;

(j) A200H, T311G, E339V, and V353S;

(k) T311G, E339V, and V353S;

(l) V59I, E339V, and V353S;

(m) L91M and E339V; and

(n) E339V and V353S.

4. The isolated, modified CGL enzyme of claim 1, wherein the modified CGL enzyme is a modified human CGL enzyme, a modified *Pan troglodytes* CGL enzyme, or a modified *Pan paniscus* CGL enzyme.

5. The isolated, modified CGL enzyme of claim 4, wherein the modified human CGL enzyme, the modified *Pan troglodytes* CGL enzyme, or the modified *Pan paniscus* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and I353S;

(b) A200P, T311G, E339V, and I353S;

(c) P193A, A200P, T311G, E339V, and I353S;

(d) H55E, E59T, L91M, N234K, T336D, and E339V;

(e) H55E, E59T, L91S, N234K, T336D, and E339V;

(f) T189S, P193G, T311G, E339V, and I353S;

(g) T163R, T311G, E339V, and I353S;

(h) A51W, H55E, E59T, T336D, and E339V;

(i) H55E, P193A, T311G, T336D, E339V, and I353S;

(j) A200H, T311G, E339V, and I353S;

(k) T311G, E339V, and I353S;

(l) E59I, E339V, and I353S;

(m) L91M and E339V; and

(n) E339V and I353S.

6. The isolated, modified CGL enzyme of claim 1, wherein the modified CGL enzyme is a modified *Macaca fascicularis* CGL enzyme.

7. The isolated, modified CGL enzyme of claim 6, wherein the modified *Macaca fascicularis* CGL enzyme comprises substitutions selected from the group consisting of:

(a) P193A, T311G, E339V, and I353S;

(b) A200P, T311G, E339V, and I353S;

(c) P193A, A200P, T311G, E339V, and I353S;

(d) H55E, E59T, L91M, N234K, T336D, and E339V;

(e) H55E, E59T, L91S, N234K, T336D, and E339V;

(f) T189S, P193G, T311G, E339V, and I353S;

(g) V163R, T311G, E339V, and I353S;

(h) A51W, H55E, E59T, T336D, and E339V;

(i) H55E, P193A, T311G, T336D, E339V, and I353S;

(j) A200H, T311G, E339V, and I353S;

(k) T311G, E339V, and I353S;

(l) E59I, E339V, and I353S;

(m) L91M and E339V; and

(n) E339V and I353S.

8. The isolated, modified CGL enzyme of any one of claims 1-7, further comprising a heterologous peptide segment or a polysaccharide.

9. The isolated, modified CGL enzyme of claim 8, wherein the heterologous peptide segment is an XTEN peptide, an IgG Fc, an albumin, or an albumin binding peptide.

10. The isolated, modified CGL enzyme of claim 8, wherein the polysaccharide comprises polysialic acid polymers.

11. The isolated, modified CGL enzyme of any one of claims 1-10, wherein the enzyme is coupled to polyethylene glycol (PEG).

12. The isolated, modified CGL enzyme of claim 11, wherein the enzyme is coupled to the PEG via one or more lysine residues.

13. A nucleic acid comprising a nucleotide sequence encoding the enzyme of any one of claims 1-7.

14. The nucleic acid of claim 13, wherein the nucleic acid is codon optimized for expression in bacteria, fungus, insects, or mammals.

15. The nucleic acid of claim 14, wherein the bacteria are *E. coli*.

16. The nucleic acid of claim 15, wherein the nucleic acid comprises a sequence according to one of SEQ ID NOs: 81-95.

17. An expression vector comprising the nucleic acid of any one of claims 13-16.

18. A host cell comprising the nucleic acid of any one of claims 13-16.

19. The host cell of claim 18, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, or a mammalian cell.

20. A therapeutic formulation comprising an enzyme of any one of claims 1-12, or the nucleic acid of any one of claims 13-16, in a pharmaceutically acceptable carrier.

21. Use of a formulation of claim 20 in the manufacture of a medicament for treating a subject having or at risk of developing cystinuria.

22. The use of claim 21, wherein the subject is maintained on a L-cystine and/or L-cysteine restricted diet.

23. The use of claim 21, wherein the subject is maintained on a methionine-restricted diet.

24. The use of claim 21, wherein the subject is maintained on a normal diet.

25. The use of claim 21, wherein the subject is a human patient.

26. The use of claim 21, wherein the formulation is to be administered intravenously, intraarterially, intraperitoneally, intramuscularly, intravascularly, subcutaneously, by injection, by infusion, by continuous infusion, or via a catheter.

27. The use of claim 21, wherein the subject has previously been treated for cystinuria and the enzyme is to be administered to prevent the recurrence of cystinuria.

28. The use of claim 21, further comprising at least a second cystinuria therapy to be administered to the subject.

29. The use of claim 28, wherein the second cystinuria therapy is a surgical therapy or a shock wave therapy.

30. The use of claim 28, wherein the second cystinuria therapy comprises an agent or drug selected from the group consisting of: bicarbonate, tris-hydroxymethylene-aminomethane (tromethamine-E), acetylcysteine, D-penicillamine, α-mercaptopropionylglycine, and captopril.

31. The use of claim 21, wherein the medicament reduces the level of cysteine and/or cystine in the subject's plasma and/or serum.

32. The use of claim 21, wherein the medicament reduces the level of cysteine and/or cystine in the subject's urine.

33. The use of claim 21, wherein the medicament prevents the formation of cystine stones in the subject's urinary tract.

34. The use of claim 21, wherein the medicament prevents the formation of cystine stones in the subject's kidneys.

35. The use of claim 21, wherein the medicament reduces the number of cystine stones in the subject's kidneys.

36. Use of an isolated or modified CGL enzyme of any of claims 1 to 12 or a composition comprising said modified CGL enzyme in the manufacture of a medicament for therapeutic application to a cystinuria patient.