Claims

1. A method of preparing a modified lysosomal protein, said method comprising:
   a) reacting a glycosylated lysosomal protein with an alkali metal periodate for a time of no more than 4 h; and
   b) reacting said lysosomal protein with an alkali metal borohydride for a time period of no more than 2 h;
   thereby modifying glycan moieties of the lysosomal protein and reducing the activity of the lysosomal protein with respect to glycan recognition receptors, provided that said protein is not sulfamidase.

2. The method of claim 1, wherein step b) is further characterized by at least one of, such as at least two, such as all of i)-iv):
   i) said alkali metal borohydride is sodium borohydride;
   ii) said borohydride is used at a concentration of no more than 4 times the concentration of said periodate, such as no more than 3 times the concentration of said periodate, such as no more than 2.5 times the concentration of said periodate, such as 0.5 to 4 times the concentration of said periodate;
   iii) said reaction is performed for a time period of no more than 2 h; such as no more than 1.5 h, such as no more than 1 h, such as no more than 0.75 h, such as around 0.5 h, and
   iv) said reaction is performed at a temperature of between 0 and 8 °C.

3. The method according to claim 1 or 2, wherein step a) is further characterized by at least one of, such as at least two, such as at least three, such as at least four, such as at least all of i)-v):
   i) said alkali metal periodate is sodium *meta*-periodate;
   ii) said periodate is used at a concentration of no more than 20 mM, such as no more than 15 mM, such as around 10 mM;
   iii) said reaction is performed at a temperature of between 0 and 22 °C, such as a temperature of 0-8 °C, such as at a temperature of 0-4 °C such as around 8 °C, such as around 0 °C;
   iv) said reaction is performed for a time period of no more than 3h, such as no more than 2 h, such as no more than 1 h, such as around 0.5 h, and
   v) said reaction of step a) is performed at a pH of 3-7.

4. The method according to any one of claims 1 to 3, wherein step a) is performed for a time period of no more than 3 h and step b) is performed for no more than 1 h, and said borohydride optionally is used at a concentration of no more than 4 times the concentration of said periodate.

5. The method according to any one of claims 1 to 4, wherein step a) and step b) are performed in sequence without performing any dialysis, ultrafiltration, precipitation or buffer exchange.

6. A method of preparing a modified lysosomal protein, said method comprising:
   a) reacting a glycosylated lysosomal protein with an alkali metal periodate, and
   b) reacting said lysosomal protein with an alkali metal borohydride;
   thereby modifying glycan moieties of the lysosomal protein and reducing the activity of the lysosomal protein with respect to glycan recognition receptors, wherein the active site or functional epitope of said lysosomal protein is made inaccessible to oxidative and/or reductive reactions during at least one of steps a) and b).

7. The method according to claim 6, wherein step b) is further characterized by at least one of, such as at least two, such as all of i)-iv):
   i) said alkali metal borohydride is sodium borohydride;
   ii) said borohydride is used at a concentration of no more than 4 times the concentration of said periodate, such as no more than 3 times the concentration of said periodate, such as no more than 2.5 times the concentration of said periodate, such as 0.5 to 4 times the concentration of said periodate;
   iii) said reaction is performed for a time period of no more than 2 h; such as no more than 1.5 h, such as no more than 1 h, such as no more than 0.75 h, such as around 0.5 h, and
   iv) said reaction is performed at a temperature of between 0 and 8 °C.

8. The method according to claim 6 or 7, wherein step a) is further characterized by at least one of, such as at least two, such as at least three, such as at least four, such as at least all of i)-v):
   i) said alkali metal periodate is sodium *meta*-periodate;
   ii) said periodate is used at a concentration of no more than 20 mM, such as no more than 15 mM, such as around 10 mM;

iii) said reaction is performed at a temperature of between 0 and 22 °C, such as a temperature of 0-8 °C, such as at a temperature of 0-4 °C such as around 8 °C, such as around 0 °C;
iv) said reaction is performed for a time period of no more than 3h, such as no more than 2 h, such as no more than 1 h, such as around 0.5 h, and
v) said reaction of step a) is performed at a pH of 3-7.

9. The method according to any one of claims 6 to 8, wherein step a) is performed for a time period of no more than 3 h and step b) is performed for no more than 1 h, and said borohydride optionally is used at a concentration of no more than 4 times the concentration of said periodate.

10. The method according to any one of claims 6 to 9, wherein step a) and step b) are performed in sequence without performing any dialysis, ultrafiltration, precipitation or buffer exchange.

11. The method according to any one of claims 1 to 10, wherein said modified lysosomal protein is a sulfatase; a glycoside hydrolase, or a protease.

12. The method according to any one of claims 1 to 10, wherein the lysosomal protein is selected from deoxyribonuclease-2-alpha; beta-mannosidase; ribonuclease T2; lysosomal alpha-mannosidase; alpha L-iduronidase; tripeptidyl-peptidase 1; hyaluronidase-3; cathepsin L2; ceroid-lipofuscinosis neuronal protein 5; glucosylceramidase; tissue alpha-L-fucosidase; myeloperoxidase; alpha-galactosidase A; beta-hexosaminidase subunit alpha; cathepsin D; prosaposin; beta-hexosaminidase subunit beta; cathepsin L1; cathepsin B; beta-glucuronidase; pro-cathepsin H; non-secretory ribonuclease; lysosomal alpha-glucosidase; lysosomal protective protein; gamma-interferon-inducible lysosomal thiol reductase; tartrate-resistant acid phosphatase type 5; arylsulfatase A; prostatic acid phosphatase; N-acetylglucosamine-6-sulfatase; arylsulfatase B; beta-galactosidase; alpha-N-acetylgalactosaminidase; sphingomyelin phosphodiesterase; ganglioside GM2 activator; N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase; iduronate 2-sulfatase; cathepsin S; N-acetylgalactosamine-6-sulfatase; lysosomal acid lipase/cholesteryl ester hydrolase; lysosomal Pro-X carboxypeptidase; cathepsin O; cathepsin K; palmitoyl-protein thioesterase 1; arylsulfatase D; dipeptidyl peptidase 1; alpha-N-acetylglucosaminidase; galactocerebrosidase; epididymal secretory protein E1; di-N-acetylchitobiase; N-acylethanolamine-hydrolyzing acid amidase; hyaluronidase-1; chitotriosidase-1; acid ceramidase; phospholipase B-like 1;

proprotein convertase subtilisin/kexin type 9; group XV phospholipase A2; putative phospholipase B-like 2; deoxyribonuclease-2-beta; gamma-glutamyl hydrolase; arylsulfatase G; L-amino-acid oxidase; sialidase-1; legumain; sialate O-acetylesterase; thymus-specific serine protease; cathepsin Z; cathepsin F; prenylcysteine oxidase 1; dipeptidyl peptidase 2; lysosomal thioesterase PPT2; heparanase; carboxypeptidase Q; β-glucuronidase, and sulfatase-modifying factor 1.

13. The method according to any one of claims 1 to 12, wherein at least one of steps a) and b) of the method is/are performed in the presence of a protective ligand.

14. The method according to any one of claims 1 to 13 wherein steps a) and b) of the method are performed while the lysosomal protein is immobilized on a resin.

15. A modified lysosomal protein having a reduced content of unmodified glycan moieties, characterized in that no more than 50 % of the glycan moieties remain unmodified as compared to an unmodified form of the lysosomal protein, said protein thereby having a reduced activity for glycan recognition receptors, provided that said protein is not sulfamidase, β-glucuronidase, tripeptidyl peptidase 1 (TPP1) or alpha L-iduronidase.

16. The modified lysosomal protein according to claim 15, said protein being selected from deoxyribonuclease-2-alpha; beta-mannosidase; ribonuclease T2; lysosomal alpha-mannosidase; hyaluronidase-3; cathepsin L2; ceroid-lipofuscinosis neuronal protein 5; glucosylceramidase; tissue alpha-L-fucosidase; myeloperoxidase; alpha-galactosidase A; beta-hexosaminidase subunit alpha; cathepsin D; prosaposin; beta-hexosaminidase subunit beta; cathepsin L1; cathepsin B;; pro-cathepsin H; non-secretory ribonuclease; lysosomal alpha-glucosidase; lysosomal protective protein; gamma-interferon-inducible lysosomal thiol reductase; tartrate-resistant acid phosphatase type 5; arylsulfatase A; prostatic acid phosphatase; N-acetylglucosamine-6-sulfatase; arylsulfatase B; beta-galactosidase; alpha-N-acetylgalactosaminidase; sphingomyelin phosphodiesterase; ganglioside GM2 activator; N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase; iduronate 2-sulfatase; cathepsin S; N-acetylgalactosamine-6-sulfatase; lysosomal acid lipase/cholesteryl ester hydrolase; lysosomal Pro-X carboxypeptidase; cathepsin O; cathepsin K; palmitoyl-protein thioesterase 1; arylsulfatase D; dipeptidyl peptidase 1; alpha-N-acetylglucosaminidase; galactocerebrosidase; epididymal secretory protein

E1; di-N-acetylchitobiase; N-acylethanolamine-hydrolyzing acid amidase; hyaluronidase-1; chitotriosidase-1; acid ceramidase; phospholipase B-like 1; proprotein convertase subtilisin/kexin type 9; group XV phospholipase A2; putative phospholipase B-like 2; deoxyribonuclease-2-beta; gamma-glutamyl hydrolase; arylsulfatase G; L-amino-acid oxidase; sialidase-1; legumain; sialate O-acetylesterase; thymus-specific serine protease; cathepsin Z; cathepsin F; prenylcysteine oxidase 1; dipeptidyl peptidase 2; lysosomal thioesterase PPT2; heparanase; carboxypeptidase Q, and sulfatase-modifying factor 1.

17. The modified lysosomal protein according to claim 15 or 16, wherein no more than 45 % of the glycan moieties remain unmodified compared to an unmodified form of the lysosomal protein, such as no more than 40 %, no more than 35 %, no more than 30 %, no more than 35 %, no more than 30 %, no more than 25 %, no more than 20 %, no more than 15 %, no more than 10 %, no more than 5 %, no more than 1 % of the epitopes for glycan recognition receptors remains from an unmodified form of the lysosomal protein.

18. The modified lysosomal protein according to any one of claims 15 to 17, wherein unmodified glycan moieties of said lysosomal protein are disrupted by single bond breaks and double bond breaks, the extent of single bond breaks being at least 60 % in oligomannose glycans.

19. The modified lysosomal protein according to any one of claims 15 to 18, wherein said unmodified glycan moieties are absent from at least one N-glycosylation site of said lysosomal protein, such as at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen of the N-glycosylation sites of said lysosomal protein, preferably said epitopes are absent from all N-glycosylation sites.

20. The modified lysosomal protein according to any one of claims 15 to 19, wherein said lysosomal protein has retained catalytic activity, such as a retained catalytic activity of at least 50 % of that of the corresponding unmodified lysosomal protein.

21. A modified lysosomal protein obtainable by the method according to any one of claims 1 to 14, provided that said protein is not sulfamidase.

22. The modified lysosomal protein according to any one of claims 15 to 21, for use in therapy.

23. The modified lysosomal protein according to claim 22, for use in treatment of a mammal afflicted with a lysosomal storage disease.