

Claims

1. A modified sulfamidase comprising substantially no epitopes for glycan recognition receptors, wherein natural glycan moieties of said sulfamidase are disrupted by single bond breaks and double bond breaks, the extent of single bond breaks per total bond breaks being at least 60 % in oligomannose glycans, thereby enabling transportation of said sulfamidase across the blood brain barrier of a mammal, wherein said sulfamidase has catalytic activity in the brain of said mammal.
2. The modified sulfamidase according to claim 1, having a relative content of natural glycan moieties of around 25 % of the content of natural glycan moieties in unmodified recombinant sulfamidase.
3. The modified sulfamidase according to any preceding claim, wherein said epitopes represent at least one type of moiety selected from a mannose-6-phosphate moiety, a mannose moiety and a galactose moiety.
4. The modified sulfamidase according to claim 3, wherein said epitopes are recognized by at least one glycan recognition receptor selected from mannose-6 phosphate receptor type 1 and 2, mannose receptor and galactose receptor.
5. The modified sulfamidase according to any preceding claim, comprising a polypeptide consisting of an amino acid sequence as defined in SEQ ID NO:1, or a polypeptide having at least 95 % sequence identity with an amino acid sequence as defined in SEQ ID NO:1, said modified sulfamidase comprising a C α -formylglycine residue in position 50 of SEQ ID NO:1 (FGly50) providing said catalytic activity.
6. The modified sulfamidase according to claim 5, wherein said epitopes are absent at at least four of the five N-glycosylation sites: N in position 21 (N(21)), N in position 122 (N(122)), N in position 131 (N(131)), N in position 244 (N(244)), and N in position 393 (N(393)) of SEQ ID NO:1.
7. The modified sulfamidase according to claim 6, wherein said epitopes are absent at N-glycosylation sites N(21), N(122), N(244), and N(393) of said modified sulfamidase polypeptide.

8. The modified sulfamidase according to any one of claims 5-7, said sulfamidase being intact in its c-terminal part, said c-terminal part optionally being represented by amino acids 436-484 of SEQ ID NO:1.

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9. The modified sulfamidase according to any preceding claim, wherein said catalytic activity provides a reduction in lysosomal storage in the brain of said mammal suffering from lysosomal storage disease.

10 10. A sulfamidase composition, comprising modified sulfamidase having substantially no epitopes for glycan recognition receptors, wherein natural glycan moieties of said sulfamidase are disrupted by single bond breaks and double bond breaks, the extent of single bond breaks per total bond breaks being at least 60 % in oligomannose glycans, thereby enabling
15 transportation of said sulfamidase across the blood brain barrier of a mammal, and having a C α -formylglycine (FGly) to serine (Ser) ratio at the active site that is greater than 1, thereby providing catalytic activity in the brain of said mammal.

20 11. The sulfamidase composition according to claim 10, comprising modified sulfamidase as defined in any one of claims 1-9.

12. The sulfamidase composition according to any one of claims 10 or 11, comprising no more than 5 % (by weight) of sulfamidase in multimeric
25 forms having a molecular weight of above 10¹⁰ kDa.

13. A method of preparing a modified sulfamidase, said method comprising:

- 30 a) reacting a glycosylated sulfamidase with an alkali metal periodate for a time period of no more than 4 h at a concentration of no more than 20 mM, and
- b) reacting said sulfamidase with an alkali metal borohydride for a time period of no more than 2 h at a concentration of between 10 and 80 mM; thereby modifying glycan moieties of the sulfamidase and reducing the
35 activity of the sulfamidase with respect to glycan recognition receptors, while retaining catalytic activity of said sulfamidase.

14. The method according to claim 13, wherein step a) 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.

5 15. The method according to claim 13 or 14, wherein step a) and step b) are performed in sequence without performing an intermediate step.

16. The method according to any one of claims 13-15, wherein step a) is further characterized by at least one of, such as at least two, such as all of i)-
10 iii):

- i) said alkali metal periodate is sodium *meta*-periodate;
- ii) said periodate is used at a concentration of no more than 15 mM, such as around 10 mM, and
- 15 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.

17. The method according to any one of claims 13-16, wherein said reaction of step a) is performed at a pH of 3-7.

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18. The method according to any one of claims 13-17, 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;
- 25 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;
- 30 iii) said reaction is performed for a time period of 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.

35 19. The method according to any one of claims 13-18, wherein each of step a) and step b) is individually performed for a time period of no more than

2 h, and said borohydride is optionally used at a concentration of 0.5-4 times the concentration of said periodate.

20. The method according to any one of claims 13-19, 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.

21. The method according to any one of claims 13-20, further comprising a2) quenching of the reaction resulting from step a).

22. The method according to any one of claims 13-21, further comprising b2) quenching of the reaction resulting from step b).

23. The method according to any one of claims 13-22, wherein said glycosylated sulfamidase polypeptide comprises glycan moieties and at least four asparagine residues.

24. The method according to claim 23, wherein said alkali metal periodate oxidizes *cis*-glycol groups of the glycan moieties to aldehyde groups.

25. The method according to claim 24, wherein said alkali metal borohydride reduces said aldehydes to alcohols.

26. The modified sulfamidase or the sulfamidase composition according to any one of claims 1-12, for use in therapy.

27. The modified sulfamidase or the sulfamidase composition according to claim 26, for use in treatment of a mammal afflicted with a lysosomal storage disease, in particular mucopolysaccharidosis IIIA (MPS IIIA).