increased. In certain aspects, the number of N-glycan branches may be increased by increasing activity of galactose transferases or GlcNAc-transferases, such as β6-GlcNAc-transferase (IGnT). The length of the N-glycan branches may be increased by, for example, increasing the number of polylactosamines. In one embodiment, the number of polylactosamines is increased by increasing expression of beta3-GlcNAC transferase (iGnT).

[0018] A method for producing rhuBChE is provided herein. The method includes culturing a mammalian cell that co-expresses huBChE, alpha2-6 sialyltransferase, and PRAD in cell culture medium, thereby producing rhuBChE. In one aspect, the rhuBChE is isolated from the culture medium. One example of a suitable culture medium is a serum-free medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figure 1 is a schematic representation showing the quaternary structure of butyrylcholinesterase (BChE). 1A. depicts the BChE monomer; 1B-~~D~~. depicts the BChE in the tetrameric assembly state.

[0020] Figure 2 is a diagram that shows the chemical structure of sialic acid. ~~1A~~2A. depicts the alpha2-3 sialic acid linkage and ~~1B~~2B. shows the alpha2-6 sialic acid linkage.

[0021] Figure 3 is a simplified schematic representation of the sialylation mammalian pathway that indicates the synthesis of sugar nucleotide CMP-Neu5Ac (CMP-sialic acid) and sialylation of BChE.

[0022] Figure 4 are graphic representations of the circulatory time of wild-type (4A) and recombinant BChE (4B) and the circulatory time after removal of sialic acid in native huBCHE (4C-D).

[0023] Figure 5 is a three-dimensional plot of mean residence time (MRT) on a molecular weight/percent acidic fraction grid.

[0024] Figure 6 is a graphic representation of the time course of FBS AChE and Eq BChE in the circulation of mice. Stability of FBS AChE (A) and Eq (B) in the circulation of mice following an i.v. injection of 50-80 units if ChE/animal are shown. Curves depict the time course of ChEs in individual mice and were generated in accordance with monoexponential or biexponential decay equations. Each data point is an average of two

What is claimed is:

1. An isolated mammalian cell comprising a heterologous alpha2-6 sialyltransferase nucleic acid sequence and a nucleic acid sequence that decreases expression of or silences an alpha2-3sialyltransferase gene or means for knock-out of the alpha2-3sialyltransferase gene.

2. The cell of claim 1, wherein the cell is a CHO cell.

3. The cell of claim 1, further comprising a nucleic acid sequence that encodes for human butyrlcholinesterase (huBChE).

~~4. The cell of claim 1, further comprising a nucleic acid sequence that decreases expression of or silences an alpha2-3sialyltransferase gene.~~

~~5~~4. The cell of claim 41, wherein the nucleic acid sequence that silences the alpha2-3sialyltransferase gene is selected from the group consisting of antisense, siRNA and miRNA.

~~6. The cell of claim 1, further comprising means for knock-out of the alpha2-3sialyltransferase gene.~~

~~7~~5. The cell of claim 1, wherein the cell further comprises the nucleic acid sequence encoding for the proline-rich attachment domain (PRAD) of the ColQ gene.

~~8~~6. A glycoprotein produced by the cell of claim 1, wherein the glycoprotein ~~comprising~~ comprises an increase in alpha2-6 sialic acid linkages and a decrease~~d level of~~ in alpha2-3 sialic acid linkages as compared with a glycoprotein produced in an isolated CHO cell.

~~9~~7. The glycoprotein of claim ~~8~~6, wherein the glycoprotein is in a tetrameric assembly state.

~~10~~8. The glycoprotein of claim ~~8~~6, wherein the glycoprotein is recombinant huBChE (rhuBChE).

~~11~~9. The glycoprotein of claim ~~8~~6, wherein the glycoprotein has an extended circulatory half-life or mean residence time (MRT).

~~12~~10. The cell of claim 1, further comprising a nucleic acid sequence encoding for an enzyme that reduces or inhibits alpha2-6 sialic acid degradation.

~~13~~11. A method for the biosynthesis of an alpha2-6-rich glycoprotein comprising culturing a cell of claim 1 under conditions to co-express a nucleic acid sequence that encodes a peptide or protein.

~~14~~12. The method of claim ~~13~~11, further comprising inhibiting expression of alpha2-3 sialyltransferase.

~~15~~13. The method of claim ~~13~~11, further comprising reducing or inhibiting degradation of alpha2-6 sialic acid.

~~16~~14. The method of claim ~~15~~13, wherein alpha2-6 sialic acid degradation is reduced or inhibited by increasing activity of an enzyme that prevents alpha2-6 sialic acid degradation.

~~17~~15. The method of claim ~~16~~14, wherein the enzyme is fucosyltransferase.

~~18~~16. The method of claim ~~17~~15, wherein the fucosyltransferase is an alpha3fucosyltransferase (alpha3FucT).

~~19~~17. The method of claim ~~18~~16, wherein alpha3FucT is encoded by a nucleic acid sequence selected from FUT4, FUT5, FUT6, FUT7, FUT8, and FUT9.

~~20~~18. The method of claim ~~17~~15, wherein the fucosyltransferase is alpha3,4 fucosyltransferase (FucTLe) or alpha2fucosyltransferase (FucTLe).

~~21~~19. The method of claim ~~15~~13, wherein alpha2-6 sialic acid degradation is reduced or inhibited by decreasing activity of an enzyme that promotes alpha2-6 sialic acid degradation.

~~22~~20. The method of claim ~~21~~19, wherein the enzyme is a sialidase or neuramidase.

~~23~~21. The method of claim ~~13~~11, further comprising increasing the number or the length of N-glycan branches.

~~24~~22. The method of claim ~~23~~21, wherein the number of N-glycan branches is increased by increasing activity of galactose transferases or GlcNAc-transferases.

~~25~~23. The method of claim ~~23~~21, wherein the length of branches is increased by increasing the number of polylactosamines.

~~26~~24. The method of claim ~~25~~23, wherein the number of polylactosamines is increased by increasing expression of beta3-GlcNAC transferase (iGnT) and/or Gal transferase.

~~27~~25. The method of claim ~~13~~11, further comprising increasing CMP-sialic acid content.

~~28~~26. The method of claim ~~13~~11, wherein the peptide is a biological protective agent.

~~29~~27. The method of claim ~~13~~11, wherein the peptide is an organophosphorus ~~OP~~ scavenger.

~~30~~28. The method of claim ~~13~~11, wherein the peptide is rhuBChE.

~~31~~29. The method of claim ~~13~~11, further comprising modifying the cell to co-express tetramer assembly chaperones, thereby generating glycoprotein tetramers.

~~32~~30. The method of claim ~~31~~29, wherein the chaperone is PRAD.

~~33~~31. A glycoprotein comprising an increase in alpha2-6 sialic acid linkages and a decreased level of alpha2-3 sialic acid linkages for use in the prevention or treatment of neurotoxin poisoning.

~~34~~32. A method for producing rhuBChE comprising culturing a mammalian cell that co-expresses huBChE, alpha2-6 sialyltransferase and PRAD in cell culture medium, thereby producing rhuBChE.

~~35~~33. The method of claim ~~34~~32, wherein the rhuBChE is isolated from the culture medium.

~~36~~34. The method of claim ~~34~~32, wherein the medium is serum-free medium.

~~37~~35. The cell of claim 41, wherein the alpha2-3sialyltransferase gene is selected from the group consisting of St3gal1 St3gal2, St3gal3, St3gal4, St3gal5, St3gal6 gene and a combination thereof.

36. The glycoprotein of claim 6, wherein the glycoprotein is an organophosphorus-scavenger protein.

37. The cell of claim 1, further comprising a nucleic acid sequence that encodes a heterologous protein.

38. The cell of claim 37, wherein the protein is a glycoprotein.

39. The cell of claim 1, wherein the means for knock-out is a zinc finger nuclease or deletion, mutation or addition of nucleotides.

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2. The cell of claim 1, wherein the cell is a CHO cell.

3. The cell of claim 1, further comprising a nucleic acid sequence that encodes for human butyrlcholinesterase (huBChE).

4. The cell of claim 1, wherein the nucleic acid sequence that silences the alpha2-3sialyltransferase gene is selected from the group consisting of antisense, siRNA and miRNA.

5. The cell of claim 1, wherein the cell further comprises the nucleic acid sequence encoding for the proline-rich attachment domain (PRAD) of the ColQ gene.

6. A glycoprotein produced by the cell of claim 1, wherein the glycoprotein comprises an increase in alpha2-6 sialic acid linkages and a decrease in alpha2-3 sialic acid linkages as compared with a glycoprotein produced in an isolated CHO cell.

7. The glycoprotein of claim 6, wherein the glycoprotein is in a tetrameric assembly state.

8. The glycoprotein of claim 6, wherein the glycoprotein is recombinant huBChE (rhuBChE).

9. The glycoprotein of claim 6, wherein the glycoprotein has an extended circulatory half-life or mean residence time (MRT).

10. The cell of claim 1, further comprising a nucleic acid sequence encoding for an enzyme that reduces or inhibits alpha2-6 sialic acid degradation.

11. A method for the biosynthesis of an alpha2-6-rich glycoprotein comprising culturing a cell of claim 1 under conditions to co-express a nucleic acid sequence that encodes a peptide or protein.

12. The method of claim 11, further comprising inhibiting expression of alpha2-3 sialyltransferase.

13. The method of claim 11, further comprising reducing or inhibiting degradation of alpha2-6 sialic acid.

14. The method of claim 13, wherein alpha2-6 sialic acid degradation is reduced or inhibited by increasing activity of an enzyme that prevents alpha2-6 sialic acid degradation.

15. The method of claim 14, wherein the enzyme is fucosyltransferase.

16. The method of claim 15, wherein the fucosyltransferase is an alpha3fucosyltransferase (alpha3FucT).

17. The method of claim 16, wherein alpha3FucT is encoded by a nucleic acid sequence selected from FUT4, FUT5, FUT6, FUT7, FUT8, and FUT9.

18. The method of claim 15, wherein the fucosyltransferase is alpha3,4 fucosyltransferase (FucTLe) or alpha2fucosyltransferase (FucTLe).

19. The method of claim 13, wherein alpha2-6 sialic acid degradation is reduced or inhibited by decreasing activity of an enzyme that promotes alpha2-6 sialic acid degradation.

20. The method of claim 19, wherein the enzyme is a sialidase or neuramidase.

21. The method of claim 11, further comprising increasing the number or the length of N-glycan branches.

22. The method of claim 21, wherein the number of N-glycan branches is increased by increasing activity of galactose transferases or GlcNAc-transferases.

23. The method of claim 21, wherein the length of branches is increased by increasing the number of polylactosamines.

24. The method of claim 23, wherein the number of polylactosamines is increased by increasing expression of beta3-GlcNAC transferase (iGnT) and/or Gal transferase.

25. The method of claim 11, further comprising increasing CMP-sialic acid content.

26. The method of claim 11, wherein the peptide is a biological protective agent.

27. The method of claim 11, wherein the peptide is an organophosphorus scavenger.

28. The method of claim 11, wherein the peptide is rhuBChE.

29. The method of claim 11, further comprising modifying the cell to co-express tetramer assembly chaperones, thereby generating glycoprotein tetramers.

30. The method of claim 29, wherein the chaperone is PRAD.

31. A glycoprotein comprising an increase in alpha2-6 sialic acid linkages and a decreased level of alpha2-3 sialic acid linkages for use in the prevention or treatment of neurotoxin poisoning.

32. A method for producing rhuBChE comprising culturing a mammalian cell that co-expresses huBChE, alpha2-6 sialyltransferase and PRAD in cell culture medium, thereby producing rhuBChE.

33. The method of claim 32, wherein the rhuBChE is isolated from the culture medium.

34. The method of claim 32, wherein the medium is serum-free medium.

35. The cell of claim 1, wherein the alpha2-3sialyltransferase gene is selected from the group consisting of St3gal1 St3gal2, St3gal3, St3gal4, St3gal5, St3gal6 gene and a combination thereof.

36. The glycoprotein of claim 6, wherein the glycoprotein is an organophosphorus-scavenger protein.

37. The cell of claim 1, further comprising a nucleic acid sequence that encodes a heterologous protein.

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