the mannose-5 glycostructure amount in the glycosylation pattern can be obtained. The DGL value or respectively the amount of glucose available per time unit has to be kept constant and at a defined reduced value per time unit.

As reported herein is a method for the production of a polypeptide, in one example of an immunoglobulin, in a eukaryotic cell, comprising the following steps

a) providing a eukaryotic cell comprising a nucleic acid encoding the polypeptide,

b) cultivating the cell under conditions wherein the degree of glucose limitation (DGL) is kept constant and wherein the DGL is less than 0.8, and

c) recovering the polypeptide from the culture,

wherein the fraction of the polypeptide with a mannose-5 glycostructure is 10 % or less of the sum comprising the amount of the polypeptide with a mannose-5 glycostructure, the amount of the polypeptide G(0) isoform, the amount of the polypeptide G(1) isoform, and the amount of the polypeptide G(2) isoform.

In one example the DGL is kept constant in the range of from 0.8 to 0.2. In a further example the DGL is kept constant in the range of from 0.6 to 0.4. In another example the fraction of the polypeptide with a mannose-5 glycostructure is 8 % or less of the sum comprising the polypeptide with a mannose-5 glycostructure, the polypeptide G(0) isoform, the polypeptide G(1) isoform, and the polypeptide G(2) isoform. In still another example the polypeptide is an immunoglobulin, in one example an immunoglobulin of class G or E.

As reported herein is a method for the production of an immunoglobulin comprising the following steps:

a) providing a mammalian cell comprising a nucleic acid encoding the immunoglobulin,

b) cultivating the cell in a cultivation medium wherein the amount of glucose available in the cultivation medium per time unit is kept constant

and limited to less than 80 % of the amount that could maximally be utilized by the cells in the cultivation medium per time unit, and

c) recovering the immunoglobulin from the cells or the cultivation medium.

In one example the amount of glucose available in the cultivation medium per time unit is kept constant and limited to a value in the range of from 80 % to 20 %. In a further example the range is of from 60 % to 40 %. In another example the cells in the cultivation medium are the viable cells in the cultivation medium.

In one example of the aspects as reported herein the eukaryotic cell is selected from CHO cells, NS0 cells, HEK cells, BHK cells, hybridoma cells, PER.C6® cells, insect cells, or Sp2/0 cells. In one example the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell. In another example of the aspects as reported herein the cultivating is at a pH value in the range of from about pH 7.0 to about pH 7.2.

In still another example of the aspects as reported herein the cultivating is a continuous or a fed-batch cultivating. The methods may comprise in another example a final step of purifying the polypeptide. In still another example the cell is cultivated for six to twenty days or for six to fifteen days. In a further example the cell is cultivated for six to eight days.

As reported herein is a composition comprising an immunoglobulin, wherein the composition has been prepared with a method as reported herein.

In one example the immunoglobulin is an anti-IL-6R antibody. In a further example the anti-IL-6R antibody comprises Tocilizumab. In another example the mannose-5 glycostructure attached to the anti-IL-6R antibody is 8 % or less. In still a further example the mannose-5 glycostructure is 6 % or less. In another example the mannose-5 glycostructure is 4 % or less. In a further example the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL-6R antibody is in the range from 9 % to 11 %.

In one aspect, the present disclosure provides a composition comprising an antibody protein that binds human interleukin-6 receptor, wherein the antibody protein is an anti-IL-6R antibody protein with mannose-5 glycostructure (M5) attached thereto, and wherein the fraction of the anti-IL-6R antibody protein with M5 attached thereto in the composition is 2.7% to 10% of the sum comprising the anti-IL-6R antibody protein with M5, G(0), G(1), and G(2) oligosaccharide attached thereto; and

wherein the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL- 6R antibody is in the range from 9 % to 11 %.

In another aspect, the present disclsoure provides a composition comprising Tocilizumab protein with mannose-5 glycostructure (M5) attached to Asn297 thereof, wherein the fraction of Tocilizumab protein with M5 attached thereto in the composition is 2.7% to less than 6 % of the sum comprising Tocilizumab protein with MS, G(0), G(1), and G(2) oligosaccharide attached thereto; and

wherein the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL- 6R antibody is in the range from 9 % to 11 %.

Patent Claims

1. A composition comprising an antibody protein that binds human interleukin-6 receptor, wherein the antibody protein is an anti-IL-6R antibody protein with mannose-5 glycostructure (M5) attached thereto, and wherein the fraction of the anti-IL-6R antibody protein with M5 attached thereto in the composition is 2.7% to 10% of the sum comprising the anti-IL-6R antibody protein with M5, G(0), G(1), and G(2) oligosaccharide attached thereto; and

   wherein the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL-6R antibody is in the range from 9 % to 11 %.

2. The composition according to claim 1, wherein the anti-IL-6R antibody protein is Tocilizumab.

3. The composition according to any one of claims 1 to 2, wherein the anti-IL-6R antibody protein with M5 attached thereto has been produced by a recombinant Chinese Hamster Ovary (CHO) cell.

4. The composition according to any one of claims 1 to 3, wherein the M5 fraction is 6% or less.

5. The composition according to any one of claims 1 to 4, wherein the M5 is attached to Asn297 of the anti-IL-6R antibody protein.

6. The composition according to any one of claims 1 to 5, further comprising anti-IL-6R antibody protein with G(0), G(1), or G(2) oligosaccharide attached thereto.

7. The composition according to any one of claims 1 to 6, wherein the M5 fraction is in the range from 2.8% to 6%.

8. The composition according to any one of claims 1 to 7, wherein the M5 fraction is 4% or less.

9. A composition comprising Tocilizumab protein with mannose-5 glycostructure (M5) attached to Asn297 thereof, wherein the fraction of Tocilizumab protein with M5 attached thereto in the composition is 2.7% to less than 6 % of the sum comprising Tocilizumab protein with MS, G(0), G(1), and G(2) oligosaccharide attached thereto; and

wherein the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL-6R antibody is in the range from 9 % to 11 %.

10. The composition according to claim 9, wherein the Tocilizumab protein with M5 attached thereto has been produced by a recombinant Chinese Hamster Ovary (CHO) cell.

11. The composition according to any one of claims 9 to 10, wherein the M5 fraction is 5% or less.

12. The composition according to any one of claims 9 to 10, wherein the M5 fraction is in the range from 2.8% to less than 5%.

13. The composition according to any one of claims 9 to 12, wherein the M5 fraction is 4% or less.

14. The composition according to any one of claims 9 to 13, further comprising the Tocilizumab protein with G(0), G(1), or G(2) oligosaccharide attached to Asn297 thereof.