

or Fc-region) affinity ligand during the enzymatic modification and the second step is performed in solution. In one embodiment the used enzyme(s) is (are) recovered after the modification of the antibody and can optionally be conditioned for re-use. The method as reported herein is, amongst other things, an improved method, especially a more economic method, for the modification of antibodies.

With the method as reported herein the enzymes used for the modification of the glycosylation of the antibody can be removed from the antibody preparation resulting in an improved preparation.

The method as reported herein is useful for the modification of any monoclonal antibody without the need of modifications to the preceding up-stream production process steps. The method as reported herein can be integrated into an existing process. Inherently no significant changes to existing antibody producing cell lines are required as the glycostructure modification is provided by the method as reported herein during down-stream processing.

It has been found that it is possible to recover the enzymes use for the modification of the glycosylation of antibodies in a form that allows re-use of the reconditioned enzymes for the same reaction without a detrimental loss of enzymatic activity. It was surprisingly found that the enzymes can be re-used at least once without significant loss of enzymatic activity and conversion efficiency.

It has further been found that an antibody light chain affinity ligand bound antibody can be effectively enzymatically modified as if the antibody would be in solution.

One aspect as reported herein is a method for the enzymatic production of an antibody with a modified glycosylation at an N-glycosylation site, comprising

- a) incubating the antibody affinity ligand-bound monoclonal antibody with a glycosylation at an N-glycosylation site with a first enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site,
- b) optionally recovering the first enzyme and producing thereby a recycled first enzyme,
- c) recovering the antibody from the antibody affinity ligand,

- d) incubating the recovered antibody in solution with a second enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site of the antibody,
- 5 e) separating the antibody with the modified glycosylation at the N-glycosylation site from the second enzyme in a cation exchange chromatography, wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and optionally a recycled second enzyme, and
- 10 f) optionally repeating step a) with the first recycled enzyme of step b) at least once and repeating step d) with the second recycled enzyme of step e) at least once.

15 As reported herein is a method for the enzymatic preparation/production of an antibody with a modified (substantially homogeneous) glycosylation at an N-glycosylation site comprising

- 20 a) incubating the antibody (light chain or Fc-region) affinity ligand-bound monoclonal antibody with a glycosylation at the N-glycosylation site with a first enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site to a defined (substantially homogeneous) form (homogeneous glycosylation),
- b) optionally recovering the first enzyme and producing thereby a recycled first enzyme,
- c) recovering the antibody from the antibody light chain affinity ligand,

As reported herein is an antibody produced with a method as reported herein.

As reported herein is the antibody as defined herein for use as a medicament.

As reported herein is a pharmaceutical formulation comprising an antibody with defined glycosylation produced by a method as reported herein.

5 As reported here is also a method for the recombinant production of an antibody or fragment thereof with defined glycosylation at an N-glycosylation site, comprising the steps of

10 a) recombinantly producing an antibody (of IgG1 isotype) or a fragment thereof in a (mammalian or CHO) cell, which comprises nucleic acids encoding the antibody, to obtain an antibody or fragment thereof with glycosylation at the N-glycosylation site,

b) isolating (recovering and optionally purifying) the antibody or fragment thereof with heterogeneous glycosylation at the N-glycosylation site,

15 c) enzymatically modifying the antibody or fragment thereof with glycosylation at the N-glycosylation site with galactosyltransferase and/or a sialyl transferase to obtain an antibody or fragment thereof with defined glycosylation at the N-glycosylation site, which comprises a relative amount of at least 70 % bi-galactosylated antibodies (G2F glycoform) (wherein 100 % corresponds to the amount of G0F, G1F and G2F glycoforms) at the N-glycosylation site, and subsequent separation of the of the modified antibody from the enzyme(s) with a method as reported herein,

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d) optionally purifying the modified antibody or fragment thereof by one or more chromatography steps,

25 and thereby producing an antibody with defined glycosylation at the N-glycosylation site.

In one embodiment of the disclosure as reported herein the first glycosylation modifying enzyme is a galactosyltransferase.

In one embodiment of the disclosure as reported herein the first glycosylation modifying enzyme is a galactosyltransferase and the second glycosylation modifying enzyme is a sialyltransferase.

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Patent Claims

1. A method for the enzymatic production of an antibody with a modified glycosylation at an N-glycosylation site, comprising
 - a) incubating the antibody affinity ligand-bound monoclonal antibody with a glycosylation at an N-glycosylation site with a first enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site,
 - b) optionally recovering the first enzyme and producing thereby a recycled first enzyme,
 - c) recovering the antibody from the antibody affinity ligand,
 - d) incubating the recovered antibody in solution with a second enzyme for a time sufficient and under conditions suitable to modify the glycosylation at the N-glycosylation site of the antibody,
 - e) separating the antibody with the modified glycosylation at the N-glycosylation site from the second enzyme in a cation exchange chromatography, wherein the cation exchange chromatography material has a matrix of cross-linked agarose with sulfopropyl cation exchange groups, and thereby producing the antibody with a modified glycosylation at the N-glycosylation site and optionally a recycled second enzyme, and
 - f) optionally repeating step a) with the first recycled enzyme of step b) at least once and repeating step d) with the second recycled enzyme of step e) at least once.
2. The method according to claim 1, wherein the antibody affinity ligand is an antibody light chain affinity ligand or an antibody Fc-region affinity ligand.
3. The method according to claim 1 or 2, wherein the incubating step a) comprises incubating the antibody affinity ligand-bound monoclonal antibody with the glycosylation at the N-glycosylation site with the first enzyme and a first activated sugar residue for a time sufficient and under

conditions suitable to modify the glycosylation at the N-glycosylation site;
and

wherein the incubating step d) comprises incubating the recovered antibody
in solution with the second enzyme and a second activated sugar residue for a
time sufficient and under conditions suitable to modify the glycosylation at
the N-glycosylation site of the antibody to a defined form.

4. The method according to any one of claims 1 to 3, wherein the first enzyme
is a galactosyltransferase and the second enzyme is a sialyltransferase.

5. The method according to claim 4, wherein the galactosyltransferase is
 β 4GalT1.

6. The method according to any one of claims 4 or 5, wherein the
sialyltransferase is ST6.

7. The method according to any one of claims 1 to 6, wherein the cation
exchange chromatography comprises the following steps

i) applying a solution comprising a galactosyltransferase and/or a
sialyltransferase and the antibody with the modified glycosylation at
the N-glycosylation site to a cation exchange chromatography material,

ii) optionally washing the cation exchange chromatography material,

iii) optionally if a galactosyltransferase is present applying a first solution
to the cation exchange chromatography material and thereby eluting the
galactosyltransferase from the cation exchange chromatography
material,

iv) applying a second solution to the cation exchange chromatography
material and thereby eluting the antibody with the modified
glycosylation at the N-glycosylation site from the cation exchange
chromatography material, and

v) applying a linear gradient to the cation exchange chromatography
material and thereby eluting the sialyltransferase from the cation
exchange chromatography material.

8. The method according to claim 7, wherein the optional washing step ii) is to remove unbound compounds from the cation exchange chromatography material but without eluting the antibody with the modified glycosylation at the N-glycosylation site; and
- 5 wherein the linear gradient in the applying step v) is a linear salt gradient.
9. The method according to claim 7 or 8, wherein
- the solution of step i) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5,
 - 10 - the solution of step ii) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5,
 - the solution of step iii) is a tris(hydroxymethyl)aminomethane buffered solution with a pH value from pH 6.6 to pH 8.0,
 - the solution of step iv) is a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising
15 about 75 mM to about 125 mM sodium chloride,
 - the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid buffered solution with a pH value from pH 5.0 to pH 6.5 comprising 750 mM to 1250 mM sodium chloride.
10. The method according to any one of claims 1 to 9, wherein the method
20 comprises the following steps instead of steps a) to c):
- applying a solution comprising the antibody with a glycosylation at the N-glycosylation site to an antibody affinity ligand bound to a solid phase whereby the antibody is bound to the ligand,
 - optionally washing the solid phase with a buffered solution,
 - 25 - enzymatically modifying the glycosylation at the N-glycosylation site of the antibody by applying a solution comprising a first glycosylation modifying enzyme for a time sufficient and under conditions suitable for the enzymatic modification to the ligand-bound antibody, optionally washing the modified ligand-bound antibody without disrupting the
30 antibody-ligand interaction,

- optionally recovering the first enzyme and producing thereby a recycled first enzyme, and
- recovering the antibody from the antibody affinity ligand.

5 11. The method according to claim 10, wherein the method comprises the following steps:

- 10 - applying a buffered solution comprising the antibody with a glycosylation at the N-glycosylation site to an antibody light chain affinity ligand or antibody Fc-region affinity ligand bound to a solid phase antibody light chain affinity ligand chromatography material or antibody Fc-region affinity ligand chromatography material whereby the antibody is bound to the ligand resulting in a ligand-bound antibody,
- optionally washing the solid phase with a buffered solution without disrupting the antibody-ligand interaction,
- 15 - enzymatically modifying the glycosylation at the N-glycosylation site of the antibody by applying a buffered solution comprising a first glycosylation modifying enzyme and a first activated sugar residue for a time sufficient and under conditions suitable for the enzymatic modification to the ligand-bound antibody, optionally washing the
- 20 modified ligand-bound antibody without disrupting the antibody-ligand interaction,
- optionally recovering the first enzyme and producing thereby a recycled first enzyme, and
- 25 - recovering the antibody from the antibody light chain affinity ligand or antibody Fc-region affinity ligand.

12. The method according to any one of claims 1 to 11, wherein the antibody is a bivalent monospecific antibody or a bivalent bispecific antibody or an antibody Fab fragment.

30 13. The method according to any one of claims 1 to 12, wherein the antibody is a chimeric or humanized or human antibody.

14. The method according to any one of claims 1 to 13, wherein the antibody is a monoclonal antibody.
15. The method according to any one of claims 1 to 14, wherein the antibody is an antibody of the human IgG1 or IgG4 subclass.
- 5 16. The method according to any one of claims 1 to 15, wherein the N-glycosylation site is a Fab region N-glycosylation site or the Fc-region N-glycosylation site at asparagine residue 297, where the numbering is according to Kabat).