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 (light chain or Fc-region) affinity ligand-bound monoclonal antibody with a glycosylation at an N-glycosylation site with a 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,

b) optionally recovering the first enzyme and producing thereby a recycled first enzyme,

c) recovering the antibody from the antibody (light chain or Fc-region) affinity ligand,

d) incubating the recovered antibody in solution with a 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),

e) separating the antibody with the modified glycosylation at the N-glycosylation site from the second enzyme in a cation exchange chromatography, 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.

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

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,

It has been found that a reduction of the pH value from 7.4 to below pH 6.0, e.g. pH 5.6, results in the elution of aggregated galactosyltransferase.

In one embodiment the linear gradient is from the solution of step iv) to a 2-(N-morpholino)ethanesulfonic acid (MES) buffered solution with a pH value from pH 5.0 to pH 6.5 comprising about 750 mM to about 1250 mM sodium chloride (NaCl). In one embodiment the linear gradient is from the solution of step iv) to a solution comprising about 50 mM MES, about 1000 mM NaCl with a pH value of about pH 6.4.

In one embodiment the repeating is for 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times. In one embodiment the repeating is for 1 to 5 times. In one embodiment the repeating is for 1 to 3 times.

In one embodiment of the disclosure the method comprises prior to the first incubation step a) the step of

- binding the monoclonal antibody with glycosylation at the N-glycosylation site to an antibody (light chain or Fc-region) affinity ligand.

In one embodiment of the disclosure the method comprises the following steps in this order instead of steps a) to c)

- applying a (buffered) solution comprising the antibody with glycosylation at the N-glycosylation site to an antibody (light chain or Fc-region) affinity ligand bound to a solid phase (antibody light chain 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,
- 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 modified ligand-bound antibody,

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

The antibodies as used in the methods as reported herein can be any antibody or antibody fragment, including Fab fragments, single chain antibodies, multispecific antibodies and antibody fusions.

Thus, in one embodiment of the disclosure as reported herein the antibody is selected from the group of antibodies consisting of a full length antibody, a bivalent monospecific antibody, a bispecific antibody, a bivalent bispecific antibody, a trivalent bispecific antibody, a tetravalent bispecific antibody, a trivalent trispecific antibody, and a tetravalent tetraspecific antibody.

In one embodiment the antibody is a bivalent monospecific antibody.

In one embodiment the antibody is a bivalent or trivalent or tetravalent bispecific antibody.

In one embodiment the antibody is a chimeric or humanized or human antibody.

In one embodiment the antibody is a polyclonal antibody preparation.

In one embodiment the antibody is a monoclonal antibody.

In one embodiment of the disclosure as reported herein the antibody (preparation) is an antibody (preparation) of the human IgG class. In one embodiment the antibody is an antibody of the human IgG1 or IgG4 subclass.

In one embodiment of the disclosure as reported herein the defined glycosylation is a glycosylation selected from the group consisting of G2 glycoform, G0 glycoform, M3 glycoform, S2 glycoform, A2B glycoform, A2BG2 glycoform and S1 glycoform.

In one embodiment of the disclosure as reported herein the defined glycosylation is a glycosylation selected from the group consisting of galactose as the terminal sugar, GlcNAc as the terminal sugar, mannose as the terminal sugar and sialic acid as the terminal sugar.

In one embodiment the antibody is a recombinantly produced antibody.

One aspect as reported herein is an antibody produced with a method as reported herein.

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

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

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

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,

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,

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

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.

In one embodiment the galactosyltransferase is β4GalT1.

In one embodiment the sialyltransferase is ST6.

In one embodiment the sialyltransferase is ST6Gal1 or ST6Gal2.

In one embodiment the (first) buffered solution comprises UDP-Gal.

In one embodiment the (second) buffered solution comprises CMP-NANA.

In one embodiment the incubation is at room temperature (20 - 25 °C, preferably about 22 °C).

In one embodiment the incubation is at 25 °C.

In one embodiment the incubation is at 37 °C.

In one embodiment the incubation is for 7 to 48 hours.

In one embodiment of the disclosure as reported herein the solution comprises a chromatographically purified antibody, the (first) glycosylation modifying enzyme is GalT1, and the incubation with the (first) glycosylation modifying enzyme is for 24 hours at 20-27 °C or 37 °C. In one embodiment the incubation is at room temperature (about 22 °C).

In one embodiment of the disclosure as reported herein the (second) glycosylation modifying enzyme is ST6, and the incubation with the (second) glycosylation modifying enzyme is for 24 hours at 20-27°C or 37 °C. In one embodiment the incubation is at room temperature (about 22 °C).

As reported herein is a method for producing an antibody or fragment thereof comprising the following steps in the following order:

- providing a cell comprising a nucleic acid encoding the antibody or a fragment thereof (optionally comprising at least an antibody light chain),
- cultivating the cell under conditions suitable for the expression of the antibody or fragment thereof with glycosylation at an N-glycosylation site,
- recovering the antibody or fragment thereof from the cell or the cultivation medium,

- optionally applying a solution comprising antibody or fragment thereof to an antibody (light chain of Fc-region) affinity chromatography column under conditions suitable for binding of the antibody or fragment thereof to the affinity chromatography material,
- modifying the glycosylation of the antibody or fragment thereof at the N-glycosylation site with a method as reported herein, and
- recovering the modified antibody or fragment thereof with a defined glycosylation at the N-glycosylation site,

and thereby producing an antibody.

In one embodiment comprises the method the following step as final step:

- purifying the modified antibody or fragment thereof with one to three chromatography steps.

In one embodiment of the disclosure the antibody affinity chromatography column is an antibody light chain affinity chromatography column.

In one embodiment of the disclosure the antibody affinity chromatography column is an antibody Fc-region affinity chromatography column.

In one embodiment the antibody affinity chromatography column is a protein A affinity chromatography column.

In one embodiment of the disclosure the N-glycosylation site is in the Fab or in the Fc-region.

Detailed Description of the Invention

DEFINITIONS

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is referred to as "numbering according to Kabat" herein. Specifically, the Kabat numbering system (see pages 647-660) of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of