Summary ~~of the Invention~~

It has been found that with the sequence of enzymatic steps as reported herein an immunoglobulin with a G1 glycostructure can be obtained in substantially pure form, i.e. with a purity of 90 % or more, with a purity of 95 % or more, or with a purity of 98 % or more.

Therefore, herein is reported a method for producing an immunoglobulin or immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment with a G1 glycostructure in substantially pure form starting from a glycosylated immunoglobulin or a glycosylated immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment which is a mixture of G0, G1 and G2 glycostructures.

Also, herein is reported a method for producing an immunoglobulin or immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment with a G1 glycostructure in substantially pure form starting from a glycosylated immunoglobulin or a glycosylated immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment which has a G0 glycostructure.

Also, herein is reported a method for producing an immunoglobulin or immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment with a G1 glycostructure in substantially pure form starting from a glycosylated immunoglobulin or a glycosylated immunoglobulin fragment or a fusion polypeptide comprising a glycosylated immunoglobulin fragment which has a G2 glycostructure.

Thus, herein is reported a method for producing an immunoglobulin with a G1 glycostructure in substantially pure form or an immunoglobulin fragment with a G1 glycostructure in substantially pure form or a fusion polypeptide comprising a glycosylated immunoglobulin fragment with a G1 glycostructure in substantially pure form comprising the following steps in the following order:

- providing an affinity chromatography column eluate containing an immunoglobulin or an immunoglobulin fragment or a fusion polypeptide, which is a mixture of a G0, G1 and G2 glycostructure,
- incubating the immunoglobulin or immunoglobulin fragment with a galactosyltransferase,

glycosylated immunoglobulin fragment with a G1 glycostructure in substantially pure form comprising the following steps in the following order:

- providing an affinity chromatography column eluate containing an immunoglobulin, which has a G2 glycostructure, or an immunoglobulin fragment, which has a G2 glycostructure, or a fusion polypeptide, which has a G2 glycostructure,
- incubating the immunoglobulin or immunoglobulin fragment or a fusion polypeptide with a sialyltransferase,
- incubating the immunoglobulin or immunoglobulin fragment or a fusion polypeptide with a beta-1,4-galactosidase or lactase,
- removing or inactivating the beta-1,4-galactoasidase or lactase, and
- incubating the immunoglobulin or immunoglobulin fragment or a fusion polypeptide with a sialidase and thereby producing an immunoglobulin or immunoglobulin fragment or fusion polypeptide with a G1 glycostructure in substantially pure form.

In an aspect, there is provided a method for producing an immunoglobulin or immunoglobulin fragment or fusion polypeptide with G1 glycostructure comprising the following steps:

- incubating an affinity chromatography column eluate containing the immunoglobulin or immunoglobulin fragment or fusion polypeptide with a galactosyltransferase,

- incubating the galactosyltransferase reaction product with a sialyltransferase,

- incubating the sialyltransferase reaction product with a beta-1,4-galactosidase,

- removing or inactivating the beta-1,4-galactosidase, and

- incubating the beta-1,4-galactosidase reaction product, in which the beta-1,4-galactosidase has been removed or inactivated, with a sialidase and thereby producing an immunoglobulin or immunoglobulin fragment or fusion polypeptide with G1 glycostructure.

In one embodiment the galactosyltransferase is added in one to ten aliquots. In one embodiment the galactosyltransferase is added in two to seven aliquots. In one embodiment the galactosyltransferase is added in three to six aliquots. In one embodiment the galactosyltransferase is added in four or six aliquots.

In one embodiment the incubating with the galactosyltransferase is for about 30 hours to about 60 hours.

In one embodiment the first galactosyltransferase aliquot is added after zero to two hours of incubating. In one embodiment the first galactosyltransferase aliquot is added at the beginning of the incubating.

In one embodiment a galactosyltransferase aliquot is added five to twenty hours after the previous aliquot has been added. In one embodiment a galactosyltransferase aliquot is added six to twelve hours after the previous aliquot has been added. In one embodiment a galactosyltransferase aliquot is added eight to ten hours after the previous aliquot has been added.

In one embodiment the immunoglobulin or immunoglobulin fragment or fusion polypeptide is not purified prior to the incubation with sialyltransferase. In one embodiment the incubation with sialyltransferase is of the crude reaction mixture of the incubation with galactosyltransferase.