Summary ~~of the Invention~~

It has been found that an (α1,3)galactosidases from plant origin, e.g. from green coffee beans (EC 3.2.1.22), can be used to selectively remove (α1,3)glycosidically bound terminal galactose residues from the oligosaccharide at amino acid Asn297 in an immunoglobulin CH2 domain. (α1,3)galactosidases from non-plant origin were found to have (β1,4)galactosidases side reactivity and/or were less or not-reactive with tri- or tetra-antennary oligosaccharides.

Thus, herein is reported a method for producing an immunoglobulin or immunoglobulin fragment with defined glycostructure comprising the following steps in the following order:

- providing an affinity chromatography column eluate containing the immunoglobulin or immunoglobulin fragment,
- incubating the affinity chromatography column eluate with an enzyme, which cleaves off the terminal monosaccharide residues of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment,
- applying the incubated affinity chromatography eluate to a protein A chromatography material under conditions suitable for binding of the immunoglobulin or immunoglobulin fragment to the protein A chromatography material and recovering the immunoglobulin or immunoglobulin fragment from the protein A chromatography material and thereby producing an immunoglobulin or immunoglobulin fragment with defined glycostructure.

One aspect as reported herein is a method for producing an immunoglobulin or polypeptide comprising at least the CH2 domain and the CH3 domain of a full length immunoglobulin heavy chain with defined glycostructure comprising the following steps:

- providing an affinity chromatography column eluate containing the immunoglobulin or immunoglobulin fragment,
- incubating the affinity chromatography column eluate with an α-galactosidase of plant origin, which cleaves off the (α1,3)glycosidically bound galactose residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, wherein said α-galactosidase is (α1,3)galactosidase (EC 3.2.1.22) from green coffee beans,
- applying the incubated affinity chromatography eluate to a protein A chromatography material and recovering the immunoglobulin or immunoglobulin fragment from the protein A chromatography material and thereby producing an immunoglobulin or immunoglobulin fragment with defined glycostructure.

In one embodiment the enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, is of plant origin. In one embodiment the enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, is selected from α-D-galactoside galactohydrolase (EC 3.2.1.22) or Melibiase. In another embodiment the enzymes is (α1,3)galactosidase from green coffee beans (EC 3.2.1.22). In another embodiment the terminal monosaccharide residue of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment is an (α1,3)glycosidically bound galactose residue.

- recovering the immunoglobulin or immunoglobulin fragment from the cell or the cultivation medium,
- applying the immunoglobulin or immunoglobulin fragment to a protein A chromatography material under conditions suitable for binding of the immunoglobulin to the protein A chromatography material and recovering the immunoglobulin from the protein A chromatography material.

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

- purifying the produced immunoglobulin or immunoglobulin fragment with a defined glycostructure with one to three chromatography steps.

In one embodiment the enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, is of plant origin. In another embodiment the enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, is selected from α-galactosidase, β-galactosidase, mannosidase, fucosidase, or sialidase. In a further embodiment the cell is a mammalian cell. In another embodiment the mammalian cell is a hamster cell, or murine cell, or a rabbit cell, or a sheep cell, or a hybridoma cell thereof. In still a further embodiment the cell is a CHO cell, a NS0 cell, a BHK cell or a SP2/0 cell.

An embodiment~~A further aspect~~ as reported herein is the use of an (α1,3)galactosidase from green coffee beans (EC 3.2.1.22) for cleaving off the terminal (α1,3)glycosidically bound galactose residues from the glycostructures in the CH2 domain of a recombinantly produced immunoglobulin or immunoglobulin fragment.

A further aspect as reported herein is the use of an (α1,3)galactosidase from green coffee beans (EC 3.2.1.22) for cleaving off the terminal (α1,3)glycosidically bound galactose residues from the glycostructures attached to the amino acid asparagine of the CH2 domain of a recombinantly produced immunoglobulin or immunoglobulin fragment.

Detailed Description of the Invention

Herein is reported that e.g. (α1,3)galactosidases from green coffee beans (EC 3.2.1.22) can be used to selectively remove (α1,3)glycosidically bound terminal galactose residues from the oligosaccharide attached at amino acid residue Asn297 to an immunoglobulin CH2 domain. (α1,3)galactosidases from other sources beside plants were found to have (β1,4)galactosidases side reactivity and/or were less or not-reactive with tri- or tetra-antennary oligosaccharides (see Table 1).