subsequent addition of a coordinating ion or molecule does precipitation take place. In a second similar application the affinity ligand is linked to a biotin-binding protein which forms a network with the mediator avidin (9).

US patent 7,083,943 describes an affinity precipitation in which a binding domain for the target protein is linked to a scaffold domain the amino acid sequence of which is intended to assist the tendency to precipitation.

Chen and Hoffman (15) describe the affinity precipitation of IgG with poly(N-isopropylacrylamide)-protein A conjugates. The precipitation of antibodies with unmodified protein A, however, has not proved effective.

One disadvantage of the methods described in the prior art is that during first order affinity precipitation, precipitation is possible only via the cross-linking molecule, or in second order affinity precipitation a stimulatable substance is required. Other disadvantages of the affinity precipitations that are best known are on the one hand a) steric hindrance in which the binding of the target protein is limited by the binding of high-molecular affinity macroligands, b) the resolubilisation of the precipitated polymer complex is slow, c) the non-specific co-precipitation of impurities, that generally require a second precipitation step, d) the additional step of binding an affinity protein to the polymer or to the cross-linker (2-5).

BRIEF SUMMARY ~~OF THE INVENTION~~

The present disclosure~~invention~~ relates to affinity precipitation using a binding protein with two binding sites, which dispenses completely with an additional fusion protein or a linker molecule. The affinity protein on its own is sufficient for the precipitation, and there is no need for a stationary phase. The disclosure~~invention~~ used here also makes it easier to recover the affinity protein.

The disclosure~~invention~~ relates in particular to a process for the selective concentration of immunoglobulins or other proteins that contain an Fc domain (target protein), comprising the following steps:

a. preparing a solution that contains the target protein;

b. incorporating an Fc-binding protein with precisely two binding sites under conditions that allow binding to occur;
c. separating the precipitate from the liquid phase;
d. undoing the binding of the target protein from the Fc-binding protein.

The disclosure may~~In another aspect the invention~~ relates to a process in which the Fc-binding protein is a dimer of an Fc-binding domain of protein A or protein G. Preferably, the two monomers of the dimer are linked to one another via a disulphide bridge.

The disclosure may~~In another aspect the invention~~ relates to a process in which the dimer is a homodimer, the monomers of which have the SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids.

In one aspect, there is provided a process for the selective concentration of immunoglobulins or other proteins that contain an Fc domain (target protein), comprising the following steps: a. preparing a solution that contains the target protein; b. incorporating an Fc-binding protein with precisely two binding sites, wherein the Fc-binding protein is a homodimer, the monomers of which have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 20, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, under conditions that allow binding to occur; c. separating the precipitate from the liquid phase; d. undoing the binding of the target protein from the Fc-binding protein.

In another embodiment~~aspect~~ the Fc-binding protein is used in a ratio of 0.5 – 20 in relation to the target protein. The solution in step a. preferably has a pH of 5.5 - 8. Preferably, the undoing of the binding in step d. takes place at a pH of 2 - 4.5.

The disclosure also~~In another aspect the invention~~ relates to an Fc-binding protein consisting of 2 identical sub-units that have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, the two sub-units being linked to each other via a covalent bond. Preferably, the covalent bond is a disulphide bond.
Hence, in another aspect, there is provided a Fc-binding protein that consists of 2 identical sub-units that have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 20, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, wherein the two-units are linked together via a disulphide bond.

PATENT CLAIMS

1. Process for the selective concentration of immunoglobulins or other proteins that contain an Fc domain (target protein), comprising the following steps:
   a. preparing a solution that contains the target protein;
   b. incorporating an Fc-binding protein with precisely two binding sites, wherein the Fc-binding protein is a homodimer, the monomers of which have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, under conditions that allow binding to occur;
   c. separating the precipitate from the liquid phase;
   d. undoing the binding of the target protein from the Fc-binding protein.

~~2. Process according to claim 1, characterised in that the Fc-binding protein is a dimer of an Fc-binding domain of protein A or protein G.~~

~~3. Process according to claim 2, characterised in that the Fc-binding domain comprises or contains one of the sequences SEQ ID NO: 1 to SEQ ID NO: 11.~~

42. Process according to claim ~~2 or 3~~ 1, characterised in that the two monomers of the dimer are linked together by a disulphide bridge

~~5. Process according to one of the preceding claims, characterised in that the dimer is a homodimer, the monomers of which have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids.~~

63. Process according to one of the preceding claims, characterised in that the Fc-binding protein is used in a molar ratio of 0.5-20 in relation to the target protein.

74. Process according to one of the preceding claims, characterised in that the solution in step a. has a pH of 5.5-9, preferably 6-8.

85. Process according to one of the preceding claims, characterised in that the undoing of the binding in step d. takes place at a pH of 2-4.5.

~~9~~6. Fc-binding protein that consists of 2 identical sub-units that have the sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO. 7 or a sequence that differs from SEQ ID NO. 1 in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids, wherein the two sub-units are linked together via a disulphide ~~covalent~~ bond.

~~10. Fc-binding protein according to claim 9, characterised in that the covalent bond is a disulphide bond.~~