proteins in mammalian cells is reported by Lo, K-M., et al. (Prot. Eng. 11 (1998) 495-500). In WO 00/40615 the expression and export of anti-obesity proteins as Fc fusion proteins is reported.

Summary of the Invention

It has been found that polypeptides, which are biologically active as n-mer and form non-defined aggregates/multimers upon expression, can be obtained in soluble form by expressing these polypeptides as fusion polypeptide with an Fc-region that does not substantially bind to an Fc-receptor. Using a fusion polypeptide for the expression of the polypeptide increases the obtainable yield of the polypeptide either in form of the fusion polypeptide or as isolated polypeptide. It has further been found that the polypeptide remains in a defined n-mer form after cleavage and removal of the Fc-region.

~~One aspect as~~There is reported herein ~~is~~ a method for the production of a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region comprising the following steps

a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to formula I

$$(B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m)_u \qquad \text{(formula I)}$$

wherein

B denotes a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC denotes a heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein u=1 or 2,

wherein FC does not substantially bind to an Fc-receptor,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

c) optionally cleaving the fusion polypeptide with a protease,

and thereby producing a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region.

One aspect as reported herein is a method for the production of a polypeptide that is biologically active as 2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region and comprises the following steps

a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to formula II

$$B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC^1\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m : FC^2 \quad \text{(formula II)}$$

wherein

B denotes a polypeptide that is biologically active as 2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC^1 denotes a first heavy chain Fc-region polypeptide,

FC^2 denotes a second heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein FC^1 and FC^2 are covalently linked by one or more disulfide bond(s) (:),

wherein FC^1 and FC^2 do not substantially bind to an Fc-receptor,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

c) cleaving the fusion polypeptide with a protease,

and thereby producing a polypeptide that is biologically active as 2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region.

There is ~~One aspect as~~ reported herein ~~is~~ a fusion polypeptide according to formula I

$$(B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m)_u \qquad \text{(formula I)}$$

wherein B denotes a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC denotes a heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein u=1 or 2,

wherein FC does not substantially bind to an Fc-receptor.

One aspect as reported herein is a fusion polypeptide according to formula II

Bn-CSo-Is-CSp-FC1-CSq-It-CSr-Bm : FC2 (formula II)

wherein

B denotes a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC1 denotes a first heavy chain Fc-region polypeptide,

FC2 denotes a second heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein the first FC and the second FC are covalently linked by one or more disulfide bond(s),

wherein FC1 and FC2 do not substantially bind to an Fc-receptor.

There is ~~One aspect as~~ reported herein ~~is~~ an n-mer polypeptide obtained by cleavage of a fusion polypeptide as reported herein obtained by a method according to the method as reported herein. One aspect as reported herein is a 2-mer or 3-mer polypeptide obtained by a method as reported herein.

wherein FC^1 and FC^2 are covalently linked by one or more disulfide bond(s) (:),

wherein FC^1 and FC^2 do not substantially bind to an Fc-receptor,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

c) optionally cleaving the fusion polypeptide with a protease,

and thereby producing a polypeptide that is biologically active as 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region.

In one embodiment the intervening amino acid sequence is selected from a first group comprising $(G_3S)_3$, $(G_3S)_4$, $(G_3S)_5$, $(G_3S)_6$, $(G_4S)_3$, $(G_4S)_4$, $(G_4S)_5$, $(G_5S)_2$, $(G_5S)_3$, $(G_5S)_4$, and any combination thereof or from a second group comprising Arg-tag, Avi-tag, His-Avi-tag, His-tag, Flag-tag, 3xFlag-tag, Strep-tag, Nano-tag, SBP-tag, c-myc-tag, S-tag, calmodulin-binding-peptide, cellulose-binding-domain, chitin-binding-domain, GST-tag, or MBP-tag, or from combinations of two elements of these group.

In one embodiment the cleavage site is selected from IgA-protease protease cleavage site, Granzyme B protease cleavage site, Tev protease cleavage site, PreScission protease cleavage site, Thrombin cleavage site, Factor10a protease site, Ides protease cleavage site, Enterokinase cleavage site, or a SUMO protease (Ulp1 (Ubl-specific protease 1) from Saccharomyces cerevisiae) cleavage site.

In one embodiment the fusion polypeptide does not comprise an additional protease cleavage site but an inherent protease cleavage site, such as e.g. a papain cleavage site, a pepsin cleavage site, or an Ides protease cleavage site.

In one embodiment the C-terminal lysine residue in the FC-region(s) has been deleted. This reduces the amount of protease cleavage side-products (removal of artificial protease cleavage site).

~~One aspect as~~ There is reported herein ~~is~~ the use of an immobilized fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein as affinity chromatography ligand. One aspect as reported herein is the use of an immobilized fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as reported herein as affinity chromatography ligand.

There is ~~One aspect as~~ reported herein ~~is~~ the use of an immobilized fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein in an immunoassay. One aspect as reported herein is the use of an immobilized fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as reported herein n an immunoassay.

In one embodiment the fusion polypeptide or the n-mer polypeptide is bound to a solid phase.

There is ~~One aspect as~~ reported herein ~~is~~ a pharmaceutical composition comprising a fusion polypeptide as reported herein or an n-mer polypeptide as reported herein. One aspect as reported herein is a pharmaceutical composition comprising a fusion polypeptide as reported herein or a 2-mer or 3-mer polypeptide as reported herein.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein in the manufacture of a medicament. One aspect as reported herein is the use of a fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as reported herein in the manufacture of a medicament.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein as immunogen. One aspect as reported herein is a fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as reported herein for use as an immunogen.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein for obtaining an animal model of a disease by applying the fusion polypeptide as reported herein or the n-mer polypeptide as reported herein to an experimental animal. One aspect as reported herein is the use of a fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as reported herein for obtaining an animal model of a disease by applying the fusion polypeptide as reported herein or the 2-mer or 3-mer polypeptide as reported herein to an experimental animal.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein for selecting an antibody that specifically binds to B or an n-mer of B. One aspect as reported herein is the use of a fusion polypeptide as reported herein or of a 2-mer or 3-mer polypeptide as

reported herein for selecting an antibody that specifically binds to B or a 2-mer or 3-mer of B.

There is ~~One aspect as~~ reported herein ~~is~~ the use of a fusion polypeptide as reported herein or of an n-mer polypeptide as reported herein for producing a disulfide-linked 2-mer of B. One aspect as reported herein is the use of a fusion polypeptide as reported herein or of a 2-mer polypeptide as reported herein for producing a disulfide-linked 2-mer of B.

Detailed Description of the Invention

Description of the Figures

Figure 1 Plasmid map of Fc-receptor fusion polypeptide expression plasmid.

Figure 2 Analytical SDS-PAGE gel of papain cleavage of Fc-receptor fusion polypeptide.

Figure 3 12% Bis Tris Gel + / -DTT; Cleavage of FcgammaRIIIaV158-Avi-Fc LALA P239G with PreScission protease (lane 4, 8) respectively IgA protease (lane 3, 7): unspecific cleavage with PP can be seen (lane 2, 6).

Patent Claims

1. A method for the production of a polypeptide that is biologically active as ~~n-mer comprising the following steps~~

~~a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to formula I~~

~~$(B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m)_u$~~
~~(formula I)~~

~~wherein~~
~~B denotes a polypeptide that is biologically active as n-mer,~~
~~FC denotes a heavy chain Fc-region polypeptide,~~
~~CS denotes a cleavage site, and~~
~~I denotes an intervening amino acid sequence,~~
~~wherein n=1 and m=0, or n=0 and m=1,~~
~~wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,~~
~~wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,~~
~~wherein u=1 or 2,~~
~~wherein FC does not substantially bind to an Fc-receptor,~~

~~b) recovering the fusion polypeptide from the cell or the cultivation medium,~~

~~c) optionally cleaving the fusion polypeptide with a protease,~~

~~and thereby producing a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region~~2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region and comprises the following steps

a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to formula II

$$B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC^1\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m : FC^2 \quad \text{(formula II)}$$

wherein

B denotes a polypeptide that is biologically active as 2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC^1 denotes a first heavy chain Fc-region polypeptide,

FC^2 denotes a second heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein FC^1 and FC^2 are covalently linked by one or more disulfide bond(s) (:),

wherein FC^1 and FC^2 do not substantially bind to an Fc-receptor,

b) recovering the fusion polypeptide from the cell or the cultivation medium,

c) cleaving the fusion polypeptide with a protease,

and thereby producing a polypeptide that is biologically active as 2-mer or 3-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region.

~~2. A fusion polypeptide according to formula I~~

~~$(B_n\text{-}CS_o\text{-}I_s\text{-}CS_p\text{-}FC\text{-}CS_q\text{-}I_t\text{-}CS_r\text{-}B_m)_u$ (formula I)~~

~~wherein B denotes a polypeptide that is biologically active as n-mer,~~
~~FC denotes a heavy chain Fc-region polypeptide,~~
~~CS denotes a cleavage site, and~~
~~I denotes an intervening amino acid sequence,~~
~~wherein n=1 and m=0, or n=0 and m=1,~~

~~wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,~~

~~wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,~~

~~wherein u=1 or 2,~~

~~wherein FC does not substantially bind to an Fc-receptor.~~

2. A fusion polypeptide according to formula II

Bn-CSo-Is-CSp-FC1-CSq-It-CSr-Bm : FC2 (formula II)

wherein

B denotes a polypeptide that is biologically active as n-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc-region,

FC1 denotes a first heavy chain Fc-region polypeptide,

FC2 denotes a second heavy chain Fc-region polypeptide,

CS denotes a cleavage site, and

I denotes an intervening amino acid sequence,

wherein n=1 and m=0, or n=0 and m=1,

wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,

wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,

wherein the first FC and the second FC are covalently linked by one or more disulfide bond(s),

wherein FC1 and FC2 do not substantially bind to an Fc-receptor.

3. A~~n~~ ~~n~~2-mer or 3-mer polypeptide obtained by ~~cleavage of a fusion polypeptide obtained by~~ a method according to claim 1.

4. The method according to claim 1 or the fusion polypeptide according to claim 2 or the ~~n~~2-mer or 3-mer polypeptide according to claim 3, characterized in that B is selected from the group of IL17, TWEAK, TNF and other TNF family members, TL1a, IL18, IL18R, IL33, and IL33R.

5. The method according to claim 1 or 4 or the fusion polypeptide according to claim 2 or 4, characterized in that FC is a variant of a heavy chain polypeptide selected from the group of human IgG1 heavy chain polypeptide, human IgG2 heavy chain polypeptide, human IgG3 heavy chain polypeptide, human IgG4 heavy chain polypeptide, murine IgG1 heavy chain polypeptide, murine IgG2 heavy chain polypeptide, murine IgG3 heavy chain polypeptide, rabbit IgG heavy chain polypeptide.

6. The method according to any one of claim 1, 4 or 5 or the fusion polypeptide according to any one of claim 2, 4 or 5, characterized in that FC is selected from human IgG1 heavy chain polypeptide with the mutations L234A, L235A and P329G, human IgG4 heavy chain polypeptide with the mutation S228P, L235E and P329G.

7. The method according to any one of claim 1, 4, 5 or 6 or the fusion polypeptide according to any one of claim 2, 4, 5 or 6, characterized in that ~~a 2 and~~ the first FC comprises the mutation T366W ~~and optionally the mutation S354C~~ and the second FC comprises the mutations T366S, L368A and Y407V~~and optionally the mutation Y349C~~.

8. The method according to claim 7 or the fusion polypeptide according to claim 7, characterized in that the first FC comprises the mutation T366W and the mutation S354C.

9. The method according to claim 7 or 8 or the fusion polypeptide according to claim 7 or 8, characterized in that the second FC comprises the mutations T366S, L368A and Y407V and the mutation Y349C.

~~8~~10. The method according to any one of claims 7 to 9 or the fusion polypeptide according to any one of claims 7 to 9, characterized in that

a) n=1 and m=0 and B fused to the first FC and B fused to the second FC are identical,

b) n=1 and m=0 and B fused to the first FC and B fused to the second FC are different,

c) n=0 and m=1 and B fused to the first FC and B fused to the second FC are identical,

d) n=0 and m=1 and B fused to the first FC and B fused to the second FC are different.

~~9~~11. Use of an immobilized fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or of ~~an~~ ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 as affinity chromatography ligand.

~~10~~12. Use of an immobilized fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or of ~~an~~ ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 in an immunoassay.

~~11~~13. The use according to any one of claims ~~9~~11 to ~~10~~12, characterized in that the fusion polypeptide or the n-mer polypeptide is bound to a solid phase.

~~12~~14. Pharmaceutical composition comprising a fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or ~~an~~ ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4.

~~13~~15. Use of a fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or of ~~an~~ ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 in the manufacture of a medicament.

~~14~~16. ~~Use of a~~A fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or of ~~an~~ ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 for use as an immunogen.

~~15~~17. Use of a fusion polypeptide according to any one of claims 2 and 4 to ~~8~~10 or of a ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 for obtaining an animal model of a disease by applying the fusion polypeptide according to

any one of claims 2 and 4 to ~~8~~ 10 or the ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 to an experimental animal.

~~16~~18. Use of a fusion polypeptide according to any one of claims 2 and 4 to ~~8~~ 10 or of a ~~n~~2-mer or 3-mer polypeptide according to any one of claims 3 to 4 for selecting an antibody that specifically binds to B or ~~an~~ ~~n~~2-mer or 3-mer of B.

~~17~~19. Use of a fusion polypeptide according to any one of claims 2 and 4 to ~~8~~ 10 or of a ~~n~~2-mer polypeptide according to any one of claims 3 to 4 for producing a disulfide-linked 2-mer of B.

~~18. The method according to any one of claims 1 and 4 to 8 for the production of a polypeptide that is biologically active as 2-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc region comprising the following steps~~

~~a) cultivating a cell comprising a nucleic acid encoding a fusion polypeptide according to formula II~~

~~B_n-CS_o-I_s-CS_p-FC^1-CS_q-I_t-CS_r-B_m : FC^2 (formula II)~~

~~wherein~~

~~B denotes a polypeptide that is biologically active as 2-mer and forms non-defined aggregates/multimers upon expression in the absence of a fused Fc region,~~

~~FC^1 denotes a first heavy chain Fc region polypeptide,~~

~~FC^2 denotes a second heavy chain Fc region polypeptide,~~

~~CS denotes a cleavage site, and~~

~~I denotes an intervening amino acid sequence,~~

~~wherein n=1 and m=0, or n=0 and m=1,~~

~~wherein if n=1 then o=0 or 1, and if o=0 then p=0 or 1, and if o=1 then p=0, and s=0 or 1, and q=0, and t=0, and r=0,~~

~~wherein if m=1 then q=0 or 1, and if q=0 then r=0 or 1, and if q=1 then r=0, and t=0 or 1, and o=0, and s=0, and p=0,~~

~~wherein the first FC and the second FC are covalently linked by one or more disulfide bond(s),~~

~~wherein FC^1 and FC^2 do not substantially bind to an Fc-receptor,~~

~~b) recovering the fusion polypeptide from the cell or the cultivation medium,~~

~~c) optionally cleaving the fusion polypeptide with a protease,~~

~~and thereby producing a polypeptide that is biologically active as 2-mer.~~