(Papadea and Check 1989). The FcRn binding site is located in the Fc region of the antibody (Martin, West et al. 2001), and thus the extended serum half-life property of the antibody is retained in the Fc fragment. The Fc region alone can be thought of as a homodimer of heavy chains comprising the hinge, CH2 and CH3 domains.

The Fc region of naturally occurring IgG antibodies is a homodimer and can be expressed and purified as a dimer. As discussed above, the Fc region of the antibody confers serum half-life via FcRn recycling mechanism. Hence, the Fc is used as a fusion partner to extend the serum half-life of therapeutic proteins, peptides (peptibody), and protein domains. However, for some therapeutic applications, it may be necessary to delete the hinge region which removes the covalent link between the two polypeptide chains that form Fc. For example, when an Fc is fused to a protein that contains internal disulfide bonds or free cysteine residues, the hinge disulfides could interfere with the folding and lead to aggregation. However, removing the hinge region eliminates the covalent link between the two polypeptide chains. This could lead to disassociation of the noncovalent interaction between the two Fc chains, either during the manufacturing stage or in-vivo, and lead to the association of the Fc chains with other proteins / molecules.

SUMMARY

As disclosed herein, by introducing a disulfide bond at the CH3 domain interface, the thermal stability of Fc-containing molecules lacking disulfide bonds within the hinge region can be improved. In addition, the covalent link keeps the two polypeptide chains that form dimer in the Fc structure intact without disassociation in-vitro or in-vivo. As shown in Figure 3, in certain embodiments, the only covalent link between the two Fc chains in the WT del hinge Fc homodimer and a mutant del hinge Fc heterodimer is the introduced disulfide bond.

In certain ~~embodiments~~examples, one or more residues that make up the CH3-CH3 interface on both CH3 domains is replaced with a sulfhydryl containing residue such that the interaction becomes stabilized by the formation of a disulfide bond (-S-S-) between the CH3 domains. In preferred examples~~embodiments~~, an amino acid in the interface, such as a leucine, threonine, serine or tyrosine, is replaced with a cysteine or methionine, preferably cysteine. In certain examples~~embodiments~~, the amino acid is replaced

with an unnatural amino acid having the desired charge characteristic, such as homocysteine or glutathione.

In a first aspect of the invention, it is referred to a polypeptide comprising an antibody Fc region, said Fc region comprising a deletion or substitution of one or more cysteines of the hinge region and substitution of one or more CH3-interface amino acids with a sulfhydryl containing residue wherein said polypeptide has a sulfhydryl containing residue substitution at residue T394 and/or residue Y407. Also described herein is a polypeptide comprises an antibody Fc region having a deletion or substitution of one or more cysteines of the hinge region and substitution of one or more CH3-interface amino acids with a sulfhydryl containing residue, preferably cysteine. The hinge region may lack cysteine residues by virtue of substitution or through deletion. In certain ~~embodiments~~examples, the Fc lacks the hinge region altogether. In other examples~~embodiments~~, only a portion of the hinge region is deleted, preferably a portion comprising the cyteine residues.

In certain examples~~embodiments of the first aspect~~, CH3-interface amino acid Y349, L351, S354, T394, or Y407 is substituted with a sulfhydryl containing residue, preferably cysteine. In preferred examples~~embodiments~~, the Fc comprises an L351C substitution. When two Fc-containing polypeptides having an L351C substitution interact under appropriate conditions, a disulfide bond is formed between the L351C residues in the two chains. Similarly, when two Fc-containing polypeptides having a T394C substitution interact under appropriate conditions, a disulfide bond is formed between the T394C residues in the two chains. Moreover, when two Fc-containing polypeptides having a Y407C substitution interact under appropriate conditions, a disulfide bond is formed between the Y407C residues in the two chains. When an Fc-containing polypeptide having a Y349C substitution interacts with an Fc-containing polypeptide having an S354C substitution under appropriate conditions, a disulfide bond is formed between the Y349C residue in one chain and the S354C residue in the other chain.

The Fc region of the polypeptide ~~of the first aspect~~as described herein may contain one or more additional amino acid substitutions in the CH2 and/or CH3 region. In preferred examples~~embodiments~~, the Fc region comprises one or more amino acid substitutions in the CH2 region that alter the effector function of an Fc-containing protein as compared to similar protein having a wild-type CH2. In other examples~~embodiments~~, the Fc region comprises one or more amino acid substitutions in the CH3 region that alter the ability of the Fc-containing polypeptide to homodimerize and/or increase the ability to heterodimerize with a Fc-containing polypeptide having reciprocal amino acid substitutions in the CH3 region.

In certain examples~~embodiments or the first aspect~~, one or more amino acids of the C-terminus of the Fc are deleted or substituted. In preferred examples~~embodiments~~, a C-terminal lysine is deleted or substituted for another amino acid. In other examples~~embodiments~~, the two or three terminal amino acids are deleted or substituted for another amino acid.

In certain examples~~embodiments of the first aspect~~, the polypeptide is an antibody heavy chain. In other examples~~embodiments~~, the polypeptide is an Fc-fusion protein. The Fc-fusion protein may contain a linker at the N-terminus and/or C-terminus of the Fc molecule.

In a second aspect of the invention, a nucleic acid encodes a polypeptide of the first aspect.

In a third aspect, an expression vector comprises the nucleic acid of the second aspect operably linked to a regulatory sequence, such as a heterologous promoter and/or enhancer.

In a fourth aspect, a host cell comprises the expression vector of the third aspect. In preferred embodiments, the host cell is a eukaryotic cell, such as a yeast or mammalian cell line. A preferred mammalian cell line is a Chinese hamster ovary (CHO) cell line.

A fifth aspect of the invention is a method of making a polypeptide of the first aspect. The methods comprise culturing a host cell of the fourth aspect under conditions in which the regulatory region is active in the host cell and isolating the polypeptide from the culture.

In a sixth aspect, a pharmaceutic composition comprises a polypeptide of the first aspect.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic diagram of IgGl antibody with the domains indicated. The IgGl antibody is a Y-shaped tetramer with two heavy chains (longer length) and two light chains (shorter length). The two heavy chains are linked together by disulfide bonds (-S-S-) at the hinge region. Fab - fragment antigen binding, Fc - fragment crystallizable, VL - variable light chain domain, VH -variable heavy chain domain, CL - constant (no sequence variation) light chain domain, CHl -constant heavy chain domain 1 , CH2 - constant heavy chain domain 2, CH3 - constant heavy chain domain 3.

CLAIMS

What is claimed is:

1. A polypeptide comprising an antibody Fc region, said Fc region comprising a deletion or substitution of one or more cysteines of the hinge region and substitution of one or more CH3-interface amino acids with a sulfhydryl containing residue wherein said polypeptide has a sulfhydryl containing residue substitution at residue T394 and/or residue Y407.

2. The polypeptide of claim 1, wherein the Fc lacks a cysteine-containing portion of the hinge region.

3. The polypeptide of claim 2, wherein the Fc lacks the hinge region.

4. The polypeptide of claim 1, wherein all cysteines within the hinge region are substituted for another amino acid.

5. The polypeptide of any one of claims 1-4, wherein CH3-interface amino acid substituted with a sulfhydryl containing residue is Y349, L351, S354, T394, or Y407.

6. The polypeptide of claim 5, wherein Y349 is substituted with cysteine (Y349C).

7. The polypeptide of claim 5, wherein L351 is substituted with cysteine (L351C).

8. The polypeptide of claim 5, wherein S354 is substituted with cysteine (S354C).

9. The polypeptide of claim 5, wherein T394 is substituted with cysteine (T394C).

10. The polypeptide of claim 5, wherein Y407 is substituted with cysteine (Y407C).

11. The polypeptide of any one of claims 1-10, wherein the Fc comprises a CH2 region comprising one or more amino acid substitutions.

12. The polypeptide of any one of claims 1-11, wherein the CH3 region further comprises one or more additional amino acids substitutions.

13. The polypeptide of any one of claims 1-12, wherein one or more amino acids of the C-terminus of the Fc are deleted.

14. The polypeptide of claim 13, wherein three, two, or one amino acid of the C-terminus of the Fc is deleted.

15. The polypeptide of claim 14, wherein the terminal amino acid of the C-terminus of the Fc is deleted.

16. The polypeptide of any one of claims 1-15, wherein the polypeptide comprises an antibody heavy chain.

17. The polypeptide of any one of claims 1-15, wherein the polypeptide comprises an Fc fusion protein.

18. A nucleic acid encoding a polypeptide of any one of claims 1-17.

19. An expression vector comprising the nucleic acid of claim 18 operably linked to a promoter.

20. A host cell comprising the expression vector of claim 19.

21. Method of making a polypeptide, said method comprising

a) culturing the host cell of claim 20 under conditions in which the promoter is active in said host cell; and

b) isolating the polypeptide from the culture.

22. A pharmaceutical composition comprising the polypeptide of any one of claims 1-17.