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; wherein the residue designation is based on the EU numbering scheme of Kabat. 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 examples, the Fc lacks the hinge region altogether. In other examples, only a portion of the hinge region is deleted, preferably a portion comprising the cyteine residues.

In certain examples, CH3-interface amino acid Y349, L351, S354, T394, or Y407 is substituted with a sulfhydryl containing residue, preferably cysteine. In preferred examples, 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 as described herein may contain one or more additional amino acid substitutions in the CH2 and/or CH3 region. In preferred examples, 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, 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.

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; wherein the residue designation is based on the EU numbering scheme of Kabat.

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 said polypeptide further comprises a CH3-interface amino acid at residue Y349, or L351, or S354 substituted with a sulfhydryl containing residue.

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 1, wherein T394 is substituted with cysteine (T394C).

10. The polypeptide of claim 1, 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 that alter the effector function of an Fc-containing protein as compared to similar protein having a wild-type CH2.

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

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.

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

14. The polypeptide of claim 13, wherein the terminal amino acid of the C-terminus of the Fc is deleted.

15. The polypeptide of any one of claims 1-14, wherein the polypeptide comprises an antibody heavy chain.

16. The polypeptide of any one of claims 1-14, wherein the polypeptide comprises an Fc fusion protein.

17. A nucleic acid encoding a polypeptide of any one of claims 1-16.

18. An expression vector comprising the nucleic acid of claim 17 operably linked to a promoter.

19. A host cell comprising the expression vector of claim 18.

20. Method of making a polypeptide, said method comprising
a) culturing the host cell of claim 19 under conditions in which the promoter is active in said host cell; and
b) isolating the polypeptide from the culture.

21. A pharmaceutical composition comprising the polypeptide of any one of claims 1-16.