Claims

1. A method for producing a heteromultimer, comprising the steps of:
a) providing a homo variant of first polypeptides each having a first antigen-binding activity and comprising an Fc region;
b) providing a homo variant of second polypeptides each having a second antigen-binding activity different from the first antigen-binding activity and comprising an Fc region;
c) incubating the homo variant of the first polypeptides and the homo variant of the second polypeptides together under a reducing condition that allows cysteines in hinge regions to cause disulfide bond isomerization; and
d) obtaining a heteromultimer comprising the first and second polypeptides, wherein

1 to 3 sets of amino acid residues selected from the following amino acid residue sets:

(1) amino acid residues at EU numbering positions 356 and 439,

(2) amino acid residues at EU numbering positions 357 and 370, and

(3) amino acid residues at EU numbering positions 399 and 409

in a CH3 region contained in the Fc region of the first and/or second polypeptide have the same type of charge,
wherein in the first and/or second polypeptide, an amino acid at EU numbering position 397 and/or 392 is substituted, and

when the amino acid residues in the same set among the amino acid residue sets (1) to (3) have the same type of charge as each other both in the CH3 region of the first polypeptide and in the CH3 region of the second polypeptide, the amino acid residues in this set in the CH3 region of the second polypeptide have a charge opposite to that of the amino acid residues in this set in the CH3 region of the first polypeptide.

2. The method according to claim 1, wherein the step a) in claim 1 comprises the step of providing a third polypeptide that forms a multimer with the first polypeptide, and the step b) comprises the step of providing a fourth polypeptide that forms a multimer with the second polypeptide.

3. The method according to claim 1 or 2, wherein the amino acid residues having the same type of charge are selected from one or more amino acid residues included in any of the following groups (A) and (B):
(A) glutamic acid (E) and aspartic acid (D); and
(B) lysine (K), arginine (R), and histidine (H).

4. The method according to any one of claims 1 to 3, wherein the set(s) of the amino acid residues having the same type of charge as each other in each of the first and second polypeptides is any one of the following amino acid residue sets (1) to (4):

(1) amino acid residues at EU numbering positions 356 and 439,

(2) amino acid residues at EU numbering positions 357 and 370,

(3) amino acid residues at EU numbering positions 399 and 409, and

(4) (i) amino acid residues at EU numbering positions 399 and 409 and

(ii) amino acid residues at EU numbering positions 356 and 439.

5. The method according to any one of claims 1 to 4, wherein the set(s) of the amino acid residues having the same type of charge as each other in each of the first and second polypeptides is the following amino acid residue sets:

(i) amino acid residues at EU numbering positions 399 and 409 and

(ii) amino acid residues at EU numbering positions 356 and 439.

6. The method according to any one of claims 1 to 5, wherein in the first and/or second polypeptide, an amino acid is altered so as to destabilize the stability of the CH3 region of the first and/or second polypeptide.

7. The method according to any one of claims 1 to 6, wherein the Fc region of the first and/or second polypeptide is of IgG1, IgG2, IgG3, or IgG4 type.

8. The method according to any one of claims 1 to 6, wherein the Fc region of the first and/or second polypeptide is a mouse-derived Fc region.

9. The method for producing a heteromultimer according to claim 8, wherein
1 to 3 sets of amino acid residues selected from the following amino acid residue sets:

(1) amino acid residues at EU numbering positions 356 and 439,

and

(2) amino acid residues at EU numbering positions 399 and 409

in the CH3 region contained in the Fc region of the first and/or second polypeptide have the same type of charge, and

when the amino acid residues in the same set among the amino acid residue sets (1) to (3) have the same type of charge as each other both in the CH3 region of the first polypeptide and in the CH3 region of the second polypeptide, the amino acid residues in this set in the CH3 region

of the second polypeptide have a charge opposite to that of the amino acid residues in this set in the CH3 region of the first polypeptide.

10. The method according to any one of claims 1 to 9, wherein in the first and/or second polypeptide,

the amino acid at EU numbering position 397 is altered to Met (M), Phe (F), or Tyr (Y), and/or

the amino acid at EU numbering position 392 is altered to Asp (D), Glu (E), Thr (T), Val (V), or Ile (I).

11. The method according to any one of claims 1 to 10, wherein in the first and/or second polypeptide, the amino acid at EU numbering position 397 is altered to Phe (F) or Tyr (Y).

12. The method according to any one of claims 1 to 11, wherein in the first polypeptide, the amino acid at EU numbering position 356 is altered to Lys (K), and the amino acid at EU numbering position 397 is altered to Phe (F) or Tyr (Y); and in the second polypeptide, the amino acid at EU numbering position 397 is altered to Phe (F) or Tyr (Y), and the amino acid at EU numbering position 439 is altered to Glu (E).

13. The method according to any one of claims 1 to 12, wherein the steps a) and b) are carried out by mixing a cell line producing the homo variant of the first polypeptides with a cell line producing the homo variant of the second polypeptides, and the step c) is carried out in the culture supernatant.

14. The method according to any one of claims 1 to 13, wherein the heteromultimer is a multispecific antibody or a hetero-Fc fusion protein.

15. The method according to any one of claims 1 to 14, wherein the heteromultimer is a bispecific antibody.

16. The method according to any one of claims 1 to 15, wherein the step c) described in claim 1 or claim 11 involves contact with a reducing agent.

17. The method according to claim 16, wherein the step c) involves the addition of an active substance selected from the group consisting of glutathione, L-cysteine, dithiothreitol, β-mercapto-ethanol, TCEP, and 2-MEA.

18. The method according to claim 17, wherein the step c) involves the addition of an active substance selected from glutathione and 2-MEA.

19. A bispecific antibody produced by a method according to any one of claims 1 to 18.

20. A composition comprising a bispecific antibody according to claim 19 and a pharmaceutically acceptable carrier.