

was not known that there are antibodies that cannot be purified by commonly-used Protein A columns.

[0009]

The present inventors discovered that there are pI-modified antibodies that cannot be efficiently purified by the commonly-used Protein A columns. An efficient purification method suitable for such antibodies is thus needed. In other words, it is an objective of the present invention to provide a highly efficient and economical purification method which enables production of an antibody on an industrial scale even when the antibody is a pI-modified antibody that cannot be efficiently purified by a common Protein A column.

[Solution to Problem]

[0010]

As a result of diligent research to achieve the above objective, the present inventors discovered that the use of a resin comprising a specific modified Protein A ligand enables efficient purification of even pI-modified antibodies that cannot be efficiently purified with a commonly-used Protein A column.

[0010a]

In one aspect, the present disclosure refers to a method of purifying an IgG antibody comprising the amino acid residue substitutions Q311R and P343R from a composition containing the antibody, wherein the method comprises the steps of:

(a) preparing an affinity column containing a carrier onto which a Protein A-modified ligand is immobilized, wherein the Protein A-modified ligand comprises a multimer of modified immunoglobulin-binding domains;

(b) loading the composition containing the IgG antibody onto the affinity column of step (a); and

(c) eluting and recovering the IgG antibody from the affinity column of step (b),

wherein the IgG antibody comprises in the heavy chain constant region an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 to 57,

wherein the multimer of modified immunoglobulin-binding domains has an ability to bind to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R, wherein the multimer of modified immunoglobulin-binding domains is represented by the following Formula (1) wherein the left end is the N-terminus and the right end is the C-terminus:

$$(R1)_n-(R2)_m \text{ or } (R2)_m-(R1)_n \quad (1)$$

wherein

(A) R1 and R2 are each the C-domain variant of *Staphylococcus* Protein A of SEQ ID NO: 1 or the Z-domain of *Staphylococcus* Protein A of SEQ ID NO: 2, and comprising

(i) a substitution of a lysine residue at position 35 with an arginine residue or a glutamine residue,

(ii) a substitution of a lysine residue at position 4 with an alanine residue, a valine residue, an isoleucine residue, an arginine residue, or a glutamic acid residue, and a substitution
5 of a lysine residue at position 35 with an arginine residue, a glutamine residue, or a valine residue, or

(iii) a substitution of a lysine residue at position 4 with an alanine residue, a valine residue, an isoleucine residue, an arginine residue, or a glutamic acid residue, a substitution of a
10 lysine residue at position 7 with a tyrosine residue, a phenylalanine residue, a glutamine residue, a leucine residue, an isoleucine residue, a proline residue, a threonine residue, an alanine residue, a valine residue, an arginine residue, or a histidine residue, and a substitution of a lysine residue at position 35 with an arginine residue, a glutamine residue, or a valine residue,

(B) n is an integer of 1 or more and 9 or less,

(C) m is 1 or 2,

(D) the total number of n + m is 2 to 10,

(E) the n number of R1 domain may all have the same sequence or may have sequences different from each other, and

(F) the m number of R2 domain may all have the same sequence or may have sequences different from each other.

[0011]

More specifically, the present disclosure provides the following [1] to [20]:

[1] a method of purifying an IgG antibody comprising the amino acid residue substitutions Q311R and P343R from a composition containing the antibody, wherein the method comprises the steps of:

(a) preparing an affinity column containing a carrier onto which a Protein A-modified ligand is immobilized, wherein the Protein A-modified ligand comprises: a modified immunoglobulin binding domain comprising a modification for substitution of any one or more originally present
25 lysine residues at positions 4, 7, and 35 of the C-domain variant of *Staphylococcus* Protein A of SEQ ID NO: 1 or Z-domain of *Staphylococcus* Protein A of SEQ ID NO: 2 with amino acid

residues other than lysine; or a multimer of these modified immunoglobulin-binding domains;

(b) loading the composition containing the IgG antibody onto the affinity column of step (a); and

(c) eluting and recovering the IgG antibody from the affinity column of step (b);

[2] the method of [1], wherein the multimer of the modified immunoglobulin-binding domains is a dimer to decamer, and wherein arranged at the first or second from the N-terminal or C-

terminal side in the multimer is an immunoglobulin-binding domain in which at least one of the
35

originally present amino acid residues at positions 40, 43, 46, 53, 54, and 56 of the C-domain variant (SEQ ID NO: 1) or Z-domain (SEQ ID NO: 2) has been substituted with a lysine residue; [3] the method of [1] or [2], wherein the substitution is a modification for substitution of any one or more originally present lysine residues at positions 4, 7, and 35 of the C-domain variant or Z-

[CLAIMS]

1. A method of purifying an IgG antibody comprising the amino acid residue substitutions Q311R and P343R from a composition containing the antibody, wherein the method comprises the steps of:
- (a) preparing an affinity column containing a carrier onto which a Protein A-modified ligand is immobilized, wherein the Protein A-modified ligand comprises a multimer of modified immunoglobulin-binding domains;
 - (b) loading the composition containing the IgG antibody onto the affinity column of step (a); and
 - (c) eluting and recovering the IgG antibody from the affinity column of step (b),
- wherein the IgG antibody comprises in the heavy chain constant region an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 to 57,
- wherein the multimer of modified immunoglobulin-binding domains has an ability to bind to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R,
- wherein the multimer of modified immunoglobulin-binding domains is represented by the following Formula (1) wherein the left end is the N-terminus and the right end is the C-terminus:
- $$(R1)_n-(R2)_m \text{ or } (R2)_m-(R1)_n \quad (1)$$
- wherein
- (A) R1 and R2 are each the C-domain variant of *Staphylococcus* Protein A of SEQ ID NO: 1 or the Z-domain of *Staphylococcus* Protein A of SEQ ID NO: 2, and comprising
- (i) a substitution of a lysine residue at position 35 with an arginine residue or a glutamine residue,
 - (ii) a substitution of a lysine residue at position 4 with an alanine residue, a valine residue, an isoleucine residue, an arginine residue, or a glutamic acid residue, and a substitution of a lysine residue at position 35 with an arginine residue, a glutamine residue, or a valine residue, or
 - (iii) a substitution of a lysine residue at position 4 with an alanine residue, a valine residue, an isoleucine residue, an arginine residue, or a glutamic acid residue, a substitution of a lysine residue at position 7 with a tyrosine residue, a phenylalanine residue, a glutamine residue, a leucine residue, an isoleucine residue, a proline residue, a threonine residue, an alanine residue, a valine residue, an arginine residue, or a histidine residue, and a substitution of a lysine residue at position 35 with an arginine residue, a glutamine residue, or a valine residue,
- (B) n is an integer of 1 or more and 9 or less,
- (C) m is 1 or 2,

(D) the total number of $n + m$ is 2 to 10,

(E) the n number of R1 domain may all have the same sequence or may have sequences different from each other, and

(F) the m number of R2 domain may all have the same sequence or may have sequences
5 different from each other.

2. The method of claim 1, wherein the R1 or R2 is each the C-domain variant of
Staphylococcus Protein A of SEQ ID NO: 1 or the Z-domain of *Staphylococcus* Protein A of
SEQ ID NO: 2, and comprising a substitution of a lysine residue at position 4 with an alanine
10 residue, a substitution of a lysine residue at position 7 with a threonine residue, and a substitution
of a lysine residue at position 35 with an arginine residue.

3. The method of claim 1 or 2, wherein the R1 or R2 further comprises 1 to 4 substitutions
among a substitution of a lysine residue at position 42 with an alanine residue, a substitution of a
15 lysine residue at position 49 with an arginine residue, a substitution of a lysine residue at position
50 with an arginine residue, and a substitution of a lysine residue at position 58 with an arginine
residue.

4. The method of claim 1 or 2, wherein the R1 or R2 further comprises a substitution of a lysine
20 residue at position 42 with an alanine residue, a substitution of a lysine residue at position 49
with an arginine residue, a substitution of a lysine residue at position 50 with an arginine residue,
and a substitution of a lysine residue at position 58 with an arginine residue.

5. The method of any one of claims 1 to 4, wherein the first and/or second R1 and/or R2 from
25 the N-terminal or C-terminal side of the multimer further comprises a substitution of at least one
amino acid residue of the originally present amino acid residues at positions 40, 43, 46, 53, 54,
and 56 with a lysine residue.

6. The method of claim 5, wherein the first and/or second R1 and/or R2 from the N-terminal or
30 C-terminal side of the multimer further comprises a substitution of the originally present amino
acid residues at positions 40, 43, 46, and 53 with a lysine residue.

7. The method of any one of claims 1 to 6, wherein the multimer of modified immunoglobulin-
binding domains is a multimer of modified immunoglobulin-binding domains wherein the R1
35 consists of the amino acid sequence of SEQ ID NO: 5 and the R2 consists of the amino acid
sequence of SEQ ID NO: 4.

8. The method of any one of claims 1 to 7, wherein the multimer of modified immunoglobulin-binding domains is a multimer of modified immunoglobulin-binding domains wherein the n is 5 and the m is 1.
- 5 9. The method of any one of claims 1 to 8, wherein the Protein A-modified ligand is immobilized onto the carrier by any one means selected from the group consisting of (1) to (5) below:
- 10 (1) a method of immobilization onto the carrier through the modified immunoglobulin-binding domain in which 1 to 6 of the amino acid residues at positions 40, 43, 46, 53, 54, and 56 in the C-domain variant or Z-domain of Protein A are additionally substituted with a lysine residue;
- (2) a method of immobilization onto the carrier through a disulfide bond or a thioether bond by introducing cysteine into the C-terminus of Protein A;
- (3) a method of immobilization onto an amino group-containing immobilization carrier by cyanation of a thiol group;
- 15 (4) a method of immobilizing the multimer of modified immunoglobulin-binding domains having a cysteine residue onto an amino group-containing carrier using 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) as a cross-linking agent; and
- (5) a method of immobilization onto the carrier through a plurality of lysine residues added to the C-terminus of a modified immunoglobulin-binding domain in which the lysine residues at
- 20 positions 42, 49, 50, and 58 of the C-domain variant of Protein A are substituted with amino acids other than lysine, or to a modified immunoglobulin-binding domain in which the lysine residues at positions 49, 50, and 58 of the Z-domain are substituted with amino acids other than lysine.
- 25 10. The method of any one of claims 1 to 9, wherein the pI value of the antibody is 4.0 to 10.0.