

increase the pI, antigen elimination from plasma can be enhanced when the antibody is administered *in vivo*.

[0006]

Protein A used for antibody purification is a protein present in the cell wall of *Staphylococcus aureus* and binds to immunoglobulins, especially to the Fc region of IgG. In general, the Protein A protein derived from *Staphylococcus* has a repeated structure including five immunoglobulin-binding domains having homology to each other, called the E-domain, D-domain, A-domain, B-domain, and C-domain, and each binding domain can bind singly to an immunoglobulin. Along with natural Protein A, recombinant proteins consisting only of immunoglobulin-binding domain(s) with partially-modified amino acids are also used as affinity ligands for affinity chromatography. For example, Protein A columns with improved antibody purification efficiency have been developed by substituting any one or more originally present lysines at positions 4, 7, and 35 of a C-domain variant or Z-domain with an amino acid other than lysine, where the C-domain variant was prepared by substituting the glycine at position 29 of the amino acid sequence of the C-domain of *Staphylococcus* Protein A with alanine, and the Z-domain was prepared by substituting the glycine at position 29 of the amino acid sequence of the B-domain of *Staphylococcus* Protein A with alanine (PTLs 4 and 5).

[Citation List]

[Patent Literature]

[0007]

[PTL 1] Japanese Patent Application *Kohyo* Publication No. (JP-A) H05-504579 (unexamined Japanese national phase publication corresponding to a non-Japanese international publication)

[PTL 2] WO 2007/11431

[PTL 3] WO 2017/104783

[PTL 4] Japanese Patent Application *Kokai* Publication No. (JP-A) 2007-252368 (unexamined, published Japanese Patent Application)

[PTL 5] WO 2015/034000

[Summary of Invention]

[Technical Problem]

[0008]

Protein A has been conventionally utilized as a ligand for antibody purification. However, evaluation of purification methods suitable for antibodies with amino acid modifications for modifying pI (hereinafter referred to as pI-modified antibodies) and issues in the purification process have not been investigated in detail so far. Therefore, for example, it

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 modified immunoglobulin-binding domain which binds to the IgG antibody comprising the amino acid residue substitutions Q311R and P343R, wherein the modified immunoglobulin-binding domain comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2 with a modification for substitution of any one or more originally present 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), wherein

the IgG antibody additionally comprises one or more amino acid residue substitutions selected from among M428L, N434A, Y436T, Q438R, and S440E in the CH3 region of the IgG antibody,

the IgG antibody comprises in the CH3 region an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 to 11, and

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.

[0011]

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

- 5 [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
10 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
 - 15 (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
20 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-

of an amino acid-substituted C-domain variant and Z-domain of Protein A, wherein the substitution is a substitution which alters any one or more originally present lysine residues at positions 4, 7, and 35 of the C-domain variant or Z-domain to amino acid residues other than lysine, and wherein the protein A-modified ligand is a modified ligand having an ability to bind to the IgG antibody;

[20] a method of producing an IgG antibody having the amino acid residue substitutions Q311R and P343R, wherein the method comprises the following steps of:

(i) providing a composition containing an IgG antibody comprising the amino acid residue substitutions Q311R and P343R;

(ii) 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 lysine residues at positions 4, 7, and 35 of 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 with amino acid residues other than lysine; or a multimer of these modified immunoglobulin-binding domains;

(iii) loading the composition containing the IgG antibody onto the affinity column of step (ii); and

(iv) eluting and recovering the IgG antibody from the affinity column loaded with the composition containing the IgG antibody in step (iii).

Alternatively, the present ~~invention~~disclosure provides:

[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;

(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 Protein A-modified ligand contains either or both of an amino acid-substituted C-domain variant and Z-domain of Protein A, wherein the substitution comprises a substitution

which alters any one or more originally present lysine residues at positions 4, 7, and 35 of the C-domain variant or Z-domain to amino acid residues other than lysine, and is a Protein A-modified ligand having an activity to bind to the IgG antibody.

Advantageous Effect of Invention

[0012]

By the present ~~invention~~disclosure, even pI-modified antibodies that cannot be successfully purified by a common Protein A column can be purified easily and efficiently.

[Brief Description of Drawings]

[0013]

Fig. 1 shows the results of measuring the dynamic binding capacity (DBC) of an antibody that does not contain a modification in the Fc region in each Protein A-immobilized resin. In the figure, the vertical axis shows DBC (g/L resin), and the horizontal axis shows residence time (minutes).

Fig. 2-1 shows the result (real-time binding curve) of evaluating the binding affinity between the ligand (structure represented by Formula (1')) for AF-rProtein A HC-650F and an antibody, using the BLItz (registered trademark) evaluation system (ForteBio).

Fig. 2-2 shows the result (real-time binding curve) of evaluating the binding affinity between the ligand for MabSelect SuRe and an antibody, using the BLItz (registered trademark) evaluation system (ForteBio).

[Description of Embodiments]

[0014]

Hereinbelow, the present invention will be described in detail.

The present invention relates to methods of purifying a composition containing a pI-modified antibody having an increased isoelectric point (pI). Specifically, the present invention relates to methods 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 following 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 containing a modification for substitution of any one or more originally present lysine residues at positions 4, 7, and 35 of 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 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 loaded in step (b).

[0015]

The IgG antibodies comprising the amino acid residue substitutions Q311R and P343R with regard to the CH2 and CH3 regions in the present invention are antibodies in which both glutamine (Q) at position 311 and proline (P) at position 343 of the CH2 and CH3 regions

[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 modified immunoglobulin-binding domain which binds to the IgG antibody comprising the amino acid residue substitutions Q311R and P343R, wherein the modified immunoglobulin-binding domain comprises the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2 with a modification for substitution of any one or more originally present 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), wherein

the IgG antibody additionally comprises one or more amino acid residue substitutions selected from among M428L, N434A, Y436T, Q438R, and S440E in the CH3 region of the IgG antibody,

the IgG antibody comprises in the CH3 region an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 to 11, and

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.

2. The method of claim 1, wherein the multimer of the modified immunoglobulin-binding domains is a dimer to decamer, and wherein the multimer of the modified immunoglobulin-binding domains comprises as ~~arranged at~~ the first or second modified immunoglobulin-binding domain from the N-terminal or C-terminal side ~~in~~ of the multimer ~~is~~ an immunoglobulin-binding domain in which at least one of the 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 claim 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-domain of Protein A with any one of amino acid residues selected from the group consisting of an alanine residue, a glutamine residue, an asparagine residue, a valine residue, a serine residue,

a threonine residue, a histidine residue, a tyrosine residue, an arginine residue, a glutamic acid residue, a phenylalanine residue, a leucine residue, an isoleucine residue, and a proline residue.

4. The method of any one of claims 1 to 3, wherein the modified immunoglobulin-binding domain ~~is (i) a modified immunoglobulin-binding domain comprising~~ comprises the amino acid sequence of SEQ ID NO: 3 or 5; ~~or (ii) a modified immunoglobulin-binding domain comprising an amino acid sequence in which one to several amino acid residues have been substituted, deleted, added, and/or inserted to the amino acid sequence of SEQ ID NO: 3 or 5 at amino acid residues other than those at positions 4, 7, and 35.~~

~~5. The method of any one of claims 1 to 4, wherein the modified immunoglobulin-binding domain has an ability to bind to an IgG antibody comprising the amino acid residue substitutions Q311R and P343R.~~

~~65.~~ The method of ~~any one of claims 1 to 54,~~ wherein the ~~Protein A-modified ligand is a modified ligand comprising a~~ modified immunoglobulin-binding domain ~~that~~ consists of ~~at least one~~ the amino acid sequence ~~selected from the group consisting of~~ SEQ ID NOs: 3, 4, ~~and~~ or 5.

~~76.~~ The method of ~~any one of claims 1 to 6,~~ 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:

(1) a method of immobilization onto the carrier through ~~a~~ 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;

(4) a method of immobilizing ~~a~~ 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 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.

5 ~~8. The method of any one of claims 1 to 7, wherein the IgG antibody is an IgG antibody additionally comprising one or more amino acid residue substitutions selected from among M428L, N434A, Y436T, Q438R, and S440E in the CH3 region of the IgG antibody.~~

10 ~~9. The method of any one of claims 1 to 8, wherein the IgG antibody is one in which the CH3 region of the IgG antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 to 11.~~

15 ~~10. The method of any one of claims 1 to 9, wherein the IgG antibody is one in which the heavy chain constant region of the IgG antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 12 to 57.~~

~~11~~7. The method of any one of claims 1 to ~~10~~6, wherein the pI value of the antibody is 4.0 to 10.0.