

mechanisms of action do not depend on the FVIII function, that is, the function of catalyzing the activation of blood coagulation factor X (FX) by activated blood coagulation factor IX (FIXa). Therefore, in some cases, bypassing agents cannot sufficiently stop the bleeding. Accordingly, there has been a strong need for pharmaceutical agents that are not affected by the presence of inhibitors and which functionally substitute for FVIII.

[0005]

As a means for solving the problem, bispecific antibodies that functionally substitute for FVIII and their use have been reported (PTLs 1, 2, 3, and 4). The bispecific antibodies against FIXa and FX can functionally substitute for FVIII by positioning the two factors close to each other to exhibit FVIII mimetic activity (NPL 5). It has been reported that the FVIII mimetic activity of the antibodies can be enhanced by optimizing the affinity towards FIXa and FX (NPL 6). Emicizumab (ACE910) having high FVIII mimetic activity, which is one of these antibodies, has been reported to exhibit hemostatic effects in monkey models of hemophilia (NPLs 7 and 8); therefore, clinical trials are being conducted on hemophilia A patients.

[Citation List]

[Patent Literature]

[0006]

[PTL 1] WO 2005/035754

[PTL 2] WO 2005/035756

[PTL 3] WO 2006/109592

[PTL 4] WO 2012/067176

[Non-Patent Literature]

[0007]

[NPL 1] Blood 58, 1-13 (1981)

[NPL 2] Nature 312, 330-337(1984)

[NPL 3] Nature 312, 337-342(1984)

[NPL 4] Biochim.Biophys.Acta 871, 268-278(1986)

[NPL 5] Nat Med. 2012 Oct;18(10):1570-4.

[NPL 6] PLoS One. 2013;8(2):e57479.

[NPL 7] J Thromb Haemost. 2014 Feb;12(2):206-213.

[NPL 8] Blood. 2014 Nov 13;124(20):3165-71.

[NPL 9] J. Appl. Cryst. 13, 577-584 (1980)

[NPL 10] IUCrJ. 2, 9-18 (2015)

[Summary]

[Technical Problem]

[0008]

The present disclosure was achieved in view of the above circumstances. An objective of the present disclosure is to provide antibody variants or isoforms with reduced FVIII mimetic activity.

[Solution to Problem]

[0009]

The present inventors performed dedicated research to solve the above-described problems and succeeded in identifying antibody variants and isoforms which are contained in a pharmaceutical composition comprising Emicizumab as an active ingredient. The present inventors also found that FVIII mimetic activity of these antibody variants and isoforms is quite low, as compared with that of Emicizumab.

[0010]

The present disclosure was made based on these findings.

In one aspect, the present disclosure refers to a variant of an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and that exhibits FVIII mimetic activity, wherein

(a) the amino acid residue R at the 12th position from the N terminus side of the sequence; or

(b) the amino acid residues YYR at the 10th to 12th position from the N terminus side of the sequence,

is deleted and the variable region is cleaved at the deletion site.

[0010a]

In another aspect, the present disclosure refers to a method for detecting the antibody variant as described herein, comprising the step of separating a sample containing an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and the variant as described herein by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof, and detecting the antibody variant.

[0010b]

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody variant as described herein, wherein the percentage of the antibody variant in the total antibody molecules in the pharmaceutical composition is 5% or less and wherein production of the antibody variant is suppressed by a method comprising the step of culturing cells producing the antibody at a pH of 7.1 or more, and/or at a culture temperature of 36°C or less.

[0010c]

In still another aspect, the present disclosure refers to a method for suppressing production of the antibody variant as described herein, comprising the step of culturing cells producing the antibody at a pH of 7.1 or more, and/or at a culture temperature of 36°C or less.

[0010d]

5 The present disclosure provides [1] to [21] below.

[1] A variant of an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2), wherein

(a) the amino acid residue R at the 12th position from the N terminus side of the sequence (the 61th position from the N terminus side of the Emicizumab Q chain: position 60 according to Kabat numbering)

10 or

(b) the amino acid residues YYR at the 10th to 12th positions from the N terminus side of the sequence (the 59th to 61th positions from the N terminus side of the Emicizumab Q chain: positions 58 to 60 according to Kabat numbering)

is deleted and the variable region is cleaved at the deletion site.

15 [2] The antibody variant of [1], wherein the sequence is a CDR sequence.

[3] The antibody variant of [1], wherein the sequence is a CDR2 sequence.

[4] The antibody variant of [1], wherein the sequence is a sequence comprised in a heavy chain.

[5] The antibody variant of [1], which is a variant of a bispecific antibody.

[6] The antibody variant of [1], which is a variant of Emicizumab.

20 [7] A method for detecting the antibody variant of any one of [1] to [6], comprising the step of separating a sample containing an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof.

25 [8] The method for detecting of [7], which uses the antibody variant of any one of [1] to [6] as a reference standard.

[8-2] The method for detecting of [8], comprising the step of conducting one or more analysis selected from the group consisting of quantitative analysis, qualitative analysis, and structural analysis.

30 [9] A pharmaceutical composition comprising the antibody variant of any one of [1] to [6], wherein the percentage of the antibody variant in the total antibody molecules in the pharmaceutical composition is 5% or less.

[10] The pharmaceutical composition of [9], wherein the antibody is Emicizumab.

35 [11] The pharmaceutical composition of [9], which is obtained by a purification process comprising purification by cation exchange chromatography (CEX).

[12] A method for suppressing production of the antibody variant of any one of [1] to [6], comprising the step of culturing antibody producing cells at a pH of 7.1 or more, and/or at a culture temperature of 36°C or less.

[12-2] The method of [12], wherein conditions for culturing the antibody producing cells are changed to pH of 7.1 or more, and/or culture temperature of 36°C or less during culturing.

[13] An isoform of a bispecific antibody comprising a first heavy chain (Q chain, SEQ ID NO: 10) and a second heavy chain (J chain, SEQ ID NO: 11), wherein disulfide bonds are formed in the following:

(1a) between cysteine at position 144 according to EU numbering of the first heavy chain (the 150th position from the N terminus side of SEQ ID NO: 10) and cysteine at position 200 according to EU numbering of the second heavy chain (the 202nd position from the N terminus side of SEQ ID NO: 11); and

(1b) between cysteine at position 200 according to EU numbering of the first heavy chain (the 206th position from the N terminus side of SEQ ID NO: 10) and cysteine at position 144 according to EU numbering of the second heavy chain (the 146th position from the N terminus side of SEQ ID NO: 11), or wherein

disulfide bonds are formed in the following:

(2a) between cysteine at position 226 according to EU numbering of the first heavy chain (the 229th position from the N terminus side of SEQ ID NO: 10) and cysteine at position 229 according to EU numbering of the second heavy chain (the 228th position from the N terminus of SEQ ID NO: 11); and

(2b) between cysteine at position 229 according to EU numbering of the first heavy chain (the 232nd position from the N terminus of SEQ ID NO: 10) and cysteine at position 226 according to EU numbering of the second heavy chain (the 225th position from the N terminus of SEQ ID NO: 11).

[14] The bispecific antibody isoform of [13], wherein disulfide bonds are formed in (1a) and (1b).

[15] An isoform of a bispecific antibody comprising a first heavy chain (Q chain, SEQ ID NO: 10) and a second heavy chain (J chain, SEQ ID NO: 11), characterized in that it elutes at a region more to the alkaline side than the bispecific antibody when separated using cation exchange chromatography.

[16] The bispecific antibody isoform of any one of [13] to [15], which is an isoform of Emicizumab.

[17] A method for detecting the antibody isoform of any one of [13] to [16], comprising the step of separating a sample containing a bispecific antibody by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof.

[18] The method for detecting of [17], which uses the bispecific antibody isoform of any one of [13] to [16] as a reference standard.

[18-2] The method for detecting of [18], comprising the step of conducting one or more analysis selected from the group consisting of quantitative analysis, qualitative analysis, and structural analysis.

[19] A pharmaceutical composition comprising the bispecific antibody isoform of any one of [13] to [16], wherein the percentage of the antibody isoform in the total antibody molecules in the pharmaceutical composition is 2% or less.

[20] A method for reducing the content percentage of the bispecific antibody isoform of any one of [13] to [16], comprising the step of purification by cation exchange chromatography.

[21] The antibody isoform or variant of [1], [13], or [15], wherein the biological activity of the antibody is markedly reduced.

[22] An isoform of an antibody having two variable regions each of which recognizes different epitopes or of a derivative thereof, wherein the isoform has an average Rg value that is smaller by 3% or more, preferably 4% or more, more preferably 5% or more, or still more preferably 6% or more, relative to the antibody or derivative thereof, and/or wherein the isoform has an average Dmax value that is smaller by 5% or more, preferably 6% or more, more preferably 7% or more, or still more preferably 7.5% or more, relative to the antibody or derivative thereof.

[23] An isoform of an antibody having two variable regions each of which recognizes different epitopes or of a derivative thereof, wherein the isoform has an average Rg value that is smaller by 0.15 nm or more, preferably 0.2 nm or more, more preferably 0.25 nm or more, or still more preferably 0.3 nm or more, relative to the antibody or derivative thereof, and/or wherein the isoform has an average Dmax value that is smaller by 0.5 nm or more, preferably 1.0 nm or more, more preferably 1.2 nm or more, or still more preferably 1.4 nm or more, relative to the antibody or derivative thereof.

[24] The isoform of [22] or [23], wherein the isoform has disulfide bond(s) different from those in the antibody or derivative thereof.

[25] The isoform of any one of [22] to [24], wherein the antibody or derivative thereof is Emicizumab (a bispecific antibody that comprises a first heavy chain (Q chain, SEQ ID NO: 10), a second heavy chain (J chain, SEQ ID NO: 11), and common light chains each one of which forms a pair with the first heavy chain or the second heavy chain (SEQ ID NO: 12)).

[26] An isoform of Emicizumab, wherein the isoform has an average Rg value of 4.9 nm or less, or preferably 4.8 nm or less, and/or wherein the isoform has an average Dmax value of 17.0 nm or less, or preferably 16.5 nm or less.

[27] The isoform of [25] or [26], wherein the isoform has disulfide bonds between cysteine at position 144 according to EU numbering of the first heavy chain (the 150th position from the N terminus side of SEQ ID NO: 10) and cysteine at position 200 according to EU numbering of the second heavy chain (the 202nd position from the N terminus side of SEQ ID NO: 11) and between cysteine at position 200 according to EU numbering of the first heavy chain (the 206th position from the N terminus side of SEQ ID NO: 10) and cysteine at position 144 according to EU numbering of the second heavy chain (the 146th position from the N terminus side of SEQ ID NO: 11).

[28] A pharmaceutical composition comprising Emicizumab and the isoform of any one of [22] to [27], wherein the percentage of the isoform in the total antibody molecules in the pharmaceutical composition is 2% or less.

[29] An isoform of a bispecific antibody (Q499-z121/J327-z119/L404-k; Emicizumab), the bispecific antibody comprising a first heavy chain (Q chain, SEQ ID NO: 10), second heavy chain (J chain, SEQ ID NO: 11), and common L chains each one of which forms a pair with the first heavy chain or the second heavy chain (SEQ ID NO: 12), wherein the isoform has a difference in molecular structure from Emicizumab in amino acid residues from position 146 according to EU numbering of the Q chain to position 174 according to EU numbering of the Q chain (the 152nd position to the 180th position from the N terminus side of SEQ ID NO: 10) and amino acid residues from position 146 according to EU numbering of the J chain to position 174 according to EU numbering of the J chain (the 148th position to the 176th position from the N terminus side of SEQ ID NO: 11).

[30] The isoform of [29], wherein the difference in molecular structure is measured as a difference in deuterium exchange rate (%D) in HDX-MS measurement.

[31] A pharmaceutical composition comprising Emicizumab and the isoform of [29] or [30], wherein the percentage of the isoform in the total antibody molecules in the pharmaceutical composition is 2% or less. [0011]

The present disclosure further provides [A1] to [A9] below.

[A1] The method for detecting of [7] or [17], comprising the step of subjecting a sample containing the antibody and/or the antibody variant or isoform to reduction reaction, hydrolysis reaction (digestion reaction), protein denaturation reaction, or a combination thereof.

[A2] The method for detecting of [A1], wherein the reduction reaction is carried out under mild reducing conditions (for example, reduction with DTT in Tris buffer (pH7.0) at 37°C).

[A3] The method for detecting of [A1], wherein the hydrolysis reaction is carried out using a site-specific cleavage enzyme (for example, a sequence-specific protease such as IdeS protease, Lys-C, papain, etc.).

[A4] The method for detecting of any one of [7], [17], and [A1] to [A3], comprising the step of separating a sample containing the antibody, the antibody variant or isoform, a reaction product thereof, or a combination thereof by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or combinations thereof.

[A5] The method for detecting of any one of [8], [18], and [A1] to [A4], comprising the step of analyzing by SE-HPLC analysis, dynamic light scattering (DLS) method, SAXS measurement, electron microscopic measurement, 3D modeling, SPR assay, HDX MS analysis, or a combination thereof.

[A6] A method for quality control of a pharmaceutical composition comprising Emicizumab, comprising the step of [7], [8], [17], [18], or [A1] to [A5] or a step of combining these methods.

[A7] A method for producing a pharmaceutical composition comprising Emicizumab, comprising a step from the method of [A6].

[A8] A method for purifying a composition comprising Emicizumab, characterized in that the method comprises a step of the Bind & Elute mode of cation exchange chromatography (CEX).

[A9] A method for producing a pharmaceutical composition comprising Emicizumab, comprising a step from the method for purifying of [A8].

Effects of the disclosure

[0012]

The present inventors succeeded in identifying antibody variants and isoforms which are contained in a pharmaceutical composition comprising Emicizumab as an active ingredient. The

[CLAIMS]

1. A variant of an antibody that comprises a variable region comprising the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and that exhibits FVIII mimetic activity,
5 wherein
 - (a) the amino acid residue R at the 12th position from the N terminus side of the sequence; or
 - (b) the amino acid residues YYR at the 10th to 12th position from the N terminus side of the sequence,
10 is deleted and the variable region is cleaved at the deletion site.
2. The antibody variant of claim 1, wherein the sequence is a CDR sequence.
3. The antibody variant of claim 1, wherein the sequence is a CDR2 sequence.
15
4. The antibody variant of claim 1, wherein the sequence is a sequence comprised in a heavy chain.
5. The antibody variant of claim 1, which is a variant of a bispecific antibody.
20
6. The antibody variant of claim 1, which is a variant of Emicizumab.
7. A method for detecting the antibody variant of any one of claims 1 to 6, comprising the step of separating a sample containing an antibody that comprises a variable region comprising
25 the amino acid sequence SISPSGQSTYYRREVKG (SEQ ID NO: 2) and the antibody variant according to any one of claims 1 to 6 by affinity chromatography, ion exchange chromatography, normal phase chromatography, reverse phase chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), separation based on charge, size exclusion chromatography (SEC), gel permeation chromatography (GPC), or
30 combinations thereof, and detecting the antibody variant.
8. The method for detecting of claim 7, which uses the antibody variant of any one of claims 1 to 6 as a reference standard.
- 35 9. A pharmaceutical composition comprising the antibody variant of any one of claims 1 to 6, wherein the percentage of the antibody variant in the total antibody molecules in the

pharmaceutical composition is 5% or less and wherein production of the antibody variant is suppressed by a method comprising the step of culturing cells producing the antibody at a pH of 7.1 or more, and/or at a culture temperature of 36°C or less.

- 5 10. The pharmaceutical composition of claim 9, wherein the antibody is Emicizumab.
- 11. The pharmaceutical composition of claim 9, which is obtained by a purification process comprising purification by cation exchange chromatography (CEX).
- 10 12. A method for suppressing production of the antibody variant of any one of claims 1 to 6, comprising the step of culturing cells producing the antibody at a pH of 7.1 or more, and/or at a culture temperature of 36°C or less.