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]

[0007a]

In one aspect, the present disclosure relates to an isoform of a bispecific antibody comprising a first heavy chain and a second heavy chain, wherein disulfide bonds are formed in the following: (1a) between cysteine at position 144 according to EU numbering of the first heavy chain and cysteine at position 200 according to EU numbering of the second heavy chain; and (1b) between cysteine at position 200 according to EU numbering of the first heavy chain and cysteine at position 144 according to EU numbering of the second heavy chain, or wherein disulfide bonds are formed in the following: (2a) between cysteine at position 226 according to EU numbering of the first heavy chain and cysteine at position 229 according to EU numbering of the second heavy chain; and (2b) between cysteine at position 229 according to EU numbering of the first heavy chain and cysteine at position 226 according to EU numbering of the second heavy chain.

[0007b]

In another aspect, the present disclosure relates to an isoform of a bispecific antibody comprising a first heavy chain and a second heavy chain, characterized in that it elutes at a region more to the alkaline side than the bispecific antibody when separated using cation exchange chromatography.

[0007c]

In another aspect, the present disclosure relates to a method for detecting the antibody isoform as disclosed herein, 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.

[0007d]

In yet another aspect, the present disclosure relates to a pharmaceutical composition comprising the bispecific antibody isoform as disclosed herein, wherein the percentage of the antibody isoform in the total antibody molecules in the pharmaceutical composition is 2% or less.

[0007e]

In another aspect, the present disclosure relates to a method for reducing the content percentage of the bispecific antibody isoform as disclosed herein, comprising the step of purification by cation exchange chromatography.

[CLAIMS]

1. An isoform of a bispecific antibody comprising a first heavy chain and a second heavy chain, wherein
disulfide bonds are formed in the following:
(1a) between cysteine at position 144 according to EU numbering of the first heavy chain and cysteine at position 200 according to EU numbering of the second heavy chain; and
(1b) between cysteine at position 200 according to EU numbering of the first heavy chain and cysteine at position 144 according to EU numbering of the second heavy chain, or
wherein
disulfide bonds are formed in the following:
(2a) between cysteine at position 226 according to EU numbering of the first heavy chain and cysteine at position 229 according to EU numbering of the second heavy chain; and
(2b) between cysteine at position 229 according to EU numbering of the first heavy chain and cysteine at position 226 according to EU numbering of the second heavy chain.

2. The bispecific antibody isoform of claim 1, wherein disulfide bonds are formed in (1a) and (1b).

3. An isoform of a bispecific antibody comprising a first heavy chain and a second heavy chain, characterized in that it elutes at a region more to the alkaline side than the bispecific antibody when separated using cation exchange chromatography.

4. The bispecific antibody isoform of any one of claims 1 to 3, which is an isoform of Emicizumab.

5. A method for detecting the antibody isoform of any one of claims 1 to 4, 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.

6. The method for detecting of claim 5, which uses the antibody isoform of any one of claims 1 to 4 as a reference standard.

7. A pharmaceutical composition comprising the bispecific antibody isoform of any one of claims 1 to 4, wherein the percentage of the antibody isoform in the total antibody molecules in the pharmaceutical composition is 2% or less.

8. A method for reducing the content percentage of the bispecific antibody isoform of any one of claims 1 to 4, comprising the step of purification by cation exchange chromatography.

9. The antibody isoform or variant of claim 1 or 3, wherein the biological activity of the antibody is markedly reduced.