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[Summary]

15 [Technical Problem]

[0011]

The above-mentioned efforts to enhance or diminish the agonistic action or effector function of antibody pharmaceuticals are still under development, and more efforts are expected. The present disclosure has been made under these circumstances. An objective of the present
 20 disclosure is to provide novel antigen-binding molecules that have activity of regulating interaction between two or more antigen molecules, or methods for producing or using such antigen-binding molecules. The present disclosure may be useful for screening and development of antibody pharmaceuticals, and may also be applicable to other various protein engineering techniques.

25 [Solution to Problem]

[0012]

In one aspect, the present disclosure relates to an antigen-binding molecule comprising a first antigen-binding domain and a second antigen-binding domain, wherein the antigen-binding domains comprise a hinge region; wherein the first and second antigen-binding domains each
 30 comprises an antibody fragment which binds to a particular antigen; wherein the first antigen-binding domain and the second antigen-binding domain are linked with each other via two or more disulfide bonds; wherein

1) at least one of the disulfide bonds linking the first antigen-binding domain and the second antigen-binding domain is formed by linking cysteine residues

35 a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,

b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or

c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

5 wherein at least one of the cysteine residues from which the disulfide bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the antibody fragment, and/or

10 (ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment, and

2) at least one disulfide bond is present within the hinge region, and wherein said antigen-binding molecule has increased resistance to protease cleavage as compared to a control
15 antigen-binding molecule, wherein the control antigen-binding molecule differs from said antigen-binding molecule only in that the control antigen-binding molecule has one less bond between the two antigen-binding domains.

In another aspect, the present disclosure relates to a pharmaceutical composition
20 comprising the antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure relates to a method for regulating interaction between two antigen molecules, comprising:

25 (a) providing an antigen-binding molecule comprising two antigen-binding domains, wherein each of the antigen-binding domains comprise a hinge region and an antibody fragment binding to a particular antigen,

(b) adding to the antigen-binding molecule at least one disulfide bond which links the two antigen-binding domains with each other such that the antigen-binding domains are linked with
30 each other via two or more disulfide bonds, wherein

1) at least one of the cysteine residues from which the disulfide bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the
35 antibody fragment, and/or

(ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment, and wherein the at least one disulfide bond is formed by linking the cysteine residue

a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,

b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or

c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

2) at least one of the two or more disulfide bonds is present within the hinge region, and (c) contacting the antigen-binding molecule produced in (b) with the two antigen molecules, and wherein

said antigen-binding molecule has increased resistance to protease cleavage as compared to a control antigen-binding molecule, wherein the control antigen-binding molecule differs from said antigen-binding molecule only in that the control antigen-binding molecule has one less bond between the two antigen-binding domains.

In another aspect, the present disclosure relates to a method for producing an antigen-binding molecule which has activity of regulating interaction between two antigen molecules, comprising:

(a) providing a nucleic acid encoding a polypeptide comprising a first antigen-binding domain and a nucleic acid encoding a polypeptide comprising a second antigen-binding domain, wherein each of the antigen-binding domains comprises a hinge region and an antibody fragment binding to a particular antigen,

(b) introducing a mutation into the nucleic acids encoding the two antigen-binding domains such that at least one disulfide bond linking the two antigen-binding domains is added, and such that the antigen-binding domains are linked with each other via two or more disulfide bonds, wherein

1) at least one of the cysteine residues from which the bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the antibody fragment, and/or

(ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment,

and wherein the at least one disulfide bond is formed by linking the cysteine residue

- a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,
- b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or
- 5 c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

2) at least one of the two or more disulfide bonds is present within the hinge region,
(c) introducing the nucleic acids produced in (b) into a host cell,

(d) culturing the host cell such that the two polypeptides are expressed, and

- 10 (e) obtaining an antigen-binding molecule which is a polypeptide comprising the first and second antigen-binding domains, wherein the two antigen-binding domains are linked with each other via two or more disulfide bonds, and wherein

said antigen-binding molecule has increased resistance to protease cleavage as compared to a control antigen-binding molecule, wherein the control antigen-binding molecule
15 differs from said antigen-binding molecule only in that the control antigen-binding molecule has one less bond between the two antigen-binding domains.

In one non-limiting example, the present inventors modified antigen-binding molecules (e.g., antibodies) which have agonist activity and contain two antigen-binding domains (e.g., Fab
20 portions) by introducing amino acid mutations into the antigen-binding domains, and produced molecules in which the antigen-binding domains were linked with each other. As a result, the inventors found that the agonist activity of the molecules was greatly improved. In addition, in one non-limiting example, the present inventors found antigen-binding molecules which acquired resistance to protease digestion through linkage between the antigen-binding domains.

25 [0013]

The present disclosure is based on these findings, and specifically encompasses examples exemplified below.

Claims

1. An antigen-binding molecule comprising a first antigen-binding domain and a second antigen-binding domain,

5 wherein the antigen-binding domains comprise a hinge region;
 wherein the first and second antigen-binding domains each comprises an antibody fragment which binds to a particular antigen;

 wherein the first antigen-binding domain and the second antigen-binding domain are linked with each other via two or more disulfide bonds;

10 wherein

1) at least one of the disulfide bonds linking the first antigen-binding domain and the second antigen-binding domain is formed by linking cysteine residues

a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,

15 b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or

c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

wherein at least one of the cysteine residues from which the disulfide bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the antibody fragment, and/or

(ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment, and

2) at least one disulfide bond is present within the hinge region, and

wherein said antigen-binding molecule has increased resistance to protease cleavage as compared to a control antigen-binding molecule, wherein the control antigen-binding molecule differs from said antigen-binding molecule only in that the control antigen-binding molecule has one less bond between the two antigen-binding domains.

2. The antigen-binding molecule of claim 1, wherein the antibody fragment is a Fab, Fab', or scFab.

3. The antigen-binding molecule of claim 1 or 2, wherein the antigen-binding domain comprises an Fc region.

4. The antigen-binding molecule of any one of claims 1 to 3, which has activity of regulating interaction between two antigen molecules.

5. A pharmaceutical composition comprising the antigen-binding molecule of any one of claims 1 to 4 and a pharmaceutically acceptable carrier.

6. A method for regulating interaction between two antigen molecules, comprising:

(a) providing an antigen-binding molecule comprising two antigen-binding domains, wherein each of the antigen-binding domains comprise a hinge region and an antibody fragment binding to a particular antigen,

(b) adding to the antigen-binding molecule at least one disulfide bond which links the two antigen-binding domains with each other such that the antigen-binding domains are linked with each other via two or more disulfide bonds, wherein

1) at least one of the cysteine residues from which the disulfide bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the antibody fragment, and/or

(ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment,

and wherein the at least one disulfide bond is formed by linking the cysteine residue

a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,

b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or

c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

2) at least one of the two or more disulfide bonds is present within the hinge region, and

(c) contacting the antigen-binding molecule produced in (b) with the two antigen molecules, and wherein

said antigen-binding molecule has increased resistance to protease cleavage as compared to a control antigen-binding molecule, wherein the control antigen-binding molecule

differs from said antigen-binding molecule only in that the control antigen-binding molecule has one less bond between the two antigen-binding domains.

7. A method for producing an antigen-binding molecule which has activity of regulating interaction between two antigen molecules, comprising:

(a) providing a nucleic acid encoding a polypeptide comprising a first antigen-binding domain and a nucleic acid encoding a polypeptide comprising a second antigen-binding domain, wherein each of the antigen-binding domains comprises a hinge region and an antibody fragment binding to a particular antigen,

(b) introducing a mutation into the nucleic acids encoding the two antigen-binding domains such that at least one disulfide bond linking the two antigen-binding domains is added, and such that the antigen-binding domains are linked with each other via two or more disulfide bonds, wherein

1) at least one of the cysteine residues from which the bonds between the antigen-binding domains originate is present

(i) at any one of positions 119 to 123, 131 to 140, 148 to 150, 155 to 167, 174 to 178, 188 to 197, 201 to 214, or 218 to 219, according to EU numbering, in the CH1 region of the antibody fragment, and/or

(ii) at any one of positions 108 to 112, 121 to 128, 151 to 156, 184 to 190, 195 to 196, 200 to 203, or 208 to 213, according to Kabat numbering, in the CL region of the antibody fragment,

and wherein the at least one disulfide bond is formed by linking the cysteine residue

a) in the CH1 region of the first antibody fragment and the CH1 region of the second antibody fragment,

b) in the CL region of the first antibody fragment and the CL region of the second antibody fragment, or

c) in the CH1 region of the first antibody fragment and the CL region of the second antibody fragment, and

2) at least one of the two or more disulfide bonds is present within the hinge region,

(c) introducing the nucleic acids produced in (b) into a host cell,

(d) culturing the host cell such that the two polypeptides are expressed, and

(e) obtaining an antigen-binding molecule which is a polypeptide comprising the first and second antigen-binding domains, wherein the two antigen-binding domains are linked with each other via two or more disulfide bonds, and wherein

said antigen-binding molecule has increased resistance to protease cleavage as

compared to a control antigen-binding molecule, wherein the control antigen-binding molecule differs from said antigen-binding molecule only in that the control antigen-binding molecule has

one less bond between the two antigen-binding domains.