[0019]

Specifically, the present invention relates to a protein having an affinity for an immunoglobulin, comprising an amino acid sequence obtained by introducing, into an amino acid sequence derived from C domain of Protein A of SEQ ID No: 5, at least one amino acid substitution at any one or more of amino acid residues at Ser as an amino acid residue corresponding to position 33 of the C domain, or Asp as an amino acid residue corresponding to position 36 of the C domain, the protein having a lower affinity for an Fab region of an immunoglobulin than a protein before introduction of the substitution, wherein the protein has a sequence identity of not less than 90% to the amino acid sequence of SEQ ID NO:5.

[0019a]

Also disclosed is a protein having an affinity for an immunoglobulin, including an amino acid sequence obtained by introducing, into an amino acid sequence derived from at least one domain selected from E, D, A, B and C domains of Protein A of SEQ ID Nos:1 to 5, at least one amino acid substitution at any one or more of amino acid residues at positions 31 to 37 of the A, B and C domains, amino acid residues at positions 29 to 35 of the E domain, and amino acid residues at positions 34 to 40 of the D domain, the protein having a lower affinity for an Fab region of an immunoglobulin than a protein before introduction of the substitution.

[0020]

Preferably, the amino acid sequence derived from at least one domain before introduction of the substitution is any of amino acid sequences of the E, D, A, B and C domains of Protein A of SEQ ID Nos:1 to 5 and amino acid sequences of SEQ ID Nos:6 to 10 derived from the E, D, A, B and C domains of Protein A.

[0021]

Preferably, the amino acid sequence derived from at least one domain before introduction of the substitution is an amino acid sequence of the C domain of Protein A of SEQ ID No:5 or an amino acid sequence of SEQ ID No:10 derived from the C domain of Protein A.

[0022]

Preferably, the domain of the amino acid sequence before introduction of the substitution contains Ser as an amino acid residue corresponding to position 33 of the C domain, Lys as an amino acid residue corresponding to position 35 of the C domain, Asp as an amino acid residue corresponding to position 36 of the C domain, or Asp as an amino acid residue corresponding to position 37 of the C domain.

[0023]

Preferably, the amino acid substitution to be introduced is a substitution of Glu, Leu or Thr for Ser corresponding to

# CLAIMS

Claim 1. A protein having an affinity for an immunoglobulin, comprising an amino acid sequence obtained by introducing, into an amino acid sequence derived from at least one domain selected from E, D, A, B and C domains of Protein A of SEQ ID Nos:1 to 5, at least one amino acid substitution at any one or more of amino acid residues at positions 31 to 37 of the A, B and C domains, amino acid residues at positions 29 to 35 of the E domain, and amino acid residues at positions 34 to 40 of the D domain,

the protein having a lower affinity for an Fab region of an immunoglobulin than a protein before introduction of the substitution.

Claim 2. The protein according to claim 1,

wherein the amino acid sequence derived from at least one domain before introduction of the substitution is any of amino acid sequences of the E, D, A, B and C domains of Protein A of SEQ ID Nos:1 to 5 and amino acid sequences of SEQ ID Nos:6 to 10 derived from the E, D, A, B and C domains of Protein A.

Claim 3. The protein according to claim 1 or 2,

wherein the amino acid sequence derived from at least one domain before introduction of the substitution is an amino acid sequence of the C domain of Protein A of SEQ ID No:5 or an amino acid sequence of SEQ ID No:10 derived from the C domain of Protein A.

Claim 4. The protein according to any one of claims 1 to 3,

wherein the domain of the amino acid sequence before introduction of the substitution comprises Ser as an amino acid residue corresponding to position 33 of the C domain, Lys as an amino acid residue corresponding to position 35 of the C domain, Asp as an amino acid residue corresponding to position 36 of the C domain, or Asp as an amino acid residue corresponding

to position 37 of the C domain.

Claim 5. The protein according to any one of claims 1 to 4, wherein the amino acid substitution to be introduced is a substitution of Glu, Leu or Thr for Ser corresponding to position 33 of the C domain,

a substitution of Arg for Lys corresponding to position 35 of the C domain,

a substitution of Arg or Ile for Asp corresponding to position 36 of the C domain, or

a substitution of Glu for Asp corresponding to position 37 of the C domain.

Claim 6. The protein according to claim 5, wherein the amino acid substitution to be introduced is a substitution of Glu for Ser corresponding to position 33 of the C domain.

Claim 7. A multi-domain protein, comprising two or more proteins according to any one of claims 1 to 6, connected together.

Claim 8. The multi-domain protein according to claim 7, wherein the proteins connected together are different from one another.

Claim 9. The multi-domain protein according to claim 7 or 8, wherein the number of the proteins connected together is 2 to 5.

Claim 10. A DNA encoding the protein according to any one of claims 1 to 9.

Claim 11. The DNA according to claim 10,

wherein ligated base sequences each comprising the domain have a sequence identity of not higher than 90% to one another.

Claim 12. A vector comprising the DNA according to claim 10 or 11.

Claim 13. A transformant which is obtainable by transformation of a host with the vector according to 12.

Claim 14. The transformant according to claim 13, wherein the host is a gram-positive bacterium.

Claim 15. The transformant according to claim 14, wherein the gram-positive bacterium is a bacterium of *Brevibacillus*.

Claim 16. The transformant according to claim 15, wherein the bacterium of *Brevibacillus* is *Brevibacillus choshinensis*.

Claim 17. A method for producing the protein according to any one of claims 1 to 9, the method comprising utilizing the transformant according to any one of claims 13 to 16, or a cell-free protein synthesis system using the DNA according to claim 10 or 11.

Claim 18. The production method according to claim 17, comprising:

accumulating the protein intracellularly and/or in a periplasmic space of the transformant; and/or

secreting the protein extracellularly from the transformant.

Claim 19. An affinity separation matrix, comprising:

the protein according to any one of claims 1 to 9 as an

affinity ligand, and

a carrier made of a water-insoluble base material on which the protein is immobilized.

Claim 20. The affinity separation matrix according to claim 19,

wherein the water-insoluble base material comprises a synthetic polymer or a polysaccharide.

Claim 21. The affinity separation matrix according to claim 20,

wherein the polysaccharide is cellulose.

Claim 22. The affinity separation matrix according to any one of claims 19 to 21,

wherein the affinity separation matrix binds to a protein containing an Fc region of an immunoglobulin.

Claim 23. The affinity separation matrix according to claim 22,

wherein the protein containing an Fc region of an immunoglobulin is an immunoglobulin G or an immunoglobulin G derivative.

Claim 24. Use of the affinity separation matrix according to any one of claims 19 to 23 for separation of a protein containing an Fc region of an immunoglobulin.

Claim 25. The use of the affinity separation matrix according to claim 24,

wherein the separation of a protein containing an Fc region of an immunoglobulin is aimed at separating and recovering a protein consisting only of the Fab region of an immunoglobulin.

CLAIMS

Claim 1. A protein having an affinity for an immunoglobulin, comprising an amino acid sequence obtained by introducing, into an amino acid sequence derived from C domain of Protein A of SEQ ID No: 5, at least one amino acid substitution at any one or more of amino acid residues at Ser as an amino acid residue corresponding to position 33 of the C domain, or Asp as an amino acid residue corresponding to position 36 of the C domain,

the protein having a lower affinity for an Fab region of an immunoglobulin than a protein before introduction of the substitution,

wherein the protein has a sequence identity of not less than 90% to the amino acid sequence of SEQ ID NO:5.

Claim 2. The protein according to claim 1,

wherein the amino acid sequence derived from at least one domain before introduction of the substitution is any of amino acid sequences of the E, D, A, B and C domains of Protein A of SEQ ID Nos:1 to 5 and amino acid sequences of SEQ ID Nos:6 to 10 derived from the E, D, A, B and C domains of Protein A.

Claim 3. The protein according to claim 1 or 2,

Wherein the amino acid sequence derived from at least one domain before introduction of the substitution is an amino acid sequence of the C domain of Protein A of SEQ ID No:5 or an amino acid sequence of SEQ ID No:10 derived from the C domain of Protein A.

Claim 4. The protein according to any one of claims1 to 3,

wherein the amino acid sequence of the domain before introduction of the substitution comprises Ser as an amino acid residue corresponding to position 33 of the C domain, Lys as an amino acid residue corresponding to position 35 of the C domain, Asp as an amino acid residue corresponding to position 36 of the C domain, or Asp as an amino acid residue corresponding to position

37 of the C domain.

Claim 5. The protein according to any one of claims 1 to 4,
Wherein the amino acid substitution to be introduced is a substitution of Glu, Leu or Thr for Ser corresponding to position 33 of the C domain,
a substitution of Arg for Lys corresponding to position 35 of the C domain,
a substitution of Arg or Ile for Asp corresponding to position 36 of the C domain, or
a substitution of Glu for Asp corresponding to position 37 of the C domain.

Claim 6. The protein according to claim 5,
wherein the amino acid substitution to be introduced is a substitution of Glu for Ser corresponding to position 33 of the C domain.

Claim 7. A multi-domain protein, comprising two or more proteins according to any one of claims 1 to 6, connected together.

Claim 8. The multi-domain protein according to claim 7,
Wherein the proteins connected together are different from one another.

Claim 9. The multi-domain protein according to claim 7 or 8,
wherein the number of the proteins connected together is 2 to 5.

Claim 10. A DNA encoding the protein according to any one of claims 1 to 9.

Claim 11. The DNA according to claim 10,
wherein ligated base sequences each comprising the domain have a sequence identity of not higher than 90% to one another.

Claim 12. A vector comprising the DNA according to claim 10 or 11.

Claim 13. A transformant which is obtainable by transformation of a host with the vector according to claim 12.

Claim 14. The transformant according to claim 13,
Wherein the host is a gram-positive bacterium.

Claim 15. The transformant according to claim 14,
wherein the gram-positive bacterium is a bacterium of *Brevibacillus*.

Claim 16. The transformant according to claim 15,
wherein the bacterium of *Brevibacillus* is *Brevibacillus choshinensis*.

Claim 17. A method for producing the protein according to any one of claims 1 to 9, the method comprising utilizing the transformant according to any one of claims 13 to 16, or a cell-free protein synthesis system using the DNA according to claim 10 or 11.

Claim 18. The production method according to claim 17, comprising:
accumulating the protein intracellularly or in a periplasmic space of the transformant; and/or
secreting the protein extracellularly from the transformant.

Claim 19. An affinity separation matrix, comprising:
the protein according to any one of claims 1 to 9 as an affinity ligand, and
a carrier made of a water-insoluble base material on which the protein is immobilized.

Claim 20. The affinity separation matrix according to claim 19, wherein the water-insoluble base material comprises a synthetic polymer or a polysaccharide.

Claim 21. The affinity separation matrix according to claim 20, wherein the polysaccharide is cellulose.

Claim 22. The affinity separation matrix according to any one of claims 19 to 21,
wherein the affinity separation matrix binds to a protein containing an Fc region of an immunoglobulin.

Claim 23. The affinity separation matrix according to claim 22, wherein the protein containing an Fc region of an immunoglobulin is an immunoglobulin G or an immunoglobulin G derivative.

Claim 24. Use of the affinity separation matrix according to any one of claims 19 to 23 for separation of a protein containing an Fc region of an immunoglobulin.

Claim 25. The use of the affinity separation matrix according to claim 24,
wherein the separation of a protein containing an Fc region of an immunoglobulin is aimed at separating and recovering a protein consisting only of the Fab region of an immunoglobulin.