CLAIMS

1. A modified biotin-binding protein comprising an amino acid sequence represented by SEQ ID NO: 2, or an amino acid sequence having an identity of not less than 95% to the amino acid sequence of SEQ ID NO: 2 and having a biotin-binding activity, the modified biotin-binding protein having replacement selected from the group consisting of:

1) replacement of the 36th serine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

2) replacement of the 80th tryptophan residue of SEQ ID NO: 2 with a hydrophilic amino acid residue;

3) replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

4) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue and replacement of the 78th threonine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

5) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue and replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid that does not form a hydrogen bond; and

6) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue, replacement of the 78th threonine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond, and replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid that does not form a hydrogen bond,

wherein modified biotin-binding protein satisfies at least one of the following properties:

i) allowing purification using biotin;

ii) maintaining a tetramer structure of a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

iii) having protease resistance; and

iv) showing high expression in a soluble fraction of Escherichia. Coli, and,

wherein modified biotin-binding protein satisfies at least one of the following provisions a) to p):

a) the 14th asparagine residue of SEQ ID NO: 2 is not modified or is replaced with glutamine or aspartic acid;

b) the 18th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

c) the 34th tyrosine residue of SEQ ID NO: 2 is not modified or is replaced with serine or threonine;

d) the 36th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

e) the 40th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with a residue other than asparagine;

f) the 69th tryptophan residue of SEQ ID NO: 2 is not modified;

g) the 76th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

h) the 78th threonine residue of SEQ ID NO: 2 is not modified or is replaced with serine or tyrosine;

i) the 80th tryptophan residue of SEQ ID NO: 2 is not modified;

j) the 96th tryptophan residue of SEQ ID NO: 2 is not modified;

k) the 108th tryptophan residue of SEQ ID NO: 2 is not modified;

l) the 116th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with glutamic acid or asparagine;

m) the 46th proline residue of SEQ ID NO: 2 is not modified;

n) the 66th alanine residue of SEQ ID NO: 2 is not modified;

o) the 97th leucine residue of SEQ ID NO: 2 is not modified or is modified to isoleucine; and

p) the 113th valine residue of SEQ ID NO: 2 is not modified,

wherein the amino acid residues specified in 1) to 6) are replaced as in specified in 1) to 6).

2. The modified biotin-binding protein according to Claim 1, selected from the group consisting of:

1-a) a modified biotin-binding protein (TM2 S36A) in which the 36th serine residue of SEQ ID NO: 2 is replaced with alanine;

2-a) a modified biotin-binding protein (TM2 W80K) in which the 80th tryptophan residue of SEQ ID NO: 2 is replaced with lysine;

3-a) a modified biotin-binding protein (TM2 D116A) in which the 116th aspartic acid residue of SEQ ID NO: 2 is replaced with alanine;

4-a) a modified biotin-binding protein (TM2 P46T-T78A) in which the 46th proline residue of SEQ ID NO: 2 is replaced with threonine and the 78th threonine residue of SEQ ID NO: 2 is replaced with alanine;

5-a) a modified biotin-binding protein (TM2 P46T-D116A) in which the 46th proline residue of SEQ ID NO: 2 is replaced with threonine and the 116th aspartic acid residue of SEQ ID NO: 2 is replaced with alanine; and

6-a) a modified biotin-binding protein (TM2 P46T-T78A-D116A) in which the 46th proline residue of SEQ ID NO: 2 is replaced with threonine, the 78th threonine residue of SEQ ID NO: 2 is replaced with alanine, and the 116th aspartic acid residue of SEQ ID NO: 2 is replaced with alanine.

3. A modified biotin-binding protein comprising an amino acid sequence represented by SEQ ID NO: 2, or an amino acid sequence having an identity of not less than 95% to the amino acid sequence of SEQ ID NO: 2 and having

a biotin-binding activity, the modified biotin-binding protein comprising:

6) replacement of the 78th threonine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond,

wherein the modified biotin-binding protein satisfies at least one of the following properties:

i) allowing purification using biotin;

ii) maintaining a tetramer structure of a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

iii) having protease resistance; and

v) having heat resistance higher than that of a protein comprising the amino acid sequence represented by SEQ ID NO: 2, and,

wherein modified biotin-binding protein satisfies at least one of the following provisions a) to p):

a) the 14th asparagine residue of SEQ ID NO: 2 is not modified or is replaced with glutamine or aspartic acid;

b) the 18th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

c) the 34th tyrosine residue of SEQ ID NO: 2 is not modified or is replaced with serine or threonine;

d) the 36th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

e) the 40th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with a residue other than asparagine;

f) the 69th tryptophan residue of SEQ ID NO: 2 is not modified;

g) the 76th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

h) the 78th threonine residue of SEQ ID NO: 2 is not modified or is replaced with serine or tyrosine;

i) the 80th tryptophan residue of SEQ ID NO: 2 is not modified;

j) the 96th tryptophan residue of SEQ ID NO: 2 is not modified;

k) the 108th tryptophan residue of SEQ ID NO: 2 is not modified;

l) the 116th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with glutamic acid or asparagine;

m) the 46th proline residue of SEQ ID NO: 2 is not modified;

n) the 66th alanine residue of SEQ ID NO: 2 is not modified;

o) the 97th leucine residue of SEQ ID NO: 2 is not modified or is modified to isoleucine; and

p) the 113th valine residue of SEQ ID NO: 2 is not modified,

wherein the amino acid residues specified in 6) are replaced as in specified in 6).

4. The modified biotin-binding protein according to Claim 3, wherein

6-a) the 78th threonine residue of SEQ ID NO: 2 is replaced with alanine residue (TM2 T78A).

5. The modified biotin-binding protein according to any one of Claims 1 to 4, showing a biotin-binding affinity lower than that of a protein comprising the amino acid sequence represented by SEQ ID NO: 2.

6. The modified biotin-binding protein according to any one of Claims 1 to 5, comprising an amino acid sequence having an identity of not less than 98% to the amino acid sequence represented by SEQ ID NO: 2.

7. A carrier to which the protein according to any one of Claims 1 to 6 is immobilized.

8. A nucleic acid encoding the protein according to any one of Claims 1 to 6.

9. A vector containing the nucleic acid according to Claim 8.

CLAIMS

1. A vector containing a nucleic acid encoding a

modified biotin-binding protein comprising an amino acid sequence represented by SEQ ID NO: 2, or an amino acid sequence having an identity of not less than 95% to the amino acid sequence of SEQ ID NO: 2 and having a biotin-binding activity, the modified biotin-binding protein having replacement selected from the group consisting of:

1) replacement of the 36th serine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

2) replacement of the 80th tryptophan residue of SEQ ID NO: 2 with a hydrophilic amino acid residue;

3) replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

4) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue and replacement of the 78th threonine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond;

5) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue and replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid that does not form a hydrogen bond; and

6) replacement of the 46th proline residue of SEQ ID NO: 2 with a threonine, serine, or tyrosine residue, replacement of the 78th threonine residue of SEQ ID NO: 2 with an amino acid residue that does not form a hydrogen bond, and replacement of the 116th aspartic acid residue of SEQ ID NO: 2 with an amino acid that does not form a hydrogen bond,

wherein modified biotin-binding protein satisfies at least one of the following properties:

i) allowing purification using biotin;

ii) maintaining a tetramer structure of a protein comprising the amino acid sequence represented by SEQ ID NO: 2;

iii) having protease resistance; and

iv) showing high expression in a soluble fraction of Escherichia. Coli, and,

wherein modified biotin-binding protein satisfies at least one of the following provisions a) to p):

a) the 14th asparagine residue of SEQ ID NO: 2 is not modified or is replaced with glutamine or aspartic acid;

b) the 18th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

c) the 34th tyrosine residue of SEQ ID NO: 2 is not modified or is replaced with serine or threonine;

d) the 36th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

e) the 40th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with a residue other than asparagine;

f) the 69th tryptophan residue of SEQ ID NO: 2 is not modified;

g) the 76th serine residue of SEQ ID NO: 2 is not modified or is replaced with threonine or tyrosine;

h) the 78th threonine residue of SEQ ID NO: 2 is not modified or is replaced with serine or tyrosine;

i) the 80th tryptophan residue of SEQ ID NO: 2 is not modified;

j) the 96th tryptophan residue of SEQ ID NO: 2 is not modified;

k) the 108th tryptophan residue of SEQ ID NO: 2 is not modified;

l) the 116th aspartic acid residue of SEQ ID NO: 2 is not modified or is replaced with glutamic acid or asparagine;

m) the 46th proline residue of SEQ ID NO: 2 is not modified;

n) the 66th alanine residue of SEQ ID NO: 2 is not modified;

o) the 97th leucine residue of SEQ ID NO: 2 is not modified or is modified to isoleucine; and

p) the 113th valine residue of SEQ ID NO: 2 is not modified,

wherein the amino acid residues specified in 1) to 6) are replaced as in specified in 1) to 6).