Claims

[Claim 1]

An EndoS mutant enzyme comprising an amino acid sequence of amino acid numbers 37 to 995 of SEQ ID NO: 2 with an additional mutation at at least one amino acid position selected from the group consisting of: amino acid 122 (H122), amino acid 184 (P184), amino acid 279 (D279), amino acid 282 (Y282), amino acid 303 (Q303), amino acid 348 (Y348), amino acid 350 (E350), amino acid 402 (Y402), amino acid 405 (D405), and amino acid 406 (R406), and exhibiting a reduced hydrolysis activity in comparison with the activity of EndoS D233Q, the activity being an activity on an N-linked sugar chain (N297-linked sugar chain) linked to Asn at position 297 in IgG.

[Claim 2]

The EndoS mutant enzyme according to claim 1, wherein the additional mutation is at 1 to 4 amino acid positions selected from the group consisting of: H122, P184, D279, Y282, Q303, Y348, E350, Y402, D405, and R406.

[Claim 3]

The EndoS mutant enzyme according to claim 1, wherein the additional mutation is a mutation at one amino acid position selected from the group consisting of: Q303, E350, and D405.

[Claim 4]

The EndoS mutant enzyme according to any one of claims 1 to 3, wherein the additional mutation is at least one selected from the following group:
a mutation at H122 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, Glu, Asp, Leu, Ile, Pro, Met, or Phe;
a mutation at P184 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, Gln, or Asn;
a mutation at D279 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, or Gln;
a mutation at Y282 to the amino acids: Arg, Lys, or His;
a mutation at Q303 to the amino acids: Met, Pro, or Leu;
a mutation at Y348 to the amino acids: His or Trp;
a mutation at E350 to the amino acids: Lys, Arg, His, Tyr, Gln, Asn, Gly, Cys, Thr, Ser, Val, Pro, or Ala;
a mutation at Y402 to the amino acids: Phe or Trp;
a mutation at D405 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, or Ala; and
a mutation at R406 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, Glu, Asp, Gln, or Asn.

[Claim 5]

The EndoS mutant enzyme according to claim 4, wherein the additional mutation is at least one selected from the following group:
a mutation at H122 to Ala (H122A) or Phe (H122F);
a mutation at P184 to Gln (P184Q);
a mutation at D279 to Ser (D279S) or Gln (D279Q);
a mutation at Y282 to Arg (Y282R);

a mutation at Q303 to Leu (Q303L);

a mutation at Y348 to His (Y348H);

a mutation at E350 to Ala (E350A), Asn (E350N), Asp (E350D), or Gln (E350Q);

a mutation at Y402 to Phe (Y402F) or Trp (Y402W);

a mutation at D405 to Ala (D405A); and

a mutation at R406 to Gln (R406Q).

[Claim 6]

The EndoS mutant enzyme according to claim 5, comprising at least one mutation selected from the group consisting of Q303L, E350A, E350N, E350D, E350Q, and D405A as the additional mutation.

[Claim 7]

The EndoS mutant enzyme according to claim 6, wherein the additional mutation is Q303L, E350A, E350N, E350D, E350Q, D405A,

H122A/Q303L, H122F/Q303L, P184Q/Q303L, D279Q/Q303L, D279S/Q303L, Y282R/Q303L, Q303L/Y348H, Q303L/E350A, Q303L/E350N, Q303L/E350D, Q303L/E350Q, Q303L/Y402F, Q303L/Y402W, Q303L/D405A, Q303L/R406Q,

H122A/E350A, H122F/E350A, P184Q/E350A, D279Q/E350A, D279S/E350A, Y282R/E350A, Y348H/E350A, E350A/Y402F, E350A/Y402W, E350A/D405A, E350A/R406Q,

H122A/E350N, H122F/E350N, P184Q/E350N, D279Q/E350N, D279S/E350N, Y282R/E350N, Y348H/E350N, E350N/Y402F, E350N/Y402W, E350N/D405A, E350N/R406Q,

H122A/E350D, H122F/E350D, P184Q/E350D, D279Q/E350D, D279S/E350D, Y282R/E350D, Y348H/E350D, E350D/Y402F, E350D/Y402W, E350D/D405A, E350D/R406Q, H122A/E350Q, H122F/E350Q, P184Q/E350Q, D279Q/E350Q, D279S/E350Q, Y282R/E350Q, E350Q/Y348H, E350Q/Y402F, E350Q/Y402W, E350Q/D405A, E350Q/R406Q, H122A/D405A, H122F/D405A, P184Q/D405A, D279Q/D405A, D279S/D405A, Y282R/D405A, Y348H/D405A, or D405A/R406Q.

[Claim 8]

The EndoS mutant enzyme according to claim 7, wherein the additional mutation is Q303L, E350A, E350N, E350D, E350Q, D405A, Q303L/E350A, Q303L/E350N, Q303L/E350D, Q303L/E350Q, Q303L/D405A, E350A/D405A, E350N/D405A, E350D/D405A, or E350Q/D405A.

[Claim 9]

The EndoS mutant enzyme according to claim 4, wherein the additional mutation is H122A, H122F, P184Q, D279Q, D279S, Y282R, Y348H, Y402F, Y402W, or R406Q.

[Claim 10]

The EndoS mutant enzyme according to claim 1, wherein the activity on the N297-linked sugar chain keeps a hydrolysis rate of 50% or less in a hydrolysis reaction in a reaction solution at pH 7.4 after 24 hours.

[Claim 11]

The EndoS mutant enzyme according to claim 1, wherein the EndoS mutant enzyme further exhibits an activity on the N297-linked sugar chain leading to a

percent transglycosylation of approximately 60% or more after 48 hours of a transglycosylation reaction with 5 equivalents of a sugar donor in a reaction solution at pH 7.4.

[Claim 12]

A polynucleotide encoding an EndoS mutant enzyme according to any one of claims 1 to 11.

[Claim 13]

A vector comprising a nucleotide complementary to the polynucleotide according to claim 12.

[Claim 14]

A host cell transformed with a vector according to claim 13.

[Claim 15]

A method of production of a molecule comprising a sugar-remodeled Fc region, comprising reacting a molecule comprising an Fc region of IgG having a core GlcNAc optionally with fucose addition as an N297-linked sugar chain with a sugar donor having a structure comprising an oxazolinated GlcNAc in the presence of an EndoS mutant enzyme according to any one of claims 1 to 11 to produce a molecule comprising an Fc region with a N297-linked sugar chain having a structure in which a sugar chain possessed by the sugar donor is transferred to the core GlcNAc of the N297-linked sugar chain.

[Claim 16]

The method of production according to claim 15, wherein the molecule comprising an Fc region is an IgG monoclonal antibody.

Claims

[Claim 1]

An EndoS mutant enzyme according to one of the following:

1) an EndoS mutant enzyme comprising an amino acid sequence of amino acid numbers 37 to 995 of SEQ ID NO: 2 with one or more mutations at at least one amino acid position selected from the group consisting of: amino acid 122 (H122), amino acid 279 (D279), amino acid 282 (Y282), amino acid 303 (Q303), amino acid 348 (Y348), amino acid 350 (E350), amino acid 402 (Y402), amino acid 405 (D405), and amino acid 406 (R406), and exhibiting a reduced hydrolysis activity in comparison with the activity of EndoS D233Q, the activity being an activity on an N-linked sugar chain (N297-linked sugar chain) linked to Asn at position 297 in IgG; or

2) an EndoS mutant enzyme according to 1) wherein 1 to 20 amino acids are substituted, deleted, inserted, and/or added at positions other than at the positions of amino acid numbers 98 to 445 and 765 to 923 of SEQ ID NO: 2;

wherein the EndoS mutant enzyme according to 1) or 2) has transglycosylation activity at a level equivalent to or higher than that of EndoS D233Q.

[Claim 2]

The EndoS mutant enzyme according to claim 1, wherein the mutations are at 1 to 4 amino acid positions selected from the group consisting of: H122, D279, Y282, Q303, Y348, E350, Y402, D405, and R406.

[Claim 3]

The EndoS mutant enzyme according to claim 1, wherein the mutation is a mutation at one amino acid position selected from the group consisting of: Q303, E350, and D405.

[Claim 4]

The EndoS mutant enzyme according to claim 1 or 2, wherein the mutation is at least one selected from the following group:

a mutation at H122 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, Glu, Asp, Leu, Ile, Met, or Phe;

a mutation at D279 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, or Gln;

a mutation at Y282 to the amino acids: Arg, Lys, or His;

a mutation at Q303 to the amino acids: Met, Pro, or Leu;

a mutation at Y348 to the amino acids: His or Trp;

a mutation at E350 to the amino acids: Lys, Arg, His, Tyr, Gln, Asn, Asp, Gly, Cys, Thr, Ser, Val, Pro, or Ala;

a mutation at Y402 to the amino acids: Phe or Trp;

a mutation at D405 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, or Ala; and

a mutation at R406 to the amino acids: Gly, Cys, Thr, Ser, Val, Pro, Ala, Glu, Asp, Gln, or Asn.

[Claim 5]

The EndoS mutant enzyme according to claim 4, wherein the mutation is at least one selected from the following group:

a mutation at H122 to Ala (H122A) or Phe (H122F);

a mutation at D279 to Ser (D279S) or Gln (D279Q);

a mutation at Y282 to Arg (Y282R);

a mutation at Q303 to Leu (Q303L);

a mutation at Y348 to His (Y348H);

a mutation at E350 to Ala (E350A), Asn (E350N), Asp (E350D), or Gln (E350Q);

a mutation at Y402 to Phe (Y402F) or Trp (Y402W);

a mutation at D405 to Ala (D405A); and

a mutation at R406 to Gln (R406Q).

[Claim 6]

The EndoS mutant enzyme according to claim 5, comprising at least one mutation selected from the group consisting of Q303L, E350A, E350N, E350D, E350Q, and D405A as the mutation.

[Claim 7]

The EndoS mutant enzyme according to claim 6, wherein the mutation is Q303L, E350A, E350N, E350D, E350Q, D405A,

H122A/Q303L, H122F/Q303L, D279Q/Q303L, D279S/Q303L, Y282R/Q303L, Q303L/Y348H, Q303L/E350A, Q303L/E350N,

Q303L/E350D, Q303L/E350Q, Q303L/Y402F, Q303L/Y402W, Q303L/D405A, Q303L/R406Q,
H122A/E350A, H122F/E350A, D279Q/E350A, D279S/E350A, Y282R/E350A, Y348H/E350A, E350A/Y402F, E350A/Y402W, E350A/D405A, E350A/R406Q,
H122A/E350N, H122F/E350N, D279Q/E350N, D279S/E350N, Y282R/E350N, Y348H/E350N, E350N/Y402F, E350N/Y402W, E350N/D405A, E350N/R406Q,
H122A/E350D, H122F/E350D, D279Q/E350D, D279S/E350D, Y282R/E350D, Y348H/E350D, E350D/Y402F, E350D/Y402W, E350D/D405A, E350D/R406Q,
H122A/E350Q, H122F/E350Q, D279Q/E350Q, D279S/E350Q, Y282R/E350Q, E350Q/Y348H, E350Q/Y402F, E350Q/Y402W, E350Q/D405A, E350Q/R406Q,
H122A/D405A, H122F/D405A, D279Q/D405A, D279S/D405A, Y282R/D405A, Y348H/D405A, or D405A/R406Q.

[Claim 8]

The EndoS mutant enzyme according to claim 7, wherein the mutation is Q303L, E350A, E350N, E350D, E350Q, D405A, Q303L/E350A, Q303L/E350N, Q303L/E350D, Q303L/E350Q, Q303L/D405A, E350A/D405A, E350N/D405A, E350D/D405A, or E350Q/D405A.

[Claim 9]

The EndoS mutant enzyme according to claim 4, wherein the mutation is H122A, H122F, D279Q, D279S, Y282R, Y348H, Y402F, Y402W, or R406Q.

[Claim 10]

The EndoS mutant enzyme according to claim 1, wherein the activity on the N297-linked sugar chain keeps a hydrolysis rate of 50% or less in a hydrolysis reaction in a reaction solution at pH 7.4 after 24 hours.

[Claim 11]

The EndoS mutant enzyme according to claim 1, wherein the EndoS mutant enzyme further exhibits an activity on the N297-linked sugar chain leading to a percent transglycosylation of approximately 60% or more after 48 hours of a transglycosylation reaction with 5 equivalents of a sugar donor relative to an acceptor molecule comprising the N297-linked sugar chain, in a reaction solution at pH 7.4.

[Claim 12]

A polynucleotide encoding an EndoS mutant enzyme according to any one of claims 1 to 11.

[Claim 13]

A vector comprising a nucleotide complementary to the polynucleotide according to claim 12.

[Claim 14]

A host cell transformed with a vector according to claim 13.

[Claim 15]

A method of production of a molecule comprising a sugar-remodeled Fc region, comprising reacting a molecule comprising an Fc region of IgG having a core GlcNAc

optionally with fucose addition as an N297-linked sugar chain with a sugar donor having a structure comprising an oxazolinated GlcNAc in the presence of an EndoS mutant enzyme according to any one of claims 1 to 11 to produce a molecule comprising an Fc region with a N297-linked sugar chain having a structure in which a sugar chain possessed by the sugar donor is transferred to the core GlcNAc of the N297-linked sugar chain.

[Claim 16]

The method of production according to claim 15, wherein the molecule comprising an Fc region is an IgG monoclonal antibody.