thrombomodulin is obtained as a flow-through fraction in a step of bringing the soluble thrombomodulin obtained by affinity column chromatography into contact with a cation exchanger under conditions of a specific conductivity of 25 to 34 ms/cm and pH 3 to 4 (Patent document 12), and a method for purifying thrombomodulin, wherein affinity column chromatography as the main purification step is followed by strong anion exchange chromatography (Patent document 13).

Prior Art References

Patent Documents

[0009]

Patent document 1: Japanese Patent Unexamined Publication (Kokai) No. 64-6219

Patent document 2: Japanese Patent Unexamined Publication No. 2-255699

Patent document 3: Japanese Patent Unexamined Publication No. 3-133380

Patent document 4: Japanese Patent Unexamined Publication No. 3-259084

Patent document 5: Japanese Patent Unexamined Publication No. 4-210700

Patent document 6: Japanese Patent Unexamined Publication No. 5-213998

Patent document 7: W092/00325

Patent document 8: W092/03149

Patent document 9: W093/15755

Patent document 10: Japanese Patent Unexamined Publication No. 3-86900

Patent document 11: Japanese Patent Unexamined Publication No. 3-218399

Patent document 12: Japanese Patent Unexamined Publication No. 11-341990

Patent document 13: W02008/117735

Non-patent Documents

[0010]

Non-patent document 1: Koji Suzuki, Igaku no Ayumi (Progress of Medicine), Vol. 125, 901 (1983)

Non-patent document 2: K. Gomi et al., Blood, 75, 1396-1399 (1990)

Non-patent document 3: M. Zushi et al., J. Biol. Chem., 24664, 10351-10353 (1989)

Non-patent document 4: D.Z. Wen et al., Biochemistry, 26, 4350-4357 (1987)

Non-patent document 5: S.M. Bates et al., Br. J. Pharmacol., 144, 1017-1028 (2005)

Non-patent document 6: H. Saito et al., J. Thromb Haemost, 5 (1), 31 (2007)

Non-patent document 7: Akio Hayakawa, Development and Security of Quality and Safety of Biomedical Products, 273-274 (2007)

What is claimed is:

[Claim 1]

Highly-purified soluble thrombomodulin which has a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of the soluble thrombomodulin, wherein the soluble thrombomodulin is produced by a transformant cell obtained by transfecting a host cell with a DNA containing a nucleotide sequence encoding the soluble thrombomodulin.

[Claim 2]

The highly-purified soluble thrombomodulin according to claim 1, wherein the soluble thrombomodulin is produced by serum-free culture of the transformant cell.

[Claim 3]

The highly-purified soluble thrombomodulin according to claim 1 or 2, wherein the host cell is a Chinese hamster ovary cell.

[Claim 4]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 3, wherein the soluble thrombomodulin has the following properties (1) to (5):

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 5]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 4, wherein molecular weight of the soluble thrombomodulin is in the range of 50,000 to 80,000.

[Claim 6]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 5, wherein the highly-purified soluble thrombomodulin is produced by a method comprising the following steps:

(a) the step of obtaining a transformant cell by transfecting a host cell with a DNA encoding soluble thrombomodulin;

(b) the step of obtaining a solution containing soluble thrombomodulin by culturing the

transformant cell, and

(c) the step of bringing the solution containing soluble thrombomodulin into contact with nylon and/or polyethersulfone to obtain highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin.

[Claim 7]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 6, wherein the soluble thrombomodulin is a peptide containing:

(i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and the peptide is soluble thrombomodulin having the following properties (1) to (5):

(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 8]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 6, wherein the soluble thrombomodulin is a peptide containing:

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

and the peptide is soluble thrombomodulin having the properties (1) to (5) mentioned below:

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 9]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 6, wherein the DNA containing a nucleotide sequence encoding soluble thrombomodulin is a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11.

[Claim 10]

A pharmaceutical composition comprising the highly-purified soluble thrombomodulin according to any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

[Claim 11]

A method for preparing highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, which comprises the step of bringing a solution containing soluble thrombomodulin, which is produced by a transformant cell obtained by transfecting a host cell with a DNA containing a nucleotide sequence encoding soluble thrombomodulin, into contact with nylon and/or polyethersulfone.

[Claim 12]

The preparation method according to claim 11, wherein the soluble thrombomodulin is prepared by serum-free culture of the transformant cell.

[Claim 13]

The method for preparing highly-purified soluble thrombomodulin according to claim 11 or 12, wherein the soluble thrombomodulin has the following properties (1) to (5);

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 14]

The preparation method according to any one of claims 11 to 13, wherein the host cell is a Chinese hamster ovary cell.

[Claim 15]

The preparation method according to any one of claims 11 to 14, wherein

~~molecular weight of the soluble thrombomodulin is 50,000 to 80,000.~~

[Claim 16]

The preparation method according to any one of claims 11 to 15, wherein the soluble thrombomodulin is a peptide containing:

(i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and the peptide is soluble thrombomodulin having the following properties (1) to (5):

(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 17]

The preparation method according to any one of claims 11 to 15, wherein the soluble thrombomodulin is a peptide containing:

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues, and

the peptide is soluble thrombomodulin having the following properties (1) to (5):

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 18]

The preparation method according to any one of claims 11 to 15, wherein the DNA containing a nucleotide sequence encoding soluble thrombomodulin is a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11.

[Claim 19]

The preparation method according to any one of claims 11 to 18, wherein the nylon and/or polyethersulfone is in the form of a filtration membrane.

[Claim 20]

The preparation method according to claim 19, wherein the filtration membrane has a membrane area of 0.01 to 0.5 m^2 for 1 mg of the host cell-originated proteins.

[Claim 21]

The preparation method according to any one of claims 11 to 20, wherein the nylon and/or polyethersulfone is nylon.

[Claim 22]

The preparation method according to any one of claims 11 to 22, which is a method for preparing highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, comprising:

(a) the step of obtaining a transformant cell by transfecting a host cell with a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11,

(b) the step of obtaining a solution containing soluble thrombomodulin by culturing the transformant cell,

(c) the step of purifying the solution containing soluble thrombomodulin to a thrombomodulin purity of 90% or higher based on the total proteins, and

(d) the step of bringing the solution containing soluble thrombomodulin into contact with nylon to isolate highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin, and

wherein the host cell is a Chinese hamster ovary cell.

Claims

[Claim 1]

Highly-purified soluble thrombomodulin which has a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of the soluble thrombomodulin, wherein the soluble thrombomodulin is produced by a transformant cell obtained by transfecting a host cell with a DNA containing a nucleotide sequence encoding the soluble thrombomodulin.

[Claim 2]

The highly-purified soluble thrombomodulin according to claim 1, which is manufactured on an industrial scale.

[Claim 3]

The highly-purified soluble thrombomodulin according to claim 1 or 2, which is used as a material for a medicament.

[Claim 4]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 3, wherein purity of the highly-purified soluble thrombomodulin is 99% or higher based on the total proteins.

[Claim 5]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 4, wherein the soluble thrombomodulin is produced by serum-free culture of the transformant cell.

[Claim 6]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 5, wherein the host cell is a Chinese hamster ovary cell.

[Claim 7]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 6, wherein the soluble thrombomodulin has the following properties (1) to (5):

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,
(4) an action of suppressing platelet aggregation caused by thrombin, and
(5) anti-inflammatory action.

[Claim 8]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 7, wherein molecular weight of the soluble thrombomodulin is in the range of 50,000 to 80,000.

[Claim 9]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 8, wherein the highly-purified soluble thrombomodulin is produced by a method comprising the following steps:
(a) the step of obtaining a transformant cell by transfecting a host cell with a DNA encoding soluble thrombomodulin;
(b) the step of obtaining a solution containing soluble thrombomodulin by culturing the transformant cell, and
(c) the step of bringing the solution containing soluble thrombomodulin into contact with nylon and/or polyethersulfone to obtain highly-purified soluble thrombomodulin having a content of host cell-originated proteins being in a ratio of less than 10 ng of the proteins per 10,000 U of soluble thrombomodulin.

[Claim 10]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 9, wherein the soluble thrombomodulin is a peptide containing:
(i) the amino acid sequence of the positions 367 to 480 in the amino acid sequence of SEQ ID NO: 9 or 11, and the amino acid sequence of (ii-1) or (ii-2) mentioned below, and the peptide is soluble thrombomodulin having the following properties (1) to (5):
(ii-1) the amino acid sequence of the positions 19 to 244 in the amino acid sequence of SEQ ID NO: 9 or 11, or
(ii-2) the amino acid sequence of (ii-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,
(1) an action of selectively binding to thrombin,
(2) an action of promoting activation of Protein C by thrombin,
(3) an action of extending thrombin clotting time,
(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 11]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 9, wherein the soluble thrombomodulin is a peptide containing:

(i-1) the amino acid sequence of the positions 19 to 516 in the amino acid sequence of SEQ ID NO: 9 or 11, or

(i-2) the amino acid sequence of (i-1) mentioned above, further including substitution, deletion or addition of one or more amino acid residues,

and the peptide is soluble thrombomodulin having the properties (1) to (5) mentioned below:

(1) an action of selectively binding to thrombin,

(2) an action of promoting activation of Protein C by thrombin,

(3) an action of extending thrombin clotting time,

(4) an action of suppressing platelet aggregation caused by thrombin, and

(5) anti-inflammatory action.

[Claim 12]

The highly-purified soluble thrombomodulin according to any one of claims 1 to 9, wherein the DNA containing a nucleotide sequence encoding soluble thrombomodulin is a DNA encoding the amino acid sequence of SEQ ID NO: 9 or 11.

[Claim 13]

A pharmaceutical composition comprising the highly-purified soluble thrombomodulin according to any one of claims 1 to 12 and a pharmaceutically acceptable carrier.