Claims

1. A method for producing soluble thrombomodulin substantially not containing a denatured product of the soluble thrombomodulin that may be generated from the soluble thrombomodulin under acidic conditions, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less, which comprises:

   (0) a step of leaving the soluble thrombomodulin under acidic conditions of pH 5 or less; (1) a step of subjecting a soluble thrombomodulin-containing material that contains or is suspected to contain a denatured product of the soluble thrombomodulin, which is obtained through the step (0) above, to an anion exchanger or hydroxyapatite; and (2) a step of obtaining a soluble thrombomodulin-containing fraction that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less, under separation conditions in which the soluble thrombomodulin can be separated from a denatured product of the soluble thrombomodulin.

2. The production method according to claim 1, wherein the step of leaving the soluble thrombomodulin under acidic conditions of pH 5 or less is a step of leaving the soluble thrombomodulin under acidic conditions of pH 4 or less.

3. The production method according to any one of claims 1 to 2, wherein the step of subjecting the soluble thrombomodulin-containing material to the anion exchanger or hydroxyapatite is the step of subjecting the soluble thrombomodulin-containing material to the anion exchanger.

4. The production method according to any one of claims 1 to 2, wherein the step of subjecting the soluble thrombomodulin-containing material to the anion exchanger or hydroxyapatite is the step of subjecting the soluble thrombomodulin-containing material to the hydroxyapatite.

5. The production method according to any one of claims 1 to 4, wherein the content of the soluble thrombomodulin is 80% or more with respect to total proteins in the soluble thrombomodulin-containing material.

6. The production method according to any one of claims 1 to 5, wherein the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

7. The production method according to any one of claim 1 to 5, wherein the step (2) is a step of obtaining a pass-through fraction, in which a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin is obtained, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

8. The production method according to any one of claims 1 to 5, wherein the step (2) is a step of carrying out isocratic elution to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

9. The production method according to any one of claims 1 to 5, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to an anion exchanger using a buffer solution of pH 4 to 9; and the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, using a buffer solution of pH 5 to 9 having a salt concentration of 0 to 1 M, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less, while confirming the presence of the soluble thrombomodulin in the elution fraction.

10. The production method according to any one of claims 1 to 5, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to an anion exchanger using a buffer solution of pH 5 to 8 having a salt concentration of 0.1 to 0.2 M; and the step (2) is a step of obtaining a pass-through fraction using a buffer solution of pH 5 to 8 having a salt concentration of 0.1 to 0.2 M, so as to obtain a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

11. The production method according to any one of claims 1 to 5, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to hydroxyapatite using a buffer solution of pH 6 to 9 having a phosphate concentration of 8 mM or less; and the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, using a buffer solution of pH 6 to 9 having a phosphate concentration of 0 to 0.5 M, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less, while confirming the presence of the soluble thrombomodulin in the elution fraction.

12. The production method according to any one of claims 1 to 5, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to hydroxyapatite using a buffer solution of pH 6 to 9 having a phosphate concentration of 5 to 20 mM or less; and the step (2) is a step of obtaining a pass-through fraction using a buffer solution of pH 6 to 9 having a phosphate concentration of 5 to 20 mM, so as to obtain a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

13. The production method according to any one of claims 1 to 12, which does not comprise a step of adjusting the pH of an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin to pH 4 or less, after the elution fraction has been obtained, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

14. The production method according to any one of claims 1 to 13, which is a production method comprising a concentration step and/or a desalination step, wherein the pH of an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin is not adjusted to pH 4 or less after the elution fraction has been obtained, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less, and wherein the production method is used to convert the soluble thrombomodulin to a pharmaceutical material.

15. The production method according to any one of claims 1 to 14, wherein the content of the denatured product of the soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 1% or less.

16. The production method according to any one of claims 1 to 15, wherein the soluble thrombomodulin is thrombomodulin obtained from transformant cells prepared by transfecting host cells with DNA encoding the amino acid sequence shown in SEQ ID NO: 9 or 11.

17. The production method according to any one of claims 1 to 16, wherein the soluble thrombomodulin is a peptide consisting of a sequence consisting of amino acids at positions 19 to 516 of the amino acid sequence as shown in SEQ ID NO: 9 or 11, or a peptide that comprises a substitution, deletion, or addition of one or multiple amino acids in the amino acid sequence of said peptide and has thrombomodulin activity.

CLAIMS

1. A method for producing soluble thrombomodulin substantially not containing a denatured product of the soluble thrombomodulin that may be generated from the soluble thrombomodulin under acidic conditions, which comprises:

(1) a step of subjecting a soluble thrombomodulin-containing material that contains or is suspected to contain a denatured product of the soluble thrombomodulin to an anion exchanger or hydroxyapatite; and

(2) a step of obtaining a soluble thrombomodulin-containing fraction that does not substantially contain a denatured product of the soluble thrombomodulin under separation conditions in which the soluble thrombomodulin can be separated from a denatured product of the soluble thrombomodulin.

2. The method for producing soluble thrombomodulin substantially not containing a denatured product of the soluble thrombomodulin that may be generated from the soluble thrombomodulin under acidic conditions according to claim 1, which comprises: (0) a step of leaving the soluble thrombomodulin under acidic conditions of pH 5 or less; (1) a step of subjecting a soluble thrombomodulin-containing material that contains or is suspected to contain a denatured product of the soluble thrombomodulin, which is obtained through the step (0) above, to an anion exchanger or hydroxyapatite; and (2) a step of obtaining a soluble thrombomodulin-containing fraction that does not substantially contain a denatured product of the soluble thrombomodulin under separation conditions in which the soluble thrombomodulin can be separated from a denatured product of the soluble thrombomodulin.

3. The production method according to claim 1 or 2, wherein the content of the soluble thrombomodulin is 80% or more with respect to total proteins in the soluble thrombomodulin-containing material.

4. The production method according to any one of claims 1 to 3, wherein the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin.

5. The production method according to any one of claim 1 to 3, wherein the step (2) is a step of

obtaining a pass-through fraction, in which a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin is obtained.

6. The production method according to any one of claims 1 to 3, wherein the step (2) is a step of carrying out isocratic elution to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin.

7. The production method according to any one of claims 1 to 3, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to an anion exchanger using a buffer solution of pH 4 to 9; and the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, using a buffer solution of pH 5 to 9 having a salt concentration of 0 to 1 M, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, either (a) after the position of a fraction from which the soluble thrombomodulin is eluted has previously been confirmed, or (b) while confirming the presence of the soluble thrombomodulin in the elution fraction.

8. The production method according to any one of claims 1 to 3, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to an anion exchanger using a buffer solution of pH 5 to 8 having a salt concentration of 0.1 to 0.2 M; and the step (2) is a step of obtaining a pass-through fraction using a buffer solution of pH 5 to 8 having a salt concentration of 0.1 to 0.2 M, so as to obtain a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin.

9. The production method according to any one of claims 1 to 3, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to hydroxyapatite using a buffer solution of pH 6 to 9 having a phosphate concentration of 8 mM or less; and the step (2) is a step of carrying out linear gradient elution, stepwise gradient elution, or gradient elution in which linear gradient elution is combined with stepwise gradient elution, using a buffer solution of pH 6 to 9 having a phosphate concentration of 0 to 0.5 M, so as to obtain an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin, either (a) after the position of a fraction from which the soluble thrombomodulin is eluted has previously been confirmed, or (b) while confirming the presence of the soluble thrombomodulin in the elution fraction.

10. The production method according to any one of claims 1 to 3, wherein the step (1) is a step of subjecting the soluble thrombomodulin-containing material to hydroxyapatite using a buffer solution of pH 6 to 9 having a phosphate concentration of 5 to 20 mM or less; and the step (2) is a step of obtaining a pass-through fraction using a buffer solution of pH 6 to 9 having a phosphate concentration of 5 to 20 mM, so as to obtain a fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin.

11. The production method according to any one of claims 1 to 10, which does not comprise a step of adjusting the pH of an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin to pH 4 or less, after the elution fraction has been obtained.

12. The production method according to any one of claims 1 to 11, which is a production method comprising a concentration step and/or a desalination step, wherein the pH of an elution fraction containing soluble thrombomodulin that does not substantially contain a denatured product of the soluble thrombomodulin is not adjusted to pH 4 or less after the elution fraction has been obtained, wherein the production method is used to convert the soluble thrombomodulin to a pharmaceutical material.

13. The production method according to any one of claims 1 to 12, wherein the content of a denatured product of soluble thrombomodulin in the soluble thrombomodulin that does not substantially contain the denatured product of soluble thrombomodulin is 3% or less.

14. The production method according to any one of claims 1 to 13, wherein the soluble thrombomodulin is thrombomodulin obtained from transformant cells prepared by transfecting host cells with DNA encoding the amino acid sequence shown in SEQ ID NO: 9 or 11.