Claims

[Claim 1] A non-peptidyl polymer-insulin multimer, comprising two or more of a non-peptidyl polymer-insulin conjugate prepared by linking a non-peptidyl polymer and insulin via a covalent bond, wherein the conjugates are complexed with cobalt ion to form a multimer.

[Claim 2] The multimer according to claim 1, wherein the non-peptidyl polymer-insulin conjugate is prepared by linking the non-peptidyl polymer to the amino terminus of the A chain, the amino terminus of the B chain, or a lysine at position 29 of the B chain of insulin, via a covalent bond.

[Claim 3] The multimer according to claim 1, wherein the insulin is a native insulin, an insulin variant prepared by substitution, addition, deletion, modification or a combination thereof of the amino acid sequences of the native insulin, an insulin derivative, or a fragment thereof.

[Claim 4] The multimer according to claim 1, wherein the non-peptidyl polymer is selected from the group consisting of biodegradable polymers, lipid polymers, chitins, hyaluronic acids, and combinations thereof.

[Claim 5] The multimer according to claim 4, wherein the biodegradable polymer is selected from the group consisting of polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, polylactic acid, and polylactic-glycolic acid.

[Claim 6] The multimer according to claim 5, wherein the biodegradable polymer is polyethylene glycol.

[Claim 7] The multimer according to claim 1, wherein the reactive group of the non-peptidyl polymer covalently binding to the insulin is selected from the group consisting of aldehyde, propion aldehyde, butyl aldehyde, maleimides, and succinimide derivative.

[Claim 8] The multimer according to claim 1, wherein the non-peptidyl polymer-insulin conjugates form a multimer via trivalent cobalt cations.

[Claim 9] The multimer according to claim 1, wherein the non-peptidyl polymer-insulin multimer is a non-peptidyl polymer-insulin hexamer.

[Claim 10] A preparation method of the non-peptidyl polymer-insulin multimer of any one of claims 1 to 9, comprising the step of reacting non-peptidyl polymer-insulin conjugates with a solution containing cobalt ions to produce non-peptidyl polymer-insulin multimers.

[Claim 11] The preparation method according to claim 10, wherein the solution contains a salt that dissociates into divalent cobalt ions by solvation in

an aqueous solution and an oxidant; a hydrate of the salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; or a salt that dissociates into trivalent cobalt ions by solvation in an aqueous solution or a hydrate thereof.

[Claim 12] The preparation method according to claim 11, wherein the salt that dissociates into divalent cobalt ions is cobalt chloride (II) ($CoCl_2$),and the salt that dissociates into trivalent cobalt ions is cobalt chloride (III) ($CoCl_3$).

[Claim 13] The preparation method according to claim 11, wherein a molar ratio of the oxidant to the divalent cobalt ion is 0.5 to 5.

[Claim 14] The preparation method according to claim 10, wherein a molar ratio of the cobalt ion to the non-peptidyl polymer-insulin conjugate is 0.1 to 1.

[Claim 15] The preparation method according to claim 10, wherein the reaction is performed in a buffer solution at pH 5 to 9.

[Claim 16] A pharmaceutical composition for the prevention or treatment of diabetes, comprising the non-peptidyl polymer-insulin multimer of any one of claims 1 to 9 as an active ingredient.

[Claim 17] The composition according to claim 16, wherein the non-peptidyl polymer-insulin multimer is included at a concentration of 0.01 μM to 100 μM.

[Claim 18] A method for preventing or treating diabetes, comprising the step of administering the pharmaceutical composition of claim 16 to a subject having diabetes or suspected of having diabetes.

[Claim 19] A kit for the preparation of the non-peptidyl polymer-insulin multimer of any one of claims 1 to 9 comprising non-peptidyl polymer-insulin conjugates prepared by linking a non-peptidyl polymer and insulin via a covalent bond; and a solution containing cobalt ions, wherein the solution contains a salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; a hydrate of the salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; or a salt that dissociates into trivalent cobalt ions by solvation in an aqueous solution or a hydrate thereof.

[Claim 20] The kit according to claim 19, wherein the salt or the hydrate thereof and the oxidant are stored separately in individual containers.

Claims

1. A non-peptidyl polymer-insulin hexamer, comprising six of a non-peptidyl polymer-insulin conjugate prepared by linking a non-peptidyl polymer and insulin via a covalent bond, wherein the six conjugates are complexed with trivalent cobalt ion to form a hexamer, and wherein the non-peptidyl polymer is selected from the group consisting of chitins, hyaluronic acids, polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, polylactic acid, and polylactic-glycolic acid.

2. The hexamer according to claim 1, wherein the non-peptidyl polymer-insulin conjugate is prepared by linking the non-peptidyl polymer to the amino terminus of the A chain, the amino terminus of the B chain, or a lysine at position 29 of the B chain of insulin, via a covalent bond.

3. The hexamer according to claim 1, wherein the reactive group of the non-peptidyl polymer covalently binding to the insulin is selected from the group consisting of aldehyde, propion aldehyde, butyl aldehyde, maleimides, and succinimide derivative.

4. A preparation method of the non-peptidyl polymer-insulin hexamer of any one of claims 1, 2, or 3, comprising the step of reacting non-peptidyl polymer-insulin conjugates with a solution containing trivalent cobalt ions to produce non-peptidyl polymer-insulin hexamers.

5. The preparation method according to claim 4, wherein the solution contains a salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; a hydrate of the salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; or a salt that dissociates into trivalent cobalt ions by solvation in an aqueous solution or a hydrate thereof.

6. The preparation method according to claim 5, wherein the salt that dissociates into divalent cobalt ions is cobalt chloride (II) ($CoCl_2$),and the salt that dissociates into trivalent cobalt ions is cobalt chloride (III) ($CoCl_3$).

7. The preparation method according to claim 5, wherein a molar ratio of the oxidant to the divalent cobalt ion is 0.5 to 5.

8. The preparation method according to claim 4, wherein a molar ratio of the cobalt ion to the non-peptidyl polymer-insulin conjugate is 0.1 to 1.

9. The preparation method according to claim 4, wherein the reaction is performed in a buffer solution at pH 5 to 9.

10. A pharmaceutical composition for the prevention or treatment of diabetes, comprising the non-peptidyl polymer-insulin hexamer of any one of claims 1, 2, or 3 as an active ingredient.

11. The composition according to claim 10, wherein the non-peptidyl polymer-insulin hexamer is included at a concentration of 0.01 μM to 100 μM.

12. Use of the pharmaceutical composition of claim 10 in the manufacture of a medicament for the treatment of diabetes.

13. A kit for the preparation of the non-peptidyl polymer-insulin hexamer of any one of claims 1, 2, or 3 comprising non-peptidyl polymer-insulin conjugates prepared by linking a non-peptidyl polymer and insulin via a covalent bond; and a solution containing cobalt ions, wherein the solution contains a salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; a hydrate of the salt that dissociates into divalent cobalt ions by solvation in an aqueous solution and an oxidant; or a salt that dissociates into trivalent cobalt ions by solvation in an aqueous solution or a hydrate thereof, wherein the non-peptidyl polymer is selected from the group consisting of chitins, hyaluronic acids, polyethylene glycol, polypropylene glycol, ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, polylactic acid, and polylactic-glycolic acid.

14. The kit according to claim 13, wherein the salt or the hydrate thereof and the oxidant are stored separately in individual containers.