Super Versatile Method For Presenting Cyclic Peptide Motif On Protein Structure

Technical Field

[0001] The present invention relates to a method of presenting a cyclic peptide on a protein having a loop structure, and the like.

Background Art

[0002] There is known a technology of modifying an amino acid residue of a protein or introducing another peptide structure into a protein with a view to providing a protein having improved activity.

Patent Document 1 discloses a chimeric polypeptide having improved bioactivity and containing a serum albumin protein having a bioactivity different peptide sequence inserted therein.

Patent Document 2 discloses that modifications to at least one loop region of an FnIII based polypeptide and also modifications to a β sheet of the FnIII based polypeptide result in an FnIII based binding molecule with improved binding ability for a target molecule.

[0003] Patent Document 3 discloses a molecule obtained by incorporating one or more bioactive peptides in the Fc domain of an antibody protein. More specifically, a bioactive peptide is inserted into amino acid residues adjacent to each other in the loop region of an Fc domain.

Patent Document 4 similar to Patent Document 3 describes a modified Fc molecule. Disclosed is an Fc molecule having an additional functionality portion covalently bound through a side chain of an amino acid residue at a conjugation site. More specifically, the amino acid residue at the conjugation site is a cysteine residue.

Patent Document 2: Japanese Translation of PCT Application No. 2013-539362

Patent Document 3: Japanese Translation of PCT Application No. 2008-514201

Patent Document 4: Japanese Translation of PCT Application No. 2009-504164

Patent Document 5: Japanese Translation of PCT Application No. 2009-541361

Patent Document 6: Japanese Translation of PCT Application No. 2009-540837

Summary

Technical Problem

[0007] An object of the present invention is to provide a method of presenting a cyclic peptide on a protein having a loop structure with the protein as a scaffold while retaining the structure and activity which the cyclic peptide has.

Solution to Problem

[0007a] In one aspect, the present disclosure refers to a method of presenting a cyclic peptide having a binding activity to an intended molecule on a protein having a loop structure, comprising: using, as the cyclic peptide, that having a chemically crosslinked structure for forming an intramolecular cyclic structure, and replacing the chemically crosslinked structure of the cyclic peptide with two amino acid residues constituting the loop structure and fusing the cyclic peptide to the protein having a loop structure while retaining the binding activity to the intended molecule.

[0007b] In another aspect, the present disclosure refers to a method of fusing a cyclic peptide having a binding activity to an intended molecule to a protein having a loop structure, thereby producing a modified protein having the cyclic peptide

presented on the protein, comprising: using, as the cyclic peptide, that having a chemically crosslinked structure for forming an intramolecular cyclic structure, selecting, from an amino acid sequence of the cyclic peptide, a partial amino acid sequence to be presented on the protein and selecting a base sequence corresponding to the partial amino acid sequence, selecting base sequences corresponding to two amino acid sequences constituting the loop structure of the protein having a loop structure, respectively, removing a base present between the selected base sequences if necessary, and preparing a nucleic acid having, inserted and incorporated therein, the base sequence corresponding to the partial amino acid sequence of the selected cyclic peptide, and translating the nucleic acid wherein the modified protein retains the binding activity to the intended molecule.

[0008] The following are the details of the present disclosure.

(1) A method of presenting a cyclic peptide on a protein having a loop structure, includes:

using, as the cyclic peptide, that having a chemically crosslinked structure for forming an intramolecular cyclic structure, and

replacing the chemically crosslinked structure of the cyclic peptide with two amino acid residues constituting the loop structure and fusing the cyclic peptide to the protein having a loop structure.

B1-Fc therein by a method similar to that described above was studied by the pulldown method.

The results of electrophoresis are shown in FIG. 23B. The pulldown results have revealed that wild type RBP (Sample No. 1) does not bind to Plexin B1-Fc, but two fusion proteins (Samples Nos. 2 and 3) having, incorporated therein, the sequence of the Plexin binder mP6-9 specifically binds to it. In other words, it has been confirmed that as in the case of Fn10-Fc, Fc, HSA, and hGH, the cyclic peptide can be converted into a fusion protein while retaining its binding activity to the binding molecule even by directly inserting it into the loop of the lipid-binding secretory protein RBP.

[0111] [Example 7] Fusion between human placental alkaline phosphatase and cyclic peptide

1. Design of cyclic peptide fusion protein

In order to replace with the chemically crosslinked structure of a physiologically active cyclic peptide, human placental alkaline phosphatase (which will hereinafter be called “PLAP”) was selected as an enzyme protein having a loop structure and serving as a scaffold protein. From the loop portion separated from the enzyme active site on the steric structure of the alkaline phosphatase, two amino acid residues Lys402 (corresponding to B_N) and Gly404 (corresponding to B_C) having a Cα atomic distance of 5.5Å as a linking residue, The steric structure of the above portions is shown in FIG. 24.

[0112] A fusion protein was designed by replacing a portion of a region sandwiched between two amino acid residues B_N and B_C selected from PLAP

What is claimed is:

1. A method of presenting a cyclic peptide having a binding activity to an intended molecule on a protein having a loop structure, comprising:

using, as the cyclic peptide, that having a chemically crosslinked structure for forming an intramolecular cyclic structure, and

replacing the chemically crosslinked structure of the cyclic peptide with two amino acid residues constituting the loop structure and fusing the cyclic peptide to the protein having a loop structure while retaining the binding activity to the intended molecule.

2. The method according to Claim 1, wherein the two amino acid residues constituting the loop structure have a Cα atomic distance within a range of from 4 to 7Å.

3. The method according to Claim 1 or 2, wherein the protein having a loop structure has from 1 to 15 amino acid residues between the two amino acid residues constituting the loop structure.

4. The method according to any one of Claims 1 to 3, wherein the cyclic peptide is composed of a proteinogenic amino acid and/or a non-proteinogenic amino acid.

5. The method according to any one of Claims 1 to 4, wherein the chemically crosslinked structure contains a thioether bond or a disulfide bond.

6. The method according to any one of Claims 1 to 5, wherein two or more cyclic peptides each fuse to respectively different loop structures of a protein having a plurality of loop structures.

7. The method according to Claim 6, wherein the two or more cyclic peptides have the same amino acid sequence.

8. The method according to Claim 6, wherein the two or more cyclic peptides have respectively different amino acid sequences.

9. The method according to any one of Claims 1 to 8, wherein in replacing the chemically crosslinked structure of the cyclic peptide with the two amino acid residues constituting the loop structure, the chemically crosslinked structure of the cyclic peptide is replaced, via a linker sequence, with the two amino acid residues constituting the loop structure.

10. The method according to any one of Claims 1 to 9, wherein the chemically crosslinked structure of the cyclic peptide is replaced with the two amino acid residues constituting the loop structure while including an amino acid sequence constituting the loop structure other than the two amino acids constituting the loop structure.

11. A method of fusing a cyclic peptide having a binding activity to an intended molecule to a protein having a loop structure, thereby producing a modified protein having the cyclic peptide presented on the protein, comprising:

using, as the cyclic peptide, that having a chemically crosslinked structure for forming an intramolecular cyclic structure,

selecting, from an amino acid sequence of the cyclic peptide, a partial amino acid sequence to be presented on the protein and selecting a base sequence corresponding to the partial amino acid sequence,

selecting base sequences corresponding to two amino acid sequences constituting the loop structure of the protein having a loop structure, respectively, removing a base present between the selected base sequences if necessary, and preparing a nucleic acid having, inserted and incorporated therein, the base

sequence corresponding to the partial amino acid sequence of the selected cyclic peptide, and

translating the nucleic acid

wherein the modified protein retains the binding activity to the intended molecule.