Still another object of the present invention is to provide a polynucleotide encoding the therapeutic enzyme fusion protein, an expression vector containing the polynucleotide, and a transformant into which the expression vector is introduced.

Still another object of the present invention is to provide a method for preparing an enzyme fusion protein including culturing the transformant.

[Technical Solution]

In one aspect, the present disclosure refers to an enzyme fusion protein, wherein an immunoglobulin Fc region is fused to a therapeutic enzyme and the therapeutic enzyme has increased *in vivo* duration compared to a therapeutic enzyme to which an immunoglobulin Fc region is not fused, wherein the enzyme fusion protein has increased stability and reduced binding affinity for lysosome receptors, thereby having a high degree of tissue distribution, compared to a therapeutic enzyme therapeutic enzyme to which an immunoglobulin Fc region is not fused, wherein the therapeutic enzyme is selected from the group consisting of beta-glucosidase, alpha-galactosidase, beta-galactosidase, iduronidase, iduronate-2-sulfatase, galactose-6-sulfatase, acid alpha-glucosidase, acid ceramidase, acid sphingomyelinsase, galactocerebrosidsase, arylsulfatase A, B, beta-hexosaminidase A, B, heparin N-sulfatase, alpha-D-mannosidase, beta-glucuronidase, N-acetylgalactosamine-6 sulfatase, lysosomal acid lipase, alpha-N-acetyl-glucosaminidase, glucocerebrosidase, butyrylcholinesterase, chitinase, glutamate decarboxylase, imiglucerase, lipase, uricase, platelet-activating factor acetylhydrolase, neutral endopeptidase, and myeloperoxidase, wherein the immunoglobulin Fc region is selected from the group consisting of (a) a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain; (b) a CH1 domain and a CH2 domain; (c) a CH1 domain and a CH3 domain; (d) a CH2 domain and a CH3 domain; (e) a combination between one or two or more domains among a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain and an immunoglobulin hinge region or a part of the hinge region; and (f) a dimer between each domain of a heavy chain constant region and a light chain constant region, wherein the immunoglobulin Fc region is derived from an IgG4 Fc region, wherein no chain exchange occurs in the immunoglobulin Fc region. An example of the present disclosure provides an enzyme fusion protein in which a therapeutic enzyme and an immunoglobulin Fc region are fused.

In a specific example, the present disclosure relates to an enzyme fusion protein, in which an immunoglobulin Fc region is fused to a therapeutic enzyme such that the therapeutic enzyme has increased *in vivo* duration compared to a therapeutic enzyme to which an immunoglobulin Fc region is not fused.

The following corresponds to a further example of the present disclosure.

Specifically, as an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the enzyme is selected from the group consisting of *beta*-glucosidase, *alpha*-galactosidase, *beta*-galactosidase, iduronidase, iduronate-2-sulfatase, galactose-6-sulfatase, acid *alpha*-glucosidase, acid ceramidase, acid sphingomyelinsase, galactocerebrosidsase, arylsulfatase A, B, *beta*-hexosaminidase A, B, heparin *N*-sulfatase, *alpha*-D-mannosidase, *beta*-glucuronidase, *N*-acetylgalactosamine-6 sulfatase, lysosomal acid lipase, *alpha-N*-acetyl-glucosaminidase, glucocerebrosidase, butyrylcholinesterase, chitinase, glutamate decarboxylase, imiglucerase, lipase, uricase, platelet-activating factor acetylhydrolase, neutral endopeptidase, and myeloperoxidase.

[CLAIMS]

1. An enzyme fusion protein, wherein an immunoglobulin Fc region is fused to a therapeutic enzyme and the therapeutic enzyme has increased *in vivo* duration compared to a therapeutic enzyme to which an immunoglobulin Fc region is not fused,

wherein the enzyme fusion protein has increased stability and reduced binding affinity for lysosome receptors, thereby having a high degree of tissue distribution, compared to a therapeutic enzyme therapeutic enzyme to which an immunoglobulin Fc region is not fused, wherein the therapeutic enzyme is selected from the group consisting of *beta*-glucosidase, *alpha*-galactosidase, *beta*-galactosidase, iduronidase, iduronate-2-sulfatase, galactose-6-sulfatase, acid *alpha*-glucosidase, acid ceramidase, acid sphingomyelinsase, galactocerebrosidsase, arylsulfatase A, B, *beta*-hexosaminidase A, B, heparin N-sulfatase, *alpha*-D-mannosidase, *beta*-glucuronidase, N-acetylgalactosamine-6 sulfatase, lysosomal acid lipase, *alpha*-N-acetyl-glucosaminidase, glucocerebrosidase, butyrylcholinesterase, chitinase, glutamate decarboxylase, imiglucerase, lipase, uricase, platelet-activating factor acetylhydrolase, neutral endopeptidase, and myeloperoxidase,

wherein the immunoglobulin Fc region is selected from the group consisting of

(a) a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain;

(b) a CH1 domain and a CH2 domain;

(c) a CH1 domain and a CH3 domain;

(d) a CH2 domain and a CH3 domain;

(e) a combination between one or two or more domains among a CH1 domain, a CH2 domain, a CH3 domain, and a CH4 domain and an immunoglobulin hinge region or a part of the hinge region; and

(f) a dimer between each domain of a heavy chain constant region and a light chain constant region,

wherein the immunoglobulin Fc region is derived from an IgG4 Fc region,

wherein no chain exchange occurs in the immunoglobulin Fc region.

2. The enzyme fusion protein of claim 1, wherein a therapeutic enzyme and an immunoglobulin Fc region are fused by a peptide linker.

3. The enzyme fusion protein of claim 1, wherein the enzyme fusion protein is a fusion of one molecule of immunoglobulin Fc region and a dimeric therapeutic enzyme.

4. The enzyme fusion protein of claim 1, wherein the immunoglobulin Fc region has a variation selected from the group consisting of substitution, addition, deletion, modification, and a combination thereof in at least one amino acid of a native immunoglobulin Fc region.

5. The enzyme fusion protein of claim 4, wherein, in the immunoglobulin Fc region having the amino sequence of SEQ ID NO: 8, the 2nd amino acid is substituted with proline; the 71st amino acid is substituted with glutamine; or the 2nd amino acid is substituted with proline and the 71st amino acid is substituted with glutamine.

6. The enzyme fusion protein of claim 1, wherein the immunoglobulin Fc region is

selected from the group consisting of (a) a region capable of forming a disulfide bond is removed, (b) a certain amino acid residue is removed at the N-terminus of a native Fc, (c) a methionine residue is added at the N-terminus of a native Fc form, (d) a complement-binding site is removed, or (e) an antibody-dependent cell-mediated cytotoxicity (ADCC) site is deleted.

7. The enzyme fusion protein of any one of claims 1 to 6, wherein the immunoglobulin Fc region is aglycosylated.

8. The enzyme fusion protein of claim 1, wherein a hinge region of the IgG4 Fc region is substituted with an IgG1 hinge region.

9. A pharmaceutical composition for use in preventing or treating lysosomal storage disorder (LSD) comprising the enzyme fusion protein of any one of claims 1 to 6.

10. Use of an enzyme fusion protein of any one of claims 1 to 6 in the manufacture of a pharmaceutical composition for preventing or treating lysosomal storage disorder (LSD).

11. The use of claim 10, wherein the lysosomal storage disorder (LSD) is selected from the group consisting of mucopolysaccharidosis (MPS), glycogen storage disease, sphingolipidosis, Niemann-Pick disease, Fabry's disease, Gaucher disease, Hunter syndrome, and Maroteaux-Lamy syndrome.

12. The use of claim 10, wherein the enzyme is iduronate-2-sulfatase (IDS) or arylsulfatase B (ARSB).

13. The use of any one of claims 10 to 12, wherein the composition reduces the binding affinity of an enzyme for lysosome receptors.

14. A polynucleotide encoding the enzyme fusion protein of any one of claims 1 to 6.

15. An expression vector comprising the polynucleotide of claim 10.

16. A transformant into which the expression vector of claim 15 is introduced.

17. A method for preparing an enzyme fusion protein, comprising:
(a) culturing the transformant of claim 16 to obtain a culture; and
(b) recovering an enzyme fusion protein from the culture.