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. 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.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the therapeutic enzyme and the immunoglobulin Fc region in the enzyme fusion protein are fused by a peptide linker.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that an immunoglobulin Fc region molecule and a dimeric therapeutic enzyme are fused.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that 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.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that, in the immunoglobulin Fc region having the amino sequence of SEQ ID NO: 8, the 2^{nd} amino acid is substituted with proline; the 71^{st} amino acid is substituted with glutamine; or the 2^{nd} amino acid is substituted with proline and the 71^{st} amino acid is substituted with glutamine.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that no chain exchange occurs in the immunoglobulin Fc region.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that it has increased stability and reduced binding affinity for lysosome receptors, thus having a high degree of tissue distribution compared to a therapeutic enzyme to which an immunoglobulin Fc region is not fused.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that 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 of 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 the heavy chain constant region and the light chain constant region.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the immunoglobulin Fc region has at least one characteristic selected from the group consisting of (a) removal of a region capable of forming a disulfide bond, (b) removal of a certain amino acid residue at the N-terminus of a native Fc, (c) addition of a methionine residue at the N-terminus of a native Fc, (d) removal of a complement-binding site, or (e) deletion of an antibody-dependent cell-mediated cytotoxicity (ADCC) site.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the immunoglobulin Fc region is aglycosylated.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the immunoglobulin Fc region is an Fc fragment derived from IgG, IgA, IgD, IgE, or IgM.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the immunoglobulin Fc region is a hybrid of domains having different origins derived from immunoglobulins selected from the group consisting of IgG, IgA, IgD, IgE, and IgM.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the immunoglobulin Fc region is an IgG4 Fc region.

As an enzyme fusion protein according to any one of the previous specific examples, the enzyme fusion protein is characterized in that the hinge region of the IgG4 Fc region is substituted with an IgG1 hinge region.

In one aspect, the present disclosure refers to a pharmaceutical composition for use in preventing or treating lysosomal storage disorder (LSD) comprising the enzyme fusion protein as described herein. In yet another aspect, the present disclosure refers to the use of an enzyme fusion protein as described herein in the manufacture of a pharmaceutical composition for preventing or treating lysosomal storage disorder (LSD). Another example of the present disclosure provides a pharmaceutical composition for preventing or treating lysosomal storage disorder (LSD).

In a specific example, the present disclosure relates to a pharmaceutical composition for preventing or treating LSD containing the enzyme fusion protein.

As a composition according to any one of the previous specific examples, the composition is characterized in that 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.

As a composition according the previous specific examples, the composition is characterized in that the enzyme is iduronate-2-sulfatase (IDS) or arylsulfatase B (ARSB).

As a composition according to any one of the previous specific examples, the composition is characterized in that it reduces the binding affinity of a therapeutic enzyme for lysosome receptors.

Still another aspect of the present disclosure provides a polynucleotide encoding the enzyme fusion protein.

Still another aspect of the present disclosure provides an expression vector containing the polynucleotide.

Still another aspect of the present disclosure provides a transformant into which the expression vector is introduced.

In another aspect, the present disclosure refers to a method for preparing an enzyme fusion protein, comprising: (a) culturing the transformant as described herein to obtain a culture; and (b) recovering an enzyme fusion protein from the culture.

Still another example of the present disclosure provides a method for preparing an enzyme fusion protein.

In a specific example, the present disclosure relates to a method for preparing an enzyme fusion protein, which includes culturing the transformant to obtain a culture; and recovering an enzyme fusion protein from the culture.

[Advantageous Effects of the Invention]

The present invention relates to a long-acting therapeutic enzyme fusion protein, and specifically, to an enzyme fusion protein in which an immunoglobulin Fc region is fused to a therapeutic enzyme such that the therapeutic enzyme has increased stability and the mechanism of enzyme removal by the kidney is reduced. The enzyme fusion protein of the present invention can be effectively used by patients due to the increased duration of time.

[Brief Description of Drawings]

FIG. 1 shows a graph confirming the expression of an IDS-Fc fusion protein.

FIG. 2 shows a graph confirming the expression of an ARSB-Fc fusion protein.

FIG. 3 shows a graph confirming the results of pharmacokinetic experiments of IDS-Fc fusion protein of the present invention.

FIG. 4 shows a graph confirming the results of pharmacokinetic experiments of ARSB-Fc fusion protein of the present invention.

FIG. 5 shows a graph confirming the *in vitro* enzyme activity of IDS-Fc fusion protein of the present invention.

FIG. 6 shows a graph confirming the *in vitro* enzyme activity of ARSB-Fc fusion protein of the present invention.

FIG. 7 shows a graph illustrating the measurement results of glycosaminoglycan (GAG) levels in urine after intravenous or subcutaneous injection of IDS-Fc fusion protein of the present invention, into an IDS-knockout mouse.

FIG. 8 shows a graph illustrating the measurement results of glycosaminoglycan (GAG) levels in tissue after intravenous or subcutaneous injection of IDS-Fc fusion protein of the

[CLAIMS]

[Claim 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.

[Claim 2]

The enzyme fusion protein of claim 1, 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.

[Claim 3]

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

[Claim 4]

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.

[Claim 5]

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.

[Claim 6]

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

amino acid is substituted with glutamine; or the 2nd amino acid is substituted with proline and the 71st amino acid is substituted with glutamine.

[Claim 7]

The enzyme fusion protein of claim 6, wherein no chain exchange occurs in the immunoglobulin Fc region.

[Claim 8]

The enzyme fusion protein of claim 1, 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.

[Claim 9]

The enzyme fusion protein of claim 1, 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 the heavy chain constant region and the light chain constant region.

[Claim 10]

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.

[Claim 11]

The enzyme fusion protein of any one of claims 1 to 10, wherein the immunoglobulin Fc region is aglycosylated.

[Claim 12]

The enzyme fusion protein of any one of claims 1 to 10, wherein the immunoglobulin Fc region is an Fc fragment derived from IgG, IgA, IgD, IgE, or IgM.

[Claim 13]

The enzyme fusion protein of claim 12, wherein the immunoglobulin Fc region is a hybrid of domains having different origins derived from immunoglobulins selected from the group consisting of IgG, IgA, IgD, IgE, and IgM.

[Claim 14]

The enzyme fusion protein of claim 13, wherein the immunoglobulin Fc region is an IgG4 Fc region.

[Claim 15]

The enzyme fusion protein of claim 14, wherein the hinge region of the IgG4 Fc region is substituted with an IgG1 hinge region.

[Claim 16]

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 10.

[Claim 17]

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

[Claim 18]

The use of claim 17, 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.

[Claim 19]

The use of claim 17, wherein the enzyme is iduronate-2-sulfatase (IDS) or arylsulfatase B (ARSB).

[Claim 20]

The use of any one of claims 17 to 19, wherein the composition reduces the binding affinity of an enzyme for lysosome receptors.

[Claim 21]

A polynucleotide encoding the enzyme fusion protein of any one of claims 1 to 10.

[Claim 22]

An expression vector comprising the polynucleotide of claim 21.

[Claim 23]

A transformant into which the expression vector of claim 22 is introduced.

[Claim 24]

A method for preparing an enzyme fusion protein, comprising:

(a) culturing the transformant of claim 23 to obtain a culture; and

(b) recovering an enzyme fusion protein from the culture.