only very costly to produce a large amount of protein, but also requires research to optimize an additional purification process.

However, the use of a heterodimeric Fc-fused protein according to the present invention makes it possible to easily produce a monovalent heterodimeric Fc-fused protein as shown in FIG. 2 without needing to optimize an additional purification process.

DISCLOSURE OF INVENTION

SUMMARY

In one aspect, the present disclosure refers to a heterodimeric Fc-fused protein comprising a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12, wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively, wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations, wherein the mutation at the CH3 domain of the first Fc region or the second Fc region includes one or more mutations selected from the group consisting of (wherein mutation positions are numbered according to the EU index): (a) glutamic acid (E) substitution of the amino acid residue at position 360 in the CH3 domain of the first Fc region; (b) tryptophan (W) substitution of the amino acid residue at position 409 in the CH3 domain of the first Fc region; and (c) threonine (T) substitution of the amino acid residue at position 405 in the CH3 domain of the second Fc region, and valine (V) substitution of the amino acid residue at position 399 in the CH3 domain of the second Fc region.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the heterodimeric Fc-fused protein as described herein and a pharmaceutically acceptable carrier.

TECHNICAL PROBLEM

It is an object of the present invention to provide a novel heterodimeric Fc-fused protein, the protein of which is composed of one, two, or more different subunits and thereby exhibits the intact biological activity by forming the assembled protein, and thus can maintain the natural physiological activity of the fused protein thereof *in vivo* for a long period of time.

In particular, the heterodimeric Fc-fused protein according to the present invention is formed such that it can retain the activity of a naturally occurring physiologically active protein, in which two or more subunits assemble together to form a protein to exhibit physiological activity, such that the fused protein can maintain the naturally occurring form and structure to the highest possible degree.

Further, the heterodimeric Fc-fused protein according to the present invention has an advantage in that the *in vivo* half-life of the physiologically active protein contained in the heterodimeric Fc-fused protein can be significantly increased due to the Fc-mediated long half-life such that the physiological activities thereof *in vivo* can be long-lasting.

【CLAIMS】

1. A heterodimeric Fc-fused protein comprising a first Fc region and a second Fc region of an immunoglobulin Fc (fragment crystallizable) pair and the p40 and p35 subunits of IL-12,

   wherein the p40 and p35 subunits of IL-12 are linked separately to the first Fc region and the second Fc region, or to the second Fc region and the first Fc region, respectively,

   wherein the p40 and p35 subunits are each linked to the N-terminus or C-terminus of the Fc regions, and

   wherein CH3 domains of the first Fc region and the second Fc region each comprise one or more mutations, wherein the mutation at the CH3 domain of the first Fc region or the second Fc region includes one or more mutations selected from the group consisting of (wherein mutation positions are numbered according to the EU index):

      (a) glutamic acid (E) substitution of the amino acid residue at position 360 in the CH3 domain of the first Fc region;

      (b) tryptophan (W) substitution of the amino acid residue at position 409 in the CH3 domain of the first Fc region; and

      (c) threonine (T) substitution of the amino acid residue at position 405 in the CH3 domain of the second Fc region, and valine (V) substitution of the amino acid residue at position 399 in the CH3 domain of the second Fc region.

2. The heterodimeric Fc-fused protein according to claim 1, wherein the p40 and p35 subunits are each linked to the N-terminus of the Fc regions.

3. The heterodimeric Fc-fused protein according to claim 2, wherein the p40 or p35 subunit is linked to its corresponding Fc region by a linker.

4. The heterodimeric Fc-fused protein according to claim 3, wherein the p35 subunit is linked to its corresponding Fc region by a linker.

5. The heterodimeric Fc-fused protein according to claim 4, wherein the linker comprises a $(G_4S)_3$ linker.

6. The heterodimeric Fc-fused protein according to claim 1, wherein each of the first Fc region and the second Fc region is from an Fc region selected from the group consisting of human IgG1, IgG2, IgG3, and IgG4.

7. The heterodimeric Fc-fused protein according to claim 1, wherein the first Fc region and the second Fc region are included in a whole antibody form selected from the group consisting of human IgG1, IgG2, IgG3, and IgG4.

8. The heterodimeric Fc-fused protein according to claim 1, wherein the IL-12 is human IL-12.

9. A pharmaceutical composition comprising the heterodimeric Fc-fused protein of claim 1 and a pharmaceutically acceptable carrier.

10. The heterodimeric Fc-fused protein according to claim 1, wherein the CH3 domain of the first Fc region includes the following residues (wherein mutation positions are numbered according to the EU index): glutamic acid (E) substitution of the amino acid residue at position 360; and tryptophan (W) substitution of the amino acid residue at position 409.

11. The heterodimeric Fc-fused protein according to claim 10, wherein the CH3 domain of the second Fc region further comprises (wherein the positions are numbered according to the EU index): arginine (R) substitution of the amino acid residue at position 347.

12. The heterodimeric Fc-fused protein according to claim 11, wherein the CH3 domains in the first Fc region and the second Fc region further include the following residues (wherein the positions are numbered according to the EU index):

    (i) cysteine (C) substitution at position 349 in the CH3 domain of the first Fc region; and

    (ii) cysteine (C) substitution at position 354 in the CH3 domain of the second Fc region.

13. The heterodimeric Fc-fused protein according to claim 1, wherein the CH3 domains in the first Fc region and the second Fc region further include the following residues (wherein the positions are numbered according to the EU index):

(i) cysteine (C) substitution at position 349 in the CH3 domain of the first Fc region; and

(ii) cysteine (C) substitution at position 354 in the CH3 domain of the second Fc region.