Variant Immunoglobulins with Improved Manufacturability

This invention relates to the expression of antibody molecules and, in particular, to the production of antibody molecules with improved manufacturability.

Summary

In one aspect, the present invention refers to a method of improving the manufacturability of an immunoglobulin, wherein the immunoglobulin is a whole antibody or an Fab antibody fragment, the method comprising; identifying a parent immunoglobulin having a VL domain and a VH domain, introducing one or both of: (i) a substitution at a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence of the parent immunoglobulin, or (ii) a substitution at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence of the parent immunoglobulin, thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays both reduced aggregation propensity and increased productivity upon expression relative to the parent immunoglobulin, wherein said substitution is introduced by mutation of the nucleic acid encoding the immunoglobulin or by synthesis of a nucleic acid encoding the immunoglobulin with a substitution at said position.

In another aspect, the present invention refers to a method of preparing an immunoglobulin with improved manufacturability comprising; providing a nucleic acid encoding a variant immunoglobulin produced by a method as disclosed herein, expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin and, recovering it.; and optionally formulating the variant immunoglobulin into a composition including a pharmaceutically acceptable excipient.

In yet another aspect, the present invention refers to a method of manufacturing an immunoglobulin, wherein the immunoglobulin is a whole antibody or an Fab antibody fragment, the method comprising; identifying a parent immunoglobulin having a VL domain and a VH domain, introducing one or both of: (i) a substitution at a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence of the parent immunoglobulin, or (ii) a substitution at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence of the parent immunoglobulin, thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays both reduced aggregation propensity and increased productivity upon expression relative to the parent immunoglobulin, wherein said substitution is introduced by mutation of the nucleic acid encoding the immunoglobulin or by synthesis of a nucleic acid encoding the immunoglobulin with a substitution at said position; expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin and recovering it.

Detailed Description

The term "aggregation" describes a broad variety of phenomena, all related to protein self-association, which can occur in multiple environments, from cell culture and fermentation, to isolation, purification and formulation processes. For example, 'aggregation' is often used when describing the formation of inclusions; the accumulation of protein in 'insoluble' fractions following cell fractionation; the appearance of turbidity, protein precipitation or formation of particles in samples; or the formation of small soluble oligomers amongst others. In this context, protein aggregation, understood as 'abnormal self-association' of polypeptide chains, can have multiple origins and manifestations which are largely dependent on both the nature of molecules involved and the environment in which polypeptides are produced, isolated, stored and administered.

The three-dimensional structure of a polypeptide determines its natural physiological function. Proteins typically acquire their native structure after being synthesised in the ribosome. The process of protein folding is fundamentally encoded in the amino acid sequence of a protein. However, folding can also be assisted *in vivo* by a number of molecular helpers and quality control mechanisms that ensure that polypeptides attain their intended biologically active structure, avoiding the population of aberrant conformations in the process. Such external helpers and quality control elements include chaperones, disulfide isomerases, post-translational modifications (i.e. proteolytic processing, acylation, glycosylation, etc.), and the ubiquitin-proteasome system.

Additionally, protein aggregation is driven by the intrinsic stability of proteins in solution. Proteins are only marginally stable in solution, and this relative low stability is crucial in conferring specific biological properties while offering good regulation mechanisms to control their action. However, taking polypeptides outside their natural environment, for example to develop them as drugs, imposes a number of strict restrictions in their production, isolation and formulation that can have a big impact on their stability and aggregation behaviour.

Alterations to the native environment of a given protein, particularly due to specific production or process requirements, can have dramatic impact on its yield and stability in solution. This problem is exacerbated by process requirements to increase yields, for example: artificial synthesis, heterologous expression (i.e. prokaryotic expression of mammalian proteins), the use of secretory versus non-secretory pathways, saturation of protein synthesis machinery; or additional hurdles posed by non-natural proteins, such as polypeptide fragments or fusions. For instance, the expression of mammalian proteins in heterologous systems poses numerous risks. This is due to the fact that such systems usually lack the adequate molecular environment to guarantee a 'native' folding and processing.

Claims:

1. A method of improving the manufacturability of an immunoglobulin, wherein the immunoglobulin is a whole antibody or an Fab antibody fragment, the method comprising;

identifying a parent immunoglobulin having a VL domain and a VH domain, introducing one or both of:

(i) a substitution at a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence of the parent immunoglobulin, or

(ii) a substitution at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence of the parent immunoglobulin,

thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays both reduced aggregation propensity and increased productivity upon expression relative to the parent immunoglobulin,

wherein said substitution is introduced by mutation of the nucleic acid encoding the immunoglobulin or by synthesis of a nucleic acid encoding the immunoglobulin with a substitution at said position.

2. A method according to claim 1 wherein the substitution is selected from the group consisting of substitution of the residue at position 37 in the VL domain sequence for Q or N; substitution of the residue at position 45 in the VL domain sequence for E, K, R, Q or V; substitution of the residue at position 46 in the VL domain sequence for L or Y; substitution of the residue at position 1 in the VH domain sequence for E, Q, D, A or V; substitution of the residue at position 17 in the VH domain sequence for R; substitution of the residue at position 94 in the VH domain sequence for R; substitution of the residue at position 95 in the VH domain sequence for D; substitution of the residue at position 96 in the VH domain sequence for A; or a substitution of the residue at position 100d in the VH domain sequence for F, G, L, P or M; and a substitution of the residue at position 102 in the VH domain sequence for A, F, H, I, L, V or Y.

3. A method according to claim 1 wherein the sequence of the variant immunoglobulin has up to 10 amino acid substitutions relative to the parent immunoglobulin sequence.

4. A method according to claim 1 wherein the sequence of the VH domain comprises substitutions at position 1 and position 102; substitutions at position 95 and position 102 or; substitutions at positions 100d and 102; and optionally further comprises substitutions at positions 94 and 100.

5. A method according to claim 4 wherein the residue at position 102 is substituted for A, F, H, I, L, V or Y and the residue at position 1 is substituted for E, Q, D, A or V; or the residue at position 102 is substituted for A, F, H, I, L, V or Y and the residue at position 95 is substituted for E or D.

6. A method according to claim 1 wherein the sequence of the VL domain of the variant immunoglobulin comprises substitutions at position 37 and position 45.

7. A method according to claim 6 wherein the residue at position 37 is substituted for Q or N and wherein the residue at position 45 is substituted for R, K or E.

8. A method according to claim 1 wherein the residue at position 94 in the VH domain is substituted for R, K or T and the residue at position 95 in the VH domain is substituted for E or D and the residue at position 46 in the VL domain is substituted for K, R, H, F or S.

9. A method according to claim 1 wherein the variant immunoglobulin is humanised; expressed in a recombinant system; isolated and/or purified following expression or formulated with a pharmaceutically acceptable excipient.

10. A method of preparing an immunoglobulin with improved manufacturability comprising;

providing a nucleic acid encoding a variant immunoglobulin produced by a method according to claim 1,

expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin and, recovering it.;

and optionally formulating the variant immunoglobulin into a composition including a pharmaceutically acceptable excipient.

11. A method of manufacturing an immunoglobulin, wherein the immunoglobulin is a whole antibody or an Fab antibody fragment, the method comprising;

identifying a parent immunoglobulin having a VL domain and a VH domain, introducing one or both of:

(i) a substitution at a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence of the parent immunoglobulin, or

(ii) a substitution at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence of the parent immunoglobulin,

thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays both reduced aggregation propensity and increased productivity upon expression relative to the parent immunoglobulin,

wherein said substitution is introduced by mutation of the nucleic acid encoding the immunoglobulin or by synthesis of a nucleic acid encoding the immunoglobulin with a substitution at said position;

expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin and recovering it.

12. The method of claim 11, further comprising formulating the variant immunoglobulin into a composition comprising a pharmaceutically acceptable excipient.