

ENGINEERING MONOCLONAL ANTIBODIES TO IMPROVE STABILITY AND PRODUCTION TITER

RELATED APPLICATIONS

5 The present application claims the benefit of U.S. Provisional App. No. 62/787,867 filed January 3, 2019, which is incorporated by reference in its entirety herein.

FIELD

10 The presented subject matter relates to the field of protein engineering. Specifically, the presented subject matter relates to engineering antibodies, especially monoclonal antibodies, and variants thereof, to improve their stability and production.

BACKGROUND

15 Monoclonal antibodies (mAbs) that are recombinantly produced (and active fragments thereof) are important therapeutic tools. However, as these molecules are complex, many challenges need to be met to facilitate production, storage, and therapeutic administration of these molecules.

Two challenges concern production and stability. mAbs are produced in bioreactors from engineered cells, such as Chinese Hamster Ovary (CHO) cells. However, production levels can be low and can vary between mAbs. Low production levels increase production costs, including production time, labor, and consumed resources, such as the necessary components for operating the bioreactor. 20 Furthermore, a lack of stability impacts the “shelf-life” of mAbs. Degraded mAbs can be less potent, and fragmented mAbs can present an immunologic risk.

Therefore there is a need to improve mAb stability and production titer.

SUMMARY

25 In one aspect, the present disclosure provides a method of increasing stability of a first antibody or antibody variant, comprising substituting a hydrophobic amino acid residue at heavy chain position 80 (Aho numbering) of the first antibody or antibody variant to create a second antibody or antibody variant, wherein the second antibody or antibody variant is more stable than the unsubstituted first antibody or antibody variant, wherein the hydrophobic amino acid residue is selected from the group 30 consisting of: alanine, isoleucine, phenylalanine, leucine, methionine, and valine.

In another aspect, the present disclosure provides an antibody or antibody variant made by the method as described herein. In another aspect, the present disclosure provides a pharmaceutical composition comprising the antibody or antibody variant as described herein.

In a first example, provided herein are methods of increasing stability of a first antibody, comprising substituting glycine, alanine, or serine at heavy chain position 56 (AHO numbering) to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. For example, glycine or serine may be substituted at heavy chain position 56. For example, glycine or alanine may be substituted at heavy chain position 56. For example, glycine may be substituted at heavy chain position 56.

In a second example, provided herein are methods of increasing stability of a first antibody, comprising substituting a hydrophobic amino acid at heavy chain position 80 (AHO numbering) of the first antibody to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. Examples of hydrophobic amino acid residues include alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, and valine. By way of example, the hydrophobic amino acid residue can comprise or consist of alanine, isoleucine, phenylalanine, leucine, methionine, or valine. By way of example, the hydrophobic amino acid residue can comprise or consist of phenylalanine, leucine, or valine.

In a third example, provided herein are methods of increasing stability of a first antibody, comprising substituting alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at heavy chain position 80 (AHO numbering) of the first antibody to create a second antibody, wherein the second antibody is more stable than the unsubstituted first antibody. For example, phenylalanine, leucine, or valine may be substituted at heavy chain position 80. For example, isoleucine or methionine may be substituted at heavy chain position 80. For example, isoleucine may be substituted at heavy chain position 80. For example, methionine may be substituted at heavy chain position 80.

In sub-examples of these first three examples, the increased stability of the second antibody is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting. In some sub-examples, the increase in titer is measured by the rate of binding to a protein A coated probe tip using an Octet Forte Bio Instrument; and/or the increased yield is measured by protein A or protein G capture; and/or the increased purity is measured by SEC of purified protein; and/or the reduction in high molecular weight

species is measured by size-exclusion chromatography (SEC) and the area under the curve of each peak for each molecular weight; and/or the increased melting point temperature is measured by differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC); and/or the increased temperature of aggregation is measured by DSF; and/or the increased temperature of the onset of melting is measured by DSF.

In some sub-examples of the first example, the second antibody is further substituted with a hydrophobic amino acid residue at heavy chain position 80 (AHo numbering). For example, the hydrophobic amino acid residue can comprise or consist of of: alanine, isoleucine, phenylalanine, leucine, methionine, or valine. For example, the hydrophobic amino acid residue can be selected from the group consisting of: phenylalanine, leucine, and valine. In some sub-examples of the first example, the second antibody is further substituted with methionine at position 80 (AHo numbering), or, alternatively, the second antibody is further substituted with isoleucine at position 80 (AHo numbering). In some sub-examples of the first example, the second antibody is further substituted with alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at position 80 (AHo numbering). In some sub-examples of the first example, the second antibody is further substituted with phenylalanine, leucine, or valine at position 80 (AHo numbering).

In some sub-examples of the second and third examples, the second antibody is further substituted with glycine, alanine, or serine at position 56 (AHo numbering). In some sub-examples of the second and third examples, the second antibody is further substituted with glycine or alanine at position 56 (AHo numbering). In some sub-examples of the second and third examples, the second antibody is further substituted with glycine or serine at position 56 (AHo numbering). In some sub-examples of the second and third examples, the second antibody is further substituted with glycine at position 56 (AHo numbering).

In these first three examples, the first antibody is a monoclonal antibody, such as, for example, a human, or humanized, antibody. Furthermore, the first antibody is an IgG antibody, such as an IgG antibody selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody. That is, the IgG antibody can be an IgG1 antibody, the IgG antibody can be an IgG2 antibody, the IgG antibody can be an IgG3 antibody, and the IgG antibody can be an IgG4 antibody.

In a fourth example, disclosed herein are methods of increasing stability of a first antibody variant, comprising substituting glycine, alanine, or serine at heavy chain position 56 (AHo numbering) to create a second antibody variant, wherein the second antibody variant is more stable than the unsubstituted first antibody variant. For example, glycine or serine can be substituted at heavy chain

position 56. For example, glycine or alanine can be substituted at heavy chain position 56. For example, glycine can be substituted at heavy chain position 56.

In a fifth example, disclosed herein are methods of increasing stability of a first antibody variant, comprising substituting a hydrophobic amino acid residue (such as alanine, isoleucine, phenylalanine, leucine, methionine, or valine) at heavy chain position 80 (AHO numbering) of the first antibody variant to create a second antibody variant, wherein the second antibody variant is more stable than the unsubstituted first antibody variant. For example, the hydrophobic amino acid residue can comprise or consist of: phenylalanine, leucine, or valine. For example, the hydrophobic amino acid residue can comprise or consist of methionine or isoleucine.

In a sixth example, disclosed herein are methods of increasing stability of a first antibody variant, comprising substituting alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at heavy chain position 80 (AHO numbering) of the first antibody variant to create a second antibody variant, wherein the second antibody variant is more stable than the unsubstituted first antibody variant. For example, phenylalanine, leucine, or valine can be substituted at heavy chain position 80. For example, methionine or isoleucine can be substituted at heavy chain position 80. For example, methionine can be substituted at heavy chain position 80. For example, isoleucine can be substituted at heavy chain position 80.

In sub-examples of these fourth, fifth, and sixth examples, the increased stability of the second antibody variant is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting. In some sub-examples, the increase in titer is measured by the rate of binding to a protein A coated probe tip using an Octet Forte Bio Instrument; and/or the increased yield is measured by protein A or protein G capture; and/or the increased purity is measured by SEC of purified protein; and/or the reduction in high molecular weight species is measured by size-exclusion chromatography (SEC) and the area under the curve of each peak for each molecular weight; and/or the increased melting point temperature is measured by differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC); and/or the increased temperature of aggregation is measured by DSF; and/or the increased temperature of the onset of melting is measured by DSF.

In some sub-examples of the fourth example, the second antibody variant is further substituted with a hydrophobic amino acid residue at heavy chain position 80 (AHO numbering). For example, the

hydrophobic amino acid residue can be selected from the group consisting of: alanine, isoleucine, phenylalanine, leucine, methionine, and valine. For example, the hydrophobic amino acid residue can be selected from the group consisting of: phenylalanine, leucine, and valine. In some sub-examples of the fourth example, the second antibody variant is further substituted with methionine at position 80 (AHO numbering), or, alternatively, the second antibody is further substituted with isoleucine at position 80 (AHO numbering). In some sub-examples of the fourth example, the second antibody variant is further substituted with alanine, phenylalanine, isoleucine, leucine, methionine, threonine, or valine at position 80 (AHO numbering). In some sub-examples of the fourth example, the second antibody variant is further substituted with phenylalanine, leucine, or valine at position 80 (AHO numbering).

In some sub-examples of the fifth and sixth examples, the second antibody variant is further substituted with glycine, alanine, or serine at position 56 (AHO numbering). For example, the second antibody variant can be substituted with glycine or alanine at position 56. For example, the second antibody variant can be substituted with glycine or serine at position 56. For example, the second antibody variant can be substituted with glycine at position 56.

In some sub-examples of these fourth, fifth, and sixth examples, the first antibody variant is a multi-specific antibody, such as a bi-specific or tri-specific antibody. In some sub-examples of these fourth, fifth, and sixth examples, first antibody variant is an antibody fragment that can bind an antigen; the antibody fragment can be selected from the group consisting of a Fab fragment, a Fab' fragment, a F'(ab)₂ fragment, an Fv fragment, a single chain antibody, diabodies), a biparatopic peptide, a domain antibody (dAb), a CDR-grafted antibody, a single-chain antibody (scFv), a single chain antibody fragment, a chimeric antibody, a diabody, a triabody, a tetrabody, a minibody, a linear antibody; a chelating recombinant antibody, a tribody, a bibody, an intrabody, a nanobody, a small modular immunopharmaceutical (SMIP), an antigen-binding-domain immunoglobulin fusion protein, a single domain antibody, and a VHH containing antibody.

Furthermore, in these fourth, fifth, and sixth examples, the first antibody variant is a monoclonal antibody variant, such as, for example, a human, or humanized, antibody variant. Furthermore, the first antibody variant is an IgG antibody variant, such as an IgG antibody variant selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody variant. That is, the IgG antibody variant can be an IgG1 antibody variant, the IgG antibody variant can be an IgG2 antibody variant, the IgG antibody variant can be an IgG3 antibody variant, and the IgG antibody variant can be an IgG4 antibody variant.

In a seventh example, disclosed herein are methods of increasing stability of a first antibody or first antibody variant, comprising

- a. identifying the germline original amino acid sequence for the heavy chain of the first antibody or of the antibody portion of the antibody variant;
- b. identifying the amino acid residues at heavy chain position 56 (AHO numbering) and heavy chain position 80 (AHO numbering) in the first antibody or of the antibody portion of the antibody variant; and
- c. substituting at heavy chain positions 56 and 80 the identified residues from the germline original amino acid sequence in the first antibody or of the antibody portion of the antibody variant, thereby creating a second antibody or second antibody variant, wherein the second antibody is more stable than the unsubstituted first antibody, or wherein the second antibody variant is more stable than the unsubstituted first antibody variant.

In a sub-example of this seventh example, the increased stability of the second antibody or second antibody variant is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting. In sub-examples of this seventh example, the increased stability of the second antibody or second antibody variant is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting. In some sub-examples, the increase in titer is measured by the rate of binding to a protein A coated probe tip using an Octet Forte Bio Instrument; and/or the increased yield is measured by protein A or protein G capture; and/or the increased purity is measured by SEC of purified protein; and/or the reduction in high molecular weight species is measured by size-exclusion chromatography (SEC) and the area under the curve of each peak for each molecular weight; and/or the increased melting point temperature is measured by differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC); and/or the increased temperature of aggregation is measured by DSF; and/or the increased temperature of the onset of melting is measured by DSF.

In this seventh example, the first antibody variant is a multi-specific antibody, such as a bi-specific or tri-specific antibody. In some sub-examples of these fourth, fifth, and sixth examples, first antibody variant is an antibody fragment that can bind an antigen; the antibody fragment can be selected from the group consisting of a Fab fragment, a Fab' fragment, a F'(ab)₂ fragment, an Fv

fragment, a single chain antibody, diabodies), a biparatopic peptide, a domain antibody (dAb), a CDR-grafted antibody, a single-chain antibody (scFv), a single chain antibody fragment, a chimeric antibody, a diabody, a triabody, a tetrabody, a minibody, a linear antibody; a chelating recombinant antibody, a tribody, a bibody, an intrabody, a nanobody, a small modular immunopharmaceutical (SMIP), an antigen-binding-domain immunoglobulin fusion protein, a single domain antibody, and a VHH containing antibody.

Furthermore, in this seventh example, the first antibody variant is a monoclonal antibody variant, such as, for example, a human, or humanized, antibody variant. Furthermore, the first antibody variant is an IgG antibody variant, such as an IgG antibody variant selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody variant. That is, the IgG antibody variant can be an IgG1 antibody variant, the IgG antibody variant can be an IgG2 antibody variant, the IgG antibody variant can be an IgG3 antibody variant, and the IgG antibody variant can be an IgG4 antibody variant.

In addition, in this seventh example, the first antibody is a monoclonal antibody, such as, for example, a human, or humanized, antibody. Furthermore, the first antibody is an IgG antibody, such as an IgG antibody selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody. That is, the IgG antibody can be an IgG1 antibody, the IgG antibody can be an IgG2 antibody, the IgG antibody can be an IgG3 antibody, and the IgG antibody can be an IgG4 antibody.

In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: GF, GI, GL, GT, GV, AF, AI, AL, AV, AA, AM, SA, SI, or ST. By way of example, it is noted that for this nomenclature "GF" would refer to a "G" at heavy chain position 56 and an "F" at heavy chain position 80 (AHO numbering). In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: GF, GL, GV, AF, AL, or AV. Such substitutions can have a higher titer and/or higher T_m compared to the first antibody (or first antibody variant). In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: AA, AL, AM, AV, GF, GL, GT, SA, or ST. Such substitutions can have a higher titer compared to the first antibody (or first antibody variant). In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: AI, AV, GI, SI, or GV.

Such substitutions can have a higher T_m compared to the first antibody (or first antibody variant). In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: GF, GL, GT, GV, AF, AL, AV, AA, AM, AV, SA, and ST. Such substitutions can have a higher titer compared to the first antibody (or first antibody variant). In some sub-examples of any of the first through eighth examples, the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: GF, GI, GL, GV, AF, AL, AV, AI, or SI. Such substitutions can have a higher T_m compared to the first antibody (or first antibody variant).

In an eighth example, provided herein are methods of making pharmaceutical compositions formulated with the second antibody or second antibody variant produced by any previous example.

In a ninth example, provided herein is an antibody or antibody variant made according to any of the first seven examples.

In a tenth example, provided herein is pharmaceutical composition comprising an antibody or antibody variant made according to any of the first seven examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D are a series of graphs showing various characteristics of two monoclonal antibodies: an engineered monoclonal antibody, and its parent monoclonal antibody.

Figure 2A is a graph showing that for mAb1, alanine and glycine at HC:56 had the highest titers. HC80 residues are shown in the top row of the X-axis labels. HC56 residues are shown in the bottom row of the X-axis labels.

Figure 2B is a graph showing that for mAb1, phenylalanine, leucine, and valine at HC:80 had the highest titers. HC56 residues are shown in the top row of the X-axis labels. HC80 residues are shown in the bottom row of the X-axis labels.

Figure 3A is a graph showing that for mAb1, the HC56 and HC80 variants with high titers also had high T_m's. HC80 residues are shown in the top row of the X-axis labels. HC56 residues are shown in the bottom row of the X-axis labels.

Figure 3B is a graph showing that for mAb1, molecules with phenylalanine, leucine, or valine at HC80 had T_m's above 65 °C. HC56 residues are shown in the top row of the X-axis labels. HC80 residues are shown in the bottom row of the X-axis labels.

Figure 4 is a graph showing that for mAb1, most high molecular weight (HMW) levels were below 5%, as determined by SEC. HC80 residues are shown in the top row of the X-axis labels. HC56 residues are shown in the bottom row of the X-axis labels.

Figure 5 is a graph showing that mAb2 expresses well with residues such as hydrophobic residues at HC56 and HC80. HC80 residues are shown in the top row of the X-axis labels. HC56 residues are shown in the bottom row of the X-axis labels.

Figure 6 is a graph showing that for substitutions at HC56 and HC80 of mAb2, T_m correlates with titer. HC80 residues are shown in the top row of the X-axis labels. HC56 residues are shown in the bottom row of the X-axis labels.

CLAIMS

1. A method of increasing stability of a first antibody or antibody variant, comprising substituting a hydrophobic amino acid residue at heavy chain position 80 (AHo numbering) of the first antibody or antibody variant to create a second antibody or antibody variant, wherein the second antibody or antibody variant is more stable than the unsubstituted first antibody or antibody variant, wherein the hydrophobic amino acid residue is selected from the group consisting of: alanine, isoleucine, phenylalanine, leucine, methionine, and valine.
2. The method of claim 1, wherein the hydrophobic amino acid residue is selected from the group consisting of: phenylalanine, leucine, and valine.
3. The method of claim 1, wherein the methionine or isoleucine is substituted at heavy chain position 80 of the first antibody or antibody variant.
4. The method of any one of claims 1-3, wherein the second antibody or antibody variant is further substituted with alanine, glycine, or serine at heavy chain position 56 (AHo numbering).
5. The method of any one of claims 1-3, wherein the second antibody or antibody variant is further substituted with alanine or glycine at heavy chain position 56 (AHo numbering).
6. The method of any one of claims 1-3, wherein the second antibody or antibody variant is further substituted with glycine at heavy chain position 56 (AHo numbering).
7. The method of any of claims 1-6, wherein the increased stability of the second antibody is demonstrated by at least one selected from the group consisting of an increase in titer during cell culture, increased yield from cell culture, increased purity after purification, a reduction in high molecular weight species, an increased melting point temperature, an increased temperature of aggregation, and an increased temperature of the onset of melting.

8. The method of any preceding claim, wherein the first antibody or antibody variant is a monoclonal antibody.

9. The method of any preceding claim, wherein the first antibody or antibody variant is a human monoclonal antibody or a humanized monoclonal antibody.

10. The method of any preceding claim, wherein the first antibody or antibody variant is an IgG antibody selected from the group consisting of an IgG1, IgG2, IgG3, and IgG4 antibody.

11. The method of any of claims 1-10, wherein the first antibody variant is a multi-specific antibody.

12. The method of claim 11, wherein the multi-specific antibody is a bispecific antibody or trispecific antibody.

13. The method of any of claims 1-12, wherein the first antibody or antibody variant is an antibody fragment that can bind an antigen, wherein the antibody fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a F'(ab)₂ fragment, an Fv fragment, a single chain antibody, diabodies), a biparatopic peptide, a domain antibody (dAb), a CDR-grafted antibody, a single-chain antibody (scFv), a single chain antibody fragment, a chimeric antibody, a diabody, a triabody, a tetrabody, a minibody, a linear antibody; a chelating recombinant antibody, a tribody, a bibody, an intrabody, a nanobody, a small modular immunopharmaceutical (SMIP), an antigen-binding-domain immunoglobulin fusion protein, a single domain antibody, and a VHH containing antibody.

14. The method of any one of claims 1-13, wherein the second antibody or second antibody variant is substituted with any one of the following pairs of residues at positions 56 and 80 of the heavy chain (AHO numbering), respectively: GF, GI, GL, GT, GV, AF, AI, AL, AV, AA, AM, SA, SI, or ST.

15. The method of any of claims 1-14, further comprising formulating the second antibody or second antibody variant into a pharmaceutical composition.

16. An antibody or antibody variant made by the method of any of claims 1-15.
17. A pharmaceutical composition comprising the antibody or antibody variant of claim 16.