METHODS OF DECREASING TRISULFIDE BONDS DURING RECOMBINANT PRODUCTION OF POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial No. 62/334,433, filed May 10, 2016, which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392036540SEQLIST.txt, date recorded: May 8, 2017, size: 34.1 KB).

FIELD OF THE INVENTION

[0003] The present disclosure relates to cell culture media and methods for decreasing trisulfide bonds in polypeptides produced recombinantly.

BACKGROUND OF THE INVENTION

[0004] Trisulfide bonds are generated by the insertion of an additional sulfur atom into a disulfide bond, thereby resulting in the covalent bonding of three consecutive sulfur atoms. Trisulfide bond formation is a source of heterogeneity in recombinantly produced therapeutic polypeptides. Such heterogeneity is undesirable, as therapeutic products must undergo extensive characterization and meet acceptable standards that ensure product quality and consistency. As such, a demand exists for methods for decreasing the levels of trisulfide bonds during the manufacture of therapeutic polypeptides. There is also a need in the art for minimizing the variability of trisulfide bond levels in therapeutic polypeptides during manufacture. The present disclosure is directed to this and other needs.

BRIEF SUMMARY

[0005] In one aspect, provided is a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or

pyridoxal (vitamin B6),iv) between about 3.4 μM to about 23 μM folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 1.58 mM methionine; (b) culturing the host cell to produce the polypeptide; and (c) harvesting the polypeptide produced by the host cell. In a related aspect, provided is a method for producing a polypeptide, comprising (a) contacting a host cell comprising a nucleic acid encoding the polypeptide with a basal medium, wherein the basal medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between about 3.4 μM to about 23 μM folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 1.58 mM methionine; (b) culturing the host cell to produce the polypeptide; and (c)harvesting the polypeptide produced by the host cell.

[0006] In certain embodiments according to (or as applied to) any of the embodiments above, the harvested polypeptide has a trisulfide bond level less than a polypeptide produced under identical conditions, except that the concentration of the one or more components differs from the concentration specified in (a). In certain embodiments according to (or as applied to) any of the embodiments above, the basal medium lacks cystine. In certain embodiments according to (or as applied to) any of the embodiments above, the basal medium comprises between about 1.4 mM to 3 mM cysteine or cystine. In certain embodiments according to (or as applied to) any of the embodiments above, the basal medium comprises between about 0 mM to about 1.58 mM methionine and between about 0 mM to about 3 mM cysteine. In certain embodiments according to (or as applied to) any of the embodiments above, the basal medium comprises about 6mM cysteine.

[0007] Also provided in one aspect is a method for decreasing trisulfide bond levels in a polypeptide comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between about 3.4 μM to about 23 μM folate/folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12),

vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell. In certain embodiments according to (or as applied to) any of the embodiments above, the concentration of one or more of the components in the cell culture medium is the cumulative concentration of one or more additions after inoculation.

[0008] In one aspect, provided is a method for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immunocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody , the method comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, ii) between about 0.11 μM to about 2 μM riboflavin (vitamin B2), iii) between about 4.5 μM to about 80 μM pyridoxine or pyridoxal (vitamin B6), iv) between about 3.4 μM to about 23 μM folate/folic acid (vitamin B9), v) between about 0.2 μM to about 2.5 μM cyanocobalamin (vitamin B12), vi) between about 9 mM and about 10 mM hypotaurine; and vii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; (c) and harvesting the polypeptide produced by the host cell.

[0009] Also provided in one aspect is a method for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immunocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody and an anti-CD40 antibody , the method comprising: (a) culturing a host cell comprising a nucleic acid encoding the polypeptide in a cell culture medium, wherein the cell culture medium comprises one or more of the following components: i) between about 2 μM to about 35μM iron, and ii) between about 0 and about 4.5 mM methionine; (b) producing the polypeptide; and (c) harvesting the polypeptide produced by the host cell.

[0010] In certain embodiments according to (or as applied to) any of the embodiments above, the method further comprises at least one feed, and wherein the feed medium lacks one or more of the following: iron, riboflavin, pyridoxine, pyridoxal, folic acid, and cyanocobalamin. In certain embodiments according to (or as applied to) any of the embodiments above, the feed is a batch feed. In certain embodiments according to (or as applied to) any of the embodiments above, the batch feed medium lacks cystine. In certain

embodiments according to (or as applied to) any of the embodiments above, the batch feed medium lacks cysteine. In certain embodiments according to (or as applied to) any of the embodiments above, the batch feed medium lacks methionine. In certain embodiments according to (or as applied to) any of the embodiments above, the iron is ferric iron (Fe^{3+}) or ferrous iron (Fe^{2+}).

[0011] In certain embodiments according to (or as applied to) any of the embodiments above, the method further comprises: (I) supplementing the culture of said host cell with a chelating agent and a reducing agent prior to harvest; (II) supplementing a pre-harvest cell culture fluid (PHCCF) of said host cell with a chelating agent and a reducing agent; or (III) supplementing a harvested cell culture fluid (HCCF) of said host cell with a chelating agent and a reducing agent following harvest.

[0012] Also provided in one aspect is a method for decreasing level of trisulfide bonds in a polypeptide produced by a host cell comprising: (i) supplementing a culture of said host cell with a reducing agent and a chelating agent prior to harvest; (ii) supplementing a pre-harvest cell culture fluid (PHCCF) of said host cell with a chelating agent and a reducing agent; or (iii) supplementing a harvested cell culture fluid (HCCF) of said host cell with a reducing agent and a chelating agent.

[0013] In certain embodiments according to (or as applied to) any of the embodiments above, the culture, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent prior to being supplemented with the reducing agent. In certain embodiments according to (or as applied to) any of the embodiments above, the PHCCF, or the HCCF of said host cell is supplemented with the chelating agent between about 60 minutes to about 30 minutes prior to being supplemented with the reducing agent. In certain embodiments according to (or as applied to) any of the embodiments above, the chelating agent and the reducing agent are maintained in the culture, the PHCCF, or the HCCF of said host cell for about 30 minutes to about 4 days. In certain embodiments according to (or as applied to) any of the embodiments above, the culture, the PHCCF, or the HCCF of said host cell is maintained at a temperature between about 15°C and about 37°C. In certain embodiments according to (or as applied to) any of the embodiments above, the culture, the PHCCF, or the HCCF of said host cell is maintained pH between about 6.5 to about 7.5. In certain embodiments according to (or as applied to) any of the embodiments above, the amount of dissolved oxygen (DO) in the culture, the PHCCF, or the HCCF of said host cell is at least about 15%. In certain embodiments according to (or as applied to) any of the embodiments

CEA, and IL-6. In certain embodiments according to (or as applied to) any of the embodiments above, the polypeptide is an antibody, and wherein the antibody is a bispecific antibody. In certain embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody is an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-CEA/anti-CD3 bispecific antibody, or an anti-Ang2/anti-VEGF bispecific antibody. In certain embodiments according to (or as applied to) any of the embodiments above, the polypeptide is an immunocytokine. In certain embodiments according to (or as applied to) any of the embodiments above, the immunocytokine is CEA-IL2v or FAP-IL2v.

[0018] Also provided in one aspect is the use of between about 0 and about 4.5 μM methionine in a cell culture medium for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immuocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody.

[0019] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the invention will become apparent to one of skill in the art.

[0020] All publications, patents, and patent applications cited herein are incorporated by reference in their entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1** provides the results of experiments performed to assess the % trisulfide bond in anti-FluB incubated in a cell-free system containing Medium 1 + cysteine (Cys); Medium 1 + Cys + Fe (iron); Medium 1 + cystine (Cys-Cys); Medium 1 + Cys-Cys + Fe; Medium 2 + Cys; Medium 2 + Cys + Fe; Medium 2 + Cys-Cys; or Medium 1 + Cys-Cys + Fe.

[0022] **FIG. 2A** provides the results of experiments performed to assess the % trisulfide bond in anti-FluB incubated in Medium 1 that was supplemented with one or more of the following components: (a) Cys-Cys, (b) Fe, and (c) B vitamins (riboflavin, pyridoxine, folic acid, and cyanocobalamin).

[0023] **FIG. 2B** provides the results of experiments performed to assess the % trisulfide bond in an anti-OX40 antibody incubated in Medium 1 that was supplemented with

33. The method of any one of claims 1-32, wherein the polypeptide is secreted into the cell culture medium.

34. The method of any one of claims 1-33, further comprising a step of purifying the harvested polypeptide.

35. The method of any one of claims 1-34, wherein the host cell is a recombinant host cell.

36. The method of any one of claims 1-35, wherein the host cell is a mammalian cell.

37. The method of claim 36, wherein the mammalian cell is a CHO cell.

38. The method of any one of claims 1-37, wherein the method further comprises measuring the level of trisulfide bonds in the polypeptide.

39. The method of any one of claims 1-38, wherein the average % trisulfide bonds in the polypeptide is less than about 20%, optionally less than about 10%, optionally less than about 5%, optionally less than about 1%, optionally less than about 0.5%, or optionally less than about 0.1%.

40. The method of any one of claims 1-9 and 12-39, wherein the polypeptide is an antibody or fragment thereof.

41. The method of claim 40, wherein the polypeptide is an antibody fragment, and wherein the antibody fragment is selected from the group consisting of: a Fab, a Fab', an $F(ab')_2$, an scFv, an $(scFv)_2$, a dAb, a complementarity determining region (CDR) fragment, a linear antibody, a single-chain antibody molecule, a minibody , a diabody, and multispecific antibody formed from antibody fragments.

42. The method of claim 40, wherein the antibody or fragment thereof binds to an antigen selected from the group consisting of: BMPR1B, E16, STEAP1, 0772P, MPF, Napi3b, Sema 5b, PSCA hlg, ETBR, MSG783, STEAP2, TrpM4, CRIPTO, CD21, CD79b, FcRH2, HER2, NCA, MDP, IL20Rα, Brevican, EphB2R, ASLG659, PSCA, GEDA, BAFF-R, CD22,

CD79a, CXCR5, HLA-DOB, P2X5, CD72, LY64, FcRH1, IRTA2, TENB2, PMEL17, TMEFF1, GDNF-Ra1, Ly6E, TMEM46, Ly6G6D, LGR5, RET, LY6K, GPR19, GPR54, ASPHD1, Tyrosinase, TMEM118, GPR172A, CD33, CLL-1, C5, OX40, α4β7 and αEβ7 integrin heterodimers, IL-13, CD-20, FGFR, influenza A, influenza B, amyloid beta, HER3, complement factor D, IL-22c, PD-L1, PD-L2, PD-1, VEGF, Angiopoietin 2, CD3, FAP, CEA, and IL-6.

43. The method of claim 40, wherein the polypeptide is an antibody, and wherein the antibody is a bispecific antibody.

44. The method of claim 43, wherein the bispecific antibody is an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-CEA/anti-CD3 bispecific antibody, or an anti-Ang2/anti-VEGF bispecific antibody.

45. The method of any one of claims 1-9 and 12-39, wherein the polypeptide is an immunocytokine.

46. The method of claim 45, wherein the immunocytokine is CEA-IL2v or FAP-IL2v.

47. Use of between about 0 and about 4.5 μM methionine in a cell culture medium for decreasing trisulfide bond levels in a polypeptide selected from the group consisting of: a CEA-IL2v immuocytokine, a FAP-IL2v immunocytokine, an anti-CEA/anti-CD3 bispecific antibody, an anti-VEGF/anti-angiopoietin bispecific antibody, an anti-Ang2/anti-VEGF bispecific antibody, an anti-C5 antibody, and an anti-CD40 antibody.