(McCormick et al., *Science* 191(4226):468-9 (1976); Wentworth et al., *Science* 293(5536):1806-11 (2001); McCormick et al., *Journal of the American Chemical Society* 100:312-313 (1978)). Additionally, tryptophan is known to produce singlet oxygen upon exposure to light (Davies, M.J., *Biochem Biophys Res Commun* 305(3):761-70 (2003)). Similar to the protein oxidation induced by autoxidation of polysorbate 20, it is possible that protein oxidation can occur upon ROS generation by other excipients in the protein formulation (*e.g.* L-Trp) under normal handling conditions.

[0005] It is apparent from recent studies that the addition of standard excipients, such as L-Trp and polysorbates, to protein compositions that are meant to stabilize the protein can result in unexpected and undesired consequences such as ROS-induced oxidation of the protein. Therefore, there remains a need for the identification of alternative excipients for use in protein compositions and the development of such compositions.

SUMMARY

[0006] Provided herein are formulations comprising a protein and a compound that prevents oxidation of the protein in the formulation, methods of making the formulations, and methods of screening compounds that prevent oxidation of a protein in a protein composition.

[0006a] In one aspect, the present disclosure refers to a liquid formulation comprising an antibody and a compound which prevents oxidation of the antibody in the liquid formulation, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

[0007] In one example, provided herein is a liquid formulation comprising a protein and a compound which prevents oxidation of the protein in the liquid formulation, wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

[0008] In some examples, the liquid formulation is a pharmaceutical formulation suitable for administration to a subject. In some examples, the formulation is aqueous.

[0009] In some examples, the compound that prevents oxidation of the protein in the formulation is from about 0.3 mM to about 10 mM, or up to the highest concentration that the compound is soluble to in the formulation. In some examples, the compound that prevents oxidation of the protein in the formulation is from about 0.3 mM to about 9 mM, from about 0.3 mM to about 8 mM, from about 0.3 mM to about 7 mM, from about 0.3 mM to about 6 mM, from about 0.3 mM to about 5 mM, from about 0.3 mM to about 4 mM, from about 0.3 mM to about 3 mM, from about 0.3 mM to about 2 mM, from about 0.5 mM to about 2 mM, from about 0.6 mM to about 1.5 mM, or from about 0.8 mM to about 1.25 mM. In some examples, the compound that prevents oxidation of the protein in the formulation is about 0.3 mM to about 1 mM. In some examples, the compound that prevents oxidation of the protein in the formulation is from about 0.3 mM to about 5 mM. In some

examples, the compound that prevents oxidation of the protein in the formulation is about 1 mM. In some examples, the compound prevents oxidation of tryptophan and/or methionine in the protein. In some examples, the compound prevents oxidation of the protein by a reactive oxygen species. In some examples, the reactive oxygen species is selected from the group consisting of singlet oxygen, a superoxide (O_2-), hydrogen peroxide, a hydroxyl radical, and an alkyl peroxide.

[0010] In some examples, the protein in the formulation is susceptible to oxidation. In some examples, tryptophan in the protein is susceptible to oxidation. In some examples, the protein is an antibody (e.g., a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment). In some examples, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0011] In some examples, the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent. In some examples, the formulation has a pH of about 4.5 to about 7.0.

[0011a] In another aspect, the present disclosure refers to a method of making a liquid formulation comprising adding an amount of a compound that prevents oxidation of an antibody to the liquid formulation comprising the antibody, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

[0011b] In another aspect, the present disclosure refers to a method of preventing oxidation of an antibody in a liquid formulation comprising adding an amount of a compound that prevents oxidation of the antibody to the liquid formulation comprising the antibody, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

[0012] In another example, provided herein is a method of making a liquid formulation comprising adding an amount of a compound that prevents oxidation of a protein to the liquid formulation, wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin. In another example, provided herein is a method of preventing oxidation of a protein in a liquid formulation comprising adding an amount of a compound that prevents oxidation of the protein to the liquid formulation, wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

[0013] In some examples of the methods described herein, the formulation is a pharmaceutical formulation suitable for administration to a subject. In some examples, the formulation is aqueous.

[0014] In some examples of the methods described herein, the compound in the formulation is from about 0.3 mM to about 10 mM, or up to the highest concentration that the compound is soluble to in the formulation. In some examples, the compound in the formulation is at a concentration from about 0.3 mM to about 9 mM, from about 0.3 mM to about 8 mM, from about 0.3 mM to about 7 mM, from about 0.3 mM to about 6 mM, from about 0.3 mM to about 5 mM, from about 0.3 mM to about 4 mM, from about 0.3 mM to about 3 mM, from about 0.3 mM to about 2 mM, from about 0.5 mM to about 2 mM, from about 0.6 mM to about 1.5 mM, or from about 0.8 mM to about 1.25 mM. In some

examples, the compound in the formulation is about 0.3 mM to about 1 mM. In some examples, the compound in the formulation is from about 0.3 mM to about 5 mM. In some examples, the compound in the formulation is from about 1 mM. In some examples, the compound prevents oxidation of tryptophan and/or methionine in the protein. In some examples, the compound prevents oxidation of the protein by a reactive oxygen species. In some examples, the reactive oxygen species is selected from the group consisting of singlet oxygen, a superoxide (O_2-), hydrogen peroxide, a hydroxyl radical, and an alkyl peroxide.

[0015] In some examples, the protein is susceptible to oxidation. In some examples, tryptophan in the protein is susceptible to oxidation. In some examples, the protein is an antibody (e.g., a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment). In some examples, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0016] In some examples, one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent are added into the formulation. In some examples, the formulation has a pH of about 4.5 to about 7.0.

[0017] In another example, provided herein is a method of screening a compound that prevents oxidation of a protein in a protein composition, comprising selecting a compound that has lower oxidation potential and less photosensitivity as compared to L-tryptophan, and testing the effect of the selected compound on preventing oxidation of the protein.

[0018] In some examples of the methods, the photosensitivity is measured based on the amount of H_2O_2 produced by the compound upon light exposure. In some examples, the compound that produces less than about 20% of the amount of H_2O_2 produced by L-tryptophan is selected. In some examples, the oxidation potential is measured by cyclic voltammetry. In some examples, the selected compound is tested for the effect on preventing oxidation of the protein by reactive oxygen species generated by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), light, and/or a Fenton reagent.

[0019] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the art. These and other examples of the disclosure are further described by the detailed description that follows.

CLAIMS

WHAT IS CLAIMED IS:

1. A liquid formulation comprising an antibody and a compound which prevents oxidation of the antibody in the liquid formulation, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

2. The formulation of claim 1, wherein the compound is N-acetyl-L-tryptophanamide.

3. The formulation of claim 1, wherein the compound is 5-amino-DL-tryptophan.

4. The formulation of claim 1, wherein the compound is 5-hydroxyindole-3-acetic acid.

5. The formulation of claim 1, which is a pharmaceutical formulation suitable for administration to a subject.

6. The formulation of any one of claims 1-5 which is aqueous.

7. The formulation of any one of claims 1-6, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

8. The formulation of any one of claims 1-7, wherein the compound in the formulation is from 0.3 mM to 1 mM.

9. The formulation of any one of claims 1-8, wherein the compound prevents oxidation of tryptophan, cysteine, histidine, tyrosine, and/or methionine in the antibody.

10. The formulation of any one of claims 1-9, wherein the compound prevents oxidation of the antibody by a reactive oxygen species.

11. The formulation of claim 10, wherein the reactive oxygen species is selected from the group consisting of singlet oxygen, a superoxide (O_2-), hydrogen peroxide, a hydroxyl radical, and an alkyl peroxide.

12. The formulation of any one of claims 1-11, wherein the antibody is susceptible to oxidation.

13. The formulation of any one of claims 1-12, wherein tryptophan in the antibody is susceptible to oxidation.

14. The formulation of any one of claims 1-13, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

15. The formulation of any one of claims 1-14, wherein the antibody concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

16. The formulation of any one of claims 1-15, which further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

17. The formulation of any one of claims 5-16, wherein the formulation has a pH of about 4.5 to about 7.0.

18. A method of making a liquid formulation comprising adding an amount of a compound that prevents oxidation of an antibody to the liquid formulation comprising the antibody, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

19. A method of preventing oxidation of an antibody in a liquid formulation comprising adding an amount of a compound that prevents oxidation of the antibody to the liquid formulation comprising the antibody, wherein the compound is selected from the group consisting of N-acetyl-L-tryptophanamide, 5-amino-DL-tryptophan, and 5-hydroxyindole-3-acetic acid, or a pharmaceutically acceptable salt thereof.

20. The method of claim 19, wherein the compound is N-acetyl-L-tryptophanamide.

21. The method of claim 19, wherein the compound is 5-amino-DL-trytophan.

22. The method of claim 19, wherein the compound is 5-hydroxyindole-3-acetic acid.

23. The method of any one of claims 18-22, wherein the formulation is a pharmaceutical formulation suitable for administration to a subject.

24. The method of any one of claims 18-23, wherein the formulation is aqueous.

25. The method of any one of claims 18-24, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

26. The method of any one of claims 18-25, wherein the compound in the formulation is from 0.3 mM to 1 mM.

27. The method of any one of claims 18-26, wherein the compound prevents oxidation of tryptophan, cysteine, histidine, tyrosine, and/or methionine in the antibody.

28. The method of any one of claims 18-27, wherein the compound prevents oxidation of the antibody by a reactive oxygen species.

29. The method of claim 28, wherein the reactive oxygen species is selected from the group consisting of singlet oxygen, a superoxide (O_2-), hydrogen peroxide, a hydroxyl radical, and an alkyl peroxide.

30. The method of any one of claims 18-29, wherein the antibody is susceptible to oxidation.

31. The method of any one of claims 18-30, wherein tryptophan in the antibody is susceptible to oxidation.

32. The method of any one of claims 18-31, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

33. The method of any one of claims 18-31, wherein the antibody concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

34. The method of any one of claims 18-33, wherein the formulation further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

35. The method of any one of claims 18-34, wherein the formulation has a pH of about 4.5 to about 7.0.