(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.

[0007] In one aspect, there is provided herein a liquid formulation comprising a protein and a compound derived from tryptophan which prevents oxidation of the protein in the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

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

[0009] In some embodiments, 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 embodiments, 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 embodiments, the compound that prevents oxidation of the protein in the formulation is about 0.3 mM to about 1 mM. In some embodiments, the compound that prevents oxidation of the protein in the formulation is from about 0.3 mM to about 5 mM. In some

embodiments, the compound that prevents oxidation of the protein in the formulation is about 1 mM. In some embodiments, the compound prevents oxidation of tryptophan and/or methionine in the protein. In some embodiments, the compound prevents oxidation of the protein by a reactive oxygen species. In some embodiments, 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 embodiments, the protein in the formulation is susceptible to oxidation. In some embodiments, tryptophan in the protein is susceptible to oxidation. In some embodiments, 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 embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0011] In some embodiments, 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 embodiments, the formulation has a pH of about 4.5 to about 7.0.

[0012] In another aspect, there is provided herein a method of making a liquid formulation comprising adding an amount of a compound derived from tryptophan that prevents oxidation of a protein to the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin. In another aspect, there is provided herein a method of preventing oxidation of a protein in a liquid formulation comprising adding an amount of a compound derived from tryptophan that prevents oxidation of the protein to the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

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

[0014] In some embodiments 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 embodiments, 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

embodiments, the compound in the formulation is about 0.3 mM to about 1 mM. In some embodiments, the compound in the formulation is from about 0.3 mM to about 5 mM. In some embodiments, the compound in the formulation is from about 1 mM. In some embodiments, the compound prevents oxidation of tryptophan and/or methionine in the protein. In some embodiments, the compound prevents oxidation of the protein by a reactive oxygen species. In some embodiments, 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 embodiments, the protein is susceptible to oxidation. In some embodiments, tryptophan in the protein is susceptible to oxidation. In some embodiments, 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 embodiments, the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

[0016] In some embodiments, 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 embodiments, the formulation has a pH of about 4.5 to about 7.0.

[0017] In another aspect, there is provided herein 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, wherein the compound that produces less than about 20% of the amount of H_2O_2 produced by L-tryptophan is selected.

[0018] In some embodiments of the methods, the photosensitivity is measured based on the amount of H_2O_2 produced by the compound upon light exposure. In some embodiments, the compound that produces less than about 20% of the amount of H_2O_2 produced by L-tryptophan is selected. In some embodiments, the oxidation potential is measured by cyclic voltammetry. In some embodiments, 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 embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the disclosure will become apparent to one of skill in the art. These and other embodiments of the disclosure are further described by the detailed description that follows.

CLAIMS

WHAT IS CLAIMED IS:

1. 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.

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

3. The formulation of claim 1 or 2 which is aqueous.

4. The formulation of any one of claims 1-3, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

5. The formulation of any one of claims 1-4, wherein the compound in the formulation is about 1 mM.

6. The formulation of any one of claims 1-5, wherein the compound prevents oxidation of tryptophan and/or methionine in the protein.

7. The formulation of any one of claims 1-6, wherein the compound prevents oxidation of the protein by a reactive oxygen species.

8. The formulation of claim 7, 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.

9. The formulation of any one of claims 1-8, wherein the protein is susceptible to oxidation.

10. The formulation of any one of claims 1-9, wherein tryptophan in the protein is susceptible to oxidation.

11. The formulation of any one of claims 1-10, wherein the protein is an antibody.

12. The formulation of claim 11, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

13. The formulation of any one of claims 1-12, wherein the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

14. The formulation of any one of claims 1-13, which further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

15. The formulation of any one of claims 3-14, wherein the formulation has a pH of about 4.5 to about 7.0.

16. 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.

17. 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.

18. The method of claim 16 or 17, wherein the formulation is a pharmaceutical formulation suitable for administration to a subject.

19. The method of any one of claims 16-18, wherein the formulation is aqueous.

20. The method of any one of claims 16-19, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

21. The method of any one of claims 16-20, wherein the compound in the formulation is about 1 mM.

22. The method of any one of claims 16-21, wherein the compound prevents oxidation of tryptophan and/or methionine in the protein.

23. The method of any one of claims 16-22, wherein the compound prevents oxidation of the protein by a reactive oxygen species.

24. The method of claim 23, 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.

25. The method of any one of claims 16-24, wherein the protein is susceptible to oxidation.

26. The method of any one of claims 16-25, wherein tryptophan in the protein is susceptible to oxidation.

27. The method of any one of claims 16-26, wherein the protein is an antibody.

28. The method of claim 27, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

29. The method of any one of claims 16-28, wherein the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

30. The method of any one of claims 16-29, 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.

31. The method of any one of claims 16-30, wherein the formulation has a pH of about 4.5 to about 7.0.

32. 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.

33. The method of claim 32, wherein the photosensitivity is measured based on the amount of H_2O_2 produced by the compound upon light exposure.

34. The method of claim 33, wherein the compound that produces less than about 20% of the amount of H_2O_2 produced by L-tryptophan is selected.

35. The method of claim 32, wherein the oxidation potential is measured by cyclic voltammetry.

36. The method of claim 32, wherein 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.

CLAIMS

WHAT IS CLAIMED IS:

1. A liquid formulation comprising a protein and a compound derived from tryptophan which prevents oxidation of the protein in the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

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

3. The formulation of claim 1 or 2 which is aqueous.

4. The formulation of any one of claims 1-3, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

5. The formulation of any one of claims 1-4, wherein the compound in the formulation is about 1 mM.

6. The formulation of any one of claims 1-5, wherein the compound prevents oxidation of tryptophan and/or methionine in the protein.

7. The formulation of any one of claims 1-6, wherein the compound prevents oxidation of the protein by a reactive oxygen species.

8. The formulation of claim 7, 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.

9. The formulation of any one of claims 1-8, wherein the protein is susceptible to oxidation.

10. The formulation of any one of claims 1-9, wherein tryptophan in the protein is susceptible to oxidation.

11. The formulation of any one of claims 1-10, wherein the protein is an antibody.

12. The formulation of claim 11, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

13. The formulation of any one of claims 1-12, wherein the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

14. The formulation of any one of claims 1-13, which further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, a surfactant, and a tonicity agent.

15. The formulation of any one of claims 3-14, wherein the formulation has a pH of about 4.5 to about 7.0.

16. A method of making a liquid formulation comprising adding an amount of a compound derived from tryptophan that prevents oxidation of a protein to the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

17. A method of preventing oxidation of a protein in a liquid formulation comprising adding an amount of a compound derived from tryptophan that prevents oxidation of the protein to the liquid formulation, wherein the compound shows reduced degradation to form reactive oxygen species compared to tryptophan, and wherein the compound is selected from the group consisting of 5-hydroxy-tryptophan, 5-hydroxy indole, 7-hydroxy indole, and serotonin.

18. The method of claim 16 or 17, wherein the formulation is a pharmaceutical formulation suitable for administration to a subject.

19. The method of any one of claims 16-18, wherein the formulation is aqueous.

20. The method of any one of claims 16-19, wherein the compound in the formulation is from about 0.3 mM to about 5 mM.

21. The method of any one of claims 16-20, wherein the compound in the formulation is about 1 mM.

22. The method of any one of claims 16-21, wherein the compound prevents oxidation of tryptophan and/or methionine in the protein.

23. The method of any one of claims 16-22, wherein the compound prevents oxidation of the protein by a reactive oxygen species.

24. The method of claim 23, 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.

25. The method of any one of claims 16-24, wherein the protein is susceptible to oxidation.

26. The method of any one of claims 16-25, wherein tryptophan in the protein is susceptible to oxidation.

27. The method of any one of claims 16-26, wherein the protein is an antibody.

28. The method of claim 27, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, or antibody fragment.

29. The method of any one of claims 16-28, wherein the protein concentration in the formulation is about 1 mg/mL to about 250 mg/mL.

30. The method of any one of claims 16-29, 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.

31. The method of any one of claims 16-30, wherein the formulation has a pH of about 4.5 to about 7.0.

32. 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, wherein the compound that produces less than about 20% of the amount of H_2O_2 produced by L-tryptophan is selected.

33. The method of claim 32, wherein the photosensitivity is measured based on the amount of H_2O_2 produced by the compound upon light exposure.

34. The method of claim 32, wherein the oxidation potential is measured by cyclic voltammetry.

35. The method of claim 32, wherein 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.