cell membrane, and which in turn leads to intravascular hemolysis and schistocyte formation. Thromboses also cause reduced blood flow, which can result in end organ damage. Symptoms typically include neurological problems, such as hallucination, bizarre behavior, altered mental status, stroke, or headaches; kidney failure; fever; and thrombocytopenia (low platelet count), resulting in bruising or purpura; and microangiopathic hemolytic anemia, involving anemia and jaundice. Current therapy involves plasmapheresis to reduce circulating antibodies against ADAMTS13, and/or replenishing blood levels of the enzyme.

[0006] Therefore, a strong need exists for providing methods of purifying recombinant ADAMTS13, particularly on a commercial production scale, which may be used as a therapeutic agent. Purification of ADAMTS13 has proven difficult and various approaches have been attempted, including chromatography. A chromatographic material that binds non-ADAMTS13 protein, allowing the ADAMTS13 protein to appear in the eluate or supernatant, would provide a useful approach for purification. A chromatography material that binds ADAMTS13 protein, while non-ADAMTS13 impurities either remain in solution or bind much more strongly, also presents an attractive approach, and may be used in tandem with other approaches. The instant disclosure provides such approaches.

[0007] Furthermore, virus contaminants have posed additional challenges in the purification of ADAMTS13 proteins, as well as other proteins and recombinant proteins. One conventional approach involved treating a sample to be purified with a solvent-detergent mixture in solution. Incubation of the sample with the solvent-detergent chemicals led to deactivation of lipid-coated viruses. This in-solution treatment, however, inefficiently required transfer of the sample to at least one other vessel, e.g., to facilitate removal of the solvent-detergent chemicals after treatment. Further, some proteins including ADAMTS13 are sensitive to the solvent-detergent chemicals, resulting in aggregate formation. The instant disclosure provides an approach involving immobilization of the protein during the solvent-detergent treatment to address such problems of virus inactivation.

SUMMARY

[0008] Disclosed is a method for purifying recombinant A Disintegrin-like and Metallopeptidase with Thrombospondin Type 1 Motif 13 (ADAMTS13) protein (particularly, human ADAMTS13) from a sample comprising ADAMTS13 protein and non-ADAMTS13 impurities. It has surprisingly been found that hydroxyapatite chromatography can be used under conditions suitable for purifying ADAMTS13 protein from non-ADAMTS13 impurities. The method comprises enriching for ADAMTS13 protein

by diafiltration exchange into a buffer comprising calcium ions and zinc ions before chromatographic contact with the hydroxyapatite; followed by chromatographically contacting the sample with hydroxyapatite under conditions that allow the ADAMTS13 protein to appear in an eluate or a supernatant from the hydroxyapatite; and then chromatographically contacting the eluate with a cation exchange/hydrophobic interaction resin that binds the ADAMTS13 protein; followed by preparing the ADAMTS13 protein for cation exchange by reducing buffer conductivity, e.g., by ultrafiltration/diafiltration; and/or by dialysis consisting of no more than 2 passes through a single dialysis module; and/or by gel filtration, optionally further comprising one or more virus inactivation steps. In some such embodiments, the virus inactivation step comprises adding a solvent-detergent mixture comprising a non-ionic detergent and an organic solvent to the ADAMTS13 protein, wherein the ADAMTS13 protein is immobilized on a cation exchange resin and the solvent-detergent mixture comprises 1% TRITONX-100, 0.3% Tri-N-butyl phosphate, and 0.3% TWEEN 80. In still another embodiment, the virus inactivation step uses, as well as or instead of the solvent-detergent treatment, a nanofilter to remove viruses and/or viral particles. In some preferred embodiments, the percent yield ADAMTS13 of the overall procedure outlined above is surprisingly 22-24% or more, and in even more preferred embodiments, aggregates surprisingly are reduced by 50%.

[0017] In some embodiments where the ADAMTS13 has been immobilized on a cation exchange resin, the method further comprises eluting the ADAMTS13 protein from the resin using step elution with a storage buffer having a pH of greater than 7.0 and comprising less than 10 mM calcium ions, a buffering compound, 0.05% non-ionic detergent, and a salt; or using gradient elution comprising a first buffer having low salt content and a second buffer having higher salt content.

[0018] Disclosed is a composition comprising a recombinant ADAMTS13 protein prepared according to any embodiment of the methods described herein. In some embodiments, the composition is a pharmaceutical composition, e.g., a composition comprising purified ADAMTS13 protein and a pharmaceutically acceptable carrier.

[0019] Further disclosed is a method for inactivating virus contaminants in a protein sample, where the protein may be any protein from a source that may have viral contaminants. In preferred embodiments, the protein is recombinant protein, particularly proteins sensitive to aggregation when exposed to organic solvents and detergents. In some embodiments, the protein may be ADAMTS13 protein, in particular

[0020a] In one aspect, there is provided an *in vitro* method for inactivating lipid-enveloped virus contaminants in a protein solution in the purification of a therapeutic protein composition, said method comprising:
providing a protein solution comprising a therapeutic protein and possibly comprising lipid-enveloped virus contaminants;
immobilizing said therapeutic protein on an ion exchange resin *in vitro*, wherein said protein is directly immobilized on said ion exchange resin;
wherein said immobilization is not in the presence of a solvent-detergent mixture, said mixture comprising a non-ionic detergent and an organic solvent; and
subsequently treating said therapeutic protein, while said therapeutic protein remains immobilized on said ion exchange resin, with a solvent-detergent mixture comprising a non-ionic detergent and an organic solvent at concentrations and for a time period suitable to effect inactivation of said lipid-enveloped virus contaminants;
wherein said solvent-detergent treatment results in reduced formation of aggregates of said therapeutic protein compared to solvent-detergent treatment when the therapeutic protein is not immobilized;
whereby said lipid-enveloped virus contaminants are inactivated.

[0021] These and other aspects of the disclosure are described in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 depicts a flow diagram of exemplary steps of the method for purifying recombinant a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) from a sample comprising ADAMTS13 and non-ADAMTS13 impurities, in accordance with the instant invention. The order of the steps set forth in FIG. 1 may be re-ordered, and/or one or more steps omitted, as disclosed herein and as understood by one of skill in the art.

[0023] FIGS. 2A-D depict variations in purification runs on a cation exchange column. FIG. 2A depicts a procedure involving cation exchange chromatography with step elution, following virus inactivation; FIG. 2B depicts a procedure involving cation exchange chromatography with step elution, but without a preceding virus inactivation; FIG. 2C depicts a procedure involving cation exchange chromatography with gradient elution, followed by virus inactivation on the chromatographic column; and FIG. 2D depicts a procedure involving cation exchange chromatography with gradient elution, but without a preceding virus inactivation.

DETAILED DESCRIPTION

[0024] One aspect of the invention relates to a method for the purification of recombinant A Disintegrin-Like and Metallopeptidase with Thrombospondin Type 1 Motif 13 (ADAMTS13) protein from a sample, which may also comprise non-ADAMTS13 impurities. The protein sample may also include virus contaminants, which may be removed and/or inactivated by one or more virus inactivation steps.

[0025] As used herein "A Disintegrin-Like and Metallopeptidase with Thrombospondin Type 1 Motif 13," "ADAMTS13," "ADAMTS13 protein," "ADAMTS13 polypeptide," and "recombinant ADAMTS13" are interchangeable (unless otherwise specified) and refer to a recombinant mammalian ADAMTS13 protein, which also may be a biologically active derivative or fragment of a full-length ADAMTS13 protein. The amino acid sequence of full-length human and murine ADAMTS13 proteins have respective UniProtKB® accession numbers of Q76LX8 and Q769J6. Structural details and sequence information on human ADAMTS13 can be found in Zheng et al. ((2001) *J. Biol. Chem.* 276:41059-63).

[0026] The term "biologically active derivative or fragment thereof" as used herein means any polypeptides with a biological function similar, or substantially similar, to that of

CLAIMS

1. An *in vitro* method for inactivating lipid-enveloped virus contaminants in a protein solution in the purification of a therapeutic protein composition, said method comprising:

providing a protein solution comprising a therapeutic protein and possibly comprising lipid-enveloped virus contaminants;

immobilizing said therapeutic protein on an ion exchange resin *in vitro*, wherein said protein is directly immobilized on said ion exchange resin;

wherein said immobilization is not in the presence of a solvent-detergent mixture, said mixture comprising a non-ionic detergent and an organic solvent; and

subsequently treating said therapeutic protein, while said therapeutic protein remains immobilized on said ion exchange resin, with a solvent-detergent mixture comprising a non-ionic detergent and an organic solvent at concentrations and for a time period suitable to effect inactivation of said lipid-enveloped virus contaminants;

wherein said solvent-detergent treatment results in reduced formation of aggregates of said therapeutic protein compared to solvent-detergent treatment when the therapeutic protein is not immobilized;

whereby said lipid-enveloped virus contaminants are inactivated.

2. The method according to claim 1, wherein said therapeutic protein is at least one selected from ADAMTS13, Factor VIII, Factor VIIa, Factor IX, von Willebrand factor, and anti-MIF antibody.

3. The method according to claim 1 or claim 2, wherein said ion exchange resin is selected from the group consisting of cation exchange resin, anion exchange resin, and mixed mode resin.

4. The method according to any one of claims 1-3, wherein said solvent-detergent mixture comprises 1% Triton X-100, 0.3% Tri-N-butyl phosphate, and 0.3% Polysorbate 80.

5. The method according to any one of claims 1-4, wherein incubation in said solvent-detergent mixture continues for 30 minutes to 1 hour.

6. The method according to any one of claims 1-5, further comprising eluting said therapeutic protein from said ion exchange resin with a storage buffer.

7. The method according to claim 6, wherein said storage buffer has a pH of greater than 7.0 and comprises less than 10 mM calcium ions, a buffering compound, 0.05% non-ionic detergent, and a salt.

8. The method according to any one of claims 1-7, wherein said ion exchange resin is a cation exchange resin; and

wherein there is no ultrafiltration, diafiltration, or buffer exchange step subsequent to the eluting step from the cation exchange resin with the storage buffer.

9. The method according to any one of claims 1-7, wherein said ion exchange resin is an anion exchange resin.