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 ~~OF INVENTION~~

[0007a] In one aspect, there is provided a method for purifying recombinant a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) protein from a sample comprising ADAMTS13 protein and non-ADAMTS13 impurities, the method comprising:

chromatographically contacting the sample with hydroxyapatite under conditions that allow said ADAMTS13 protein to appear in a flow through fraction from said hydroxyapatite by said ADAMTS13 protein not binding to said hydroxyapatite while said non-ADAMTS13 impurities are retained on said hydroxyapatite;

subsequently chromatographically contacting said flow through fraction with a mixed mode cation exchange/hydrophobic interaction resin that binds said ADAMTS13 protein; and

eluting said ADAMTS13 protein from said mixed mode cation exchange/hydrophobic interaction resin with an elution buffer.

[0008] One ~~aspect~~embodiment of the ~~invention~~disclosure relates to 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] Another ~~aspect~~embodiment of the ~~invention~~disclosure relates to 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] Still a further ~~aspect~~embodiment of the ~~invention~~disclosure relates to 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

[0021] These and other ~~aspects~~embodiments of the ~~invention~~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. A method for purifying recombinant a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) protein from a sample comprising ADAMTS13 protein and non-ADAMTS13 impurities, the method comprising:

chromatographically contacting the sample with hydroxyapatite under conditions that allow said ADAMTS13 protein to appear in a flow through fraction from said hydroxyapatite by said ADAMTS13 protein not binding to said hydroxyapatite while said non-ADAMTS13 impurities are retained on said hydroxyapatite;

subsequently chromatographically contacting said flow through fraction with a mixed mode cation exchange/hydrophobic interaction resin that binds said ADAMTS13 protein; and

eluting said ADAMTS13 protein from said mixed mode cation exchange/hydrophobic interaction resin with an elution buffer.

2. The method according to claim 1, further comprising chromatographically contacting said sample with an anion exchange resin and eluting said ADAMTS13 protein from said anion exchange resin before chromatographic contact with said hydroxyapatite.

3. The method according to any one of claims 1-2, further comprising concentrating said ADAMTS13 protein in said sample by ultrafiltration; and stabilizing said ADAMTS13 protein by diafiltration exchange into a buffer comprising calcium ions and zinc ions before chromatographic contact with said hydroxyapatite.

4. The method according to any one of claims 1~~2~~-3, further comprising, following contact with said hydroxyapatite or said mixed mode cation exchange/hydrophobic interaction resin, a preparing step for preparing said ADAMTS13 protein for cation exchange by reducing buffer conductivity.

5. The method according to claim 4, wherein said preparing step is performed by ultrafiltration/diafiltration.