of the body. Manifesting initially as a thickening of the wall of the heart, lungs and airways, and abnormal enlargement of the liver, spleen and kidneys, these profound changes can ultimately lead to widespread catastrophic organ failure. As a result, Hunter syndrome is always severe, progressive, and life-limiting.

[0005] Enzyme replacement therapy (ERT) is an approved therapy for treating Hunter syndrome (MPS II), which involves administering exogenous replacement I2S enzyme to patients with Hunter syndrome.

SUMMARY

[0006] The present disclosure provides, among other things, improved methods for purifying I2S protein produced recombinantly for enzyme replacement therapy. The present disclosure is, in part, based on the surprising discovery that recombinant I2S protein can be purified from unprocessed biological materials, such as, I2S-containing cell culture medium, using a process involving as few as four chromatography columns. Approved existing purification process of recombinant I2S for enzyme replacement therapy involves 6 chromatography columns. As described in the Examples section, recombinant I2S proteins purified using a four-column process according to the invention conforms with the marketing purity requirements in the US and many other countries. In addition, the recombinant I2S enzyme purified according to the present invention retains high percentage of C_{α}-formylglycine (FGly) (e.g., higher than 70% and up to 100%), which is important for the activity of I2S enzyme, and distinct characteristics such as sialic acid content and glycan map that may facilitate bioavailability and/or lysosomal targeting of the recombinant I2S protein. Therefore, the present disclosure provides an effective, cheaper, and faster process for purifying recombinant I2S protein. The present disclosure is particularly useful for purifying recombinant I2S protein produced in serum-free medium.

[0006a] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), wherein the purified recombinant I2S contains less than 150 ng/mg Host Cell Protein (HCP).

[0006aa] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), wherein the composition contains less than 150 ng/mg Host Cell Protein (HCP).

[0006b] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S contains at least contains at least 10% bis-phosphorylated oligosaccharides per molecule.

[0006bb] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S contains at least 10% bis-phosphorylated oligosaccharides per molecule.

[0006c] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 40 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

[0006d] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 20 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

[0006e] In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S

(i) comprises at least about 70% conversion of the cysteine residue corresponding to

Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

(ii) contains on average at least 16 sialic acids per molecule.

[0006f]In one aspect, there is provided a composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified I2S is characterized with a glycan map comprising seven or fewer peak groups selected from the peak groups indicative of neutral (peak group 1), mono-sialylated (peak group 2), di-sialylated (peak group 3), monophosphorylated (peak group 4), tri-sialylated (peak group 5), tetra-sialylated (peak group 6), or diphosphorylated (peak group 7) I2S protein.

[0007] Thus, in one aspect, the present invention provides a method of purifying recombinant I2S protein from an impure preparation using a process based on one or more of anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography. In some embodiments, an inventive method according to the present invention involves less than 6 (e.g., less than 5, less than 4, or less than 3) chromatography steps. In some embodiments, an inventive

Claims

1. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), wherein the purified recombinant I2S contains less than 150 ng/mg Host Cell Protein (HCP).

2. The composition of claim 1, wherein the purified recombinant I2S comprises at least 75% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

3. The composition of claim 1, wherein the purified recombinant I2S comprises at least 85% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

4. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S contains at least contains at least 10% bis-phosphorylated oligosaccharides per molecule.

5. The composition of claim 4, wherein the purified recombinant I2S contains at least 50% bis-phosphorylated oligosaccharides per molecule.

6. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 40 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

7. The composition of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 50 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

8. The composition of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 60 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

9. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 20 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

10. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 30 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

11. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 40 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

12. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 50 U/mg as determined by an *in vitro* 4-MUF-SO_4 to 4-MUF conversion assay.

13. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 60 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

14. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S

(i) comprises at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

(ii) contains on average at least 16 sialic acids per molecule.

15. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified I2S is characterized with a glycan map comprising seven or fewer peak groups selected from the peak groups indicative of neutral (peak group 1), mono-sialylated (peak group 2), di-sialylated (peak group 3), monophosphorylated (peak group 4), tri-sialylated (peak group 5), tetra-sialylated (peak group 6), or diphosphorylated (peak group 7) I2S protein.

16. The composition of claim 1, wherein the purified recombinant I2S protein contains less than 100 ng/mg HCP.

17. The composition of claim 1, wherein the purified recombinant I2S protein contains less than 80 ng/mg HCP.

CLAIMS

1. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), wherein the composition contains less than 150 ng/mg Host Cell Protein (HCP).

2. The composition of claim 1, wherein the purified recombinant I2S comprises at least 75% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

3. The composition of claim 1, wherein the purified recombinant I2S comprises at least 85% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

4. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S contains at least 10% bis-phosphorylated oligosaccharides per molecule.

5. The composition of claim 4, wherein the purified recombinant I2S contains at least 50% bis-phosphorylated oligosaccharides per molecule.

6. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 40 U/mg as determined by an in vitro sulfate release activity assay using heparin disaccharide as substrate.

7. The composition of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 50 U/mg as determined by an in vitro sulfate release activity assay using heparin disaccharide as substrate.

8. The composition of claim 6, wherein the purified recombinant I2S protein has specific activity of at least 60 U/mg as determined by an in vitro sulfate release activity assay using heparin disaccharide as substrate.

9. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified recombinant I2S protein has specific activity of at least 20 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

10. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 30 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

11. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 40 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

12. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 50 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

13. The composition of claim 9, wherein the purified recombinant I2S protein has a specific activity of at least 60 U/mg as determined by an in vitro 4-MUF-SO_4 to 4-MUF conversion assay.

14. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1, wherein the purified recombinant I2S

(i) comprises at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

(ii) contains on average at least 16 sialic acids per molecule.

15. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having the amino acid sequence of SEQ ID NO:1,

wherein the purified recombinant I2S comprises at least 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly), and

wherein the purified I2S is characterized with a glycan map comprising seven or fewer peak groups selected from the peak groups indicative of neutral (peak group 1), mono-sialylated (peak group 2), di-sialylated (peak group 3), monophosphorylated (peak group 4), tri-sialylated (peak group 5), tetra-sialylated (peak group 6), or diphosphorylated (peak group 7) I2S protein.

16. The composition of claim 1, wherein the composition contains less than 100 ng/mg HCP.

17. The composition of claim 1, wherein the composition contains less than 80 ng/mg HCP.