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 ~~OF THE INVENTION~~

[0006] The present ~~invention~~ disclosure provides, among other things, improved methods for purifying I2S protein produced recombinantly for enzyme replacement therapy. The present ~~invention~~ 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 ~~invention~~ disclosure provides an effective, cheaper, and faster process for purifying recombinant I2S protein. The present ~~invention~~ 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).

[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.

[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

method according to the present ~~invention~~ disclosure involves 2, 3, 4 or 5 chromatography steps. In some embodiments, an inventive method according to the present ~~invention~~ disclosure involves 4 chromatography steps. In some embodiments, the purified recombinant I2S protein according to the present invention contains less than 100 ng/mg Host Cell Protein (HCP) (e.g., less than 90 ng/mg HCP, less than 80 ng/mg HCP, less than 70 ng/mg HCP, less than 60 ng/mg HCP, less than 50 ng/mg HCP, less than 40 ng/mg HCP, less than 30 ng/mg HCP, less than 20 ng/mg HCP, less than 10 ng/mg HCP).

[0008] In some embodiments, a suitable anion-exchange chromatography is Q chromatography. In some embodiments, a suitable cation-exchange chromatography is SP chromatography. In some embodiments, a suitable mixed-mode chromatography is hydroxyapatite (HA) chromatography. In some embodiments, a suitable hydrophobic interaction chromatography is phenyl chromatography.

[0009] It is contemplated that anion-exchange chromatography (e.g., O column), cation-exchange chromatography (e.g., SP column), mixed-mode chromatography (e.g., HA column), and hydrophobic interaction chromatography (e.g., phenyl column) can be carried out in any order. In some embodiments, a method according to the present invention carries out anion-exchange chromatography (e.g., O column), cation-exchange chromatography (e.g., SP column), mixed-mode chromatography (e.g., HA column), and hydrophobic interaction chromatography (e.g., phenyl column) in that order.

[0010] In some embodiments, an impure preparation or an intermediate eluate or flow-through is adjusted to pH of about 5.0-7.0 (e.g., about 5.0, 5.5, 6.0, 6.5 or 7.0) and the conductivity of about 10-20 mS/cm (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mS/cm) prior to loading to the anion-exchange chromatography column (e.g., Q column). In some embodiments, the pH is adjusted using 1M sodium acetate. In some embodiments, the conductivity is adjusted using 5 M sodium chloride. In some embodiments, the anion-exchange chromatography column, once loaded, is washed using a wash buffer comprising salt (e.g., NaCl) concentration ranging from about 140 mM to 200 mM (e.g., about 140 mM, 145 mM, 150 mM, 155 mM, 160 mM, 165 mM, 170 mM, 175 mM, 180 mM, 185 mM, 190 mM, 195 mM, or 200 mM) with pH of about 5.0-7.0 (e.g., about 5.0, 5.5, 6.0, 6.5 or 7.0). In some embodiments, the anion-exchange chromatography column is eluted using a elution buffer comprising a linear salt (e.g., NaCl) gradient. In some embodiments, a suitable linear NaCl gradient contains a range from about 0-500 mM NaCl (e.g., about 0-400 mM, about 0-

chromatography column has a height ranging from 14-25 cm (e.g., 15-25 cm, 15-20 cm, 14-24 cm, 14-22 cm, 14-20 cm, or 16-18 cm). In some embodiments, each of the anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography column has a height of approximately 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 cm.

[0015] In some embodiments, an inventive method according to the present ~~invention~~ disclosure includes a step of viral inactivation before loading the impure preparation onto the first chromatography column. In some embodiments, the step of viral inactivation includes adding a detergent to the impure preparation. In some embodiments, an inventive method according to the ~~invention~~ disclosure further includes a step of viral removal after the last Chromatography column. In some embodiments, a method of the ~~invention~~ disclosure further includes a step of ultrafiltration and/or dialfiltration. In some embodiments, the step of ultrafiltration and/or dialfiltration includes exchanging the purified recombinant I2S protein into a drug formulation buffer.

[0016] In some embodiments, the present ~~invention~~ disclosure is used to purify a recombinant I2S protein having an amino acid sequence at least about 50% (e.g., at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) identical to SEQ ID NO:1. In some embodiments, the present invention is used to purify a recombinant I2S protein having an amino acid sequence identical to SEQ ID NO:1.

[0017] In some embodiments, the present ~~invention~~ disclosure is used to purify a recombinant I2S protein produced by mammalian cells cultured in suspension in a serum-free medium. In some embodiments, a serum-free medium suitable for the ~~invention~~ disclosure lacks animal-derived components. In some embodiments, a serum-free medium suitable for the invention is a chemically-defined medium. In some embodiments, the mammalian cells are cultured in a bioreactor. In some embodiments, the mammalian cells co-express the recombinant I2S protein and formylglycine generating enzyme (FGE). In some embodiments, the mammalian cells are human cells.

[0018] In some embodiments, an impure preparation used in a method of the invention is prepared from the serum-free medium containing recombinant I2S protein secreted from the mammalian cells. In some embodiments, an impure preparation used in a method of the invention is thawed from a frozen medium preparation.

[0019] In some embodiments, a purified recombinant I2S protein according to the present invention contains, on average, 16-22 (e.g., 16-21, 16-20, 16-19, 17-22, 17-21, 17-20, 17-19) sialic acids per molecule. In some embodiments, a purified recombinant I2S protein according to the present invention contains, on average, 16, 17, 18, 19, 20, 21, or 22 sialic acids per molecule.

[0020] In some embodiments, a purified recombinant I2S protein according to the present ~~invention~~ disclosure has at least about 70% (e.g., at least about 77%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) conversion of the cysteine residue corresponding to Cys59 of human I2S (SEQ ID NO:1) to C_α-formylglycine (FGly). In some embodiments, a purified recombinant I2S protein according to the present invention has substantially 100% conversion of the cysteine residue corresponding to Cys59 of human I2S (SEQ ID NO:1) to C_α-formylglycine (FGly). In some embodiments, a purified recombinant I2S protein according to the present invention has specific activity of at least 20 U/mg, 30 U/mg, 40 U/mg, 50 U/mg, 60 U/mg, 70 U/mg, 80 U/mg, 90 U/mg, or 100 U/mg as determined by an *in vitro* sulfate release activity assay using heparin disaccharide as substrate.

[0021] In some embodiments, a purified recombinant I2S protein according to the present ~~invention~~ disclosure is characterized with cellular uptake of greater than 70%, 75%, 80%, 85%, 90%, 95%, as determined by an *in vitro* uptake assay.

[0022] In some embodiments, a purified recombinant I2S protein according to the present invention is characterized with a glycan map comprising seven 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), and diphosphorylated (peak group 7) I2S protein, respectively. In some embodiments, the glycan map is generated following a neuraminidase digestion. In other embodiments, the glycan map is generated following an alkaline phosphatase digestion.

[0023] Among other things, the present ~~invention~~ disclosure provides purified recombinant I2S protein as described herein, and pharmaceutical compositions or formulation containing the same. In some embodiments, a formulation is formulated for intravenous, subcutaneous and/or intrathecal administration. The present ~~invention~~ disclosure also provides methods of treating Hunter syndrome by administering into a subject in need of treatment a purified recombinant I2S, pharmaceutical composition or formulation containing the same.

[0024] As used herein, the terms "I2S protein," "I2S," "I2S enzyme," or grammatical equivalents, refer to a preparation of recombinant I2S protein molecules unless otherwise specifically indicated.

[0025] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0026] Other features, objects, and advantages of the present ~~invention~~ disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present ~~invention~~ disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0027] The Figures described below, that together make up the Drawing, are for illustration purposes only, not for limitation.

[0028] *Figure 1* depicts an exemplary purification scheme for recombinant I2S produced in serum free medium.

[0029] *Figure 2* depicts an exemplary peptide maps of purified recombinant I2S AF as compared to a reference I2S.

[0030] *Figure 3* depicts an exemplary SDS-PAGE (Silver) analysis of purified recombinant I2S AF.

[0031] *Figure 4* depicts an exemplary charge profile analysis of purified recombinant I2S AF assessed by ion-exchange chromatography.

[0032] *Figure 5* depicts exemplary glycan map profiles of purified recombinant I2S AF.

[0033] *Figure 6* depicts an exemplary analysis of activity (U/mg) after a viral inactivation UPB step of a clarified harvest of recombinant I2S.

What is claimed is:

1. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having an amino acid sequence at least 70% identical to SEQ ID NO:1,

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

further 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 contains less than 100 ng/mg HCP.

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

4. The composition of claim 1, wherein the purified recombinant I2S contains less than 60 ng/mg HCP.

5. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having an amino acid sequence at least 70% identical to SEQ ID NO:1

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

further wherein the purified recombinant I2S contains, on average, at least 16 sialic acids per molecule.

6. The composition of claim 5, wherein the purified recombinant I2S contains, on average, at least 18 sialic acids per molecule.

7. The composition of claim 5, wherein the purified recombinant I2S contains, on average, at least 20 sialic acids per molecule.

8. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having an amino acid sequence at least 70% identical to SEQ ID NO:1

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

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

9. The composition of claim 8, wherein the purified recombinant I2S contains at least 20% bis-phosphorylated oligosaccharides per enzyme.

10. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having an amino acid sequence at least 70% identical to SEQ ID NO:1

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

further 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.

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

12. The composition of any one of claims 1-10, wherein the purified recombinant I2S comprises at least about 80% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

13. A composition comprising purified recombinant iduronate-2-sulfatase (I2S) having an amino acid sequence at least 70% identical to SEQ ID NO:1, wherein the purified recombinant I2S comprises greater than about 85% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

14. The composition of claim 13, wherein the purified recombinant I2S comprises greater than about 90% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

15. The composition of claim 13, wherein the purified recombinant I2S comprises greater than about 95% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

16. The composition of claim 13, wherein the purified recombinant I2S comprises substantially 100% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

17. The composition of any one of the preceding claims, wherein the purified recombinant I2S has an amino acid sequence at least 80% identical to SEQ ID NO:1.

18. The composition of any one of the preceding claims, wherein the purified recombinant I2S has an amino acid sequence at least 90% identical to SEQ ID NO:1.

19. The composition of any one of the preceding claims, wherein the purified recombinant I2S has an amino acid sequence at least 95% identical to SEQ ID NO:1.

20. The composition of any one of the preceding claims, wherein the purified recombinant I2S has an amino acid sequence identical to SEQ ID NO:1.

21. A formulation comprising the composition of any one of the preceding claims and a physiologically acceptable carrier.

22. The formulation of claim 21, wherein the formulation is suitable for intravenous administration.

23. The formulation of claim 21, wherein the formulation is suitable for intrathecal administration.

24. The formulation of claim 21, wherein the formulation is suitable for subcutaneous administration.

25. The formulation of claim 21, wherein the formulation is for treating Hunter syndrome.

26. A method comprising

purifying recombinant iduronate-2-sulfatase (I2S) protein from an impure preparation by conducting one or more of anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography, and

wherein the method involves less than 6 chromatography steps and wherein the purified recombinant I2S protein contains less than 100 ng/mg Host Cell Protein (HCP).

27. The method of claim 26, wherein the anion-exchange chromatography is Q chromatography.

28. The method of claim 26 or 27, wherein the cation-exchange chromatography is SP chromatography.

29. The method of any one of claims 26-28, wherein the mixed-mode chromatography is hydroxyapatite (HA) chromatography.

30. The method of any one of claims 26-29, wherein the hydrophobic interaction chromatography is phenyl chromatography.

31. The method of any one of claims 26-30, wherein the method involves 5 chromatography steps or less.

32. The method of any one of claims 26-31, wherein the method involves 4 chromatography steps or less.

33. The method of any one of claims 26-32, wherein the method conducts the anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography in that order.

34. The method of any one of claims 26-33, wherein the anion-exchange chromatography column, once loaded with the impure preparation or an intermediate eluate, is washed using a

wash buffer comprising NaCl at a concentration ranging between about 140 mM and 200 mM with pH 5.5.

35. The method of any one of claims 26-34, wherein the anion-exchange chromatography column is eluted using a elution buffer comprising a linear NaCl gradient.

36. The method of claim 33, wherein the linear NaCl gradient comprises a range from 0-500 mM NaCl.

37. The method of any one of claims 26-36, wherein the impure preparation or an intermediate eluate is adjusted to a conductivity ranging between about 1 mS/cm and 20 mS/cm prior to loading to the cation-exchange chromatography column.

38. The method of claim 37, wherein the cation-exchange chromatography column is run at a pH of about 5.5.

39. The method of any one of claims 26-38, wherein the impure preparation or an intermediate eluate is adjusted to phosphate concentration ranging from about 0.001-0.01 M and pH 5.5 prior to loading to the mixed-mode chromatography column.

40. The method of claim 39, wherein the mixed-mode chromatography column, once loaded, is washed using a wash buffer comprising phosphate concentration ranging between about 10-20 mM, pH 5.5.

41. The method of claim 40, wherein the mixed mode chromatography column is eluted using a elution buffer comprising phosphate concentration ranging between about 50-150 mM, pH 5.5.

42. The method of any one of claims 26-41, wherein the impure preparation or an intermediate eluate is adjusted to a salt concentration ranging from about 0.5-2.0 M, pH of about 4.5-6.0 prior to loading on to the hydrophobic interaction chromatography column.

43. The method of claim 42, wherein the hydrophobic interaction chromatography column, once loaded, is washed using a wash buffer comprising salt concentration ranging between about 0.5-2.0 M, pH of about 4.5-6.0.

44. The method of claim 43, wherein the hydrophobic interaction chromatography column is eluted using a elution buffer comprising salt concentration ranging between about 0.1-0.5 M, pH of about 4.5-6.0.

45. The method of any one of claims 26-44, wherein each of the anion-exchange chromatography, cation-exchange chromatography, mixed-mode chromatography, and hydrophobic interaction chromatography column has a height ranging from 14-25 cm.

46. The method of any one of the preceding claims, wherein the method further comprises a step of viral inactivation.

47. The method of claim 46, wherein the step of viral inactivation is before loading the impure preparation onto the first chromatography column.

48. The method of claim 46, wherein the step of viral inactivation comprises adding a detergent to the impure preparation.

49. The method of any one of claims 26-48, wherein the method further comprises a step of viral removal after the last Chromatography column.

50. The method of any one of claims 26-49, wherein the method further comprises a step of ultrafiltration and/or dialfiltration.

51. The method of claim 50, wherein the step of ultrafiltration and/or dialfiltration comprises exchanging the purified recombinant I2S protein into a drug formulation buffer.

52. The method of any one of claims 26-51, wherein the recombinant I2S protein has an amino acid sequence at least 70% identical to SEQ ID NO:1.

53. The method of any one of claims 26-52, wherein the recombinant I2S protein has an amino acid sequence identical to SEQ ID NO:1.

54. The method of any one of claims 26-53, wherein the recombinant I2S protein is produced by mammalian cells cultured in suspension in a serum-free medium.

55. The method of claim 54, wherein the mammalian cells are cultured in a bioreactor.

56. The method of claim 54 or 55, wherein the serum-free medium lacks animal-derived components.

57. The method of any one of claims 54-56, wherein the serum-free medium is a chemically-defined medium.

58. The method of any one of claims 54-56, wherein the mammalian cells co-express the recombinant I2S protein and formylglycine generating enzyme (FGE).

59. The method of claim 58, wherein the mammalian cells are human cells.

60. The method of any one of claims 54-59, wherein the impure preparation is prepared from the serum-free medium containing recombinant I2S protein secreted from the mammalian cells.

61. The method of claim 60, wherein the impure preparation is thawed from a frozen medium preparation.

62. The method of any one of claims 26-61, wherein the purified recombinant I2S protein contains less than 80 ng/mg HCP.

63. The method of any one of claims 26-62, wherein the purified recombinant I2S protein contains less than 60 ng/mg HCP.

64. The method of any one of claims 26-63, wherein the purified recombinant I2S protein contains, on average, 16-22 sialic acids per molecule.

65. The method of any one of claims 26-63, 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.

66. A pharmaceutical composition comprising a recombinant I2S protein purified according to a method of any one of claims 26-65.

67. A method of treating Hunter syndrome comprising administering into a subject in need of treatment a pharmaceutical composition of claim 66.

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.