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 and compositions for production of recombinant I2S protein that allows more effective enzyme replacement therapy for Hunter syndrome. The present disclosure encompasses the discovery that more potent recombinant I2S protein can be produced by mammalian cells engineered to co-express a recombinant I2S protein and a formylglycine generating enzyme (FGE). Unexpectedly, recombinant I2S protein produced by such engineered cells has an unusually high level of C_{α}-formylglycine (FGly) conversion percentage (e.g., greater than 70% and up to 100%), resulting in significantly improved enzymatic activity of recombinant I2S protein. In addition, mammalian cells co-expressing I2S and FGE proteins according to the present disclosure have been successfully adapted to grow in suspension culture at a large scale. Therefore, the present disclosure allows more efficient large scale production of highly potent recombinant I2S protein.

[0006a] In one aspect, the present disclosure refers to a cell comprising a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:1; and a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:5, wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition, produces I2S protein comprising at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly), and wherein the level of iduronate-2-sulfatase mRNA expressed by the cell is between 0.1-fold and 10-fold higher than the level of formylglycine generating enzyme mRNA expressed by the cell.

[0006b] In another aspect, the present disclosure refers to a cell comprising a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid

sequence at least 90% identical to SEQ ID NO:1; and a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:5, wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition, produces I2S protein comprising at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly) and at a specific productivity rate of greater than about 5 picogram/cell/day.

[0006c] In yet another aspect, the present disclosure refers to a method of producing recombinant iduronate-2-sulfatase (I2S) protein comprising cultivating a cell as described herein under conditions such that the recombinant I2S and FGE proteins are co-expressed in the cell.

[0007] Thus, in one example, the present disclosure provides a cell containing a first nucleic acid encoding an iduronate-2-sulfatase (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%, or 99%) identical to SEQ ID NO:1; and a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising 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%, or 99%) identical to SEQ ID NO:5, wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition (e.g., suspension or adherent culture), produces the I2S protein comprising at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly).

[0008] In another example, the present disclosure provides a cell containing a first nucleic acid encoding an iduronate-2-sulfatase (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%, or 99%) identical to SEQ ID NO:1; and a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising 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%, or 99%) identical to SEQ ID NO:5, wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition, produces I2S protein comprising at least about 50% (e.g., at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to Cα-formylglycine (FGly) and at

a specific productivity rate of great than about 10 picogram/cell/day (e.g., greater than about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 picogram/cell/day).

[0009] In some examples, the first nucleic acid encodes an I2S protein having an amino acid sequence identical to SEQ ID NO:1. In some examples, the second nucleic acid encodes an FGE protein having an amino acid sequence identical to SEQ ID NO:5.

[0010] In some examples, the first and/or the second nucleic acid is operably linked to a hCMV promoter.

[0011] In some examples, the first and/or second nucleic acid are codon optimized. In some examples, the first nucleic acid has a 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:7. In particular examples, the first nucleic acid has a sequence of SEQ ID NO:7.

[0012] In some examples, the second nucleic acid comprises a 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:8. In some examples, the second nucleic acid has a sequence identical to SEQ ID NO:8.

[0013] In some examples, both of the first and second nucleic acids are exogenous (also referred to as recombinant). In some examples, the first and/or second nucleic acids are integrated (e.g., stably) in the genome of the cell. In some examples, the first and/or second nucleic acids are present in one or more extra-chromosomal constructs.

[0014] In some examples, a cell of the present disclosure is a mammalian cell. In certain examples, a suitable mammalian cell is a human cell. In certain examples, a suitable mammalian cell is a CHO cell.

[0015] In some examples, a cell according to the disclosure is adaptable to suspension culture. In other examples, a cell according to the disclosure is adherent.

[0016] In a further example, the present disclosure provides a method of producing recombinant iduronate-2-sulfatase (I2S) protein by cultivating a cell described in various examples herein under conditions such that the recombinant I2S and FGE proteins are co-expressed in the cell. In some examples, the cell is cultivated at a large scale. In some examples, a large scale suitable for the present disclosure is a bioreactor process. In some

examples, a bioreactor suitable for the disclosure is at a scale selected from 10L, 200L, 500L, 1000L, 1500L, 2000L. In some examples, a large scale (e.g., bioreactor) process suitable for the present disclosure involves a perfusion process. In some examples, a large scale (e.g., bioreactor) process suitable for the present disclosure involves a batch culture. In some examples, a large scale process suitable for the present disclosure is a roller bottle process. In some examples, a cell according to the present disclosure is cultivated in suspension. In other examples, a cell according to the present disclosure is cultivated adherent.

[0017] In some examples, a cell according to the present disclosure is cultivated in a serum-free medium (e.g., animal-free, chemically-defined, or protein-free medium). In other examples, a cell according to the present disclosure is cultivated in a serum-containing medium.

[0018] In various examples, a method according to the disclosure further includes a step of purifying the recombinant I2S protein.

[0019] In still another example, the present disclosure provides a recombinant iduronate-2-sulfatase (I2S) protein produced by a cell or method described in various examples herein.

[0020] In some examples, the present disclosure provides a preparation of recombinant iduronate-2-sulfatase (I2S) protein, in which said recombinant I2S protein has 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; and containing at least about 70% (e.g., at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%) conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C_{α}-formylglycine (FGly). In some examples, the recombinant I2S protein has an amino acid sequence identical to SEQ ID NO:1. In some examples, the recombinant I2S protein has specific activity of at least about 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] Among other things, the present disclosure also provides a pharmaceutical composition containing a recombinant I2S protein described in various examples herein and a pharmaceutically acceptable carrier and a method of treating Hunter syndrome by

administering into a subject in need of treatment recombinant I2S protein described herein or a pharmaceutical composition containing the same.

[0022] 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.

[0023] 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.

[0024] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating examples of the present disclosure, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art from the detailed description.

CLAIMS

1. A cell comprising a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:1; and

a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:5,

wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition, produces I2S protein comprising at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly), and wherein the level of iduronate-2-sulfatase mRNA expressed by the cell is between 0.1-fold and 10-fold higher than the level of formylglycine generating enzyme mRNA expressed by the cell.

2. A cell comprising a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:1; and

a second nucleic acid encoding a formylglycine generating enzyme (FGE) protein comprising an amino acid sequence at least 90% identical to SEQ ID NO:5,

wherein the first and/or the second nucleic acid are exogenous and wherein the cell, once cultivated under a cell culture condition, produces I2S protein comprising at least about 70% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly) and at a specific productivity rate of greater than about 5 picogram/cell/day.

3. The cell of claim 1, wherein the cell, once cultivated under a cell culture condition, produces the I2S protein comprising at least about 80% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly).

4. The cell of claim 1, wherein the cell, once cultivated under a cell culture

condition, produces the I2S protein comprising at least about 90% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly) .

5. The cell of claim 1, wherein the first and/or the second nucleic acid is operably linked to a hCMV promoter.

6. The cell of claim 1, wherein the second nucleic acid comprises a sequence at least 70% identical to SEQ ID NO:8.

7. The cell of claim 1, wherein both of the first and second nucleic acids are exogenous.

8. The cell of claim 1, wherein the first and/or second nucleic acids are integrated in the genome of the cell.

9. The cell of claim 1, wherein the first and/or second nucleic acids are present in one or more extra-chromosomal constructs.

10. The cell of claim 1, wherein the cell is a mammalian cell.

11. A method of producing recombinant iduronate-2-sulfatase (I2S) protein comprising cultivating a cell of claim 1 under conditions such that the recombinant I2S and FGE proteins are co-expressed in the cell.

12. The method of claim 11, wherein the cell is cultivated at a large scale.

13. The method of claim 12, wherein the large scale is a bioreactor process.

14. The method of claim 13, wherein the bioreactor process is a perfusion process.

15. The method of claim 13, wherein the bioreactor is at a scale selected from 10 L, 200 L, 500 L, 1000 L, 1500 L, or 2000 L.

16. The method of claim 11, wherein the cell is cultivated in a serum-free medium.

17. The method of claim 11, wherein the cell is cultivated in suspension.

18. The method of claim 11, wherein the method further comprises a step of purifying the recombinant I2S protein.

19. The cell of claim 1, wherein the cell, once cultivated under a cell culture condition, produces the I2S protein comprising at least about 95% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly).

20. The cell of claim 1, wherein the cell, once cultivated under a cell culture condition, produces the I2S protein comprising at least about 97% conversion of the cysteine residue corresponding to Cys59 of SEQ ID NO:1 to C.alpha.-formylglycine (FGly).

21. The cell of claim 1 , wherein the first nucleic acid comprises a sequence at least 70% identical to SEQ ID NO:7.

22. The cell of claim 10, wherein the mammalian cell is a CHO cell.

23. The cell according to claim 1, wherein the first nucleic acid encodes an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 95% identical to SEQ ID NO:1.

24. The cell according to claim 1, wherein the second nucleic acid encodes a

formylglycine generating enzyme (FGE) protein comprising an amino acid sequence at least 95% identical to SEQ ID NO:5.

25. The cell according to claim 1, wherein the first nucleic acid encodes an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence identical to SEQ ID NO:1.

26. The cell according to claim 1, wherein the second nucleic acid encodes a formylglycine generating enzyme (FGE) protein comprising an amino acid sequence identical to SEQ ID NO:5.