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] In one aspect, there is provided 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 Cα-formylglycine (FGly), and wherein the level of iduronate-2-sulfatase protein expressed by the cell is between 0.3-fold and 10-fold higher than the level of formylglycine generating enzyme protein expressed by the cell.

[0006a] In one aspect, there is provided 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 Cα-formylglycine (FGly) and at a specific productivity rate of greater than about 30 picogram/cell/day.

[0006b] The present invention 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 invention 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 invention have been successfully adapted to grow in suspension culture at a large scale. Therefore, the present invention allows more efficient large scale production of highly potent recombinant I2S protein.

[0007] Thus, in one aspect, the present invention 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).

We claim:

1. A cell comprising

a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 70% 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 70% 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 Cα-formylglycine (FGly).

2. A cell comprising

a first nucleic acid encoding an iduronate-2-sulfatase (I2S) protein comprising an amino acid sequence at least 70% 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 70% 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 60% 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 30 picogram/cell/day.

3. The cell of claim 1 or 2, 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 Cα-formylglycine (FGly).

4. The cell of any one of the preceding claims, 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 Cα-formylglycine (FGly).

5. The cell of any one of the preceding claims, 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 Cα-formylglycine (FGly).

6. The cell of any one of the preceding claims, 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 Cα-formylglycine (FGly).

7. The cell of any one of the preceding claims, wherein the first and/or the second nucleic acid is operably linked to a hCMV promoter.

8. The cell of any one of the preceding claims, wherein the first nucleic acid encodes an I2S protein having an amino acid sequence identical to SEQ ID NO:1.

9. The cell of any one of the preceding claims, wherein the second nucleic acid encodes an FGE protein having an amino acid sequence identical to SEQ ID NO:5.

10. The cell of any one of the preceding claims, wherein the first nucleic acid comprises a sequence at least 70% identical to SEQ ID NO:7

11. The cell of any one of the preceding claims, wherein the first nucleic acid comprises a sequence of SEQ ID NO:7.

12. The cell of any one of the preceding claims, wherein the second nucleic acid comprises a sequence at least 70% identical to SEQ ID NO:8

13. The cell of any one of the preceding claims, wherein the second nucleic acid comprises a sequence identical to SEQ ID NO:8.

14. The cell of any one of the preceding claims, wherein both of the first and second nucleic acids are exogenous.

15. The cell of any one of the preceding claims, wherein the first and/or second nucleic acids are integrated in the genome of the cell.

16. The cell of any one of the preceding claims, wherein the first and/or second nucleic acids are present in one or more extra-chromosomal constructs.

17. The cell of any one of the preceding claims, wherein the cell is a mammalian cell.

18. The cell of claim 17, wherein the mammalian cell is a human cell.

19. The cell of claim 17, wherein the mammalian cell is a CHO cell.

20. The cell of any one of the preceding claims, wherein the cell is adaptable to suspension culture.

21. A method of producing recombinant iduronate-2-sulfatase (I2S) protein comprising cultivating a cell of any one of the preceding claims under conditions such that the recombinant I2S and FGE proteins are co-expressed in the cell.

22. The method of claim 21, wherein the cell is cultivated at a large scale.

23. The method of claim 22, wherein the large scale is a bioreactor process.

24. The method of claim 23, wherein the bioreactor process is a perfusion process.

25. The method of claim 23 or 24, wherein the bioreactor is at a scale selected from 10L, 200L, 500L, 1000L, 1500L, or 2000L.

26. The method of claim 22, wherein the large scale is a roller bottle process.

27. The method of any one of claims 21-26, wherein the cell is cultivated in a serum-free medium.

28. The method of any one of claims 21-26, wherein the cell is cultivated in a serum-containing medium.

29. The method of any one of claims 21-26, wherein the cell is cultivated in suspension.

30. The method of any one of claims 21-26, wherein the cell is cultivated adherent.

31. The method of any one of claims 21-30, wherein the method further comprises a step of purifying the recombinant I2S protein.

32. A recombinant iduronate-2-sulfatase (I2S) protein produced by a method of any one of claims 21-31.

33. A recombinant iduronate-2-sulfatase (I2S) protein produced by a cell of any one of claims 1-20.

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 Cα-formylglycine (FGly), and wherein the level of iduronate-2-sulfatase protein expressed by the cell is between 0.3-fold and 10-fold higher than the level of formylglycine generating enzyme protein 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 Cα-formylglycine (FGly) and at a specific productivity rate of greater than about 30 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 Cα-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 Cα-formylglycine (FGly).

5. 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 Cα-formylglycine (FGly).

6. 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 Cα-formylglycine (FGly).

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

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

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

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

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

12. The cell of claim 11, wherein the mammalian cell is a CHO cell.

13. 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.

14. The method of claim 13, wherein the cell is cultivated in a large scale bioreactor process at a scale selected from 10L, 200L, 500L, 1000L, 1500L, or 2000L.

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

16. The method of claim 13, wherein the cell is cultivated in a serum-free medium.

17. The method of claim 13, wherein the cell is cultivated in suspension.

18. The method of claim 13, wherein the method further comprises a step of purifying the recombinant I2S protein.

19. 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.

20. 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.

21. 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.

22. 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.