and *in vivo* clearance of therapeutic glycoproteins have made monitoring and control of glycosylation a critical parameter for biopharmaceutical manufacturing. The high mannose glycoform content of therapeutic proteins is a critical quality attribute that has been found to affect pharmacokinetic properties of certain therapeutic antibodies (Goetze, et al., (2011) Glycobiology 21, 949-59; Yu, et al., (2012) *MAbs 4*, 475-87). Therefore, methods for controlling the high mannose glycoform content of therapeutic proteins would be beneficial.

There is a need in the pharmaceutical industry to manipulate and control the high mannose glycoform content of recombinant therapeutic glycoproteins and methods for doing such would be useful. The invention provides a method for manipulating the high mannose glycoform content of recombinant glycoproteins by regulating ornithine metabolism in the host cells.

SUMMARY

The invention provides in one aspect a method for decreasing the high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and the recombinant protein has a decreased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking the arginase inhibitor, wherein in case the arginase inhibitor is N^G-hydroxy-L-arginine monoacetate, the concentration of N^G-hydroxy-L-arginine monoacetate is between 1 μM and 10 μM.

In another aspect, the present invention refers to a method of decreasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising spermine, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture with a greater concentration of spermine, and the recombinant protein has a decreased high mannose glycoform content than the recombinant protein expressed by the cell culture having the greater concentration of spermine.

In yet another aspect, the present invention refers to a method of increasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine production in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor, and the recombinant protein has an increased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor.

In yet another aspect, the present invention refers to a method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and the recombinant protein has a decreased high mannose glycoform content when compared to the recombinant protein in the cell culture lacking the arginase inhibitor.

In yet another aspect, the present invention refers to a method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine production in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide

synthase inhibitor and an arginine decarboxylase inhibitor; and the recombinant protein has an increased high mannose glycoform content when compared to the recombinant protein expressed in the cell culture lacking the one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor.

Also disclosed herein is a method for manipulating the high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture under conditions that regulate ornithine metabolism in the host cell.

In one example ornithine metabolism in the host cell is regulated by decreasing the accumulation of ornithine in the host cell. In a related example ornithine accumulation in the host cell is regulated by culturing the host cell in a cell culture medium containing an arginase inhibitor or spermine. In another related example ornithine accumulation in the host cell is regulated through the addition of an arginase inhibitor to cell culture medium. In another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine) or DL-α-Difluoromethylornithine. In another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine). In another related example the arginase inhibitor is DL-α-Difluoromethylornithine. In another related example the concentration of the arginase inhibitor is at least 10 μM. In another related example the concentration of the arginase inhibitor is from 10 μM to 2 mM. In yet another related example the concentration of the arginase inhibitor is 10 μM. In yet another related example the concentration of the arginase

inhibitor is 0.5 mM. In yet another related example the concentration of the arginase inhibitor is 1 mM. In yet another related example the concentration of the arginase inhibitor is 2 mM.

In another example ornithine accumulation in the host cell is regulated by adding 35 μM or less of spermine to the cell culture medium. In a related example the concentration of spermine is 7 μM to 35 μM. In another related example the concentration of spermine is 17 μM to 35 μM. In another related example the concentration of spermine is 7 μM to 17 μM. In another related example the concentration of spermine is 35 μM. In another related example the concentration of spermine is 17 μM. In another related example the concentration of spermine is 7 μM.

In another example ornithine metabolism in the host cell is regulated by increasing the accumulation of ornithine in the host cell. In a related example ornithine accumulation in the host cell is regulated by culturing the host cell in a cell culture medium containing ornithine, arginine, an ornithine decarboxylase inhibitor, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor or an arginine decarboxylase inhibitor. In a yet another related example ornithine accumulation in the host cell is regulated by the addition of at least 0.6 mM ornithine to the cell culture medium. In a yet another related example the concentration of ornithine is from 0.6 to 14.8 mM. In a yet another related example the concentration of ornithine is from 6 to 14.8 mM. In a yet another related example the concentration of ornithine is 0.6 mM. In a yet another related example the concentration of ornithine is 6 mM.

In a yet another related example the concentration of ornithine is 14.8 mM. In another example ornithine accumulation in the host cell is regulated by the addition of at least 8.7 mM arginine to cell culture medium. In a yet another related example the concentration of arginine is from 8.7 mM to 17.5 mM. In a yet another related example the concentration of arginine is 8.7 mM. In a yet another related example the concentration of arginine is 17.5 mM.

In another example ornithine accumulation in the host cell is regulated through the addition of an ornithine decarboxylase inhibitor, a nitric oxide synthase inhibitor, an ornithine aminotransferase inhibitor, or an arginine decarboxylase inhibitor

to the cell culture medium. In a related example ornithine accumulation in the host cell is regulated through the addition of an ornithine decarboxylase inhibitor to the cell culture medium. In yet another related example the ornithine decarboxylase inhibitor is alpha-defluoromethylornithine (DMFO). In yet another related example the ornithine decarboxylase inhibitor is piperonyl butoxide (PBO).

In another related example ornithine accumulation in the host cell is regulated through the addition of an ornithine aminotransferase inhibitor to the cell culture medium. In yet another related example the ornithine aminotransferase inhibitor is 5-fluoromethylornithine (F-FMOrn). In yet another related example the host cell is regulated through the addition of a nitric oxide synthase inhibitor to the cell culture medium. In yet another related example the nitric oxide synthase inhibitor is 2-ethyl-2-thiopseudourea or N-Nitro-L-arginine and L^G-monomethyl-L-arginine. In yet another related example the nitric oxide synthase inhibitor is N-Nitro-L-arginine and L^G-monomethyl-L-arginine.

In another related example ornithine accumulation in the host cell is regulated through the addition of an arginine decarboxylase inhibitor to the cell culture medium. In yet another related example the arginine decarboxylase inhibitor is asymmetric dimethyl-arginine (ADMA).

The disclosure provides a method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture wherein ornithine metabolism is regulated by reducing ornithine accumulation in the host cell. In a related example ornithine accumulation in the host cell is reduced by culturing the host cell in a cell culture medium containing an arginase inhibitor or spermine.

In a related example ornithine accumulation in the host cell is reduced through the addition of an arginase inhibitor to the cell culture medium. In yet another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine) or DL-α-Difluoromethylornithine. In yet another related example the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine). In yet another related example the arginase inhibitor is DL-α-Difluoromethylornithine. In yet another related example the arginase inhibitor is at least 10 μM. In yet another related example the arginase

inhibitor is from 10 μM to 2 mM. In yet another related example the arginase inhibitor is from 10 μM to 20 μM. In yet another related example the arginase inhibitor is 10 μM. In yet another related example the arginase inhibitor is 0.5 mM. In yet another related example the arginase inhibitor is 1 mM. In yet another related example the arginase inhibitor is 2 mM.

In another example ornithine accumulation in the host cell is reduced by culturing the host cell in a cell culture medium containing 35 μM or less of spermine in the cell culture medium. In yet another related example the concentration of spermine is 7 μM to 35 μM. In yet another related example the concentration of spermine is 17 μM to 35 μM. In yet another related example the concentration of spermine is 0.07 mL/L to 0.17 mL/L. In yet another related example the concentration of spermine is 35 μM. In yet another related example the concentration of spermine is 17 μM. In yet another related example the concentration of spermine is 7 μM.

The disclosure provides a method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture wherein ornithine metabolism is regulated by increasing ornithine accumulation in the host cell. In a related example ornithine accumulation in the host cell is increased by adding ornithine, arginine, an ornithine decarboxylase inhibitor, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor or an arginine decarboxylase inhibitor to the cell culture medium.

In another related example ornithine accumulation in the host cell is increased by the culturing the host cell in a cell culture medium containing of at least 0.6 mM ornithine. In yet another related example the concentration of ornithine is from 0.6 to 14.8 mM. In yet another related example the concentration of ornithine is from 6 to 14.8 mM. In yet another related example the concentration of ornithine is 0.6 mM. In yet another related example the concentration of ornithine is 6 mM. In yet another related example the concentration of ornithine is 14.8 mM.

In another related example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing at least 8.7 mM arginine. In yet another related example the concentration of arginine is from 8.7 mM to 17.5

mM. In yet another related example the concentration of arginine is 8.7 mM. In yet another related example the concentration of arginine is 17.5 mM.

In another related example ornithine accumulation is increased in the host cell by culturing the host cell in a cell culture medium containing an ornithine decarboxylase inhibitor, a nitric oxide synthase inhibitor, an ornithine aminotransferase inhibitor, or an arginine decarboxylase inhibitor. In another related example ornithine accumulation in the host cell-cell is increased by culturing the host cell in a cell culture medium containing an ornithine decarboxylase inhibitor. In yet another related example the ornithine decarboxylase inhibitor is alpha-defluoromethylornithine (DMFO). In yet another related example the ornithine decarboxylase inhibitor is piperonyl butoxide (PBO).

In another related example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing an ornithine aminotransferase inhibitor. In yet another related example the ornithine aminotransferase inhibitor is 5-fluoromethylornithine (F-FMOrn).

In another related example ornithine accumulation in a host cell is increased by culturing the host cell in a cell culture medium containing a nitric oxide synthase inhibitor. In yet another related example the nitric oxide synthase inhibitor is 2-ethyl-2-thiopseudourea or N-Nitro-L-arginine and L^G-monomethyl-L-arginine.

In another example ornithine accumulation in the host cell is increased by culturing the host cell in a cell culture medium containing an arginine decarboxylase inhibitor. In yet another related example the arginine decarboxylase inhibitor is asymmetric dimethyl- arginine (ADMA).

In another example the host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof. In yet another related example the culture is a perfusion culture. In yet another related example perfusion comprises continuous perfusion. In yet another related example the rate of perfusion is constant. In yet another related example the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day. In yet another related example the perfusion is accomplished by alternating tangential flow.

In another example the host cell expressing the recombinant protein is cultured in a bioreactor. In yet another related example the bioreactor has a capacity of at least 500L. In yet another related example the bioreactor has a capacity of at least 500L to 2000L. In yet another related example the bioreactor has a capacity of at least 1000L to 2000L. In yet another related example the bioreactor is inoculated with at least 0.5 x 10^6 cells/mL.

In another example the host cell expressing the recombinant protein is cultured in a serum-free cell culture medium. In another related example the serum-free culture medium is a perfusion cell culture medium. In yet another related example the host cells are mammalian cells. In yet another related example the host cells are Chinese Hamster Ovary (CHO) cells.

In another example the recombinant protein is a glycoprotein. In another example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In another example the methods above further comprise a step of harvesting the recombinant protein produced by the host cell.

In another example the recombinant protein produced by the host cell is purified and formulated into a pharmaceutically acceptable formulation.

In another example is provides a recombinant protein produced by any of the methods above. In a related example the recombinant protein is purified. In another example the recombinant protein is formulated into a pharmaceutically acceptable formulation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Ornithine metabolism overview. ARG, Arginase; AZ, Antizyme; AZIN, Antizyme inhibitor; P5C, Pyrroline-5-carboxylate; ASP, Aspartate; ORNT, Ornithine transporter; GATM, Glycine amidinotransferase; NOS, Nitric oxide synthase; OAT, Ornithine aminotransferase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamoylase; SMS, Spermine synthase; SRS, Spermidine synthase.

production levels. However, the effectiveness of arginase inhibitors may be compromised due to the high level of arginine in cell culture medium. In addition, there are other metabolic precursors of ornithine (i.e. glutamate and proline, see Figure 1) that may contribute to ornithine accumulation.

As described herein, it was found that high mannose glycoform content of a recombinant protein expressed by the cultured host cell could be modulated by adding an arginase inhibitor to the cell culture medium. Blocking the activity of arginase reduced the amount of ornithine production in the host cells, lowered the high mannose glycan level of the expressed recombinant glycoproteins.

Arginase also inhibits ornithine transcarbamoylase (OTC) (Vissers et al., (1982) J. Gen. Microbio. 128:1235-1247). Blocking the activity of arginase not only reduces ornithine production from arginine but could potentially relieve the repression of OTC activity, allowing ornithine conversion to citrulline, allowing for additional reduction of ornithine accumulation (see Figure 1).

In patients with OTC deficiency, ammonia levels are increased. If enhanced arginase expression or activity in host cell lines expressing recombinant proteins with high levels of high mannose glycans induces OTC inhibition, this m also result in ammonia level increase. Increase in the intracellular levels of ammonia could potentially alter pH gradient in Golgi apparatus and induce suboptimal relocalization of glycosyltransferases, resulting in higher levels of high mannose glycans due to incomplete glycan branching in Golgi complex (Campbell et al, (1973) NJM 288 (1):1-6; Hopkins et al., (1969) Archive of Disease in Childhood 44(234):143-148; Mühling et al, (2001)Amino Acids 21(3):303-318; Park et al., (2000) J. Biotechnol 81(2):129-140; Rivinoja et al., (2009) J. Cell Physiol. 220(1):144-154; and Axelsson et al., (2001) Glycobiology 11(8):633-644).

Useful arginase inhibitors are known in the art and available from commercial sources. Such arginase inhibitors include, BEC Hydrochloride; DL-α, Diflouromethylornithine Hydrochloride; N^G-Hydroxy-L-arginine and Nω- Hydroxy-nor-arginine. In one embodiment the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine) or DL-α-Difluoromethylornithine. In one embodiment of the invention the

arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine). In one embodiment of the invention the arginase inhibitor is DL-α-Difluoromethylornithine.

Arginase inhibitors can be added to cell culture medium at concentrations of at least 10 μM to decrease high mannose glycan levels of expressed recombinant proteins without significantly affecting productivity. In one embodiment, the concentration of the arginase inhibitor is from 10 μM to 2 mM. In another embodiment, the concentration of the arginase inhibitor is 10 μM. In another embodiment the concentration of the arginase inhibitor is 0.5 mM. In another embodiment the concentration of the arginase inhibitor is 1mM. In another embodiment the concentration of the arginase inhibitor is 2 mM.

Ornithine accumulation in the host cell can also be regulated by culturing the host cell in a cell culture medium containing spermine, as described in the Examples below. Ornithine, via the action of ornithine decarboxylase (ODC), is the starting point for the polyamine pathway and synthesis of the polyamines putrescine, spermidine and spermine. Exogenously added spermine can be used to inactivate ODC directly, or through antizyme, see Figure 1. Inactivation of ODC can lead to accumulation of ornithine as described below. By limiting the amount of exogenously added spermine, suppression of ODC activity can be relieved and ornithine can be metabolized through the polyamine pathway, thereby reducing the overall ornithine accumulation in the host cell.

Spermine can be added to cell culture medium at concentrations of less than or equal to 35 μM to decrease high mannose glycan levels of expressed recombinant proteins without significantly affecting productivity. In one embodiment the concentration of spermine is 7 μM to 35 μM. In one embodiment the concentration of spermine is 17 μM to 35 μM. In one embodiment the concentration of spermine is 7 μM to 17 μM. In another embodiment the concentration of spermine is 35 μM. In another embodiment the concentration of spermine is 17 μM. In another embodiment the concentration of spermine is 7 μM.

Another method for regulating ornithine metabolism is to increase the accumulation of ornithine. In one embodiment the invention provides regulating ornithine accumulation in a host cell by culturing the host cell in a cell culture medium

containing ornithine, arginine, an ornithine decarboxylase inhibitor, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor or an arginine decarboxylase inhibitor.

As described herein, levels of extracellular ornithine in conditioned cell culture media was found to correlate to high mannose glycan levels on recombinant glycoproteins. It was found that culturing host cells expressing recombinant proteins in a cell culture medium containing ornithine resulted in recombinant glycoproteins having increased levels of high mannose glycans.

As described above, ornithine accumulation in cell culture media likely reflects alterations in ornithine metabolism which can lead to accumulation of ammonia, similar to patients carrying defective genes of ornithine metabolism (eg. OTC deficiency or ORNT1 mutation). While the cellular mechanism behind ammonia induced high mannose increase is not known, it has been suggested that alteration in the pH gradient in Golgi can lead to suboptimal relocalization of glycosyltransferases. These changes could lead to decreased availability of glycosylation enzymes to complete glycan branching, and thus result in higher levels of high mannose glycan levels.

Another possibility is that ornithine accumulation potentially induces disturbance of redox homeostasis (Zanatta et al., (2013) Life sciences 93(4): 161-168). Ornithine correlates positively with high mannose suggesting the possibility that high mannose glycoform content is regulated by cellular redox state. Ornithine can increase the levels of lipid oxidation. Since many of the glycosylation regulating enzymes are lipid membrane bound, alterations in lipid oxidation caused by ornithine accumulation could potentially alter the integrity and activity of glycosylation regulating enzymes in Golgi and ER, and subsequently affect high mannose glycoform contents.

Ornithine can be added to cell culture medium at concentrations of at least 0.6 mM, to increase high mannose glycan levels of expressed recombinant proteins without significantly affecting productivity. In one embodiment the concentration of ornithine is from 0.6 mM to 14.8 mM. In one embodiment the concentration of ornithine is from 6 mM to 14.8 mM. In another embodiment the concentration of ornithine is 0.6 mM. In another embodiment the concentration of ornithine is 6 mM. In another embodiment the concentration of ornithine is 14.8 mM.

What is claimed is:

1. A method for decreasing the high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and

the recombinant protein has a decreased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking the arginase inhibitor, wherein in case the arginase inhibitor is N^G-hydroxy-L-arginine monoacetate, the concentration of N^G-hydroxy-L-arginine monoacetate is between 1 μM and 10 μM.

2. The method according to claim 1, wherein ornithine accumulation in the host cell is regulated through the addition of an arginase inhibitor to the cell culture.

3. The method according to claim 2, wherein the arginase inhibitor is selected from the group consisting of N^G-hydroxy-L-arginine monoacetate, Nω-hydroxy-nor-arginine diacetate, BEC (S-(2-boronoethyl)-l-cysteine) and DL-α-difluoromethylornithine.

4. The method according to claim 2, wherein the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine).

5. The method according to claim 2, wherein the arginase inhibitor is DL-α-difluoromethylornithine.

6. The method according to claim 2, wherein the arginase inhibitor is:

(a) N^G-hydroxy-L-arginine monoacetate, having a concentration of 1 μM or 10 μM;

(b) Nω-hydroxy-nor-arginine diacetate, having a concentration of 10 μM or 20 μM;

(c) BEC (S-(2-boronoethyl)-l-cysteine), having a concentration of 1 μM, 10 μM, 20 μM or 0.5 mM; or

(d) DL-α-difluoromethylornithine, having a concentration of 1 μM, 10 μM, 20 μM, 1 mM or 2 mM.

7. The method according to claim 6, wherein concentration of the arginase inhibitor is selected from the group consisting of at least 10 μM, from 10 μM to 2 mM, 0.5 mM to 2 mM, 0.5 mM to 1 mM and 1 mM to 2 mM.

8. The method according to claim 7, wherein the arginase inhibitor is selected from the group consisting of Nω-hydroxy-nor-arginine diacetate, BEC (S-(2-boronoethyl)-l-cysteine); and DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 10 μM.

9. The method according to claim 7, wherein the arginase inhibitor is BEC (S-(2-boronoethyl)-l-cysteine), wherein the concentration of the arginase inhibitor is 0.5 mM.

10. The method according to claim 7, wherein the arginase inhibitor is DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 1mM.

11. The method according to claim 7, wherein the arginase inhibitor is DL-α-difluoromethylornithine, wherein the concentration of the arginase inhibitor is 2mM.

12. A method of decreasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising spermine, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture with a greater concentration of spermine, and

the recombinant protein has a decreased high mannose glycoform content than the recombinant protein expressed by the cell culture having the greater concentration of spermine

13. The method according to claim 12, wherein 35 μM or less of spermine is added to the cell culture medium.

14. The method according to claim 13, wherein the concentration of spermine is 7 μM to 35 μM.

15. The method according to claim 13, wherein the concentration of spermine is 17 μM to 35 μM.

16. The method according to claim 13, wherein the concentration of spermine is 7 μM to 17 μM.

17. The method according to claim 13, wherein the concentration of spermine is 35 μM.

18. The method according to claim 13, wherein the concentration of spermine is 17 μM.

19. The method according to claim 13, wherein the concentration of spermine is 7 μM.

20. A method of increasing high mannose glycoform content of a recombinant protein comprising culturing a host cell expressing the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine production in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor, and

the recombinant protein has an increased high mannose glycoform content than when the recombinant protein is expressed in the cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor.

21. The method according to claim 20, wherein ornithine accumulation in the host cell is increased by the addition of at least 0.6 mM ornithine to the cell culture.

22. The method according to claim 21, wherein the concentration of ornithine is from 0.6 to 14.8 mM.

23. The method according to claim 21, wherein the concentration of ornithine is from 6 to 14.8 mM.

24. The method according to claim 21, wherein the concentration of ornithine is 0.6 mM.

25. The method according to claim 21, wherein the concentration of ornithine is 6 mM.

26. The method according to claim 21, wherein the concentration of ornithine is 14.8 mM.

27. The method according to claim 20, wherein ornithine accumulation in the host cell is regulated by the addition of at least 8.7 mM arginine to cell culture medium.

28. The method according to claim 27, wherein the concentration of arginine is from 8.7 mM to 17.5 mM.

29. The method according to claim 27, wherein the concentration of arginine is 8.7 mM.

30. The method according to claim 27, wherein the concentration of arginine is 17.5 mM.

31. A method of producing a recombinant protein wherein the high mannose glycoform content is reduced comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising an arginase inhibitor, wherein ornithine production in the host cell is decreased when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking the arginase inhibitor, and

the recombinant protein has a decreased high mannose glycoform content when compared to the recombinant protein in the cell culture lacking the arginase inhibitor.

32. A method of producing a recombinant protein wherein the high mannose glycoform content is increased comprising culturing a host cell which expresses the recombinant protein in a cell culture comprising one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor, wherein ornithine production in the host cell is decreased by increasing the accumulation of ornithine when compared to the host cell expressing the recombinant protein is cultured in a cell culture lacking one selected from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor and an arginine decarboxylase inhibitor; and

the recombinant protein has an increased high mannose glycoform content when compared to the recombinant protein expressed in the cell culture lacking the one selected

from the group consisting of ornithine, arginine, an ornithine aminotransferase inhibitor, a nitric oxide synthase inhibitor, and an arginine decarboxylase inhibitor.

33. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof.

34. The method according to claim 33, wherein the culture is a perfusion culture.

35. The method according to claim 34, wherein perfusion comprises continuous perfusion.

36. The method according to claim 34, wherein the rate of perfusion is constant.

37. The method according to claim 34, wherein the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day.

38. The method according to claim 34, wherein the perfusion is accomplished by alternating tangential flow.

39. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a bioreactor.

40. The method according to claim 39, wherein the bioreactor has a capacity of at least 500L.

41. The method according to claim 39, wherein the bioreactor has a capacity of at least 500L to 2000L.

42. The method according to claim 39, wherein the bioreactor has a capacity of at least 1000L to 2000L.

43. The method according to claim 39, wherein the bioreactor is inoculated with at least 0.5 x 10^6 cells/mL.

44. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cell expressing the recombinant protein is cultured in a serum-free cell culture medium.

45. The method according to any of claim 44, wherein the serum-free culture medium is a perfusion cell culture medium.

46. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cells are mammalian cells.

47. The method according to any of claims 1, 12, 20, 31, or 32, wherein the host cells are Chinese Hamster Ovary (CHO) cells.

48. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein is a glycoprotein.

49. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

50. The method according to any of claims 1, 12, 20, 31, or 32, further comprising a step of harvesting the recombinant protein produced by the host cell.

51. The method according to any of claims 1, 12, 20, 31, or 32, wherein the recombinant protein produced by the host cell is purified and formulated into a pharmaceutically acceptable formulation.