In accordance with the present disclosure, reducing the occurrence of α-amidation at the C-terminal amino acids can either be facilitated by decreasing the formation of C-terminal α-amidated Pro by using a medium with a selenium (Se) concentration from 0.175 μM to about 2.98 μM, or by increasing the proportion of the C-terminal lysine residue on one or preferably all C-termini(us) of the expressed polypeptide(s) by using a medium comprising zinc (Zn) in a concentration ranging from about 0.2 μM to about 20 μM. In a preferred embodiment, a medium comprising 1 μM to 2.4 μM selenium (Se) as well as 3 μM to 15 μM zinc (Zn) is used.

In a preferred embodiment of the present disclosure, the expression is a heterologous expression and takes place in a eukaryotic cell-based expression system, wherein the eukaryotic cell-based system comprises insect cells, plant cells, mammalian cells, animal cells and lower eukaryotic cells comprising fungi and yeast cells.

In accordance with the present disclosure, the expression of the polypeptide, *i.e.*, the process of the present disclosure, takes place in at least one bioreactor (BR) or culture vessel comprising shake flasks (SF), T-flasksrollers, bottles, bags, BRs, and/or spinner flasks.

It is another aspect of the present disclosure that selenium or a salt thereof is used as an inhibitor for peptidylglycine [alpha]-amidating monooxygenase (PAM) enzyme.

In another aspect, the present disclosure provides a cell culture medium for controlling [alpha]-amidation and/or C-terminal cleavage of amino acid residue of at least one expressed polypeptide in a cell culture, wherein the medium comprises at least one essential trace element or a compound comprising the element which acts on peptidylglycine [alpha]-amidating monooxygenase (PAM) enzyme or carboxypeptidase (CP).

In one aspect, the present disclosure provides a cell culture medium for controlling [alpha]-amidation and C-terminal cleavage of amino acid residue of at least one expressed polypeptide in a cell culture, wherein the medium comprises selenium ions or a salt thereof or a compound comprising selenium ions in a concentration ranging from 1 μM to 2.98 μM, and zinc ions or a salt thereof or a compound comprising zinc ions in a concentration ranging from 3 μM to 20 μM.

In another aspect, the present disclosure provides a cell culture process for controlling [alpha]-amidation and/or C-terminal cleavage of amino acid residue of at least one expressed polypeptide, the process comprising as least the steps of:

i) adding at least one essential trace element or a compound comprising the same to the culture medium comprising a host cell producing the polypeptide; and

ii) fermenting and recovering the polypeptide.

In another aspect, the present disclosure provides a cell culture process for controlling [alpha]-amidation and/or C-terminal cleavage of amino acid residue of at least one expressed polypeptide, the process comprising:

i) adding to the culture medium, which comprises a host cell producing the polypeptide, selenium ions or a salt thereof or a compound comprising selenium ions in a concentration ranging from 1 μM to 2.98 μM, and zinc ions or a salt thereof or a compound comprising zinc ions in a concentration ranging from 3 μM to 20 μM; and

ii) fermenting and recovering the polypeptide.

In another aspect, the present disclosure provides a polypeptide obtainable by the process as disclosed herein.

In another aspect, the present disclosure provides the use of selenium or a salt thereof as inhibitor for peptidylglycine [alpha]-amidating monooxygenase (PAM) enzyme.

In another aspect, the present disclosure provides the use of zinc or a salt thereof as an activator for carboxylpeptidase (CP).

In another aspect, the present disclosure provides the *ex vivo* and/or *in vitro* use of selenium or a salt thereof as an inhibitor for peptidylglycine [alpha]-amidating monooxygenase (PAM) enzyme.

In another aspect, the present disclosure provides the *ex vivo* and/or *in vitro* use of zinc or a salt thereof in a concentration ranging from 3 μM to 20 μM as an activator for carboxypeptidase (CP).

Definitions

Unless otherwise stated, a term as used herein is given the definition as provided in the Oxford dictionary of biochemistry and molecular biology, Oxford University Press, 2006, Print ISBN-13: 9780198529170 Current Online Version: 2008; eISBN: 9780191727641.

For further elaboration of general techniques useful in the practice of this invention, the practitioner can refer to standard textbooks and reviews in cell biology and tissue culture; see also the references cited in the examples. General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook *et al.*, Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel *et al.* eds., John Wiley & Sons 1999); Protein Methods (Bollag *et al.*, John Wiley & Sons 1996); Non-viral Vectors for Gene Therapy (Wagner *et al.*

Claims

1. A cell culture medium for controlling [alpha]-amidation and C-terminal cleavage of amino acid residue of at least one expressed polypeptide in a cell culture, wherein the medium comprises selenium ions or a salt thereof or a compound comprising selenium ions in a concentration ranging from 1 μM to 2.98 μM, and zinc ions or a salt thereof or a compound comprising zinc ions in a concentration ranging from 3 μM to 20 μM.

2. The cell culture medium of claim 1, further comprising copper ions or a salt thereof or a compound comprising copper ions in a concentration ranging from 0.02 μM to 1 μM.

3. The cell culture medium of claim 2, wherein the concentration of copper ions or the salt thereof, or of the compound comprising copper ions is in the range from 0.1 μM to 0.8 μM.

4. The cell culture medium of any one of claims 1 to 3, wherein the cell culture medium comprises:
   i) selenium in a concentration ranging from 1 μM to 2.4 μM; and
   ii) zinc in a concentration ranging from above 3 μM to 15 μM.

5. A cell culture process for controlling [alpha]-amidation and/or C-terminal cleavage of amino acid residue of at least one expressed polypeptide, the process comprising:
   i) adding to the culture medium, which comprises a host cell producing the polypeptide, selenium ions or a salt thereof or a compound comprising selenium ions in a concentration ranging from 1 μM to 2.98 μM, and zinc ions or a salt thereof or a compound comprising zinc ions in a concentration ranging from 3 μM to 20 μM; and
   ii) fermenting and recovering the polypeptide.

6. The cell culture process of claim 5, further comprising adding to the culture medium copper ions or a salt thereof or a compound comprising copper ions in a concentration ranging from 0.02 μM to 1 μM.

7. The cell culture process of claim 6, wherein the concentration of copper ions or the salt thereof or of the compound comprising copper ions is in the range from 0.1 µM to 0.8 µM.

8. The cell culture process of any one of claims 5 to 7, wherein the adding to the culture medium decreases the amount of C-terminal [alpha]-amidated proline and/or increases the amount of C-terminal lysine.

9. The cell culture process of any one of claims 5 to 8, wherein selenium is used for inhibiting [alpha]-amidation, and wherein zinc is used for activating C-terminal cleavage of amino acid residue and/or for activating [alpha]-amidation.

10. The cell culture process of any one of claims 5 to 9, wherein the medium comprises:
    i) selenium in a concentration ranging from 1 µM to 2.4 µM; and
    ii) zinc in a concentration ranging from above 3 µM to 15 µM.

11. The cell culture process of any one of claims 5 to 10, wherein the amount of
    i) C-terminal alpha-amidated Proline of the expressed polypeptides is decreased compared to the use of standard media containing 2.04 µM copper and 0.173 µM selenium; and/or
    ii) C-terminal lysine of the expressed polypeptides is increased compared to the use of standard media containing 22 µM zinc.

12. The cell culture process of any one of claims 5 to 11, wherein said expressed polypeptide is
    i) a heterologously expressed polypeptide; and/or
    ii) selected from the group consisting of:
        a) an antibody, or a fragment or derivative thereof
        b) a fusion protein, and/or
        c) non-antibody proteins.

13. The cell culture process of claim 12, wherein the heterologous expression takes place in a eukaryotic cell-based expression system.

14. The cell culture process of claim 12, wherein the eukaryotic cell-based expression system comprises a eukaryotic cell selected from the group consisting of: insect cell, plant cell, algae cell, fungus cell, mammalian cell and animal cell.

15. The cell culture process of any one of claims 14, wherein said eukaryotic cell-based expression system is a mammalian expression system, being at least one mammalian cell line selected from the group consisting of:

- Baby hamster Kidney cell lines (*e.g.*, BHK21)
- Chinese hamster ovary cell lines (*e.g.*, CHO-K1, CHO-DG44, CHO-DXB, or CHO-dhfr$^-$)
- Murine myeloma cell lines (*e.g.*, SP2/0)
- Mouse myeloma cell lines (*e.g.*, NS0)
- Human embryonic kidney cell lines (*e.g.*, HEK-293)
- Human-retina-derived cell lines (*e.g.*, PER-C6), or
- Amniocyte cell lines (*e.g.*, CAP).

16. A polypeptide obtainable by the process of any one of claims 5 to 15.

17. The polypeptide of claim 16, wherein the polypeptide comprises less than 20% C-terminal alpha amindated proline.

18. The *ex vivo* and/or *in vitro* use of selenium or a salt thereof as an inhibitor for peptidylglycine [alpha]-amidating monooxygenase (PAM) enzyme.

19. The *ex vivo* and/or *in vitro* use of claim 18, wherein selenium or the salt thereof is used in a cell culture medium in the presence of zinc ions.

20. The *ex vivo* and/or *in vitro* use of zinc or a salt thereof in a concentration ranging from 3 μM to 20 μM as an activator for carboxypeptidase (CP).