The solution to said technical problem is achieved by providing the embodiments characterized in the independent claims of the present invention. The dependent claims are related to preferred embodiments. It is to be understood that value ranges delimited by numerical values are to be understood to include the said delimiting values.

Summary of the ~~invention~~Disclosure

The present ~~invention~~ disclosure provides means and methods for producing non-immunogenic polypeptides, wherein the means and methods are suitable for large scale bioprocesses.

The present disclosure~~invention~~ solves the above mentioned problem by means and methods for modulating (*e.g.* reducing) C-terminal heterogeneity of a post-translational modification, essentially consisting of α-amidation and/or C-terminal cleavage of amino acid residues of expressed polypeptides by use of a determined concentration of at least one essential trace element or a compound comprising the element as an additive in cell culture medium.

The present disclosure~~invention~~ is based on the surprising finding that essential trace elements are capable of regulating the C-terminal heterogeneity of recombinantly expressed polypeptides.

Put in other words, the present disclosure~~invention~~ relates to a cell culture medium for controlling α- [alpha]-amidation and/or C-terminal cleavage of amino acid residues 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/modulates the activity of peptidylglycine [alpha]-amidating monooxygenase (PAM) and/or carboxypeptidase (CP).

In another aspect, the present disclosure~~invention~~ provides a cell culture process for or of controlling α-[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 to the cell culture medium comprising a host cell expressing the polypeptide; and ii) fermenting and recovering the polypeptide produced by the host cell. In a preferred embodiment of the present disclosure~~invention~~, the essential trace element is selected from the group consisting at least of zinc (Cu) selenium (Se) and/or copper (Cu).

In accordance with the present disclosure~~invention~~, 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~~invention~~, 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~~invention~~, the expression of the polypeptide, *i.e.*, the process of the present disclosure~~invention~~, 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~~invention~~ 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 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 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).

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.*