~~To provide a comprehensive disclosure without unduly lengthening the specification, the applicant hereby incorporates by reference each of the patents and patent applications referenced above.~~

The particular combinations of elements and features in the above detailed embodiments are exemplary only; the interchanging and substitution of these teachings with other teachings in this and the patents/applications ~~incorporated by reference~~ referred to are also expressly contemplated. As those skilled in the art will recognize, variations, modifications, and other implementations of what is described herein can occur to those of ordinary skill in the art without departing from the spirit and the scope of the invention as claimed. Accordingly, the foregoing description is by way of example only and is not intended as limiting. The invention's scope is defined in the following claims and the equivalents thereto. Furthermore, reference signs used in the description and claims do not limit the scope of the invention as claimed.

Brief description of the examples and drawings

Additional details, features, characteristics and advantages of the object of the invention are disclosed in the subclaims, and the following description of the respective figures and examples, which, in an exemplary fashion, show preferred embodiments of the present invention. However, these drawings should by no means be understood as to limit the scope of the invention.

Experiments: Effect of PAM inhibitors on amino acid amidation/proline amid formation

In their experiments, the inventors focused on published PAM inhibitors as potential medium components. Among the screened components are, i.e., sodium sulfite, tiopronin, captopril, EDTA, ammonium sulfite, L-histidine, D-histidine, ammonium meta thiomolybdate, L-carnosine, penicillamine, 4-phenyl-3 butenoic acid (PBA).

Two recombinant proteins were used to investigate the effect of PAM inhibitors on amino acid amidation/proline amid formation, i.e., a monoclonal antibody (human-mouse-chimeric IgG), and an Fc-fusion protein. CHO cells were used as expression system.

PBA was shown to have the strongest effect on proline amide formation, while not impacting growth or other product quality attributes significantly.

Based on these results, the inventors concluded that the composition of a cell culture medium can be adapted by addition of PBA (e.g., 100 μM). In another approach, PBA can be supplemented to the medium as well as to a feed solution. For example, the culture can be started without, or with low amounts of, PBA, and increasing amounts of PBA can be added after the cell culture has reached its peak cell densities, but before the main amount of recombinant protein is being produced. This may support higher peak cell densities and higher product titers at harvest compared to having the final amount of PBA already present during the growth phase.

Drawings

Fig. 1: Influence of PAM inhibitors on proline amide (PA) formation, quantified as 'pseudo 1K' in CEX-CPB analysis, on a monoclonal antibody (chimeric IgG) expressed in CHO cells. PBA was shown to have the strongest effect on proline amide formation when supplemented to the cell culture, with concentrations of 10μM or 100μM in the cell culture medium decreased the amount of pseudo 1K from app. 10% to app. 8%, or app. 5%, respectively. 5% pseudo 1K in CEX-CPB corresponds to 0-2% proline amide per heavy chain as confirmed by peptide mapping.

Fig. 2: Influence of PAM inhibitor PBA on proline amide formation on an Fc-fusion protein expressed in CHO cells, as quantified by peptide mapping. Supplementing the cultures of recombinant CHO cells with various concentrations of PBA up to 100μM decreased the amount of proline amide (% per heavy chain) in a dose-dependent effect curve from app. 2% (no PBA) to app. 0.1 % (100μM PBA).

Fig. 3: Influence of PAM inhibitor PBA on proline amide formation on an Fc-fusion protein expressed in CHO cells, as quantified by peptide mapping. Supplementing the culture of recombinant CHO cells with 50μM or 100μM PBA resulted in a decrease of proline amide (% per heavy chain) from >4% to <1% and <0.5% respectively.

What is claimed is:

1. A cell culture medium for the expression of a protein, which medium comprises a PAM inhibitor, or a ~~physiological equivalent~~ salt or an ester thereof, wherein said expression is a heterologous protein expression, and wherein the salt or ester dissolves, or is hydrolysed as applicable, to the PAM inhibitor in a physiological setting.

2. A cell culture process for the expression of a protein, in which process a PAM inhibitor, or a ~~physiological equivalent~~ salt or an ester thereof, is used, wherein said expression is a heterologous protein expression, and wherein the salt or ester dissolves, or is hydrolysed as applicable, to the PAM inhibitor in a physiological setting.

~~3. The process or the cell culture medium according to claim 1 or 2, wherein said expression is a heterologous protein expression.~~

43. The process or the cell culture medium according to ~~any of~~ claims ~~1 – 3~~1 or 2, wherein the heterologous expression takes place in a mammalian cell based expression system

~~5~~4. The process or the cell culture medium according to any of claims 1 - 43, wherein said protein is at least one protein selected from the group consisting of:

- an antibody, or a fragment or derivative thereof
- a fusion protein, and/or
- non-antibody proteins.

~~6~~5. The process or the cell culture medium according to ~~any of~~ claims ~~4 – 5~~ 3 or 4, wherein said mammalian cell-based expression system is at least one 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), and/or
- Amniocyte cell lines (e.g., CAP).

~~7~~6. The process according to any of claims 2 - ~~6~~5, wherein said process takes place in at least one bioreactor or culture vessel selected from the group consisting of:

- shake flasks
- T-flasks
- roller bottles
- bags
- bioreactors, and/or
- spinner flasks.

~~8~~7. The process or the cell culture medium according to any of claims 1 - ~~7~~6, wherein the PAM inhibitor, or ~~a physiological equivalent~~ the salt or ester thereof, serves to ~~affect~~ inhibit the formation of amidated amino acid residues, particularly C-terminal proline amide residues.

~~9~~8. The process or the cell culture medium according to any of claims 1 - ~~8~~7, wherein the PAM inhibitor is 4-phenyl-3-butenoic acid (PBA)~~, or a physiological equivalent thereof~~.

~~10~~9. The process or the cell culture medium according to claim ~~9~~8, wherein 4-phenyl-3-butenoic acid (PBA), or a ~~physiological equivalent~~ salt or an ester thereof, is used in a concentration of ≥ 0,01 µM and ≤ 3M.