Improved fed-batch bioprocesses using chemically modified amino acids in concentrated feeds

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Introduction:

Fed-batch culture bioprocesses are essential for the production of therapeutic proteins. In these cultures, concentrated feeds are added during cultivation to prevent nutrient depletion and to extend the growth phase, thus increasing product concentration. One limitation arises from the low solubility of some amino acids at high concentrations, in particular tyrosine. This amino acid is commonly solubilized in separate feeds at basic pH inducing pH spikes, precipitation and finally early cell death when added in the bioreactor. This work describes the evaluation of several chemically modified tyrosines as alternative to simplify fed-batch bioprocesses by using single feeding strategies at neutral pH (Figure 1).

Figure 1. Evolution of fed-batch processes from multiple feeds to single feed strategies.

Experimental strategy

- Synthesis of chemically modified tyrosines
- Solubility and stability in concentrated feeds or media
- Batch culture (screening, proof of concept)
- Fed-batch culture (dose finding, process optimization)
- Impact on the mAb cQAs

Materials and Methods

Solubility and stability: Increasing concentrations of modified tyrosines were solubilized in a Merck proprietary feed at pH 7.0 until reaching the maximum solubility. Stability was assessed during 6 months in Merck proprietary medium and amino acids (including modified tyrosine) were quantified by UPLC using ACQ•Tag™ Ultra reagent.

Batch cultures: Modified tyrosines were solubilized at a concentration of 4.5 mM in a Merck medium depleted in normal tyrosine. The control medium contained 1 mM tyrosine disodium salt. CHO-S cells were seeded at 1 × 10^5 cells/mL in 50 mL spin tubes and incubated at 37 °C, 5% CO_2, 80% humidity and a rotation speed of 320 rpm. Growth and viability were monitored during 11 days using Beckman Coulter ViCell®.

Fed-batch cultures: CHO-S cells expressing a human mAb were seeded at 2 × 10^5 cells/mL in medium containing tyrosine disodium salt. Feeds were added every other day starting at day 3. In the control, tyrosine disodium salt was added in a separate feed at pH 11 whereas modified tyrosines were solubilized in the main feed at pH 7.0. Glucose was maintained to 3-4 g/L using a separate feed. Growth and viability were monitored during 14 days using Beckman Coulter ViCell®.

Antibody analysis: IgG concentration was determined by a turbidometric method using Roche Cedex Bio HT®. Glycan analysis was performed on samples from day 14 using 2-AB labeling and UPLC.

Results

1. Solubility in concentrated feed:

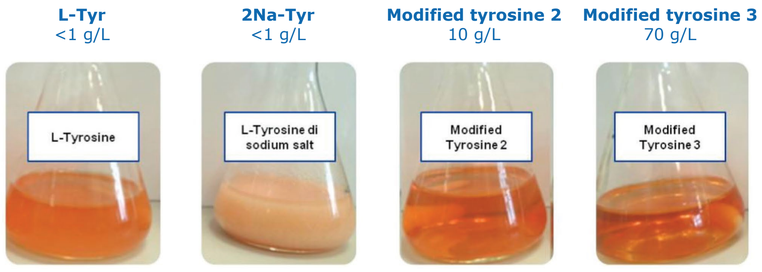

Figure 2. Maximum solubility of different tyrosine derivatives in a concentrated Merck feed at pH 7.0. The maximum solubility of L-tyrosine or its disodium salt was below 1 g/L in concentrated feed at pH 7.0 whereas 10 g/L and 70 g/L concentrations were reached using modified tyrosines 2 and 3.

2. Stability in chemically defined medium

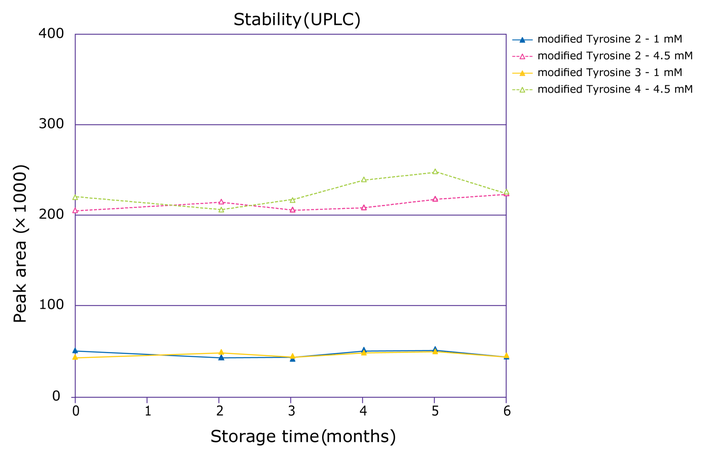

Figure 3. The stability of modified tyrosines was monitored during 6 months in chemically defined medium (CDM) stored at 4 °C. No decrease of peak area was observed indicating a good stability of the two derivatives.

3. Batch culture

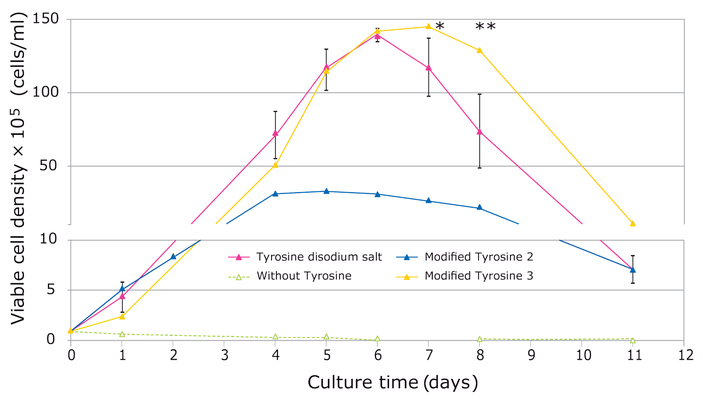

Figure 4. Batch culture of CHO-S cells in medium containing tyrosine disodium salt or modified tyrosines 2 and 3. The growth of CHO-S cells was inhibited with modified tyrosine 2 whereas an extended growth was observed with modified tyrosine 3. Representative experiment: mean ± SD (n=5). * p-value < 0.05 (Kruskal–Wallis Test).

4. Fed-batch culture

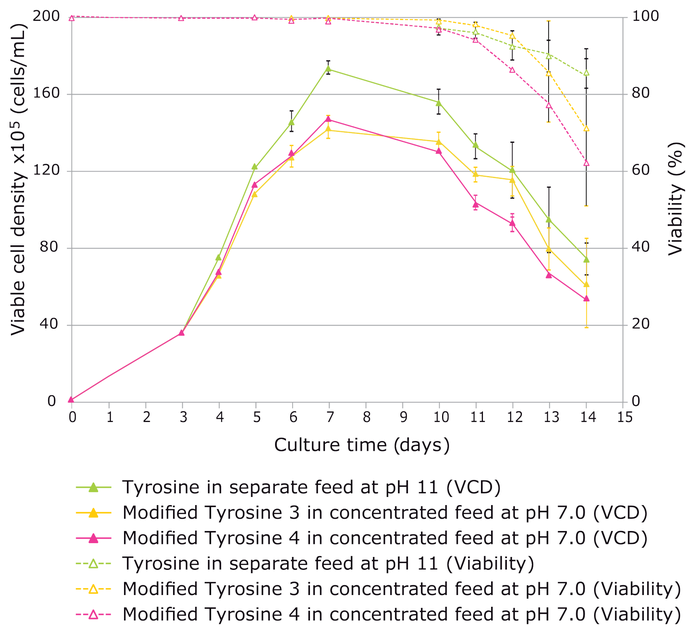

Figure 5. Fed-batch culture of recombinant CHO-S cells. Representative experiment: mean ± SD (n=5).* p-value < 0.05 (Kruskal–Wallis Test). Modified tyrosine 3 and 4 were successful at replacing tyrosine in the main feed at pH 7.0. The maximum VCD was lower than the control but could be optimized through process modifications (not shown).

4. mAb characterization

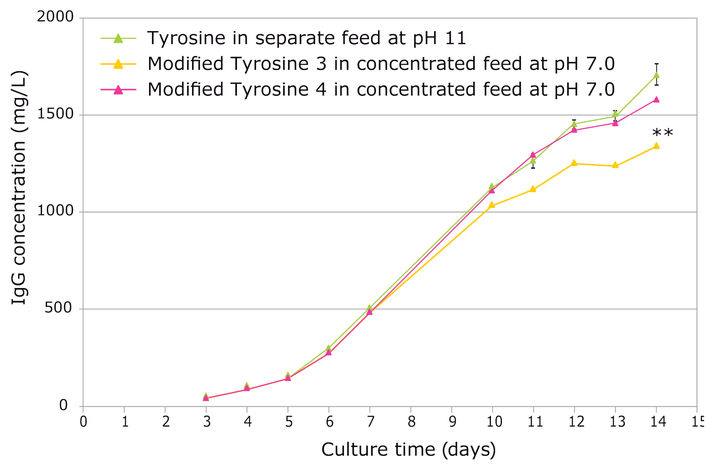

Figure 6. Quantification of IgG in the cell culture supernatant by turbidometric method. Representative experiment: mean ± SD (n=5); **p-value < 0.01 (Kruskal–Wallis test). Modified tyrosine 3 had a significant negative impact on the productivity (may result from a suboptimal process). The IgG concentration obtained with the modified tyrosine 4 was equivalent to the control indicating an overall higher specific productivity.

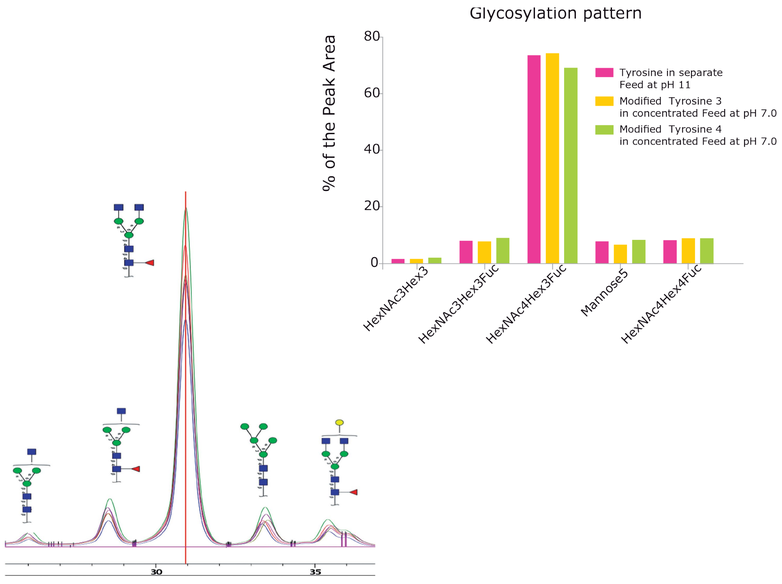

Figure 7. Glycosylation pattern on day 14 of the fed-batch process. The main glycosylation form of this mAb is a G0F (HexNac4Hex3Fuc, 70%) whereas approximately 8% are either Man5, HexNac3Hex3Fuc or G1F. The modified tyrosines had no significant impact on the observed glycoforms.

Discussion

Chemically modified tyrosines demonstrated an excellent solubility in concentrated feed and a good stability over 6 months in medium.

The performance in batch was dependent on the chemical modification, but some derivatives could extend the growth period.

In fed-batch mode, the modified tyrosine 4 showed interesting performance with similar titers as the control, but lower VCD resulting in a higher specific productivity.
Intact mass analysis (not shown), peptide mapping (not shown) and glycosylation analysis were performed on the mAb to study the impact of modified tyrosines on the final molecule. No difference was found between conditions, indicating that the use of tyrosine derivatives in feeds did not induce any detectable modification on the mAb.

Altogether, this study demonstrates that modified amino acids can be used successfully in highly concentrated feeds to improve the next generation of fed-batch processes.

Summary:

- The chemical modification of tyrosine can improve the solubility of the amino acid by up to 70 fold.
- Chemically modified tyrosines are stable in CDM and can be used in batch and fed-batch mode.
- The use of this modified amino acid in fed-batch bioprocesses has no detectable impact on the mAb or the recombinant protein produced.
- Chemically modified tyrosines are alternatives to simplify fed-batch bioprocesses by using single feeding strategies at neutral pH.