BIOPROCESS INTENSIFICATION WITH PEPTIDE-BASED CELL CULTURE MEDIA OPTIMIZATION USING TYROSINE AND/OR CYSTINE (DI)PEPTIDES

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INTRODUCTION

Over the past decade, cell culture media (CCM) optimization has been a key strategy for obtaining high yields and improving productivity, while ensuring product quality in biopharmaceutical production. However, further bioprocess intensification with chemically defined media is limited by undesired CCM chemistry such as the formation of reactive oxygen species (ROS) that negatively impact cellular metabolism and the final protein quality. ROS are formed during aerobic metabolism but also by chemical reactions of various media components such as transition metal ions (e.g., Fenton-based reactions) and photosensitive vitamins. Intra- and extracellular ROS react with and damage biomolecules, including DNA, lipids, and proteins as well as single amino acids. Therefore, the oxidation and degradation of media components and cellular biomolecules can strongly impact the productivity and product quality of bioprocesses [1].

Studies have shown that the essential amino acid L-tryptophane (Trp), as a single media component or as an individual residue in the final product, can be rapidly oxidized and degraded. Some of the end products show toxicity as well as contribute to undesirable CCM and product colorization, and product micro heterogeneity [2]. Similarly, L-cysteine is highly reactive and can generate hydroxyl free radicals and sulfide free radicals that promote oxidative stress leading to an insufficient process performance. Moreover, L-cysteine can oxidize to L-cystine, which can precipitate in CCM due to its low solubility. In this regard, various antioxidants as well as cysteine/cystine derivatives such as s-sulfocysteine or *N*-acetyl-cysteine have already been tested to secure CCM stability through ROS scavenging and/or oxidative stress prevention [3].

Chemically defined (di)peptides such as L-alanyl-L-tyrosine (Ala-Tyr) and glycyl-L-tyrosine (Gly-Tyr) as well as *N,N'*-di-L-alanyl-L-cystine [$(Ala\text{-}Cys)_2$] and *N,N'*-di-L-lysyl-L-cystine [$(Lys\text{-}Cys)_2$] are now commonly used to formulate more concentrated media due to their superior solubility. However, their involvement in media chemistry and impact on bioprocesses has not been investigated in detail.

RESULTS AND DISCUSSION

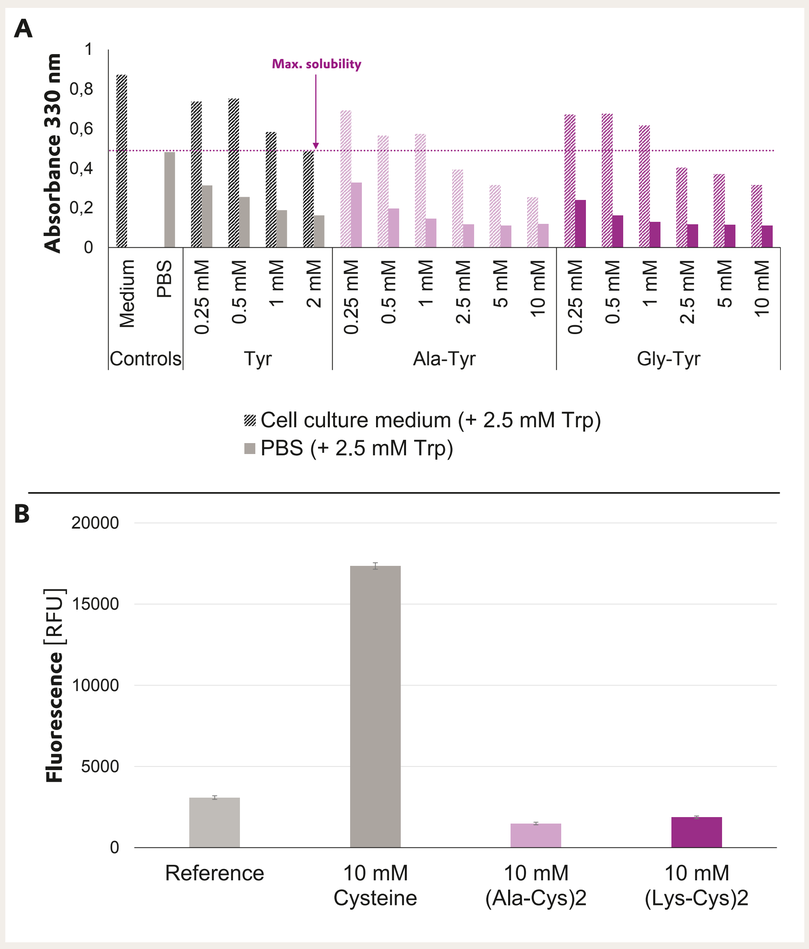


Fig 1: (**A**) Light induced colorization of Trp-containing model solutions (PBS) and CCM could be strongly reduced using Tyr-dipeptides. (**B**) Quantification of ROS using the fluorescence probe 2',7'-dichlorodihydrofluorescein di-acetate (H_2DCFDA) [3] in cell culture media containing different cysteine (Cys) derivatives.

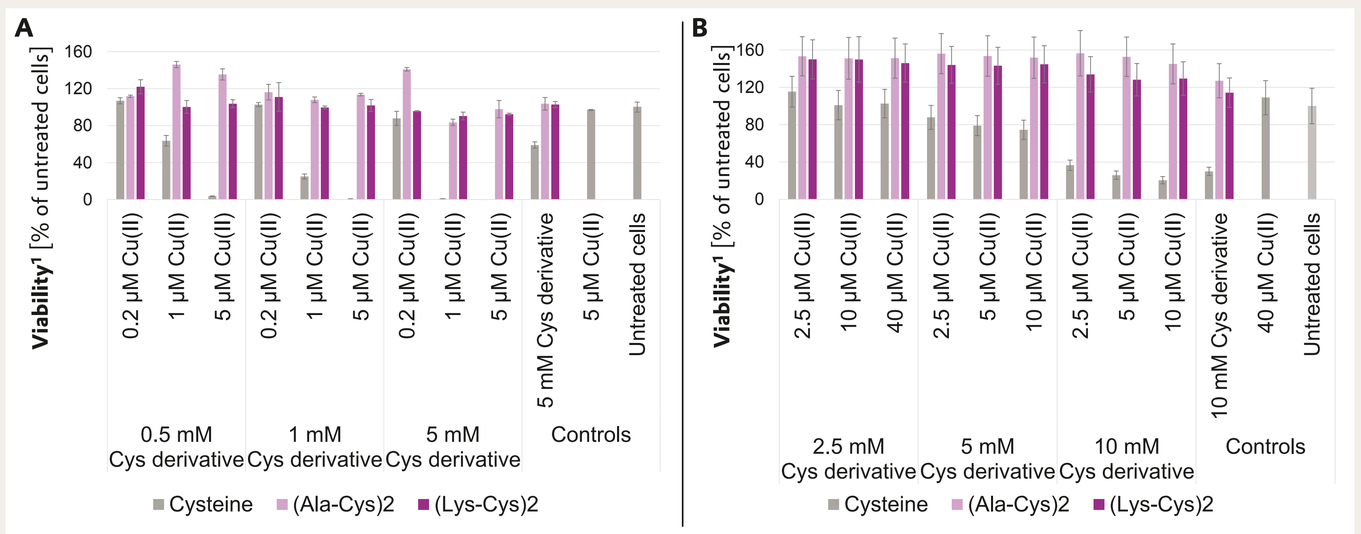


Fig 2: Viability assay of MSCs (**A**) and CHO cells (**B**) cultivated in 96-WPs in CCM with increased L-cysteine, $(Ala\text{-}Cys)_2$, or $(Lys\text{-}Cys)_2$ concentration in the presence of Cu(II) ions. A decrease in cell viability with increasing L-cysteine concentration was observed whereas no such effect was observed in the presence of peptides. The decrease is cause by increased formation of ROS. [1] Viability was determined quantifying the ATP present per well using the CellTiter-Glo® Luminescent Cell Viability Assay.

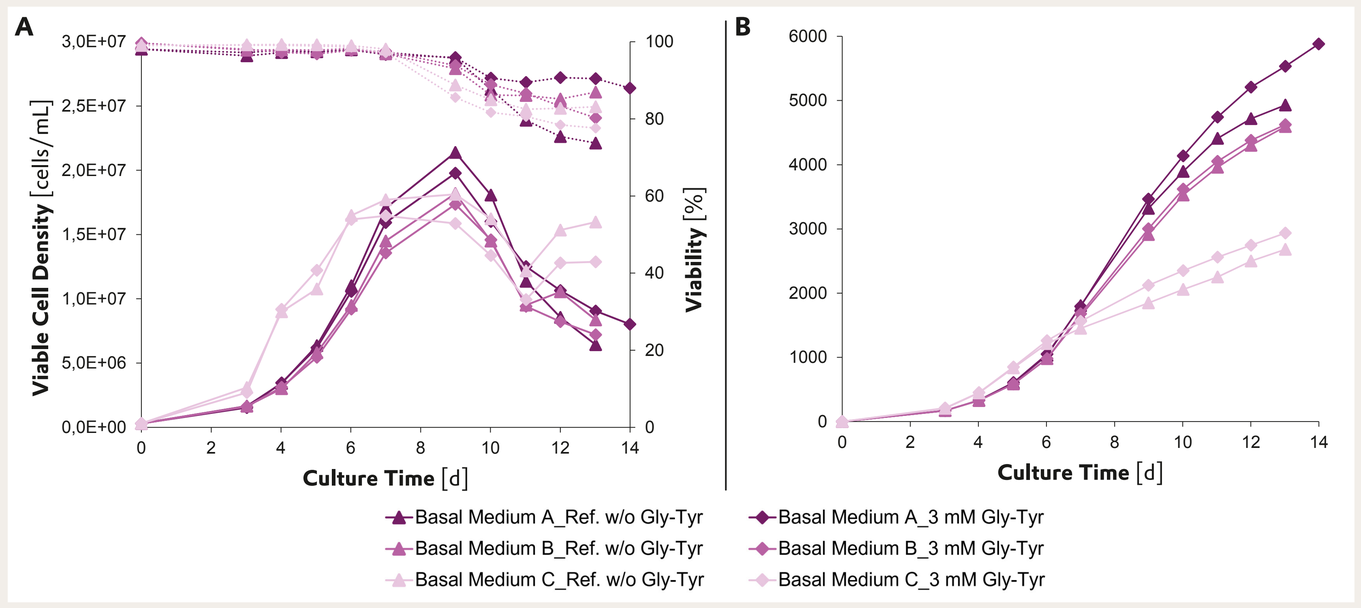


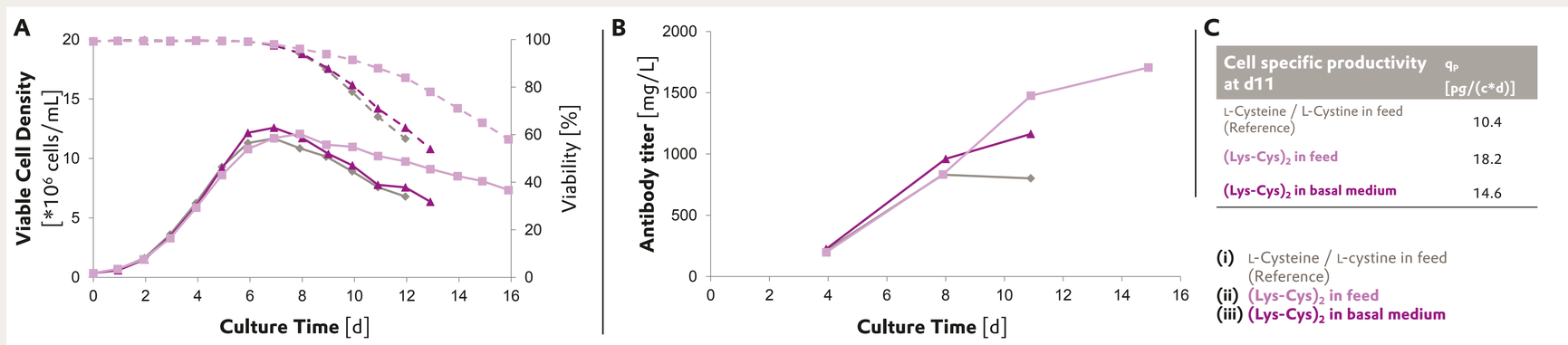
Fig 3: Small scale spinning tube fed-batch performance data (n=2; **A**, viable cell density; **B**, antibody titer) of a (common) IgG1-producing CHO-K1 GS cell line cultivated in three different commercial media systems (basal medium + feed media). Media systems A and B are dual-feed systems, while medium system C is a single-feed system. All three corresponding basal media were supplemented with or without (references) 3 mM glycyl-L-tyrosine (Gly-Tyr). Several positive effects depending on the medium system used were observed, ranging from e.g., higher cell viability throughout the process, extended cultivation time, increased cell specific productivity (data not shown, and/or strong increase in titer (e.g., > 20 %).

Fig 4: Bioreactor (2 L) fed-batch performance data (**A**, viable cell density; **B**, antibody titer; **C**, cell specific productivity at day 11) of a (common) IgG1-producing CHO-GS cell line cultivated in different peptide containing media and feed combinations:

(i) Reference was a commercial single feed containing L-cysteine/L-cystine.

(ii) Complete replacement of L-Cysteine/L-cystine in the feed by equimolar conc. of $(Lys\text{-}Cys)_2$. Free L-lysine in the feed was reduced accordingly to maintain a comparable L-lysine concentration.

(iii) Feed did not contain any Cys-derivative. Instead, an equivalent quantity of $(Lys\text{-}Cys)_2$ was added to the basal medium.



CONCLUSION

Light induced colorization of Trp-containing model solutions and CCM could be strongly reduced by addition of Tyr-dipeptides. This can be attributed to photoprotective effects and the natural ROS scavenger ability of the Tyr residue in combination with a much higher solubility of the Tyr-dipeptides compared to the free amino acid Tyr. ROS formation and cell viability was also studied as a function of Cu(II) ion concentration and increasing concentrations of L-cysteine or highly soluble L-cystine peptides. We found that viability strongly decreased with increasing L-cysteine concentration in the presence of Cu(II) ions whereas no such effect was observed in the presence of peptides. Thus, Tyr-dipeptides can be used as light stabilizers while Cys-peptides can be applied in CCM to reduce/prevent ROS formation catalyzed by free Cys. We demonstrated that their application in bioprocesses resulted in improved cell culture performance. Therefore, in addition to their nutritional function, these peptides can be used to control media chemistry and stabilize CCM to further improve media formulations and enable more efficient bioprocessing.

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