Plant Peptones: Nutritional Properties Sustaining Recombinant Protein Secretion by CHO Cells

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Abstract: Plant protein hydrolysates or plant peptones have been widely utilized as culture media additives to support cell growth and recombinant protein production. Unfortunately, their molecular targets remain largely unknown and it remains controversial whether plant peptones act as simple nutritive supplements providing various amino-acids to the cells or as inducers of some signal transduction pathways.

Key words: CHO cells, plant peptones, peptide transporters, protein-free, suspension.

1. INTRODUCTION

Chinese hamster ovary (CHO) cells are considered as one of the most versatile cell line for the production of recombinant proteins and as such are widely utilized. When the protein production is directed towards therapeutic purposes, biosafety concerns have to be taken into account. As such, serum and animal-derived proteins are progressively banned from the composition of culture media, while plant proteins and plant protein hydrolysates appear as very attractive substitutes.

In this study, an interferon-gamma (IFN-γ) secreting CHO cell line (CHO-320 cells) was utilized as a model to study the effect of plant peptones on the physiology of the cells during cultivation in suspension and in protein-free media.

R. Smith (ed.), Cell Technology for Cell Products, 599–601.

2. RESULTS AND DISCUSSION

CHO-320 cells were inoculated in a homemade protein-free medium designated as BDM for *Basal-defined medium* with or without rice peptones (2g $.l^{-1}$) at 3. 10^5 ml^{-1} and cultivated in suspension at 100 rpm. Living cell density was determined by Trypan blue assisted manual counting and IFN-γ secretion by ELISA (R&D Systems) (Figure 1). Mainly, rice peptones induce a doubling of IFN-γ concentration in the culture medium after ~120 hours of culture whereas the cell density remains weakly affected by the addition of these peptones to the culture medium.

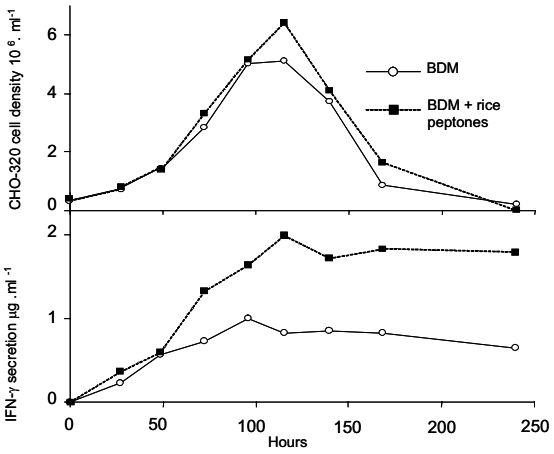

Figure 1. Plant peptones support IFN-γ secretion.

To investigate whether some peptides present in the rice peptones could be directly internalized as di- or tripeptides or if some larger peptides could be partially digested and afterwards taken up by the cells, some substrate competition assays were undertaken (Figure 2).

The activities of two membrane exopeptidases (dipeptidyl peptidase IV, DPPIV and tripeptidyl peptidase II, TPPII) were measured using para-nitroanilide (*p*NA)-conjugated peptides. It appears that only the TPPII activity was reduced by an increase of rice peptones concentration in the extracellular medium, suggesting that these peptones could be substrates of TPPII. As such, this suggests also that TPPII could release some tripeptides in the medium. Therefore, the peptide transport activity (Figure 2B) was measured. It appears that the cultivation of CHO-320 cells in the presence of the rice peptones does not dramatically modify the transport activity as peptones were removed for the assay by washing the cells, suggesting that the expression of the transporters were not modified. In addition

(Figure 2D), the presence of rice peptones during the assay strongly affect the peptide transport activity suggesting that some peptide present in the peptones could act as substrates of the peptide transport proteins. Finally, Figure 2C shows that a typical peptide resulting from the activity of TPPII (Ala-Ala-Phe) has a similar inhibitory activity towards the peptide transport.

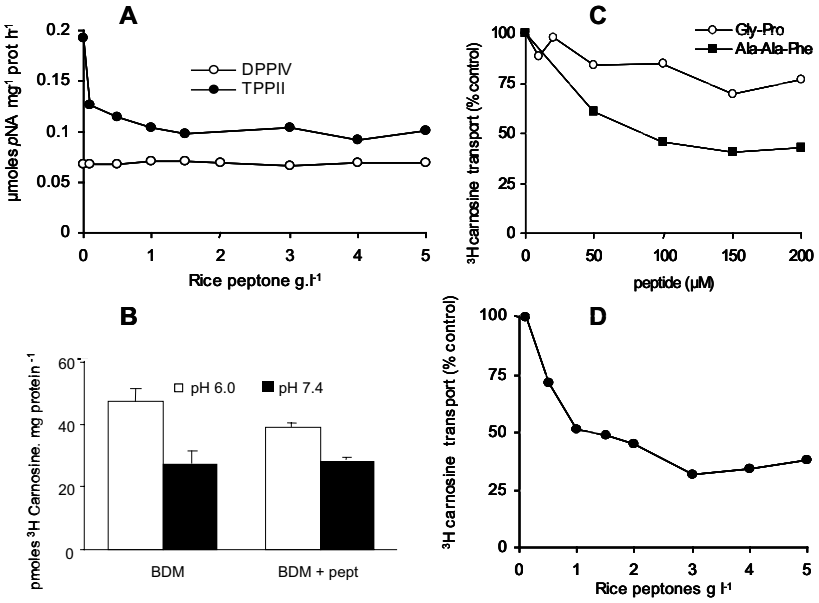

Figure 2. Rice peptones interfere with the activity of TPPII as well as with the peptide transport activity through the plasma membrane.

To conclude, these results strongly suggest that some peptides present in the rice peptones could be directly taken up by some peptide transporters or indirectly after partial digestion into tripeptides by TPPII. Consequently, these results support the idea that at least rice peptones have some nutritive properties.

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