PURIFICATION AND CHARACTERIZATION OF RECOMBINANT GLYCOSYLATED HUMAN PROLACTIN SYNTHESIZED IN CHO CELLS

Oliveira TL; Heller SR; Oliveira JE; Arthuso FS; Goulart HR; Sousa JM; Suzuki MF; Bartolini P; Soares CRS.

Centro de Biotecnologia - IPEN-CNEN/SP
crsoares@ipen.br

Introduction: Human prolactin (hPRL) is a 199 amino acid protein hormone (23.000Da) with a wide spectrum of biological activities being, however, best known for its stimulation of lactation and development of the mammary gland. About 10% of hPRL is glycosylated and this form (G-hPRL) is an ideal model for glycosylation studies because, as it contains only one potential asparagine-linked glycosylation site (Asp^{31}-Leu-Ser-Ser), it exhibits a simple type of macroheterogeneity: one protein population with and one without a single N-linked oligosaccharide.

Objetive: Development of a practical two-step purification process to obtain pure G-hPRL from conditioned culture medium of CHO cells transfected with a vector containing hPRL cDNA.

Materials and Methods: The purification process consists of a concentration step based on SP-Sepharose fast flow followed by a high performance size exclusion chromatography (HPSEC) used as a preparative step that can efficiently separate G-hPRL from non-glycosylated hPRL (NG-hPRL). To increase G-hPRL produced by CHO cells, the culture medium was supplemented with 0.6μg/mL cycloheximide which increases the extent of glycosylation. The G-hPRL obtained showed a purity exceeding 95% and was characterized by physico-chemical and biological assays including mass spectrometry; SDS-PAGE, Western blotting, HPSEC, RPHPLC, Nb2 and BaF3-LLP.

Conclusion: Our results show that this purification process can be an important tool for the production of G-hPRL, whose physiological actions need to be studied and better defined.

Supported by: FAPESP