Purification of Glycosylated Human Prolactin from CHO Cells Adapted to Serum-free Suspension Culture, by Reverse-Phase HPLC

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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. In the present study, G-hPRL obtained from CHO cultured in suspension in spinner flasks was finally purified by reverse-phase HPLC (RP-HPLC). The purification process consists of a first concentration step based on SP-Sepharose fast flow followed by RP HPLC used as a preparative step that can efficiently separate G-hPRL from non-glycosylated hPRL (NG-hPRL). The mass fraction of G-hPRL present in total prolactin was also greatly enriched with the addition of cycloheximide, an inhibitor of protein synthesis that favours the glycosylation reaction, facilitating its purification. Our results show that RP HPLC can be an important tool for G-hPRL production, facilitating the purification and characterization of this important isoform of prolactin, whose physiological action has not been defined. The next step will be the analysis of this carbohydrate (N-glycan) that is present in G-hPRL and whose structure has never been reported.

Key words: CHO Cells, Glycosylated Human Prolactin, RP-HPLC.

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