Abstract to be considered for: Poster presentation
Preferred Session:Glycoengineering

Cycloheximide increases the synthesis of recombinant glycosylated human prolactin (G-hPRL) secreted by CHO cells

Soares CRJ, Heller SR, Oliveira TL, Arthuso FS, Goulart HR, Bartolini P
Biotechnology Department, IPEN-CNEN, University of São Paulo, Brazil

Human prolactin hPRL is a 199 aminoacid protein hormone (MM ~23.000 Da) with a wide spectrum of biological activities being, however, best known for its stimulation of lactation and development of mammary gland. Besides proteolytic cleavage, the majority of prolactin variants can be the result of other posttranslational processing of the mature molecule in the anterior pituitary gland or the plasma. These include dimerization and polymerization, phosphorylation, deamidation, sulfation, and glycosylation. This protein contains only one potential asparagine-linked glycosylation site which is partially (~10%) occupied when the protein is synthesized in eukaryotic cells. Although the biological activity of glycosylated hPRL (G-hPRL) has been found ~ 4-fold lower compared to that of hPRL, its physiological function is not well defined yet, and the carbohydrate moiety seems to play an important role in the biosynthesis, secretion, biological activity, and plasma clearance of glycohormones. In order to better characterize and study this hormone variant, we carried out its laboratory scale purification from genetically modified CHO cells condtioned medium that had been supplemented with cycloheximide, increasing thus ~4-fold its absolute concentration and ~12-fold the glycosylated versus non-glycosylated hPRL concentration ratio (Fig.1). G-hPRL purification was carried via a simple and effective two-step process based on a cationic exchanger and a preparative size-exclusion HPLC column (HPSEC). Characterization was carried out by reversed-phase and size-exclusion HPLC, SDS-PAGE, western blotting, MALDI-TOF_MS and *in vitro* bioassay utilizing Nb2 and BaF3-LLP cells. Ours results show that cycloheximide can be an important tool to increase the production of glycosylated proteins facilitating the purification and characterization of these isoforms.

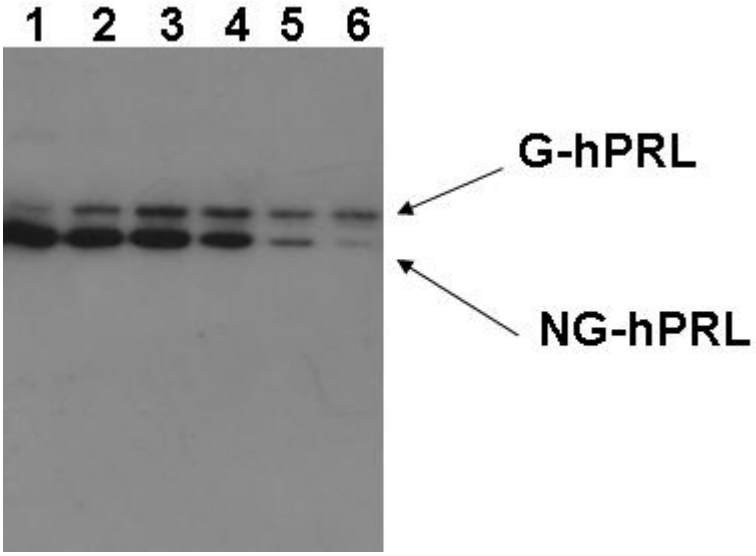

Fig. 1. Effect of cycloheximide on prolactin glycosylation. Confluent cell cultures incubated with α-MEM without fetal bovine serum were collected daily. The collections were analysed by western blot. Lane 1, cultivation without cycloheximide. Lane 2 -6, cultivation with 0.02 - 0.06 - 0.2 - 0.6 -2.0 μg cycloheximide mL^{-1}, respectively.