
**N-glycoproteomic analysis in a simple
glycoprotein model: Glycosylated human
prolactin**



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Human prolactin (hPRL) is a polypeptide hormone occurring in the non-glycosylated (NG-hPRL) and glycosylated (G-hPRL) forms, with molecular weights of approximately 23 and 25 kDa, respectively. It has a single, partially occupied N-glycosylation site located at Asn-31: the physiological function of G-hPRL is, however, not clear. Carbohydrate moieties generally play an important role in the biosynthesis, secretion, biological activity, and plasma survival of glyco hormones and can vary depending on the host cell. The main objective of this study was to determine the N-glycan structures present in native, pituitary G-hPRL and compare them with those present in the recombinant hormone. CHO cell-derived G-hPRL was then compared to pituitary G-hPRL especially with regard to N-glycoproteomic analysis. Among the main differences found in the pituitary sample were an extremely low presence of sialylated (1.7%) and a high percentage of sulfated (74.0%) and of fucosylated (90.5%) glycans. A ~4-fold lower bioactivity and a higher clearance rate in mice were also found for pituitary G-hPRL. N-glycan proteomic analysis, moreover, proved to be a useful and accurate methodology for molecular mass and carbohydrate content determination for the two G-hPRL preparations, in good agreement with the values obtained directly via MALDI-TOF-MS.

<http://dx.doi.org/10.1016/j.jbiotec.2014.07.097>
