PRODUCTION AND CHARACTERIZATION OF AUTHENTIC HUMAN PROLACTIN (hPRL) SECRETED IN THE PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

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A bacterial expression vector, p3SN8-hPRL-2, containing the tac promoter-controlled sequences for a bacterial cellulase leader joined to those coding for a previously described form of human prolactin with a 12 amino acids peptide tag including ala-ser, six histidines and a factor Xa cleavage site (tag-hPRL) (Biotechnol. Appl. Biochem. 1996; 23:67-75), was modified by site-specific mutagenesis in order to delete the tag sequence.

The secretion of immunologically active hPRL into the bacterial periplasmic space occurred, even though at relatively low levels (0.1 µg/ml/A600). Immunoassay, Western blot and SDS-PAGE confirmed, though, the correct processing and quality of the obtained authentic form of hPRL. An affinity purification step is being carried out in order to prepare a certain amount of hPRL for more extensive characterization, including N-terminal amino acid sequencing.

This is the first report describing periplasmic secretion of a bioactive form of authentic hPRL.

A study carried out comparing the periplasmic secretion levels of hPRL and tag-hPRL reproducibly showed an approximately 1:5 secretion ratio, the higher efficiency of the last vector being apparently only due to the presence of the extra N-terminal amino acids.

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