G - 3

SOLUBILIZATION, RENATURATION AND HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY (HPSEC) QUALITY CONTROL OF A CYTOPLASMIC FORM OF RECOMBINAT HUMAN PROLACTIN.

R. Affoso, I.M.C. Camargo, E.K. Gimbo-Vianna and P. Bartolini
Department of Application of Nuclear Techniques in Biological Sciences, IPEN-CNEN/São Paulo - Brazil.

A form of human prolactin having an N-terminal 13 amino acids additional peptide (taghPRL) has been expressed in *E. coli* cytoplasm in very large amounts: approximately 90 $\mu g/ml.A_{600}$. This expression yield is about 100 times higher than *E. coli* periplasmic secretion of this same protein with the disadvantage, though, that in the cytoplasm the protein is present in insoluble inclusion bodies which require solubilization and refolding, in order to provide the biologically active form of the hormone.

A modification of the solubilization and renaturing technique described by Luck et al. for bovine prolactin (Prot. Engineer. 1992; 5:559) has been set up in our laboratory reaching yields of the order of 70% of the total taghPRL cellular content. This recovery yields are much higher than those reported in general for cytoplasmic recombinant proteins. The renatured form of this hormone, however, when analysed through an HPSEC technique originally described by Riggin et al. (J. Chromatogr. 1988; 435:307) for human growth hormone, turned out to be mostly aggregated. A modification of the HPSE chromatographic conditions led to the full recovery of the monomeric form of taghPRL, showing that the analytical technique was partly responsible for aggregation. Studies are in progress in order to define what fine modification can be responsible for the altered behaviour of renatured cytoplasmic taghPRL in comparison with the periplasmic form of the same hormone.

Supported by CNPq (Brasilia), IAEA (Vienna) and FAPESP (São Paulo)