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ISOLATION AND PURIFICATION OF BIOSYNTHETIC HUMAN GROWTH HORMONE EXPRESSED IN TRANSFORMED E.coli CELLS.

Y. Murata*, L.E.M.F. Dias, M.T.C.P. Ribela, C.R.J. Soares and P. Bartolini,

Departamento de Aplicações de Técnicas Nucleares em Ciências Biológicas. IPEN/CNEN-SP. C.P. 11049 São Paulo-SP.

An E.coli strain was transformed with plasmids engeneered for the expression of human growth hormone (hGH) as periplasmic space secretion. We have studied the secretion, extraction and purification of hGH and the quality of the product obtained was compared with pituitary hGH. The expression and yields obtained with the use of different culture media was also studied.

In a first extraction step we submited the E.coli cells to an osmotic shock to obtain the desired protein (Koshland,1980). The periplasmic fluid was than purified by hydrophobic interaction chromatography, an Octyl-Sepharose, in a 10:1 protein:gel ratio. The selective interaction of hGH with the hydrophobic gel increased the specific activity 4.7 times (from 17±3 mg hGH/mg protein in the periplasmic fluid to 80±15 mg hGH/mg protein in the 30% acetonitrile elution step of Octyl-Sepharose gel).

The fractions containing hGH were pooled and loaded directly onto Sephadex G-100 column, after having determined the protein and hGH radioimmunoassayable content.

The quality of the hGH obtained was also controlled by SDS-PAGE, Native-PAGE, isoeletric focusing, size exclusion HPLC, triptic mapping reverse-phase HPLC analysis and biological assay. The results of a comparison with pituitary hGH demonstrated a perfect identity between the natural and the recombinant hormone (authentic-hGH). The degree of purity obtained up to this step is considered satisfactory.