

Extraction and purification of recombinant intact human Parathyroid Hormone (hPTH) from bacterial cell

ABSTRACT

Objective: The intact human Parathyroid hormone (hPTH) is one of the biopharmaceutical drug produced by biotechnology, this hormone can be provided in a good amount using simple batch culture, and therefore the main purpose of this study was the production of purified bioactive intact hPTH.

Methods: A cloning BL21(DE3) strain (which already cloned in our Lab. by synthetic human Parathyroid hormone (*shPTH*) gene) was grown in LB medium and the cloning gene expression was induced by addition of IPTG to the medium. The recombinant fused protein was purified from the bacterial cell lysate by affinity chromatography and underwent proteolysis by Enterokinase enzyme to obtain the target hPTH, and it's further purified by DEAE and SP Sepharose ion exchange chromatography. RP-HPLC analysis and biological activity on the MCF-7 breast cancer cells were done for both the standard and produced hPTH. Furthermore, the haemolysis ability of the latter was tested on the human RBC.

Results: 225ng/ml of hPTH was produced after SP chromatography which detected by specific PTH ELISA kit, standard and produced hPTH showed the same peaks in the same retention time when analyzed by HPLC. The produced hPTH haemolysis assay showed the ability of the produced hPTH to partially haemolyze the RBC in a concentration above 200µg/ml. The bioactivity assay for the produced and standard rhPTH demonstrated that both compounds had a biological activity on the MCF-7 cells with IC₅₀ of about 84.4 and 75.7 µg/ml for

standard and produced hPTH respectively, and no significant difference was detected between them.

Conclusions: Via suitable purification processes, the biologically active hPTH with structure similar to the standard ones as detected by RP-HPLC and bioactivity assay, can be obtained by using already cloned isolate with *shPTH* gene for hormone production. The hormone showed haemolysis effect on the RBC similar to the normal secreted hPTH.