

EFFECT OF PROPEPTIDE ALTERATION ON THE EXPRESSION OF RECOMBINANT HUMAN FACTOR IX IN DROSOPHILA S2 CELL LINE

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Production of biologically active human vitamin K-dependent proteins (VKDPs) in heterologous hosts is challenging, due to insufficient carboxylation. In VKDPs, an N-terminal propeptide containing recognition site for γ -glutamyl carboxylase (GGCX) is required for carboxylation. The weak-binding of the propeptide to GGCX, increases the carboxylation rate of the protein. The human prothrombin (hPT) is highly carboxylated and its propeptide affinity to GGCX is 10-fold weaker than that of the human factor IX (hFIX). To study the function of the hPT propeptide on the carboxylation efficiency of hFIX in *Drosophila* cell, we generated three constructs, based on a *Drosophila*-specific expression vector, carrying a chimeric hFIX cDNA next to the hPT pre-pro sequence, a mutant hFIX cDNA carrying an R-9N substitution in its propeptide, and a normal hFIX cDNA. The three constructs were subjected for transient expression analysis of hFIX in a *Drosophila* cell line, by performing coagulation test, ELISA and γ -carboxylation assay, on the cultured media after various post transfection time. Based on the results obtained, the functional impact of the hPT propeptide on the hFIX γ -carboxylation, in a distantly related host, was addressed. Our finding suggested the saturation of *Drosophila* GGCX, as a result of accumulation of hFIX in endoplasmic reticulum. These results demonstrated the functional importance of amino acid at position -9 in a mammalian derived propeptide on the expression efficiency of its cognate protein.

Biography

Samira Bahrami has completed her MSc in the field of Biochemistry from Tehran University. Her thesis was about Studying the expression hFIX, when its signal/propeptide is replaced with that of the human propeptide. This study was supported by a grant (Project No. 372) from the National Institute of Genetic Engineering and Biotechnology (NIGEB) of Iran. She has worked at the NIGEB as a Research Assistant for about two years. Now, she is a PhD candidate in Molecular Medicine at Shahid beheshti University of Medical Sciences, Tehran, Iran. Her PhD thesis is about Assessment of protein expression pattern in muscle-invasive bladder tissue using 2-DE and MS techniques. This study is performing for the first time in Iran and is supported by a grant from the National Institute for Medical Research Development (NIMAD).

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