

Expression and purification of recombinant rabbit tissue factor in pichia pastoris expression system to produce and evaluate PT reagent

Z. Naseri*, H. Tehrani

department of medical biotechnology, school of medical science, tarbiat modares university, tehran, iran

Background and Aims: Tissue Factor is a transmembrane glycoprotein that binds Factor VII and initiates the blood coagulation cascades. Using it as reagent in blood coagulation test such as prothrombin time (PT) is the most important application of this protein. PT is a screening test that is used to diagnose disfunctions in the blood coagulation system of patients and for monitoring oral anticoagulant therapy using Warfarin.

Methods: In this research, for production of the recombinant rabbit tissue factor (rRTF) protein an already-prepared pPICZ-TF construct was used. The pPICZ-TF construct transformed by electroporation into pichia pastoris. Subsequently, PCR-amplification of the RTF and flanking regions of the AOX1 gene confirmed the phenotype using genomic DNA extracts. For expression of rRTF, recombinant strains were grown in BMMY medium. SDS-PAGE and western Blot were performed. Cell extract was purified by Nickel Affinity Chromatography. Purified rRTF was relipidated by reconstituting a synthetic phospholipid blend composed of phosphatidylcholine and phosphatidylserine.

Results: the pPICZ-TF construct contain a full length cDNA of the rabbit tissue factor gene which cloned downstream the AOX1 promoter and upstream the His-tag. The pPICZ-TF construct inserted in downstream the AOX1 gene on chromosome 4 and Phenotype of recombinant strains was Mut+. Expression of rRTF (43KD) was confirmed by performing of SDS-PAGE and Western Blot. Empty liposomes were made in Rotary evaporator and their sizes were decreased by extrusion method. For obtaining optimal activity purified rRTF was incubated with empty liposomes. Then the activity of the relipidated rRTF was confirmed by PT test.

Conclusions: these findings confirm that P. pastoris is suitable expression system for production active rRTF protein. Relipidated RTF was used as PT reagent and there were little differences in results of PT test on normal and patient plasma compared with control reagent and therefore the activity of relipidated rRTF was favorable.

Keywords: Tissue factor; Pichia pastoris; Prothrombin time; pPICZ-TF construct