Production and activity assay of recombinant micro-plasminogen in *Pichia pastoris*

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Abstract

Introduction: Plasmin is a trypsin like serine protease that can catalyze the hydrolytic cleavage of peptide bonds at the COOH sides of arginines and lysines in extracellular matrix (ECM) components including fibrin, laminin and fibronectin. Microplasminogen (µPlg) is the COOH-terminal portion of plasminogen from 531 to 791, and has molecular weight of about 30kDa. µPlg consists of the catalytic domain of plasminogen and can be converted by many plasminogen activators, for example, streptokinase (SK) into microplasmin, which shares the same catalytic properties as human plasmin. µPlg can be used as a therapeutic agent in vitreoretinopathies and thrombotic diseases.

Methods and Results: The purpose of this study was production and activation of recombinant µPlg. After codon optimization and synthesis of the µPlg gene, it was inserted into expression vector pPICZα and this plasmid was transformed into *Pichia pastoris* X-33 cells by electroporation. The selected positive transforments were transferred into YPM (yeast extract-peptone-methanol) medium and were induced by methanol. The recombinant protein (33kDa) was successfully secreted into the supernatant of the induction medium and was purified by Ni-sepharose column. The presence of the recombinant µPlg was confirmed by SDS-PAGE. The activation of µPlg by streptokinase was performed at a 1:1 molar ratio of streptokinase to µPlg at 37°C for 2hr in Tris-HCl buffer, pH=7.8. Microplasminogen activity was measured by FDP (Fibrinogen Degradation Product) ELISA kit. In a blood sample the clot is broken down by plasmin. Some of These broken fibrin fragments are called d-dimers. In general, D-dimer elevation indicates increased fibrin turnover. In this procedure, 30 µL of active enzyme was added. And the rate of D-dimers was measured at 380 µg/mL. While the natural amount of D-dimers in the blood is less than 40 µg/mL.

Conclusions: The availability of microplasminogen with lower molecular weight can be a valuable pharmaceutical tool for treatment of thrombotic diseases and vitreoretinopathies because the isolation of autologous plasmin is an expensive and time-consuming process. In this study we managed to produce microplasminogen and successfully turn it into active microplasmin. However more effort is needed to increase the yields of Protein Production.

Key words: Plasmin, Microplasminogen, Streptokinase, *Pichia pastoris*