纤维蛋白肽与低分子量尿激酶原融合蛋白的构建及性质

Construction and Characterization of a Fusion Protein with Fibrin Peptide and scuPA-32k

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中文摘要：

将人工合成的寡核苷酸片段进行定向连接后，得到编码纤维蛋白β链N端(β15～42)多肽的基因片段及连接区片段(linker)，再与低分子量尿激酶原(scuPA-32k) cDNA分子进一步连接后，得到了Fβ(15～42)/scuPA-32k的融合基因.在大肠杆菌中经过IPTG诱导表达，经过变性及复性，Zn^{2+}螯合层析及Sephacryl S200凝胶层析后，目的蛋白被纯化.SDS-聚丙烯酰胺凝胶电泳(PAGE)显示为一条蛋白质纯化条带，分子质量为35 ku. 经纤维蛋白平板法测定比活为87 000 U/mg.经纤溶酶活化后的融合蛋白与低分子量尿激酶相比，对显色底物S2444酶促动力学性质相似.同时Fβ(15～42)/scuPA-32k具有较高的纤维蛋白的亲和性并能抑制纤维蛋白凝块的形成.

英文摘要：

A novel plasminogen activator containing low molecular single-chain urokinase (scuPA-32k) and fibrin β chain polypeptide (Fβ 15～42) was designed and constructed. ScuPA-32k cDNA was obtained by polymerase chain reaction (PCR) from pro-urokinase gene; while Fβ (15～42) cDNA was generated by joining synthesized oligonucleotide fragments together. Through suitable linker and approximately restriction site, scuPA-32k and Fβ (15～42) cDNA were ligated together. The fusion protein was expressed by IPTG induced in *E.coli*. After denaturation and renaturation, the aim protein was purified to homogeneity by Zn^{2+} chelating chromatography and Sephacryl S200 chromatography. The apparent molecular mass was 35 ku shown by SDS-PAGE analysis. The special activity was 87 000 U/mg detected by fibrin plate determination. The enzyme had similar kinetic parameters to that of natural uPA-32k when was assayed with the chromognic substrate S2444. However Fβ(15～42)/scuPA-32k had higher fibrin affinity than that of natural scuPA-32k and had antifibrin polymerization. These results showed that the fusion protein had good respects.

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