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研究论文

强阴离子色谱法从毕赤酵母培养液中分离纯化重组巴西日圆线虫乙酰胆碱酯酶

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摘要 提出了一种从基因工程毕赤酵母(Pichia storis)培养液中分离纯化重组巴西日圆线虫(Nippostrongylus brasiliensis)乙酰胆碱酯酶(NbAChE)的方法。采用Q-Sepharose Fast Flow强阴离子交换色谱柱对重组NbAChE进行了分离纯化。十二烷基硫酸钠聚丙烯酰胺凝胶电泳分析表明,纯化物的活性峰为单一蛋白质带,其相对分子质量约为66000。该方法的活性回收率为52.6%,纯化因子为3.87;纯化后AChE的比活为 2837 U/mg。结果表明,该法是一种理想的分离纯化重组NbAChE的方法。

关键词 阴离子交换色谱 乙酰胆碱酯酶 毕赤酵母 培养液 分离 纯化

分类号

Separation and Purification of Recombinant Nippostrongylus brasiliensis Acetylcholinesterase from Culture Medium of Genetic Engineering Pichia pastoris

Abstract

To develop a simple, fast and highly efficient method for the separation and purification of recombinant Nippostrongylus brasiliensis acetylcholinesterase (NbAChE) from culture medium of genetic engineering Pichia pastoris, Q-Sepharose Fast Flow chromatographic medium was used. The chromatographic column was 20 cm×3.5 cm i.d. The elution buffer A was 20 mmol/L NaH2PO4-Na2HPO4 (pH 8) and buffer B was 1 mol/L NaCl+20 mmol/L NaH2PO4-Na2HPO4 (pH 8). The elution gradient was nonlinear. It was firstly eluted with 10%B for 300 min, then with 30%B for 300 min, finally with 100%B for 300 min. The flow rate of mobile phase was 6 mL/min. The obtained recombinant NbAChE was proved to be homogeneous on sodium-dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and its relative molecular mass was estimated to be approximately 66000, which was consistent with that reported in literature. The total activity recovery of this purification method was 52.6% and the purification factor was 3.87. The final specific activity of recombinant NbAChE was 2837 U/mg. This chromatographic process is simple and highly efficient. It can be used to separate and purify recombinant NbAChE from culture medium of Pichia pastoris harboring NbAChE gene.

Key words anion exchange chromatography acetylcholinesterase Pichia pastoris culture medium separation purification

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