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论著

恶性疟原虫谷氨酸脱氢酶在大肠埃希菌中的可溶性表达、纯化和鉴定

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摘要

目的 在大肠埃希菌 (*E .coli*)中尝试非融合蛋白技术可溶性表达恶性疟原虫谷氨酸脱氢酶 (GDH) ,以获得具有相对完整空间表位的重组非融合GDH。 方法 将恶性疟原虫GDH基因克隆到pET-23 (a)表达载体中 ,转化*E .coli* BL21菌株 ,异丙基-β-D-硫代半乳糖苷诱导表达 ,菌体反复冻融后 ,通过十二烷基硫酸钠 聚丙烯酰胺凝胶电泳 (SDS-PAGE)分析表达产物的存在形式 ,可溶性表达产物经Source-Q及Source-S层析纯化并用SDS PAGE分析纯度。通过蛋白质印迹试验鉴定表达和纯化产物的免疫学活性。结果 可溶性重组GDH分子占宿主蛋白 15 %左右 ,相对分子质量为 5 2 0 0 0。经过阴离子和阳离子交换层析纯化后 ,GDH纯度达 90 %以上。该蛋白质具有良好的抗原性。 结论 通过*E .coli*表达系统和柱层析分离技术可获得高纯度、具有相对完整空间表位的重组GDH分子。

关键词 恶性疟原虫 谷氨酸脱氢酶 基因表达 蛋白质印迹试验

分类号

Soluble Expression of Plasmodium falciparum Glutamate Dehydrogenase in *Escherichia coli*, and its Purification and Identification

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Abstract

Objective To make soluble expression of *Plasmodium falciparum* (FCC1/HN) glutamate dehydrogenase(GDH) in *Escherichia coli*, purification and immunocompetence identification of the recombinant non-fusion GDH. Methods The GDH gene was cloned into prokaryotic expression vector pET23(a) to form recombinant expression vector pET23(a)/GDH. pET23(a)/GDH was transformed into *E.coli* BL21(DE3). Induced by IPTG (isopropyl-beta D-thiogalactoside), GDH was highly expressed in the supernatant after sonication. The soluble recombinant GDH was purified by Source-Q and Source-S chromatography. Enzyme-linked immunosorbent assay and Western blotting were carried out to identify the immunocompetence of the purified product. Results SDS-PAGE analysis showed that the soluble GDH protein accounted for approximately 15% of the total bacterial protein. By two-step ion-exchange chromatography, the purity of GDH reached more than 90% and the GDH possessed high antigenicity. Conclusion The soluble expression of GDH results in an integral three-dimensional structure epitope with high biological activity.

Key words *Plasmodium falciparum* glutamate dehydrogenase gene expression Western blotting

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