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实验报道

华支睾吸虫3-磷酸甘油醛脱氢酶重组蛋白的纯化、酶学活性及免疫学研究

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摘要

目的体外制备华支睾吸虫3-磷酸甘油醛脱氢酶重组蛋白(CsGAPDH),分析其酶学活性及免疫学特性。方法常规表达重组质粒pGEX-4T-1-GAPDH,用谷胱甘肽-S-转移酶(GST)亲和纯化试剂盒纯化重组蛋白,经十二烷基磺酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE),用凝血酶和纯化柱酶切、纯化的CsGAPDH免疫BALB/c小鼠,制备抗血清。ELISA检测抗IgG抗体滴度,浓缩型S-P免疫组化3步法检测CsGAPDH抗原在免疫小鼠体内的分布和表达。蛋白质印迹法(Western blotting)鉴定其抗血清的特异性。建立酶反应体系测定重组蛋白CsGAPDH的酶催化活性。结果纯化蛋白样品呈单一条带。免疫动物获取CsGAPDH抗血清,在免疫过程中抗体滴度呈连续上升趋势。CsGAPDH抗原分布和表达于小鼠肌细胞膜部位。免疫小鼠血清具有抗CsGAPDH特异性抗体。重组蛋白CsGAPDH具有酶生理活性,其酶活力单位为2 872 U $min^{-1}.ml^{-1}$。结论制备的重组蛋白CsGAPD H具有较好的酶活性和免疫原性。

关键词 华支睾吸虫 3-磷酸甘油醛脱氢酶 蛋白质印迹法 免疫原性

分类号

Purification, Enzyme Activity and Immunology Study of Recombinant Protein Glyceraldehyde-3-phosphate Dehydrogenase of *Clonorchis sinensis*

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Abstract

Objective To produce prokaryotic recombinant protein glyceraldehyde-3-phosphate dehydrogenase of *Clonorchis sinensis* (CsGAPDH), analyze its enzyme activity and immunological function. Methods The recombinant CsGAPDH was purified according to the protocol of GST·BindTM kit and was digested with thrombin proteinase and eluted with wash buffer. The BALB/c mice were inoculated with the purified protein. The antisera collected from the mice were used to detect the titres of IgG antibodies by ELISA, and Western blotting was used to identify the specificity of the antisera with the purified CsGAPDH. S-P immunohistochemistry method was used to confirm the expression and distribution of CsGAPDH in adult *Clonorchis sinensis* with the polyclonal antibodies from immunized BALB/c mice. The CsGAPDH catalytic activity was evaluated employing the conventional substrate glyceraldehydes-3-phosphate (3-GAP). Results SDS-PAGE showed a single purified protein band. Gel scanning analysis revealed that the protein purity of CsGAPDH was 90%. ELISA analysis showed an increased IgG value. S-P immunohistochemistry analysis demonstrated that the recombinant plasmid pGEX-4T-1-GAPDH expressed and distributed in muscle cell membrane of immune mice. Western blotting result suggested that CsGAPDH protein contained essential epitopes with high antigenic activities. This protein CsGAPDH could catalyzed 3-GAP with enzymatic active unit of 2 872 U $min^{-1}ml^{-1}$. Conclusion The recombinant protein CsGAPDH shows a proper enzymatic activity and immunogenicity.

Key words *Clonorchis sinensis* Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Western blotting Immunogenicity

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