生物物理学报(2007) 23(1), 20-28

PDF文档

云南阿昌族G6PD基因突变G487A在DF213中的表达

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为获得阿昌族G6PDWT和G6PDG487A重组蛋白，研究G6PDG487A的结构和功能改变，从云南省德宏州梁河县杞木寨乡湾中村阿昌族聚集地的G6PD缺陷家系先证者和正常阿昌族个体全血提取RNA，经RT-巢式PCR得cDNA，将cDNA克隆至pMD18-T simple载体中并测序；错配碱基经定点突变修复后，目的基因亚克隆至pThioHis(A)载体，构建了阿昌族G6PD基因野生型和G487A突变型原核表达载体：pThioHis(A)-AChang-G6PDWT和pThioHis(A)-AChang-G6PDG487A。用重组质粒转化E.coli Competent Cells DF213 (G6PD defeciency)，经IPTG诱导G6PD表达、10% SDS-PAGE电泳检测表达蛋白和紫外340 nm定量测定G6PD活性的分析表明，pThioHis(A)-AChang-G6PDWT和pThioHis(A)-AChang-G6PDG487A在DF213中成功表达，分子量约为59 kDa。IPTG诱导0、3、6、9、和12 h后，G6PD活性逐渐增高，G6PD基因WT表达的酶活性约是G487A的20~25倍。表达载体的构建以及G6PD cDNA在DF213中成功表达，为重组酶G6PDG487A的进一步研究奠定了基础。

Expression of G6PD Gene G487A Mutation in DF213 from AChang People of Yunnan

In order to get the recombinant protein of G6PDWTand G6PDG487A and study the changes on the structure and function of G6PDG487A in AChang People, full-length cDNA coding human G6PD gene was obtained by RT-nest PCR from the proband of G6PD deficiency and normal subject in AChang people. G6PD cDNAs were cloned into pMD18-T simple vector and the mismatch bases were corrected by using the site-mutation technique; then cDNA were sub-cloned into the expression vector pThioHis (A) and expressed in E.coli DF213 (G6PD deficiency). 10 % SDS-PAGE electrophoresis analysis showed that the expressional G6PD native protein molecular mass was about 59 kDa. G6PD activity increased gradually after 0、3、6、9、and 12 hours of IPTG induction by monitoring the rate of reduction of NADP+ to NADPH at 340 nm spectrophotometrically. G6PDWT activity was about 20-25 times of G6PDG487A activity. In Conclusion, the prokaryotic expressional vectors, pThioHis(A)-AChang-G6PDWT and pThioHis(A)-AChang-G6PDG487A, are constructed and expressed in DF213 successfully. It can be helpful for further study of recombinant enzyme with G6PDG487A.

关键词

阿昌族(AChang people)；葡萄糖-6-磷酸脱氢酶(Glucose-6-phosphate dehydrogenase)；基因(Gene)；表达载体(Expressional vector)