遗传 2005 27 (2): 249-254 ISSN: 0379-4172 CN: 11-5450/R

研究报告

双点突变核糖核酸酶抑制因子在毕赤酵母中的表达及对抗氧化能力的影响

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收稿日期 2004-2-16 修回日期 2004-3-22 网络版发布日期 接受日期

摘要 人胎盘核糖核酸酶抑制因子(HRI)是一种存在于细胞浆中的50 kDa的酸性蛋白质，富含亮氨酸和半胱氨酸。作为胞浆蛋白可保护细胞不受外来的胰RNase的侵袭。HRI有32个半胱氨酸残基，且多数半胱氨酸残基是成对的并在序列上相连。文章用丙氨酸同时取代cys328/cys329，并将此双突变的HRI的cDNA片段构建于质粒pPIC9K，电击转化入毕赤酵母(Pichia pastoris)GS115中，进行分泌型表达。对表达产物进行亲和层析纯化及抗氧化活性检测。实验结果表明，双点突变后的HRI对RNase A的亲和力几乎没有影响，但其抗氧化能力却增加7～9倍。此种抗氧化能力的提高可能是因为在cys328-cys329之间不能形成二硫键而稳定了HRI的三维结构所致。

关键词 核糖核酸酶抑制因子 定点突变 毕赤酵母 半胱氨酸 二硫键

分类号

Anti-oxidative Effect of Ribonuclease Inhibitor by Site-Directed Mutagenesis and Expression in Pichia pastoris

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Abstract

Human placental ribonuclease inhibitor is an acidic protein of Mr~50 kDa with unusually high contents of leucine and cysteine. It is a cytosolic protein that protects cells from the adventitious invasion of pancreatic-type ribonuclease. HRI has 32 cysteine residues, and the oxidative formation of disulfide bonds from those cysteine residues is a rapid cooperative process that inactivates HRI. The most proximal cysteine residues in native HRI are two pairs that are adjacent in sequence. In the present paper, two molecules of alanine to substitute for cys328/cys329 were performed by site-directed mutagenesis. The site-mutated RI cDNA was constructed into plasmid pPIC9K, and then transformed Pichia pastoris GS115 by electroporation. After colony screening , the bacterium was cultured and the product was purified with affinity chromatography. The affinity of the recombinant human RI with double site mutation was examined for RNase A and its anti-oxidative effect. The results indicated that there was no much change in the affinity for RNase A detected when compared with the wild type of RI. But the capacity of anti-oxidative effect was increased by 7~9 times. The enhance in anti-oxidative effect might be the reason for preventing the formation of disulfide bond between cys328 and cys329 and the three dimensional structure of RI was thereby maintained.

Key words ribonuclease inhibitor site-directed mutagenesis Pichia pastoris cysteine disulfide bond

DOI:

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