化学学报 2001 59 (10): 1769-1775 ISSN: 0567-7351 CN: 31-1320/06

金属硫蛋白家族内的结构域拼接

季清洲,曾卫华,陈巧林,任宏伟,茹炳根

北京大学生命科学学院;北京大学蛋白质工程国家重点实验室

收稿日期 修回日期 网络版发布日期 接受日期

摘要 金属硫蛋白(Metallothioneins,MTs)由结构独立且功能明显区别的β，α两个结构域组成。神经生长抑制因子(NeuronalGrowthInhibitoryFactor,GIF)双名金属硫蛋白-Ⅲ(MT-Ⅲ)，是神经系统中第一个被鉴定的具有神经元生长抑制功能的蛋白，而β－结构域为其功能结构域。为深入系统地研究MTs，尤其是GIF及其结构域的结构与功能，我们构建了金属硫蛋白家族内结构域拼接体βGIF-αMT-1(βⅢ-αⅠ)和βMT-1-αGIF(βⅠ-αⅢ):PCR扩增得各个结构域的cDNA序列，酶切后克隆入原核表达载体pGEX-4T-1，经发酵、诱导表达、亲和层析、凝血酶切和进一步纯化，得率约为80mg蛋白/L菌液。测其电泳行为、氨基酸组成、质谱、金属巯基含量等，证明得到了目的蛋白。紫外吸收图谱和圆二色性图谱显示，结构域拼接体拥有金属硫蛋白家族成员的特征金属巯基簇结构域，初步功能实验表明，βⅢ－αⅠ也具有抑制神经元生长的功能。

关键词 金属硫蛋白 发酵 神经生长抑制因子 结构

分类号 0627

Domain splicing within metallothionein family

Ji Qingzhou,Zeng Weihua,Chen Qiaolin,Ren Hongwei,Ru Binggen

Abstract metalothioneins(MTs) fold into two separate domains: β-domain and α -domain which obviously differ in function. Neuronal growth inhibitory factor (GIF), named as Metallothionein -Ⅲ(MT-Ⅲ), is first characterized to be capable of inhibiting the growth of neuronal cell in nervous system, and β-domina is its functional domina. To study deep the the structure and function of MTs, GIF and their domains; we construct the splicing-domains within metallothioneins family, βGIF- αMT-1(βⅢ-αⅠ) and βMT-1-αGIF(βⅠ-αⅢ): cDNAs were amplified by polymerase chain reaction (PCR), inserted into vector pGEX-4T-1, expressed in Esherichia coli as carboxyl teminal extension of glutathione-S-transferase (GST) by ⅠPTG's induction. After the fusion protein had been digested by thrombin on a Glutathione-Sephacryl- S100. Eighty mg of column, recombinat βⅢ－αⅠ and βⅠ－αⅢ were purified by gel fitration on Sephacryl-S100. Eighty mg of the protein can be obtained from evergy liter medium after fermentation. The results of SDS-PAGE, amino acids composition, molecualr mass, the ratio of metal/protein and sulfhydryl group/protein confirm that the purified protein is the desired one. Utraviolet (UV) absorption spectroscopy and circular dichroism (CD) spectroscopy show splicing- domains have the characteristic metal-sulfhydryl group clusters of metallothionein family. βⅢ－αⅠ, similar with GIF, displays the neuronal growth inhibitory activity.

Key words METALLOTHIONEIN FERMENTATION STRUCTURE

DOI:

通讯作者