色谱 2006 24 (3): 279-283 ISSN: 1000-8713 CN: 42-1226/O

研究论文

亲和柱色谱原位酶切法纯化重金属镉结合的金属硫蛋白-3

郑伟娟 杨锋 吴芳 陆纯 华子春

南京大学生命科学院 医药生物技术国家重点实验室, 江苏 南京 210093

收稿日期 2005-6-1 修回日期 2005-8-16 网络版发布日期 2006-6-1 接受日期

摘要 按照人金属硫蛋白-3(hMT-3)的基因序列,选用大肠杆菌偏爱的密码子合成了全长hMT-3基因,并将其插入大肠杆菌融合表达质粒pALEX的多克隆位点中,在谷胱甘肽-硫-转移酶(GST)下游与GST融合表达。通过异丙基-β-D-硫代半乳糖苷(IPTG)诱导在大肠杆菌表达菌株BL21 (DE3) LysS中表达了与重金属离子镉结合的融合蛋白GST-Cd2+-hMT-3。经十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)分析表明融合蛋白主要在超声上清液中。分别通过“先纯化、后酶切”和“亲和柱色谱原位酶切”两种方法纯化了Cd2+-hMT-3,比较了两种方法的纯化效率和得率,表明原位酶切法操作简便,较之“先纯化、后酶切”法减少了洗脱、透析、冻干等步骤,从而也减少了样品的损失,提高了样品的纯度和得率。从摇瓶培养菌液中纯化获得了结合有Cd2+的完整的人金属硫蛋白-3,得率为1.8%。氨基酸组成分析结果表明所获得的Cd2+-hMT-3不含芳香族氨基酸和组氨酸,符合金属硫蛋白的特征;直读电感耦合等离子体发射光谱分析其硫镉原子比为21∶(7.5±0.1),与理论值21∶7基本吻合。

关键词 亲和柱色谱 人金属硫蛋白-3;融合表达 Factor Xa原位酶切 纯化

分类号

Purification of Cadmium Ion Binding Metallothionein-3 by Proteinase Digestion on Affinity Chromatographic Column

ZHENG Weijuan, YANG Feng, WU Fang, LU Chun, HUA Zichun

State Key Laboratory of Pharmaceutical Biotechnology, School of Life Science,Nanjing University, Nanjing 210093, China

Abstract

The gene encoding human metallothionein-3 (hMT-3) was synthesized and inserted into the poly-cloning sites of fusion expression vector pALEX, and fused downstream to its glutathione S-transferase (GST) fusion partner. Fusion protein GST-Cd2+-hMT-3 was expressed after isopropyl-β-D-thiogalactopyranoside (IPTG) induction and addition of 0.1 mmol/L CdSO4 into the culture medium, and mainly existed in cellular soluble fraction as revealed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Recombinant MT was purified by two purification procedures: “proteinase digestion after purification” method, e.g. by elution of GST-Cd2+-hMT-3 from GST affinity chromatography first, then proteinase digestion and GST affinity chromatographic purification again subsequently; and “proteinase digestion in situ” method, e.g. digestion of GST-Cd2+-hMT-3 directly on the column while its binding to the GST affinity chromatographic resin and collection of Cd2+-MT directly from the flow through fraction after the digestion. It was confirmed that the later procedure exhibited more effective and more convenient by avoiding the conventional elution, dialysis and lyophilization processes and increasing the purity, recovery or yield of the final product. After further purification by a SuperdexTM 75 HR 10/30 column, finally 6-7 mg of Cd2+-hMT-3 was obtained from 3 L of flask culture with the recovery of about 1.8%. SDS-PAGE, amino acid composition and inductively coupled plasma atomic emission spectrometer (ICP-AES) analysis showed that the relative molecular mass of Cd2+-hMT-3 is about 7000, with a purity above 90%. Its amino acid composition is consistent with the expected value of natural hMT-3, particularly no aromatic amino acid and histidine, and the atomic ratio of 21∶ (7.5±0.1) for S∶ Cd, is also consistent with the theoretical value of 21∶ 7.

Key words affinity column chromatography human metallothionein-3 (hMT-3) fusion expression Factor Xa digestion on column purification

DOI:

通讯作者 华子春