色谱 2006 24 (5): 425-431 ISSN: 1000-8713 CN: 42-1226/O

研究论文

伴刀豆球蛋白亲和色谱柱的制备及其在糖蛋白核糖核酸酶B结构分析中的应用

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收稿日期 2006-5-15 修回日期 2006-7-10 网络版发布日期 2006-10-13 接受日期

摘要 通过对硅胶基质进行化学改性键合伴刀豆球蛋白(Con A),制备了对糖蛋白具有特异亲和作用的亲和色谱固定相;该固定相非特异性吸附弱,对于糖蛋白和糖肽的分离效果良好。对亲和色谱的分离条件进行了优化,以标准糖蛋白核糖核酸酶B(RNase B)为模型,对其进行了纯化;用糖苷酶切除糖链,并对切除糖链前后的RNase B用胰蛋白酶酶解;用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF MS)对亲和色谱分离得到的糖蛋白、糖链及糖肽进行了分析,确定了RNase B的一级结构、糖含量、糖基化位点及糖连接方式。该方法快速准确,适于糖蛋白和糖肽的分离表征。将其应用于血清中糖蛋白及酶解后血清中糖肽的分离富集,取得了很好的效果。

关键词 伴刀豆球蛋白 亲和色谱 基质辅助激光解吸电离飞行时间质谱 糖蛋白 核糖核酸酶B 蛋白质组学

分类号

Preparation of a Concanavalin A Immobilized Affinity Column and Its Application in the Structural Analysis of Ribonuclease B

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Abstract

The research on glycoproteomes represents an interesting field in the functional proteomics research. Affinity chromatography and mass spectrometry are powerful techniques that are used for gaining valuable information on glycoproteomes because glycoproteins and their unusual forms resulting from protein glycosylation can be important indicators of several diseases. In this study, the concanavalin A (Con A) immobilized silica packing was prepared and used for the separation of glycoprotein and glycopeptides. A very low, non-specific adsorption on the Con A affinity column was demonstrated by mass recovery of bovine serum albumin at more than 98.5%. The effect of concentration of methyl-α-D-mannopyranoside (α-Me-D-Man) in the mobile phase and the effect of flow rate on the retention behavior of ribonuclease B (RNase B) were also investigated. The standard glycoprotein RNase B was separated under optimized conditions using 0.2 mol/L α-Me-D-Man in the mobile phase at a flow rate of 0.5 mL/min. Meanwhile, the oligosaccharides and glycopeptides were enriched using a Con A column after digestion of the purified RNase B with peptide-N-glycosidase F (PNGase F) and trypsin. The structure of N-linked glycan and the rate and the site of glycosylation of RNase B were determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). Glycoproteins and glycopeptides in human serum and digest solution could be separated by this method. The results showed that this method is rapid and sensitive for the purification and characterization of glycoproteins and glycopeptides.
Fig.8 Tab.1 Ref.22

Key words

concanavalin A (Con A) affinity chromatography matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) glycoprotein ribonuclease B (RNase B) proteomics

DOI:

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