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论文

蚕丝蛋白固相萃取分离富集细胞色素c的研究

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摘要:

在特定实验条件下, 蚕丝蛋白对细胞色素c表现出选择性吸附. 以蚕丝蛋白微填充柱为载体, 在流动系统中建立了细胞色素c的分离富集方法, 以分光光度法在410 nm处检测分离富集过程. 在进样流速低于10 μL/s时, 2 mL样品溶液(pH=5.6的水溶液)中5 μg/mL的细胞色素c可被蚕丝蛋白微柱完全吸附, 而在洗脱流速低于15 μL/s时, 200 μL NaCl溶液(1.0 mol/L)可将吸附的细胞色素c完全洗脱, 分离富集系数为10. 用本方法测定细胞色素c的线性范围为1.0~10.0 μg/mL, 检出限为0.33 μg/mL, 精密度RSD为2.5%(5 μg/mL, *n*=9). 此外, 还采用本文方法对人全血中的蛋白质进行了分离富集, 并用SDS-聚丙烯酰胺凝胶电泳验证了分离后蛋白的纯度.

关键词: 蚕丝蛋白; 固相萃取; 细胞色素c

Adsorption and Separation of Cytochrome c on Silk Fibroin

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Abstract:

Selective adsorption of cytochrome c using silk fibroin as adsorbent was observed under certain experimental conditions. A novel separation and preconcentration approach for cytochrome c was thus developed via a silk fibroin packed micro-column in a sequential injection system. The entire process of separation and elution was monitored by spectrophotometry at 410 nm. At pH 5.6, cytochrome c in 2 mL of aqueous solution at 5 g/mL were quantitatively adsorbed by the silk fibroin micro-column, and a NaCl solution of 1.0 mol/L suffices the complete elution of the adsorbed cytochrome c from the micro-column. An enrichment factor of 10 was achieved by fixing a sample volume of 2 mL and an eluent volume of 200 μL. A linear calibration graph within 1.0—10.0 μg/mL cytochrome c was obtained with a detection limit of 0.33 μg/mL and a precision(RSD) of 2.5%(5 μg/mL, *n*=9). The practical applicability of this system was demonstrated by processing human whole blood for preconcentration of cytochrome c, and the results were verified by assay with SDS-PAGE.

Keywords: Silk fibroin; Solid phase extraction; Cytochrome c

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