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还原变性核糖核酸酶在疏水性液-固界面上的复性

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Refolding of reduced/denatured RNase A on the hydrophobic liquid-solid interface

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摘要 采用疏水相互作用色谱(HIC)对还原变性核糖核酸酶A (RNase A)在疏水性液-固界面上的复性进行了研究。详细讨论了流动相中脲的浓度、还原型谷胱甘肽/氧化型谷胱甘肽(GSH/GSSG)的比例、流动相pH和变性蛋白质浓度对还原变性RNase A复性效率和质量回收率的影响。结果表明,在最优化的复性条件(流动相中含有2.0 mol/L脲,GSH/GSSG的浓度比为8:1,流动相pH为8.0)下,还原变性RNase A能完全复性。当变性蛋白质质量浓度为5.0 mg/mL时,还原脲变性RNase A的活性回收率和质量回收率分别为98.0%和61.9%,还原胍变性RNase A分别为100.1%和66.8%。研究表明HIC是还原变性蛋白质复性的有力工具之一,可为蛋白质复性研究提供新方法和新思路。

关键词： 蛋白质复性 疏水作用色谱 还原变性 核糖核酸酶A

Abstract： The renaturation of the reduced/denatured RNase A on the hydrophobic liquid-solid interface was investigated using hydrophobic interaction chromatography (HIC). The effects of urea concentrations, the ratios of reduced and oxidized glutathiones (GSH and GSSG), the pH of mobile phase and protein concentrations on the refolding efficiency and mass recovery of the reduced/denatured RNase A were investigated in detail. The results indicated that the reduced/denatured RNase A can be refolded completely under the optimized conditions of pH 8.0, 2.0 mol/L urea and the concentration ratio of GSH/GSSG of 8:1 in mobile phase. When the denatured protein was at the concentration of 5.0 mg/mL, the bioactivity efficiency and mass recoveries were 98.0% and 61.9% for 8.0 mol/L urea-denatured RNase A, respectively; and 100.1% and 66.8% for 7.0 mol/L guanidine hydrochloride (GuaHCl)-denatured RNase A, respectively. It proves that HIC is a powerful tool and new approach for protein refolding.

Keywords： protein renaturation hydrophobic interaction chromatography (HIC) reduced/denatured RNase A

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