於逆流層析儀中以反微胞法萃取及濃縮細胞色素分子

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摘 要

在本實驗室早期的研究中，曾使用反微胞結合逆流層析儀來萃取及濃縮細胞色素分子，其正向萃取使用 0.2M KCl，反向萃取使用在 5mL sample loop 中的高 pH 值的鹼液，但是由於反向萃取的速率很慢，而且回收率不高，必需將所使用的反微胞系統，在反向萃取的過程中，以高 pH 值的鹼液破壞，才能得到不錯的回收率，但是反微胞系統將因此而無法重覆使用，而使用高 pH 值，也可能破壞蛋白質分子，因而沒有實用的價值。而我們實驗的目的，即是使用新的溶劑系統，使細胞色素分子的反向萃取，在反微胞系統未被破壞的情況下，也能夠進行，且可大幅提高回收率。

我們的新系統是在反微胞有機相中添加輔助溶劑乙酸乙酯，使細胞色素分子容易進出反微胞系統，如此提升了反向萃取的速率及回收率。此外，我們使用緩衝溶液做反向萃取，使細胞色素分子帶電性的改變，成為萃取的主要作用力，如此一來，反微胞系統仍然保有萃取的能力，可以再次進行萃取;此外，在實驗中，進行大量濃縮，將 10.8 mg/540mL 的 cytochrome c，濃縮為 10.1 mg/60mL，回收率為 93.2%，濃縮為原來體積的九分之一，使反微胞結合逆流層析的技術能有在應用上的價值。

Extraction and concentration of cytochrome c using reversed-micelle in countercurrent chromatograph

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Abstract

Recently we have applied a countercurrent chromatograph to extract and concentrate cytochrome c molecules in our laboratory. The forward extraction was accomplished using an AOT-containing hexane solution retented in the countercurrent chromatograph by eluting a 0.2 M KCl cytochrome c aqueous solution and the backward extraction was performed by injecting a 5 mL alkaline solution through a sample loop to elute and liberate the protein molecules seized by the reverse micellar solution. The protein molecules were thus transferred from a low pH to a high pH aqueous solution via a micellar solution. Due to slow backward extraction rate and low recovery, a high pH, such as 12.8, solution was required. However, this alkaline solution would spoil the reverse micellar system permanently; therefore it could not be reused. The second drawback was the possibility of denature of the protein molecules.

In this study, we discovered that the addition of ethyl acetate to the

micellar solution may help improve the backward extraction as well as the sample recovery rate. In addition, we applied a buffer solution to replace the un-buffered solution used previously during the backward extraction. The outcome showed that a buffered solution with a lower pH (such as 11) could recover higher yield than an un-buffered solution of a higher pH. Accordingly, the micellar solutions could remain intact longer than those treated un-buffered solutions with higher pH values during the backward process. Finally, we achieved to concentrate a 10.8 mg in 540 mL cytochrome c aqueous solution to a 10.1 mg in 60 mL aqueous solution. The recovery was 93.2% with a volume reduction of one ninth to the original volume.