Separation and purification of horseradish peroxidase by membrane affinity chromatography

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Abstract

Concanavalin A (con A) was immobilized on Mercerized macroporous cellulose membranes and used as a ligand for membrane affinity chromatography. Three activation and immobilization methods involving 2,4,6-trichloro-1,3,5-triazine, glutaraldehyde or diazotization were employed and compared. To obtain information about the effectiveness of the membranes, the adsorptions of ovalbumin and γ-globulin on the prepared affinity membranes were investigated under a variety of conditions. The prepared affinity membranes were employed for the separation and purification of peroxidase from horseradish and commercial peroxidase, respectively. The separation and purification were monitored by determining the peroxidase activity, using 3,3′,5,5′-tetramethylbenzidine (TMB) as substrate. For the separation from horseradish, a 24.5% recovery and a 142-fold enrichment was achieved; for the purification of a commercial sample, a 71% recovery and a 2.3-fold enrichment was reached.

Keywords: Affinity chromatography; Cellulose membranes; Concanavalin A; Peroxidase

1. Introduction

Compared with the classical column chromatography, the affinity membrane chromatography possesses a number of advantages, such as higher flow rate, faster binding rate, lower pressure drop, higher productivity and easier scale-up [1–4]. After more than 10 years of development, the affinity membrane chromatography is now well accepted as a novel technology for separation and purification. Since abundant information was accumulated regarding the classical column affinity chromatography, the main focus of the membrane affinity chromatography is the preparation of suitable membranes. Numerous materials, such as nylon, polysulfone, chitosan, cellulose and cellulose derivatives [5–8], were used to prepare matrixes for membrane affinity chromatography. However, while the synthetic polymeric materials are more easily processable as membranes, they are less suitable for ligand immobilization, because their lower compatibility increases the probability for the biomolecules denaturation. In contrast, the natural macromolecular materials are compatible with the usual ligands, but often difficult to be processed as membranes.

In previous papers [9,10], macroporous cellulose membranes were prepared from high quality filter paper by Mercerization followed by chemical cross-linking. The prepared membranes possessed a high porosity (~50%) and large pores (0.4–1.0 μm), and were proved to be suitable as membranes for affinity chromatography.

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