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Affinity Chromatographic Purification of Lentil Lectin Using Immobilized Yeast Cells

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Abstract

A model system for evaluating macroporous supports containing immobilized whole cells in affinity chromatographic applications is described. Whole cells were immobilized in a polyacrylamide network in a two-step polymerization process. The affinity system discussed consists of immobilized cells of *Saccharomyces cervisiae* in the purification of lentil lectin from *Lens culinaris*.

Index Entries: Affinity chromatographic purification, of lentil lectin; chromatography, affinity, of lentil lectin; lentil lectin, affinity purification of; lectin, lentil, affinity purification of; immobilized yeast cells, affinity chromatography with; yeast, immobilized; polyacrylamide entrapment, of yeast cells; *Saccharomyces cervisiae*, immobilized; *Lens culinaris*, lentil lectin from.

Introduction

The use of immobilized whole cells in analytical and preparative applications has increased during the last years (*1*, *2*). This development has been made possible by the fact that cell walls containing specific structures interact specifically with macromolecules. These interactions can be used in affinity chromatography where the specific structures act as ligands, which are often expensive and hard to synthesize. Many systems have been described where the immobilized cells are attached to the matrix by adsorption, but a major drawback of these systems is loss of cells during use.

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Experimental Procedure and Discussion

We have studied a number of methods for the immobilization of cells including covalent coupling to Sepharose and entrapment in various polymers, such as agarose, alginate, alginate/collagen, and polyacrylamide (*3*). The method that has given the best result in our hands is entrapment of cells in polyacrylamide. Using a two-step bead polymerization process, allowing the small beads first formed to aggregate and form raspberry-like clusters in a second step, a gel with good flow properties and high binding capacities was obtained.

The cells were suspended in a monomer solution consisting of acrylamide and *N,N'*-methylenebisacrylamide. The suspension was stirred slowly under nitrogen gas. This was followed by addition of a catalytic system. The mixture was then transferred to an Ehrlenmayer flask containing a hydrophobic phase stirred under high speed on a magnetic stirrer. This hydrophobic phase, with the same density as the cell suspension, consisted of a toluene/chloroform mixture and a small amount of a surfactant medium. Nitrogen gas was bubbled through the phase before the addition of the cell suspension. Small droplets of the cell suspension were formed in the hydrophobic phase and began to polymerize within 15 min.

When the small beads began to form the stirring speed was reduced, allowing the beads to aggregate and form clusters (Fig. 1). The polymerization was complete within 30 min, after which the clusters were separated from the hydrophobic phase by decantation and washed with several large portions of luke warm water containing a small amount of detergent. The gel was finally suspended in phosphate buffer containing 0.02% sodium azide.

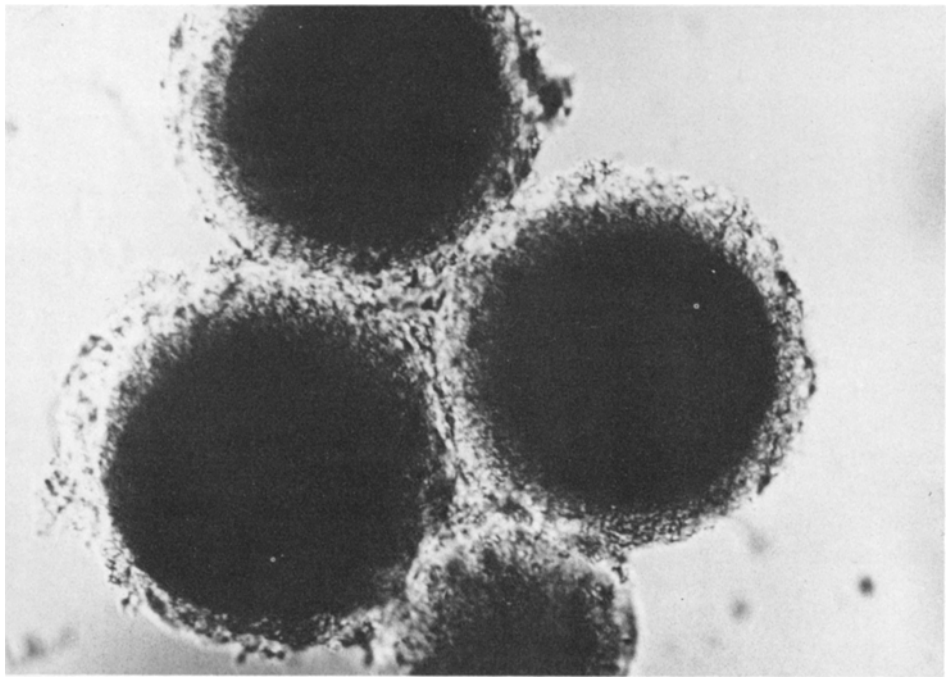

Fig. 1. Micrograph photo of clustered beads containing entrapped yeast cells.

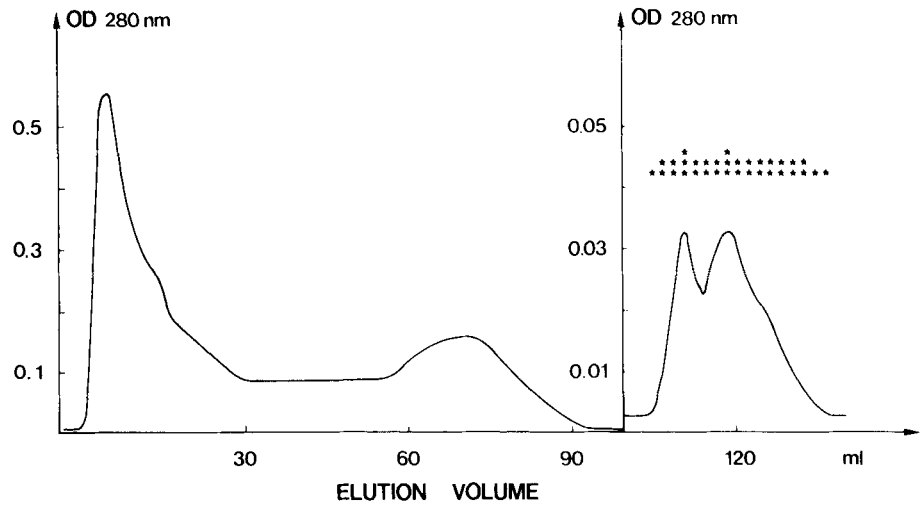

Fig. 2. Elution pattern obtained from a column containing immobilized cells of *Saccharomyces cervisiae* on applying a crude extract of *Lens culinaris*. The hemagglutinative activity is denoted by asterisks.

Results

A "berry-bead" gel obtained in the way described above and containing cells of *Saccharomyces cervisiae* was packed on a column (1.8 × 8.0 cm) and equilibrated in a flow (0.5 mL/min) of 0.1*M* phosphate buffer (pH 7.00). A crude homogenate of *Lens culinaris* was prepared by soaking seeds in 0.9% saline overnight at +4°C. The mixture was homogenized in a Waring Blendor, left for 30 min at room temperature and finally centrifuged. The supernatant was applied to the column and eluted with phosphate buffer until all the protein not bound to the column was washed out. The bound protein was eluted by a change of buffer to 0.2*M* glycine-HCl (pH 2.2). As seen in Fig. 2, two peaks appeared on measurement of the absorbance at 280 nm. The contents of these peaks were analyzed with SDS-gel electrophoresis. The first peak gave only one band identical with that of pure lectin in a reference sample. The second peak showed one major band and three weaker ones. The activity in the peaks was determined by the ability to agglutinate red blood cells. The cell column could be re-used for several mounts provided bacteriostatics were added to the perfusion buffer.

In conclusion, cells immobilized in polyacrylamide, produced as described here, retain their capacity to bind proteins and provide a gel with good flow properties.

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