Effect of Nucleic Acid Contamination on Partitioning of Proteins in Two-Phase PEG-Dextran System

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ABSTRACT

Downstream processing of bioproducts results in considerable losses of compounds of interest in a large number of cases. For the intracellular enzyme tartrate dehydrogenase, an analysis of the laboratory process for enzyme recovery revealed that maximum losses occur in the initial stages of purification when the enzyme is separated from nucleic acids and other undesirable enzymes. Hence, aqueous two-phase extraction was studied to investigate the separation of several enzymes from nucleic acids. Single-component and binary equilibria for three commercially available enzymes (bovine serum albumin, trypsin, chymotrypsin) and yeast RNA were studied in a two-phase system consisting of dextran and polyethylene glycol (PEG). The effects of pH and concentrations of the components and salts (NaCl) were investigated.

Index Entries: Nucleic acid contamination; protein partitioning; two-phase PEG-Dextran system; two-phase aqueous partitioning; partition coefficient.

INTRODUCTION

Several industrially important biomolecules are formed as intracellular proteins. Their recovery involves disruption of cells, as a result of which these products must be purified from a mixture of proteins and nucleic acids. A typical recovery process for an intracellular enzyme is presented

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Fig. 1. Schematic of downstream process for recovering intracellular tartrate dehydrogenase from *P. putida* cells.

in Fig. 1. Similar schemes are commonly used for other intracellular products. A number of published reports suggest that significant losses of protein products occur during their separation from other proteins and nucleic acids (1-5). Activities of an intracellular enzyme tartrate dehydrogenase, produced by *Pseudomonas putida*, at different stages of its recovery process (Fig. 1) are listed in Table 1. Clearly, the maximum losses of the enzyme occur during precipitation of nucleic acids and during preliminary purification using ammonium sulfate fractionation. Therefore, efficient methods for separation of proteins from each other and from nucleic acids are needed for improvement of the economics of production of protein bioproducts. It is also desirable if the engineering principles for design and scale-up of the methods are well established.

Liquid-liquid extraction is a well-established unit operation for separating compounds on the basis of their affinities in two immiscible phases (6). Aqueous solutions of polyethylene glycol (PEG) in the presence of salts or another water-soluble polymer (dextran) have been known to form a two-phase system under a wide range of concentrations (7,8). These have been suggested to be an effective tool for separating proteins not only from each other, but also from nucleic acids (9). The polymers have been found to stabilize the tertiary structures and the biological activities (10). The extraction process is also amenable to easy scale-up. As a