Recovery of microsomal cytochrome P-450 from *Saccharomyces cerevisiae* using poly(ethylene glycol) precipitation

ANDREW M. SADLER,* MICHAEL A. WINKLER* and ALAN WISEMAN†

*Department of Chemical Engineering and †Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

For immobilization of cytochrome P-450 and other uses, the microsomal fraction of the yeast containing the enzyme has hitherto been prepared by a lengthy multi-step process requiring high-speed centrifugation at 108000 g for 1 h (Azari & Wiseman, 1982). Alternative microsome preparation methods we have tried were those of Cinti et al. (1965) (Ca2+ precipitation followed by centrifuging at 10000 g for 10 min). The low volume capacity of centrifuges operating at such speeds causes a serious bottleneck in the large-scale production of the enzyme. We now report a successful rapid method for the recovery of microsomal cytochrome P-450 using a convenient low-speed centrifugation of a poly(ethylene glycol) (PEG) precipitate. The method gives the enzyme in a relatively high-concentration low-purity form, but in this instance enzyme purity is of secondary importance to enzyme concentration.

Yeast (*Saccharomyces cerevisiae* N.C.Y.C. no. 754, derived from N.C.Y.C. no. 240) was grown under glucose repression (Wiseman & Lim, 1975) to a final biomass concentration of 70 g wet wt./litre, containing 8–10 nmol of cytochrome P-450/g wet wt. of yeast. Cytochrome P-450 was assayed spectrophotometrically by the method of Omura et al. (1975) and protein by that of Lowry et al. (1951). The yeast broth was centrifuged at 1000 g for 5 min to sediment the cells. The supernatant was discarded and the yeast resuspended in 0.1 M-phosphate buffer, pH 7.2, containing 1 mM-EDTA and 1 mM-dithiothreitol, to give a concentration of 300 g wet wt. of yeast/litre. The yeast suspension was then passed twice through an APV MantonGaulin homogenizer operating at 60 MPA (600 bar). The disrupted material was centrifuged at 1500 g for 5 min to sediment intact cells and cell debris. The separated supernatant contained 25 to 35% of the enzyme originally in the yeast, and to this was added 12% PEG (average mol.wt. 60000) at room temperature. The solution was placed in a cold-room at 8°C and stirred for 1 h, then centrifuged in a low-speed centrifuge at 2300 g for 20 min. This gave a slurry-like sediment rather than a solid pellet. Cytochrome P-450 was not detected in the supernatant, and the recovery of microsomal cytochrome P-450 in the precipitation stage was approx. 90%. By using a control sample of enzyme prepared by the multi-step method, it was demonstrated that PEG did not significantly affect the spectrophotometric assay of cytochrome P-450. The sediment was a relatively high-concentration low-purity enzyme preparation suitable for immobilization, the required enzyme concentration being obtained by resuspending the sediment in the appropriate volume of buffer. Further investigation of the precipitation using different PEG concentrations between 1% and 20% (Fig. 1) showed that although the amount of cytochrome P-450/g sediment indicating percentage recovery, was highest when PEG concentrations of 8% and above were used, the amount of cytochrome P-450 in the microsomal particles per mg of protein is highest with PEG concentrations between 4 and 8%. These precipitation profiles show that the cytochrome P-450 in the microsomal particles is precipitated separately from the bulk of the protein.

![Fig. 1. Precipitation profiles of microsomal cytochrome P-450 from S. cerevisiae with different proportions of PEG 6000](image)

Disrupted yeast was centrifuged at 15000 g for 5 min, and the supernatant was treated with PEG and stirred for 1 h at 8°C. The PEG-treated solution was centrifuged at 23000 g for 20 min and the resulting sediment and supernatant assayed for cytochrome P-450 and protein. • Specific concentration of enzyme in nmol of cytochrome P-450 per mg of protein in sedimented PEG precipitate. ▲ Cytochrome P-450 present in sedimented PEG precipitate expressed as a percentage of that originally present in the supernatant from the centrifuged disrupted material.

The multi-step method for producing a preparation rich in microsomal cytochrome P-450 gives 60–70% recovery of enzyme from the disrupted material, with 0.036 nmol of cytochrome P-450/mg of protein. The rapid method described here, taking about the same time of 1 h, gives 85–90% recovery, with 0.02 nmol of cytochrome P-450/mg of protein.

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