Short Communications

A mild procedure for separating enzymes and cofactors

The successful use of the polydextran Sephadex[1] in separating large molecules from small molecules indicated that this material might be useful in the separation of enzymes from cofactors. The present report illustrates an instance of such a separation. The enzymes involved were obtained from a water extract of acetone-dried *Escherichia coli* PA15, a mutant which requires either serine or glycine for growth[2]. The organism was grown on minimal medium plus glycine[3] and the extracts were prepared as described[3]. Such extracts can synthesize methionine from homocysteine and serine when supplemented as indicated in Table I. In addition a coenzyme form of folic acid found in heated cell extracts is required[4,5].

TABLE I

THE RESTORATION OF METHIONINE SYNTHESIS IN SEPHADEX-TREATED AND DIALYSED ENZYME EXTRACTS BY COFACTOR ELUTED FROM SEPHADEX

Each incubation mixture contained 4.6 mg protein, 1 μmole diphosphopyridine nucleotide, 10 μmoles adenosine triphosphate, 10 μmoles fructose 1,6-diphosphate, 1 μmole pyridoxal phosphate, 10 μmoles L-serine, 10 μmoles $MgSO_4$, 20 μmoles DL-homocysteine, 100 μmoles potassium phosphate, pH 7.8. Total volume 2 ml. Incubated 3 h at 37° under H_2 in Thunberg tubes. Methionine was determined microbiologically[7] with *Leuconostoc mesenteroides* P60 (ATCC 8042). Methionine values are corrected for the small amount of methionine present in the eluted cofactor preparation (0.02 μmole methionine in 0.1 ml).

Enzyme preparation	*Addition*	*Methionine (μmole)*
Untreated	None	0.71
Sephadex-treated	None	0.00
Sephadex-treated	0.1 ml eluted cofactor	0.65
Sephadex-treated	0.2 ml eluted cofactor	0.83
Sephadex-treated	0.4 ml eluted cofactor	0.84
Dialysed	None	0.38
Dialysed	0.1 ml eluted cofactor	1.00
Dialysed	0.2 ml eluted cofactor	0.95

A 2 × 12 cm Sephadex G-50* column was prepared as described[1] and washed with 100 ml 0.01 *M* potassium phosphate buffer, pH 7.8 and chilled to 5° in the cold room where subsequent elution was carried out. A 10-ml portion of an extract of acetone-dried cells containing 7.8 mg protein/ml[6] was pipetted onto the column and 5-ml fractions were collected using the above-mentioned buffer as eluant. 95 % of the added protein was recovered in combined fractions 2, 3 and 4. A distinct yellow band could be seen remaining on the column. Continued elution with the same buffer removed this band. Fractions showing an absorbancy (Beckman DU Spectrophotometer, 1-cm light path) at 260 mμ greater than 1.0 (fractions 8 through 14) were combined and lyophilized. The elution of enzyme and cofactor was complete in 30 min.

* Obtained from Pharmacia, Uppsala, Sweden or Pharmacia Laboratories Inc., 501 Fifth Avenue, New York 17, N. Y., U.S.A.

The lyophilized material (total dry wt. 30 mg excluding buffer) was taken up in 2 ml water and tested for its ability to reactivate depleted enzyme preparations (Table I). The cofactor solution showed an absorption peak at 260 mμ with a ratio of absorption at 280 mμ to 260 mμ of 0.43.

A second 10-ml portion of the extract of acetone-dried cells was enclosed in Visking cellulose casing (8/32 inches). The casing was tied to a mechanical stirrer and suspended in 4 l of the above-mentioned buffer. Stirring was continued for 20 h at 5°.

The results shown in Table I indicate that the folic acid coenzyme was completely removed by the Sephadex treatment whereas the dialysed preparation still retained 54 % of the activity of the untreated extract. 92 % of the activity could be restored to the Sephadex treated extract by adding the amount of cofactor preparation (0.1 ml) derived from 0.5 ml of the untreated enzyme extract. Doubling the amount of the cofactor preparation increased the activity to 117 % that of the untreated extract. Further addition of cofactor did not result in increased methionine synthesis. The eluted cofactors also restored activity to the partially depleted dialysed extract.

This procedure for separating enzymes and soluble cofactors is more satisfactory than dialysis in that it is more rapid, cofactors are removed more completely and the cofactors are obtained in a relatively small volume.

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The prosthetic group of a chromoprotein from mycobacteria

Several yellowish pigments soluble in aqueous media have been isolated from acid-fast bacteria, *e.g.*, phthiocol[1], riboflavin[2] and pterins[3,4].

During the isolation of a lactic acid oxidase from *Myco. smegmatis*[5] a yellow chromoprotein which accompanies the oxidase through several stages of purification was separated in a partly purified state.

This protein, which is not known to have enzymic activity, showed a characteristic absorption spectrum with maxima at 276 mμ and 403 mμ, the ratio $E_{403\ m\mu}/E_{276\ m\mu}$ being 1:3 for the purified preparation (Fig. 1). Acidified solutions of the protein are very pale yellow and this colour is intensified on making alkaline, the change being