A facile method for the isolation of porcine heart mitochondrial malate dehydrogenase by affinity elution chromatography on Procion Red HE3B

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A quick, simple method has been devised for isolating pig heart mitochondrial malate dehydrogenase in apparently homogeneous state and good yield. It entails the adsorption of the enzyme to agarose-linked Procion Red HE3B and specific elution of a ternary complex consisting of the malate dehydrogenase, NAD\(^+\), and L-malate.

Of all the malate dehydrogenase (MDH; L-malate-NAD\(^+\) oxido-reductase, EC 1.1.1.37) species, those from porcine heart are perhaps the most extensively investigated. In this system, as in eukaryotic tissues generally, two distinct forms of MDH, cytoplasmic and mitochondrial, occur. The two iso-enzymes from pig heart are similar in molecular weight and are both dimers, but they differ significantly in other properties. In the majority of prokaryotes MDH is a dimeric protein, but in some bacteria, notably those belonging to the genus Bacillus, the enzyme exists as a tetramer (Sundaram et al., 1980).

Methods employed for the purification of porcine heart mitochondrial MDH have generally been laborious, involving several steps and producing low yields. We have recently devised procedures for purifying MDHs from a number of mesophilic and thermophilic bacteria, in which the triazinyl dye, Procion Red HE3B, immobilized on agarose is used as an adsorbent and the elution of MDH is rendered selective by being dependent on the presence of both NAD\(^+\) and L-malate. For the majority of these MDHs the procedure involves but one step and for a few two easy steps (Smith et al., 1982; Smith et al., in press). We have observed previously that antibodies to the pig heart mitochondrial MDH cross-react poorly with several bacterial MDHs with the exception of Escherichia coli MDH, with which significant cross-reaction occurs (Sundaram et al., 1980). Thus the pig heart mitochondrial enzyme does not appear to bear appreciable native structural homology to bacterial MDHs in general. It was therefore important to investigate whether the mitochondrial MDH, like the bacterial MDHs, could be isolated pure by a simple method involving selective elution by NAD\(^+\) and L-malate from Procion Red. The results reported here show that the principle of selective elution can indeed be successfully adapted to the efficient purification of the mitochondrial MDH.
Materials and Methods

Materials

Porcine heart mitochondrial MDH, NAD⁺, and NADH were obtained from Boehringer Corporation (London) Ltd.; L-malate and pig heart acetone powder from Sigma London Chemical Company Limited; oxaloacetic acid from Calbiochem-Boehringer Corp., and agarose-linked Procion Red HE3B (Mätrex Gel Red A) from Amicon Ltd. The other chemicals were purchased from various commercial sources.

Electrophoresis

Electrophoresis of native and denatured MDH in polyacrylamide, staining of the polyacrylamide gels for protein and for MDH activity, and densitometric scanning of the gels were performed as described previously (Smith et al., 1982). The system for the electrophoresis of native MDH was based on the discontinuous technique developed by Davis (1966); the pH of the stacking gel was about 6.7, that of the separating gel was about 8.9, and that of the electrophoresis buffer was 8.3. Denatured MDH was electrophoresed in the discontinuous system containing sodium dodecyl sulphate described by Laemmli (1970). The MDH was denatured in a boiling sodium-dodecyl-sulphate/2-mercaptoethanol buffer mixture containing the protease inhibitor phenylmethanesulphonyl fluoride (120 μg/ml).

Sedimentation equilibrium centrifugation

The centrifugation was done at 10°C in a Beckman Model L8-70 ultracentrifuge using a 13-mm double-sector EPON centrepiece in a cell with quartz windows and an AnF rotor. The absorbance (A) at 280 nm was scanned at intervals to ascertain the attainment of equilibrium. The molecular weight (Mr) of the purified porcine mitochondrial MDH was derived from the slope of the plot of log A against r² by using the equation

\[ M_r = \frac{d \ln A}{d (r^2)} \cdot \frac{2RT}{\omega^2(1-\bar{v}\rho)} \]

where \( r \) = radial distance in centimeters, \( R \) = gas constant, \( T \) = absolute temperature, \( \omega \) = angular velocity in radians per second, \( \bar{v} \) = partial specific volume of the enzyme, and \( \rho \) = density of the solvent. The enzyme sample was dialyzed against 100 mM sodium potassium phosphate buffer, pH 7, before the centrifugation; the protein concentration was 4 mg/ml. The partial specific volume was calculated (Cohn & Edsall, 1943) from the amino acid composition of the MDH deduced from its amino acid sequence (Fernley et al., 1981).

Amino acid composition

The MDH samples were hydrolyzed in vacuo at 110°C for 24 h in 6 M HCl (containing a small amount of phenol to protect tyrosine residues) and the hydrolysates were analyzed in a Locarte Amino Acid