NH₄Cl. None of the adaptations could be attributed to the high-protein diet per se since they were not evident in those rats fed a high-protein diet and given NaHCO₃ solution to drink. Of particular interest is the increased rate of gluconeogenesis found in kidney slices from rats fed on a high-protein diet. Krebs et al. (1963) have observed an increased rate of renal gluconeogenesis in rats fed on a low-carbohydrate diet. However, their low-carbohydrate diet was enriched in protein and it seems that the increased rate of gluconeogenesis observed may be attributed, at least in part, to the acidogenic effects of the diet.


Affinity Chromatography of Potato Lactate Dehydrogenase

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Many plants produce lactic acid during the period of anaerobiosis that occurs during seed germination. The lactate is produced from pyruvate by the action of NAD-linked lactate dehydrogenases (EC 1.1.1.27). This enzyme has been purified from a number of plants, and appears to be similar in many respects to animal lactate dehydrogenases (D'Auzac & Jacob, 1968; Davies & Davies, 1972; Barthova et al., 1976).

The activity of lactate dehydrogenase in plant tissues is very low, and purification of the enzyme by multi-step conventional procedures results in poor yields. Lactate dehydrogenase from animal and bacterial sources has been purified by affinity chromatography with a variety of adsorbents including several based on the sulphated aromatic dye Cibacron Blue F3GA (Ryan & Vestling, 1974; Thompson et al., 1975; Gordon & Doelle, 1976; Anderson & Jervis, 1977). It has been proposed that this dye interacts specifically with the NAD-binding domain present in lactate dehydrogenase and many other dehydrogenases and kinases (Thompson & Stellwagen, 1976; Stellwagen, 1977). Immobilized derivatives of the dye provide inexpensive and effective affinity adsorbents for those enzymes with which it interacts.

Potato lactate dehydrogenase is competitively inhibited by Cibacron Blue F3GA with respect to NADH ($K_i = 26\mu M$). However, the enzyme is not inhibited by the dextran–dye conjugate Blue Dextran 2000. This behaviour contrasts with that of those animal and bacterial lactate dehydrogenases that have been purified with immobilized dye derivatives (Thompson et al., 1975; Gordon & Doelle, 1976). It has been suggested that lack of inhibition by Blue Dextran indicates the absence of a dinucleotide fold as the NAD-binding site (Wilson, 1976; Stellwagen, 1977). Enzymes lacking this type of NAD-binding site do not bind to immobilized derivatives of either Blue Dextran or Cibacron Blue F3GA.

Lactate dehydrogenase from potato, as expected from the lack of inhibition by Blue Dextran, failed to bind to either Blue Dextran–Sepharose or to Blue Sepharose. Thus it appears that either the potato enzyme does not possess a dinucleotide fold as the NAD-binding domain, or that the nicotinamide nucleotide-binding region of the fold is more deeply buried within the protein than is usual with lactate dehydrogenases.

The potato lactate dehydrogenase also differs from the animal and bacterial enzymes in that it is inhibited by ATP in an apparently allosteric manner (Davies & Davies, 1972). This behaviour suggested that immobilized derivatives of ATP might provide an effective affinity adsorbent for the enzyme.
Potatoes (Solanum tuberosum var. Golden Wonder) were extracted into 50 mM-potassium phosphate buffer, pH 7.4, containing 5 mM-β-mercaptoethanol. The extract was subjected to (NH₄)₂SO₄ fractionation, and the precipitate obtained between 25 and 50% saturation was found to contain lactate dehydrogenase activity. The precipitate was suspended in the above buffer and dialysed against several changes of 50 mM-potassium phosphate buffer, pH 6.2. Insoluble material was removed by centrifugation and the clear enzyme solution was applied to a column (1.5 cm × 10 cm) of ATP-Sepharose equilibrated and eluted with 50 mM-potassium phosphate buffer, pH 6.2. The column was operated at 20°C at a flow rate of 20 ml/h. After removal of unbound proteins, lactate dehydrogenase was eluted by addition of NADH (50 μM) to the irrigating buffer. The column effluent was monitored for protein (●), lactate dehydrogenase activity (○), and malate dehydrogenase activity (●).

ATP was coupled to Sepharose hydrazide through the ribose moiety of the nucleotide as described by Lamed et al. (1973). The resulting gel removed the potato enzyme from solution under batch-incubation conditions at pH 6.0-6.4, but not at pH 7.0. Under column conditions, the adsorbent selectively removed lactate dehydrogenase from partially purified preparations of the enzyme. The bound enzyme could be eluted from the column by addition of 50 μM-NADH to the irrigating buffer (Fig. 1). Considerable purification of the enzyme was achieved, and recovery of bound enzyme was high (Table 1). Other plant dehydrogenases that are inhibited by ATP, notably malate dehydrogenase and alcohol dehydrogenase (D’Auzac & Jacob, 1968), were not adsorbed on to the ATP-Sepharose gel and were easily separated from the bound lactate dehydrogenase.

The unusual properties of potato lactate dehydrogenase may be characteristic of plant lactate dehydrogenases (D’Auzac & Jacob, 1968; Barthova et al., 1976), and the ATP-Sepharose gel may prove to be generally applicable to the purification of these enzymes. The low activity of the enzyme occurring in plant tissues presents considerable problems of purification. The development of an efficient affinity purification system for these enzymes would greatly aid investigation of the apparently unusual NAD-binding site present in plant lactate dehydrogenases.
Table 1. *Affinity purification of potato lactate dehydrogenase on ATP-Sepharose*

Lactate dehydrogenase was assayed spectrophotometrically as described by Davies & Davies (1972). One unit of activity is defined as the amount of enzyme causing the oxidation of 1.0 μmol of NADH/min. Protein was assayed colorimetrically as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Purification (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>800</td>
<td>10400</td>
<td>193</td>
<td>0.018</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>25–50% Satd. (NH₄)₂SO₄ precipitate</td>
<td>100</td>
<td>4200</td>
<td>141</td>
<td>0.033</td>
<td>1.8</td>
<td>73</td>
</tr>
<tr>
<td>ATP-Sepharose eluate</td>
<td>36</td>
<td>10.3</td>
<td>120</td>
<td>11.6</td>
<td>644</td>
<td>62</td>
</tr>
</tbody>
</table>
P-Phenylethanolamine as a Substrate for Monoamine Oxidase

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Monoamine oxidase [amine-oxygen oxidoreductase (deaminating) (flavin containing), EC 1.4.3.41] is thought to exist in at least two forms, A and B, which differ in substrate specificity and inhibitor-sensitivity. Houslay & Tipton (1974) have suggested structural criteria for predicting the substrate specificity of each form of the enzyme from rat liver. For phenethylamine derivatives these are: (a) type-A substrates must carry a \( \beta \)-hydroxy group; (b) type-B substrates must be devoid of a \( \beta \)-hydroxy group in the side chain. Amines fulfilling both these conditions are substrates for both enzymes. This classification appears to preclude \( \alpha \)-phenylethanolamine, which satisfies neither criterion, although there are reports of oxidation of this amine by monoamine oxidase (e.g. Pratesi & Blaschko, 1959).

The use of whole-tissue preparations in some studies allows the possibility of oxidation by enzymes other than the mitochondrial monoamine oxidase. The oxidation of \( \alpha \)-phenylethanolamine and its enantiomers by a mitochondrial preparation from rat liver has therefore been reinvestigated.

Mitochondria were prepared by the method described by Hawkins (1952) and finally suspended in phosphate buffer, pH 7.4, prepared by mixing 0.05M solutions of \( \text{KH}_2\text{PO}_4 \) and \( \text{Na}_2\text{HPO}_4 \). \(-(-)\) and \((+)-\beta\)-Phenylethanolamines were prepared from the corresponding mandelic acids. The purified hydrochlorides had optical rotations, \([\alpha]_D \), of \(-44.9 \pm 1.6 \) and \(+46.9 \pm 1.4 \), measured in water. Monoamine oxidase activity was measured by following \( \text{O}_2 \) uptake in an oxygen electrode at 30°C and at pH 7.4 (Williams, 1974). The reaction was started by adding substrate in 0.05ml of the above-mentioned buffer to an incubation medium containing \( \text{KCN} \) (1 mm), mitochondrial protein (6mg), in 1.95ml of the same buffer. Preliminary studies showed that maximum velocities were obtained at phenylethanolamine concentrations of 3 mM. This concentration was used in all experiments. Oxidation of the amine was studied both in the presence and absence of the selective inhibitors \( \text{N-3-(2,4-dichlorophenoxy)propyl-N-methylprop-2-ynylamine (Clorgyline) and N-(2-phenylisopropyl)-N-methylprop-2-ynylamine (Deprenil). Results are shown in Table 1.}

It is clear that the amine is a substrate for monoamine oxidase, although rates of oxidation are slow compared with those obtained with substrates such as tyramine or benzylamine. Under comparable conditions the latter amines were turned over at rates 3-4 times those shown in Table 1. The \(-(-)\)-isomer of \( \beta \)-phenylethanolamine is oxidized more readily than the \((+)-\)-form. The use of Clorgyline and Deprenil produced noteworthy results. At concentrations of the former that produced 50% inhibition of monoamine oxidase A, but which left the B enzyme unaffected, the rate of oxidation of the \(-(-)\)-isomer was halved, whereas the \((+)-\)-isomer was barely affected. When Deprenil