PRELIMINARY NOTES

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Simplification of mouse lactate dehydrogenase electrophoretic patterns

Gel electrophoresis of mouse tissue extracts shows many bands of lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) activity[1]. There is strong evidence that beef heart lactate dehydrogenase is a tetramer composed of two different kinds of subunits[2]. On this basis, the many electrophoretic bands of the mouse enzyme have been explained in terms of different numbers of NAD molecules bound to the tetramer[1] and as the expression of separate genes producing three different kinds of polypeptide subunits[3]. More recently, the multiple bands have been suggested to represent conformational isomers of lactate dehydrogenase[4].

The present communication indicates that mouse lactate dehydrogenase band patterns can be simplified to a set of 5 isoenzyme bands, that the original multiple bands are not due to a plethora of genes, and that, as extracted, the mouse isoenzymes bind varying numbers of an anionic residue which can be removed by thiols, but which is apparently not NADH, nor pyruvate.

Tissues of Quackenbush albino mice *(Mus musculus)* were excised immediately after slaughter and frozen until used. The electrophoretic band patterns were not affected by freezing the tissues, which were thawed as required and homogenised in ice-cold 10 mM Tris–20 mM glycine buffer (pH 8.8) at a ratio of about 1:6 (w/v). The homogenates were centrifuged at 30 000 × *g* for 30 min at 4°, and the clear supernatant fluids were used as samples.

Electrophoresis was carried out on vertical polyacrylamide gels (6%) in the Tris–glycine buffer described above. Before being loaded with enzyme, the gels were electrophoresed until a bromophenol blue marker had run the length of the gels. Enzyme preparations were then electrophoresed at a constant 19 $V \cdot cm^{-1}$ and stained for lactate dehydrogenase activity[5]. Unless otherwise specified, dialyses were carried out in the Tris–glycine buffer already described, containing added thiols or substrates.

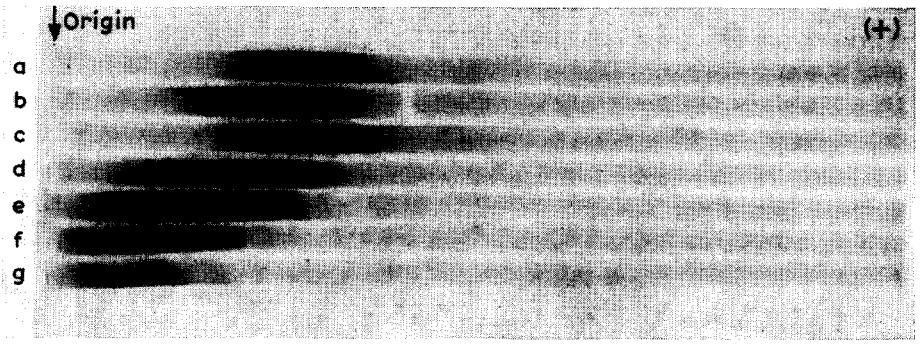

Fig. 1. Polyacrylamide gel electrophoresis patterns of mouse liver lactate dehydrogenase. The heavy bands all belong to isoenzyme 5. Before electrophoresis the mouse liver extract was dialysed in 2-mercaptoethanol of the following concentrations (in μM): a, 0; b, 7.6; c, 25; d, 76; e, 250; f, 760; g, 2.5 mM.

Electrophoresis of mouse liver extract, dialysed in 2-mercaptoethanol of several different concentrations from 7.6 μM to 2.5 mM (Fig. 1), produced some new bands of activity with lower mobility than the slowest band of isoenzyme 5 from the untreated extract (*cf.* ref. 6). Two of the most prominent sub-bands from isoenzyme 5 disappeared preferentially, leaving a pattern of widely spaced bands, retarded by increasing concentrations of thiol. By independent electrophoresis of untreated and of 2-mercaptoethanol-treated liver extracts on polyacrylamide gels of differing concentrations (5–10%)[7], the new slow bands, formed in 2-mercaptoethanol, were shown to come from molecules of a size similar to those from the untreated extract. Thus the slow bands do not arise from mouse lactate dehydrogenase by oligomer formation.

Subsequent dialysis in 0.1 mM or in 10 mM NADH and pyruvate had no effect on the band pattern of the 2-mercaptoethanol-treated liver extract.

Individual sub-bands of mouse liver isoenzyme 5 were prepared by electrodialysis[8] of corresponding slices from a large number of polyacrylamide electrophoretograms. After dialysis of these purified fractions in 25 μM 2-mercaptoethanol, electrophoresis showed (Fig. 2) that each sub-band was giving rise to a part of the same

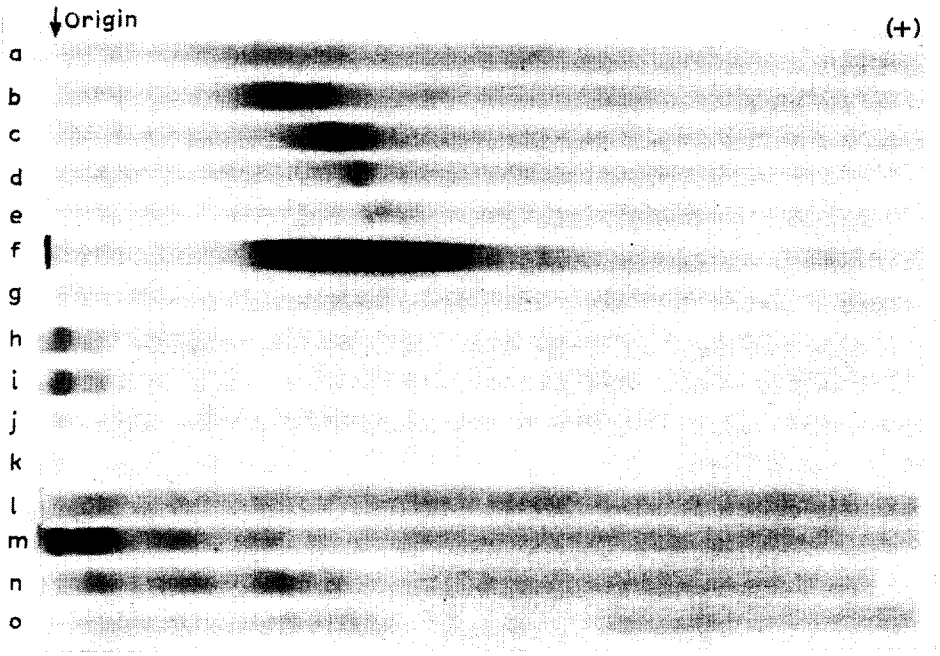

Fig. 2. The effect of 2-mercaptoethanol on individual electrophoretic sub-bands of mouse liver lactate dehydrogenase isoenzyme 5. a–e, purified sub-bands 6, 5, 4, 3 and 2, respectively, of isoenzyme 5; f, untreated mouse liver extract; g–k, sub-bands 6, 5, 4, 3 and 2, respectively, after dialysis in 250 μM 2-mercaptoethanol; l–o, sub-bands 6, 5, 4 and 3, respectively, after dialysis in 25 μM 2-mercaptoethanol.

pattern of slower bands; sub-bands 4 and 5 produced the full pattern consisting of 5 bands. However, when the purified fractions were dialysed in 250 μM 2-mercaptoethanol, sub-bands 2 and 6 each produced only the slowest band of the new pattern (Fig. 2), while sub-bands 3, 4 and 5 each produced the slowest two bands. These results

imply that the isoenzyme 5 sub-bands all give rise to the same lactate dehydrogenase molecule at high 2-mercaptoethanol concentration and suggest that in crude tissue extracts, this molecule is bound to a varying number of anionic residues by disulphide bonds.

Dithiothreitol and H_2S (pH 7.0) produced results similar to those obtained with 2-mercaptoethanol, but dialysis of liver extract in 2 mM ethanolamine at pH 8.0, or in 2 mM 8-hydroxyquinoline at pH 5.0, to complex any dissociating anions, failed to affect the original band pattern, supporting the view that the anionic residues are bound to the lactate dehydrogenase molecule by disulphide bonds.

Similarly, when extracts of mouse leg muscle, heart or kidney were electrophoresed after dialysis in 12.6 mM 2-mercaptoethanol each isoenzyme reduced to a single band or two bands. These new bands were progressively retarded, isoenzyme 5 being most affected, and isoenzyme 1 not at all.

Goldfish[9] gives an electrophoretic pattern of many bands of lactate dehydrogenase activity, and preliminary investigations in this laboratory have shown that the pattern can be simplified by prior dialysis in 2-mercaptoethanol. Whiting[10] also shows a large number of bands on starch gel electrophoresis, and a simplification of this pattern such as observed for the mouse seems not unlikely. Thus the need to invoke extra genes to account for the multiplicity of bands may be eliminated.

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