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Fig. 1. Electrophoretic patterns of L-lactate dehydrogenase activity from various tissues of the squid (L. vulgaris)

A, digestive gland; B, buccal cavity; C, mantle muscle; D, eye; E, brain. The lower margin of the photograph is the position reached by bromophenol blue. The L-LDH was specifically isolated by affinity chromatography before application to the tops of gels (see text). Electrophoresis was carried out in tubes of 7.5% (w/v) polyacrylamide at pH 8.3 according to the method of Davis [5]. LDH was visualized using the zymogram-staining cocktail of Epstein et al. [6] containing either L-lactate (left-hand gel of each pair) or DL-lactate (right-hand gels).

isolation of LDH using 'conventional' purification methods, and we have used the efficient oxamate affinity chromatographic system [2] to extricate, and purify to homogeneity, the L-LDH from various tissues of normal squid, despite the presence of a large preponderance of ODH in some of these tissues, particularly mantle muscle, and the presence also of the D-lactate-specific LDH noted by Cullina [3].

We confirm Gade's conclusion [1] that the L-LDH is a subunit dimer, but we find that in most normal animals it exists as three isoenzymic forms, probably formed from two variant LDH polypeptides.

Distribution of lactate dehydrogenase isoenzymes in potato (Solanum tuberosum) and other plants

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Lactate dehydrogenase (LDH) of most plants has been reported to exist as a single electrophoretic form [1-3], but barley seedlings [4] and potato tubers [5-7] express isoenzymic forms of LDH, the functional significance of which has been the subject of some discussion [8]. Differential tissue distribution is usually considered to be suggestive of differential functions of isoenzymes (e.g. in the case of the mammalian LDH isoenzymes), but the only information of this kind for plant LDH seems to be that of Asker & Davies [8] who reported that only one of the isoenzymes expressed by potato tubers appeared in extracts of potato leaves. We confirm a difference between tuber and leaf, although we find predominantly the fastest electrophoretic form (isoenzyme-1) in leaf extracts, rather than isoenzyme-2 as reported by Asker & Davies [8]. (The isoenzyme numbering

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Squid (Loligo vulgaris) were from Galway Bay and were obtained fresh from local fishermen (within 2-3 h of being caught, and kept on ice in the interim). The animals were dissected carefully and the individual tissues were extracted by homogenization in 0.05 m-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA. These extracts were subjected to ammonium sulphate fractionation. The fraction precipitating between 35 and 80% saturation of ammonium sulphate contained the t-LDH and was redissolved and subjected to affinity chromatography essentially as described (for mammalian LDH) by O'Carra & Barry [2]. This procedure yielded t-LDH preparations that seemed to contain no detectable contaminating proteins, as judged by SDS/PAGE electrophorograms [4] stained for protein with Amido Black. The LDH appeared as a single sharp band of M, 36000. Gel filtration of the native enzyme indicated an M, of 70000. Thus the enzyme seems to be a subunit dimer, in agreement with the findings of Gade [1].

Using non-denaturing electrophoresis, however, we found that the enzyme usually appears as three distinct bands of activity (Fig. 1), contrary to Gade's finding of a single electrophoresis species [1]. This suggests an isoenzymic composition based on two different, but similarly-sized, versions of the polypeptide: A, AB and B, The splitting of the lower band in the preparations from mantle muscle (Fig. 1) suggests the existence of a further form. Otherwise the three isoenzymic forms occur in approximately similar proportions in all the tissues examined; those shown in Fig. 1, and also sexual tissues (ovaries and spermatophores). Extracts from the tissues of most specimens showed the three-banded pattern, but Gade, as already mentioned, found only a single t-LDH form in one, apparently mutant animal, and in the early stages of this study, we confirmed Gade's conclusion [1] that the L-LDH is a subunit dimer, but we find that in most normal animals it exists as three isoenzymic forms, probably formed from two variant LDH polypeptides.

We thank Jane Dorman, Department of Zoology, Galway, for help with the dissection of the animals, McDonaghs of Galway (fish merchants) for their assistance in obtaining supplies of squid, and Carlow Vocational Education Committee for a Maintenance Grant to P.M.


Received 14 September 1989

Abbreviation used: LDH, lactate dehydrogenase (EC 1.1.1.27).
system of Jervis [7] is used here.) Previous electrophoretic examination of the LDH of most other plants was carried out with extracts from leaves [1, 2]. Such a study of potato leaves would not have revealed the existence of isoenzyme forms in this species. We have, therefore, examined extracts from various regions of a number of plants: roots, stem-base, upper stems, leaves and any special tissue, such as the tubers of the potato.

The plants used were grown under normal horticultural conditions, harvested before flowering, and immediately extracted. They were first washed thoroughly, but quickly, with ice-cold water, and representative portions were finely chopped and ground in a pestle and mortar with acid-washed sand and 50 mm-micron phosphate buffer, pH 7.4, containing 0.8% (w/v) suspension of polyvinylpyrrolidone (1–4 volumes of this buffer were used, depending on the consistency of the homogenate). The homogenates were then centrifuged at 8000 g for 30 min and the supernatants were subjected to ammonium sulphate fractionation (largely to concentrate the LDH activity, which is orders of magnitude lower in plants than in animals and bacteria). The fraction precipitating between 30 and 70% saturation of ammonium sulphate was redissolved in a minimal volume of 50 mm-Tris/glycine buffer, pH 8.6, in 7.5% (w/v) polyacrylamide gels either by the ‘disc-gel’ method of Davis [9] or using the ‘Mighty Small’ II SE 250 slab gel apparatus (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.). The LDH activity was visualized using the zynogram-staining ‘cocktail’ of Epstein et al. [10].

We have experienced problems with many plants due, apparently, to interfering substances that may adsorb the LDH. These substances interfere with its extraction or electrophoresis. With special extraction procedures we have been able to demonstrate LDH activity in extracts from all higher plants [11], even those previously thought to lack this enzyme (see, e.g. [1]). However, we have been less successful in overcoming interference with electrophoresis. The LDH activity in extracts from many plants did not penetrate properly into the polyacrylamide gels, remaining as a diffuse streak near the point of application and this limited the number of species we could usefully include in this study.

Of these, the majority showed only a single electrophoretically identical form in all tissues. Such species include onion (Allium cepa), leek (Allium porrum) and turnip (Brassica rapa). But multiple forms were found in potato (Solanum tuberosum) and in the related tomato (Lyco-persicum esculentum). Other members of the Solanaceae, Capsicum annuum and Solanum dulcamara, were also examined, but although extracts from the tissues of these species have LDH activity, we were unable to ‘find’ it on electrophoregrams, only diffuse activity near the origin being detectable, as described above. The same problem, probably attributable to interfering factor(s) in the extracts, was encountered with extracts from leaves of the two tomato cultivars studied, ‘Moneymaker’ and ‘Hildares’, but the other tissues gave clear electrophoretic patterns with up to four LDH bands, the number and intensity of the bands varying with tissue and with cultivar.

In addition to the leaves and tubers mentioned above, other regions of potato plants showed variations of isoenzyme pattern. With the cultivar ‘Kerr’s Pink’, the tubers showed all five isoenzymes, with isoenzymes 2 and 3 greatly predominating. In the stems and roots, there was a predominance of isoenzymes 1 and 2, but the others were also detectable, while only isoenzyme-1 could be detected in extracts from the leaves. In another potato cultivar, ‘British Queen’, the leaf extracts again had predominantly isoenzyme-1, but also a trace of isoenzyme-2, while isoenzyme-5 appeared missing from the tubers and other tissues whose LDH patterns were otherwise similar to those found in the ‘Kerr’s Pink’ cultivar. Only three isoenzymes could be detected in tubers of the cultivar ‘Homeguard’. Such variation among cultivars seems odd, if the isoenzyme forms fulfil any important differential function.

A recent survey by Hoffman et al. [12] of LDH isoenzymes in barley seedlings shows somewhat similar variation among cultivars. More significantly, these authors describe one strain that seemed to have only a single isoenzyme form of LDH without suffering any apparent disadvantage. This seems to cast doubt on any important physiological role for the isoenzymic multiplicity in barley. We have considered the possibility that the variation of isoenzyme pattern among potato tissues and cultivars might be an artifact produced by the interfering substances so evident in our experiments with other plants. However, we have been unable to find any evidence for this. Co-extraction of tissues having different isoenzyme patterns (e.g. a half-and-half mixture of leaf and tuber tissue) gave seemingly additive isoenzyme patterns.

We thank Eolas (The Irish Board for Science and Technology) and Carlow Vocational Education Committee for Maintenance Grants to M. O. D. and P. M., respectively.

Received 14 September 1989

Tissue distribution of mammalian lactate dehydrogenase isoenzymes

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Although the mammalian lactate dehydrogenase (LDH) isoenzymes were among the first isoenzyme phenomena to be characterized, and have been the subjects of a very extensive literature, the functional significance of this isoenzymic differentiation remains obscure. Excluding the more recently discovered isoenzyme, LDH-X, which seems to be confined to spermazaoa, the ‘classic’ electrophoretically separable isoenzymes 1–5 are tetramers composed of a random association of two different types of kinetically non-interacting polypeptide subunits, variously distinguished as A and B or