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On the ubiquity of aldolase C in ruminant tissues

Recently, considerable research interest has been displayed in the occurrence of multiple forms of the enzyme fructose-1,6-diphosphate aldolase (EC 4.1.2.7, 4.1.2.13). Several groups of investigators have explored the implications of this heterogeneity in relation to the structural and biological properties of the enzyme. As a result, it is now recognised that the enzyme exists as a tetramer (rather than a trimer as previously thought), and the multiplicity of the enzyme seems to be explicable on the basis of four membered hybridization sequences between three parental forms, A, B and C (refs. 1, 2).

Tissue distributions have played a significant role in determining previous interpretations of the physiological roles and gene expression of the aldolase heteromorphs. For example, in view of its predominant localization and catalytic properties, it has been suggested that aldolase A (the classical muscle enzyme) is tailored for glycolysis, whilst aldolase B (the liver enzyme) seems more suited to gluconeogenesis and fructose metabolism in that tissue[3]. As yet, however, no distinctive biological role for aldolase C has become evident.

During an investigation of the properties of aldolase in ruminant tissues, it was noticed that these species exhibited an unusual tissue distribution of aldolase heteromorphs; quite distinct from that in any mammal previously investigated. In view of the possible significance of this behaviour in relation to physiological functions and gene expression of aldolase C, this phenomenon has been investigated in some detail and reported herein.

Fresh tissues were excised from sheep and cattle, frozen, and stored at $-10°$ until required for analysis. Homogenates of these tissues were prepared and analyzed as described previously[4].

Zone electrophoresis was carried out in starch gels at 4° with a current density of 3 mA/cm and the gels stained by a methodology which involved linking the aldolase substrate, fructose 1,6-diphosphate, with NAD^+ and glyceraldehyde phosphate dehydrogenase[4]. To obviate the necessary involvement of NAD^+ in the reaction, zymograms were also developed using 2,4-dinitrophenyl hydrazine as the aldolase reactant[5].

As confirmatory analyses in the determination of the substrate activity ratios, assays, involving hydrazine and the adsorption at 240 mμ were included[6].

In our investigations, the first indication that muscle aldolase in sheep may be of a different type to that normally present in the skeletal muscle of mammalian species (*i.e.* the parental A form), came from atypical parameters of the enzyme. Consequent electrophoretic examination revealed the presence of an anodic array of five aldolase forms in sheep muscle and a similarity of pattern to that of sheep brain (Fig. 1). Hence the presence of the parental and hybrid forms of aldolase C were indicated. Extending these observations, all the major tissues of both sheep and cattle were studied and found to display similar sets of five anodic forms, corresponding in mobility to the brain A-C set of isoenzymes. Liver and kidney, in addition, exhibited the A-B set of multiple forms normally present in these mammalian organs (Fig. 1).

In view of the extremely unusual nature of this aldolase distribution, and in order to exclude the possibility of interference in the zymogram positions by non-

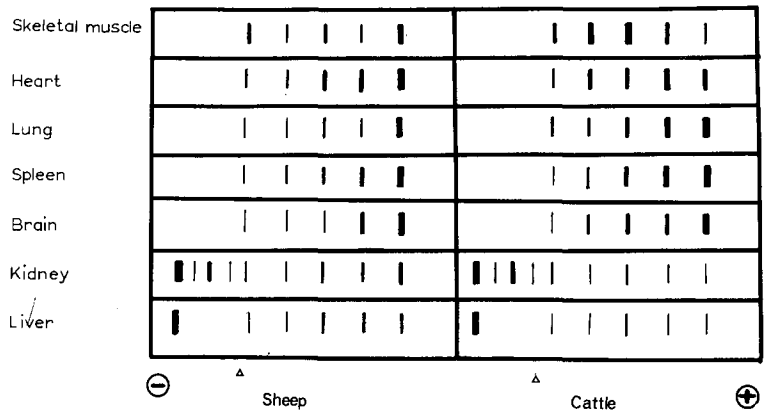

Fig. 1. Diagrammatic representation of the aldolase zymograms from adult tissues of sheep and cattle.

specific dehydrogenases, gels from ruminant tissues were also stained for aldolase activity by reaction with 2,4-dinitrophenyl hydrazine. Identical banding was revealed by this methodology, as well, and this would appear to substantiate the identity of these bands as a true aldolase A-C set.

Further characterization of the aldolase activity in ruminant tissues is provided by the substrate specificity results listed in Table I. Perhaps most significant is the correlation observable between values for brain and skeletal muscle in both species. The values for liver and kidney are low by comparison, but this is in accordance with the predominant properties of aldolase B in these tissues.

An additional noteworthy feature of the enzyme activity in many of these ruminant tissues may be noted in regard to the low values of the ratios given by the usual analytical procedures. This is caused by significant interference by an enzyme responsibile for the utilization of NADH in the presence of aldolase and fructose 1,6-diphosphate in these tissues (glycerol phosphate dehydrogenase) and results in apparent fructose 1,6-diphosphate cleavage activities which are considerably lower than the true values. When these tissues were tested by the hydrazine assay, which does not involve a coenzyme linked assay procedure, true ratios in line with the

TABLE I

SUBSTRATE ACTIVITY RATIOS OF RUMINANT TISSUES

Relative activities against fructose 1,6-diphosphate and fructose 1-phosphate [4,6]. NAD-linked assay is listed first with the corresponding hydrazine assay in parentheses.

Tissue	*Sheep*	*Cattle*
Skeletal muscle	50 (50)	65 (65)
Heart	1.0 (approx. 50)	2.8 (approx. 70)
Lung	1.0 (approx, 50)	2.2 (approx. 70)
Spleen	0.8 (approx. 50)	1.8 (approx. 70)
Brain	52 (54)	70 (70)
Kidney	2.1 (2)	2.5 (3)
Liver	0.8 (1)	0.8 (1)

values obtained for akeletal muscle were indicated (Table I). In view of the wide utilization of substrate specificity ratios in the characterization of aldolase activity in animal tissues, it seems pertinent that the possibility of similar interference should be considered in future studies.

Although the characteristics of aldolase heterogeneity in mammalian tissues have been studied quite intensively in recent years[1,4,7] and the occurrence of A-C isoenzyme sets established for beef brain[7], no previous reports on the distribution of aldolase heteromorphs in other ruminant tissues have appeared in the available literature. The data in the present communication are strikingly different from the findings in other mammalian species, in that all the major tissues of cattle and sheep exhibit considerable C-type aldolase activity (Fig. 1, Table I), whereas in these same tissues in non-ruminant mammals, appreciable aldolase C activity is normally restricted to brain, only[1,4]. These results would appear to broaden our understanding of the biological significance and gene expression of this type of activity significantly. The high content of C-type activity in all the ruminant tissues, taken together with the major role of aldolase C in mammalian ontogeny, seem to show that any interpretation of the biological role of aldolase C must extend beyond a mere neural role, and must take into account the wide variety of carbohydrate metabolism and other environmental conditions encompassed by the tissues exhibiting C-type activity[1,4]. Again it is quite evident from these results that expression of the aldolase C gene is not restricted to a few cell types only in mammalian species as previously proposed[3].

Another point of unusual interest in relation to ruminant aldolase is the occurrence of all three types of activity in kidney and liver of these species. This behaviour entails the expression of three aldolase genes in one tissue, a situation which has not been reported for mammals previously. Theoretically, this would appear to allow the possibility of three sets of hybrids (AB, AC, BC) in these tissues, but the mobility characteristics of the isoenzymes point to AC and AB hybridization only. BC hybrids have been reported as formed by dissociation–association experiments *in vitro*[1] but have not been observed in mammalian tissues to this time. Apparently, synthetic compartmentalization in kidney and liver exclude the possibility of BC hybridization in these tissues *in vivo*.

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