CHARACTERISTICS OF ALDOLASE VARIFORMITY[1]

Colin J. Masters[2]

Graduate Department of Biochemistry
Brandeis University, Waltham, Massachusetts 02154
(Publication No. 516)

Received August 14, 1967

Although the comparative enzymology and structural characteristics of aldolase (E.C. 4.1.2.7) have been closely studied for many years, it is only recently that this enzyme has been recognized as possessing an extensive heterogeneity (Penhoet et al., 1966; Herskovits et al., 1967) and coincident with this realization, a reassessment of the compositional conceptions for this enzyme has become necessary. Whereas the aldolase molecule previously was widely considered to be a trimer, the more recent evidence appears to favor a four subunit model (Stellwagen and Schachman, 1962; Chan et al., 1967; Penhoet et al., 1966; Kawahara and Tamford, 1966). At present, however, considerable uncertainty remains in this area, and this communication was prompted by the need for clarification and reconciliation. Variant characteristics of the heteromorphs in some vertebrate tissues are described, and the occurrence of a widespread microheterogeneity of this enzyme is reported.

---

[1] Supported in part by research grants from the U. S. Air Force Office of Scientific Research (AF OSR 732-67) and the American Cancer Society, New Jersey Division (P-77I) and a research contract from the National Aeronautics and Space Administration (NSG 375).

[2] On leave from the Department of Biochemistry, University of Queensland, Brisbane, Australia.

METHODS--Fresh tissues were excised and homogenized in two vols. of 0.01 M Tris-HCl buffer, pH 7.4, which contained EDTA (0.001 M) and β-mercaptoethanol (0.01 M). The homogenates were then centrifuged at 100,000 g and 2° for 60 minutes. The starch gel electrophoresis and assay procedures for these extracts have been described in full previously (Masters et al., 1967). Disc gel electrophoresis was carried out in vertical columns of polyacrylamide gel (5.5%) at 4° using a Tris-glycine buffer (pH 8.6, I 0.03), and a constant current of 2 mA per gel for 3 hours (Holmes and Masters, 1967).

RESULTS AND DISCUSSION--Aldolase C and its hybrid forms exhibit a broad tissue distribution in avian species (Herskovits et al., 1967). During an investigation of the characteristics of these heteromorphs, however, an anomalous behavior of the enzyme in liver and intestine of the chicken was recognized. The electrophoretic mobilities of the anodic forms in these tissues were greater than for heart or brain (Fig. 1a and 1b) and the fructose-1,6-diphosphate per fructose-1-phosphate[3] ratios were lower than usually observed for the brain type (Masters et al., 1967)

One possible explanation of these observations lay in the involvement of an additional aldolase gene, responsible for the synthesis of a polypeptide which was distinct from those previously considered (A, B, and C) for this enzyme. Two lines of evidence were considered to point against this interpretation, however. First, it had been observed previously, that the developmental isozyme redistributions in chicken liver displayed a switch from normal C type to a form showing increased mobilities

[3] Abbreviations: FDP = fructose-1,6-diphosphate; FIP = fructose-1-phosphate.

during embryonic maturation, and this behavior appeared to be inconsistent with replacement of a new polypeptide chain (Masters et al., 1967). Secondly, although no immunological cross reaction exists between the established parental forms of aldolase in this species (Wassarman, Herskovits, Masters and Kaplan, unpublished results) pretreatment of intestine (Fig. 1b) or liver with anti-chicken aldolase reduced the total aldolase activity and completely removed the anodic bands from the resulting zymogram.

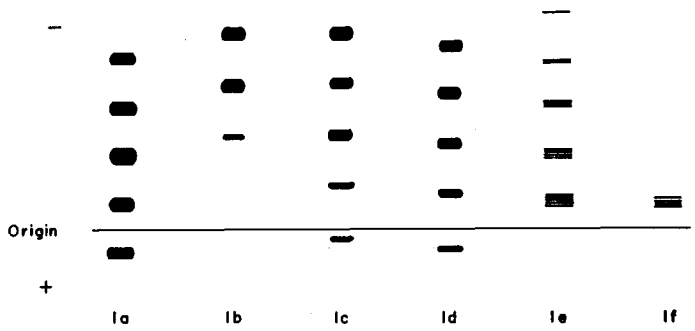

Fig. 1. Ia-Id. Starch gel zymograms of chicken tissue aldolase. Heart (Ia), intestine (Ib), mixed extracts of heart and intestine after (Ic) and before (Id) incubation at 37°. Ie and If represent acrylamide gel zymograms of mouse brain aldolase and rabbit muscle aldolase, respectively.

These facts suggested an alternative interpretation - namely, that the atypical aldolase variants were attributable to modification of the isozymes in situ. In order to test this possibility further, appropriate tissue extracts were incubated at 37° for 15 min. Chicken heart homogenate by itself showed no appreciable loss of activity nor alteration of electrophoretic mobility with this treatment, but addition of intestinal supernatant fractions resulted in an increased mobility of the isozymes and a marked reduction in FDP aldolase activity and the FDP/F1P ratio (Figs. 1 and 2).

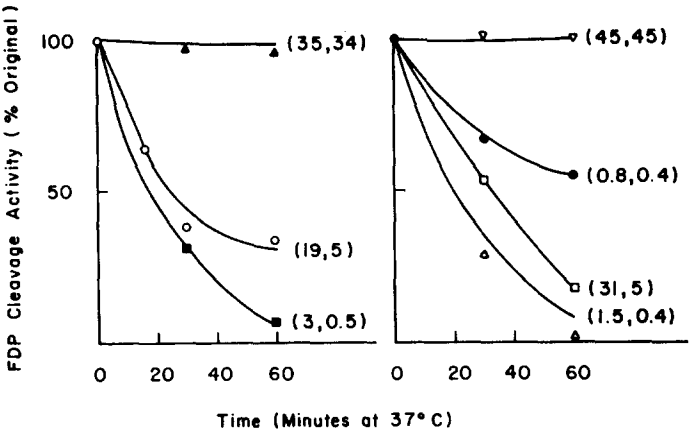

Fig. 2. The activities during the incubation of supernatant fractions from the following chicken tissue extracts:- ▲ heart, o heart plus intestine, ■ intestine, ▽ breast muscle, ● liver, 0 breast muscle plus intestine, Δ liver plus intestine. The figures in parentheses represent the substrate activity ratios (FDP/F1P) before and after incubation.

Liver supernatant fractions had a similar effect. These results support the interpretation of a modification of aldolase C in these tissues. In addition, the activities of muscle extracts and purified aldolases A, B and C, were altered in a similar manner by intestinal or liver supernatant fractions, although no mobility change was detected with the B form. Aldolase A was the most susceptible of the aldolases to this type of change.

Blostein and Rutter (1963) have ascribed the degradation of muscle aldolase (type A) by liver extracts to peptidase activity. The present findings support a broadening of these conclusions to aldolases B and C. Although the response of all three parental forms to this treatment is reminiscent of the action of carboxypeptidase on these enzymes, the inhibitory characteristics point to differences in the mode of action of these two sources. The degradation of aldolases by liver extracts was inhibited by iodoacetamide (10^{-3} M) but not by FDP (10^{-3} M), whereas the intestinal action was partly inhibited

by FDP but not by iodoacetamide. Again, the FDP cleavage activities of both intestine and liver were capable of further degradation by incubation (Fig. 2), but even fresh extracts prepared in the presence of inhibitors showed altered characteristics. Hence, these aldolase variants must be accorded an *in vivo* significance as well as an *in vitro* recognition.

Furthermore, although other major tissues such as heart, brain and muscle have not been demonstrated to exhibit appreciable degradative activity in these short term experiments, the more dramatic results in liver and intestine invoke the need for caution in excluding the possibility of long term *in vivo* modification. Certainly, mobility differences are observable for the aldolase isozymes both between separate tissues in the same animal and in the same tissues at different developmental stages (Herskovits *et al.*, 1967; Christen *et al.*, 1966). Modification of aldolase structure either *in situ* or during isolation of the enzyme from tissue extracts is, of course, of considerable significance to the structural analysis of this enzyme. It is apparent that end group determinations or tests of subunit identity could readily be distorted, and these factors must be held relevant to previous divergences from theoretical results.

Compounding the difficulties of analytical interpretation in this situation are the unusual mobility characteristics of aldolase in mixed tissue extracts. When equivalent activities of intestine and heart are mixed, for example, they migrate on starch not as additive patterns, but as single bands of intermediate mobility (Fig. 1c). This behavior is suggestive of association between similar proteins, and indicates that some of the common tests of protein homogeneity may be inadequate for aldolase. In this situation the presence of non-identical poly-

peptides in a single electrophoretic band might indicate not hybridization but, rather, the presence of distinct protein entities.

Another observation of considerable relevance to the problem of aldolase structure is the microheterogeneity which has been resolved in several vertebrate tissues by means of polyacrylamide gel electrophoresis. In mouse tissues, for example, Fig. 1c the pattern of multiplicity suggests the existence of two alleles or duplicates of the aldolase A gene. It is interesting that such a phenomenon would require the presence of polypeptides with different primary structures in the aldolase A region. This may explain the recent reports concerning nonidentical subunits in rabbit muscle aldolase C (Chan et al., 1967).

Microheterogeneity has been reported previously for mouse lactate dehydrogenase (Costello and Kaplan, 1963; Shaw and Barto, 1963) but as far more widespread in the vertebrate aldolase system, with most species so far investigated displaying multiple forms of aldolase A or aldolase C activity. It is also interesting that during the recent sequencing of carboxypeptidase, allelic forms were detected (Walsh et al., 1966).

In summary, then, these observations on the unusual properties of aldolase heteromorphs are preferred at this time in order to emphasize the complexity of this enzyme's multiplicity, the effects of tissue modification, isozyme association and microheterogeneity on the structural interpretations for this enzyme. Some of these characteristics of aldolase variformity may have wider implications in enzymology.

ACKNOWLEDGMENT -- The author is sincerely grateful to Prof. N. O. Kaplan for continued interest and encouragement.

References

Blostein, R. and Rutter, W. J., J. Biol. Chem. 238, 3280 (1963).
Chan, W., Morse, D. E., and Horecker, B. L., Proc. Natl. Acad. Sci. U. S. 57, 1013 (1967).
Christen, Ph. von, Hensing, U., Schmid, A., and Leuthardt, F., Helv. Chim. Acta 49, 1972 (1966).
Costello, L. A. and Kaplan, N. O., Biochim. Biophys. Acta 73, 658 (1963).
Herskovits, J., Masters, C. J., Wassarman, P. M., and Kaplan, N. O., Biochem. Biophys. Res. Commun. 26, 24 (1967).
Holmes, R. S., and Masters, C. J., Biochim. Biophys. Acta 132, 379 (1967).
Kawahara, K., and Tanford, C., Biochemistry 5, 1578 (1966).
Masters, C. J., Herskovits, J., and Kaplan, N. O. (1967). Submitted for publication.
Penhoet, E., Rajkumar, T., and Rutter, W. J., Proc. Natl. Acad. Sci. U. S. 56, 1275 (1966).
Shaw, C. R. and Barto, E., Proc. Natl. Acad. Sci. U. S. 50, 211 (1963).
Stellwagen, E., and Schachman, H. K., Biochemistry 1, 1056 (1962).
Walsh, K. A., Ericsson, L. H., and Neurath, H., Proc. Natl. Acad. Sci. U. S., 56, 1339 (1966).