STUDIES ON ALDOLASE ISOZYMES DURING AMPHIBIAN DEVELOPMENT

I. EVIDENCE FOR THE PRESENCE OF TWO TYPES OF ALDOLASE IN EGGS FROM Rana pipiens*

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The many enzymes which exist in multiple forms represent an interesting area of investigation with regard to possible changes during differentiation and development. Early studies in this field were concerned primarily with lactate dehydrogenase (Markert and Møller, 1959; Cahn et al., 1962). Recently, reports on the types of fructose-1,6-diphosphate+ aldolase present in fetal tissues of the fowl (Herskovitz et al., 1967) and rat (Rensing et al., 1967a) have appeared. On the basis of molecular and catalytic properties, three distinct types of animal FDP aldolase have been demonstrated. These are aldolase A, the classical muscle enzyme, aldolase B from liver, and aldolase C from rabbit brain (Penhoet et al., 1966) or from several of the major tissues of the chicken (Herskovitz et al., 1967) and of the rat (Rensing et al., 1967a). Five forms of aldolase have been demonstrated by chromatography and electrophoresis either after in vitro dissociation and reassociation of mixtures of each two-membered set of parental aldolases or in vivo in extracts of tissues (Penhoet et al., 1966). These results are

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+ The abbreviations used are: FDP, fructose-1,6-diphosphate; F1P, fructose-1-phosphate.

explained most readily on the basis of a molecule with four rather than three subunits.

Studies on the relationship between the types of aldolase and the stage of development in the amphibian are under investigation in this laboratory. In this report, evidence is presented for the presence of two types of aldolase in the eggs of Rana pipiens and these are compared to those found in adult tissues.

Materials and Methods. Gravid female grassfrogs (Rana pipiens) were killed by decapitation and brain, liver and muscle were removed. The fresh tissues were homogenized in two volumes (w/v) of 0.05 M Tris-HCl buffer, pH 7.5, containing 0.001 M EDTA and 0.01 M 2-mercaptoethanol. The two types of egg preparations used in these studies were eggs from the ovary of noninduced females (eggs from ovary) and those obtained after ovulation (eggs, spawned). In the latter case, gravid females were induced to ovulate by injection of a pituitary suspension according to the method of Rugh (1961). Eggs were stripped 24 to 48 hours later. The jelly coat was removed from the eggs before homogenization by the following procedure which is a slight modification of the method described by Gusseck and Hedrick[1]. Eggs were shaken gently with four volumes of 0.125 M 2-mercaptoethanol in 0.005 M Tris-HCl buffer, pH 7.5, for about 20 minutes. The buffer was changed three times during this treatment. Following the dejellying procedure, eggs were homogenized in an equal volume of 0.05 M Tris-HCl buffer, pH 7.5, containing 0.001 M EDTA and 0.01 M 2-mercaptoethanol. All homogenates were centrifuged at 100,000g for 40 minutes. The resulting supernatant solutions were passed through a column of Sephadex G-25, previously equilibrated with the same buffer, before enzymatic assay. Aldolase activity was assayed by the method of Blostein and Rutter (1963) except that 0.008 M FDP was used. Zone

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1 Gusseck, D. J., and Hedrick, J. L., personal communication.

electrophoresis was performed in 0.06 M sodium barbital buffer, pH 8.5, which contained 0.001 M EDTA and 0.0025 M 2-mercaptoethanol, on cellulose acetate strips (Gelman Sepraphore III, 2.5 X 17 cm) at 25 volts per cm for two hours at 4°C. The strips were stained for aldolase activity as follows: The staining solution contained 0.1 M Tris-HCl buffer, pH 7.5, 0.00015 M sodium arsenate, 0.001 M EDTA, 0.01 M FDP, or 0.03 M F1P, 0.001 M NAD, 0.12 mg per ml of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (Sigma), 24 µg per ml of phenazine methosulfate, and 0.5 mg per ml of nitroblue tetrazolium. A strip of cellulose acetate paper was soaked in this staining solution and then placed on a glass plate in a plastic box. After electrophoresis, the cellulose acetate strips containing the samples were placed on the staining strip and incubated in the dark at 37°C for 10 to 20 minutes for color development. After staining, both sample and staining strips were soaked in 1 per cent formalin for fixing.

Results and Discussion The FDP/F1P activity ratios for the various tissue extracts are given in Table I. In their studies, Blostein and Rutter (1963) used 0.01 M F1P in their assay system, however, as shown in Fig. 1 and Table I, aldolase in the tissues examined here is not saturated by this concentration of F1P. Therefore, as suggested by Rensing et al. (1967b), the activity ratios have been calculated on the basis of the apparent $V_{max.}$ for F1P obtained from the data of Fig. 1. The velocity of the reaction was demonstrated to be independent of FDP concentration at the level used in the assay system. The FDP/F1P activity ratios were lowest for liver and highest for brain aldolases. In these studies, muscle (aldolase A) was found to have a much lower activity ratio than values reported previously for this tissue (Blostein et al., 1963; Rensing et al., 1967a,b). The activity ratios for the egg preparations were intermediate between those of muscle and of brain. The apparent K_m values for F1P of aldolase from muscle, brain and egg were of the same order of magnitude, however, that from liver was

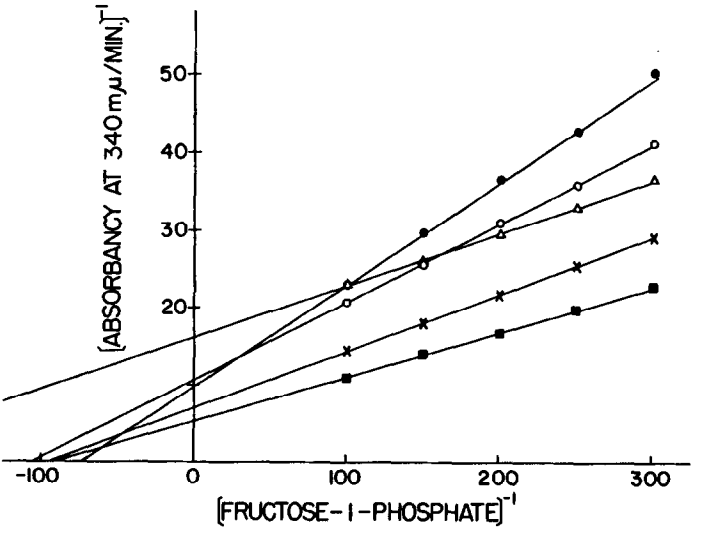

Fig. 1. Double reciprocal plots of 1/v with respect to 1/[F1P].
■, muscle; Δ, liver; x, brain; o, eggs from ovary; •, eggs, spawned.

Table I

Aldolase activity, FDP/F1P activity ratio, and K_m for F1P of frog tissue extracts.

Organ	Activity for FDP Units/mg tissue*	FDP/F1P activity ratio	K_m for F1P
Muscle	52	5.7	1.1×10^{-2} M
Liver	2.7	1.3	0.41 "
Brain	5.5	12	1.1 "
Eggs from ovary	1.1	8.6	0.95 "
Eggs, spawned		7.9	1.4 "

* One unit of aldolase activity was defined as that amount of enzyme which catalyzed the aldol cleavage of 1 micromole of FDP per minute under the conditions described in the assay procedure.

lower.

The electrophoretic patterns of aldolase from various organs of the

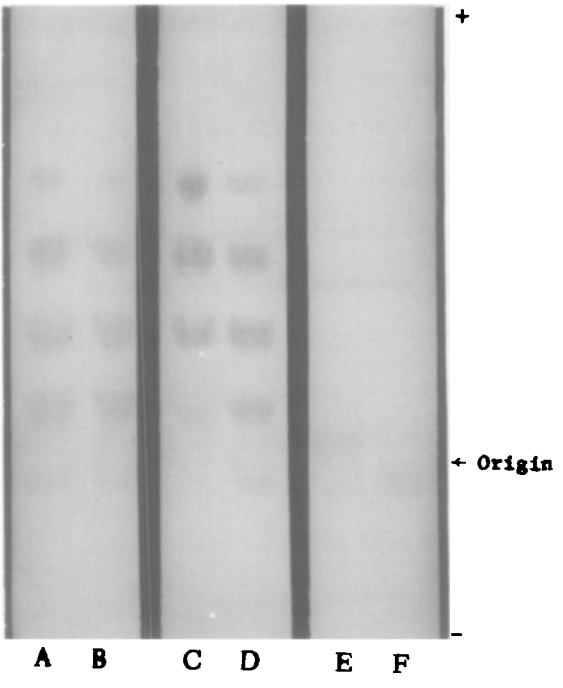

Fig. 2. Electrophoretic patterns of frog tissue aldolases.
A: Eggs from ovary.
B: Spawned eggs.
C: Brain.
D: Spawned eggs.
E: Liver.
F: Muscle.

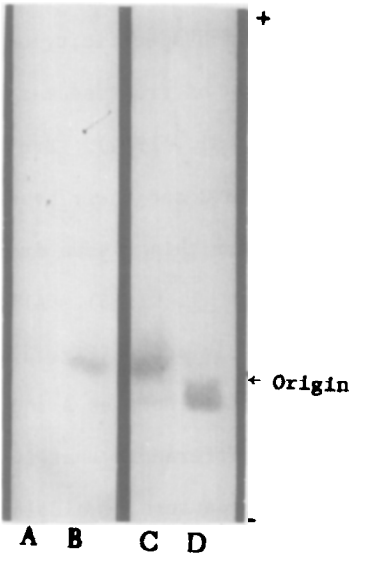

Fig. 3. Electrophoretic patterns of aldolase stained for F1P and FDP.
A: Muscle, stained for F1P.
B: Liver, stained for F1P.
C: Liver, stained for FDP.
D: Muscle, stained for FDP.

frog are shown in Fig. 2. The muscle preparation showed only one band of activity, which was localized at the most cathodic site, whereas liver gave two bands of activity, one coinciding with that of muscle (aldolase A) and a second, more anodic band, which was specific for liver (aldolase B). These results are in contrast to those found with aldolase B from rabbit (Penhoet et al., 1966) and rat (Rensing et al., 1967a), which had more cathodic mobility than aldolase A. The possible presence of hybrids between aldolase A and B was not detected in these experiments. The localization of aldolase

activity for F1P (Fig. 3) was the same as that of liver aldolase B. The muscle preparation could not be stained for F1P activity because of the low activity in this tissue. This observation also suggests that aldolase B from liver catalyzes the cleavage of both FDP and F1P. It has been proposed that the high specificity of aldolase B from liver for F1P may be related to the pathway of fructose metabolism in this tissue (Rutter et al., 1963; Spolter et al., 1964). Brain aldolase from the frog was composed of aldolases A and C and their hybrids. This is in agreement with the results obtained for this tissue from either rat (Rensing et al., 1967a) or rabbit (Penhoet et al., 1966). Aldolase C was present in greater concentration than aldolase A in brain (Fig. 2). Aldolase from egg was similar to that of brain in that both aldolases A and C and their hybrid forms were present. However, it differed in that type A predominated over type C. The electrophoretical pattern of aldolase from egg was intermediate between that of muscle and of brain as was the FDP/F1P activity ratio. In order to provide additional evidence that the aldolases from egg consists of types A and C, the egg preparation was mixed with either brain or muscle extract and then subjected to electrophoresis. The distribution of all bands of egg aldolase coincided with those of brain and muscle and resulted in an increased intensity of enzymatic staining. This observation suggests that the aldolases of egg are of the A and C types. Additional proof must await the results of immunochemical and other studies which are in progress.

There have been several reports concerning the changes in the electrophoretical patterns of aldolase isozymes during later stages of development. However, to our knowledge, the forms of aldolase present at earlier stages, i.e. in egg, have not been investigated previously. Rensing et al. (1967a) have demonstrated the predominance of aldolase A (muscle type) in most fetal tissues of the rat followed by a shift to the organ-specific type of aldolase with development. On the other hand, Kaplan's group has reported the presence of aldolase C, as well as A, in embryonic muscle of the

chick (Herskovitz et al., 1967). The present studies provide evidence for the existence of two parent types of aldolases, A and C, together with their three hybrids, in the egg of the frog. Similarly, multiple forms of malate dehydrogenase have been demonstrated in unfertilized sea urchin eggs (Patton et al., 1967). However, a single lactate dehydrogenase isozyme (heart type) has been observed in mouse ova from the unfertilized ovum to the blastocyst stage (Auerbach and Brinster, 1967; Rapola and Koskimies, 1967), although the early embryonic tissues of mammals contain predominantly the muscle type (Markert and Ursprung, 1962). The number of malate dehydrogenase isozymes in sea urchin eggs was shown to decrease following fertilization (Patton et al., 1967). Possible changes in aldolase types in frog eggs upon fertilization and during early developmental stages are under investigation.

REFERENCES

Auerbach, S. and Brinster, R. L., Exp. Cell Res., 46, 89 (1967).
Blostein, R. and Rutter, W. J., J. Biol. Chem., 238, 3280 (1963).
Cahn, R. D., Kaplan, N. O., Levine, L., and Zwilling, E., Science, 136. 962 (1962).
Herskovitz, J. J., Masters, C. J., Wasserman, P. M., and Kaplan, N. O., Biochem. Biophys. Res. Commun., 26, 24 (1967).
Markert, C. L. and Møller, F., Proc. Natl. Acad. Sci., 45, 753 (1959).
Markert, C. L. and Ursprung, H., Develop. Biol., 5, 363 (1962).
Patton, G. W., Jr., Mets, L., and Villee, C. A., Science, 156, 400 (1967).
Penhoet, E., Rajikumar, T. V., and Rutter, W. J., Proc. Natl. Adac. Sci. U. S., 56, 1275 (1966).
Rapola, J. and Koskimies, O., Science, 157, 1311 (1967).
Rensing, U., Schmid, A., and Leuhardt, F., Hoppe-Seyler's Z. Physiol. Chem., 348, 921 (1967a).
Rensing, U., Schmid, A., Christen, P., and Leuhardt, F., Hoppe-Seyler's Z. Physiol. Chem., 348, 1004 (1967b).
Rugh, R., "Experimental Embryology: A Manual Techniques and Procedures", Burgess, Minneapolis, Minn., 1961, p. 102.
Rutter, W. J., Blostein, R. E., Woodfin, B. M., and Weber, C. S., in Weber, G. (Editor), Advances in Enzyme Regulation, vol. 1, Pergamon Press Ltd., London, 1963, p. 39.
Rutter, W. J., Federation Proc., 23, 1248 (1964).
Spolter, P. D., Adelman, R. C., DiPietro, D. L., and Weinhouse, S., in Weber, G. (Editor), Advances in Enzyme Regulation, vol. 2, Pergamon Press Ltd., London, 1964, p. 79.