ALDOLASE ISOZYMES IN RAT TUMOR CELLS

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We have previously reported that the activity ratios (fructose 1,6-diphosphate/fructose 1-phosphate = FDP/FIP) of aldolase (EC 4.1.2.13) in Morris minimum deviation hepatomas were 3-6 and those of Yoshida ascites hepatomas were 40-60, and suggested that Morris hepatoma contained both liver- and muscle-types of aldolase, and Yoshida hepatoma contained muscle-type aldolase instead of liver-type aldolase (Sugimura, et al., 1966). Recently, the multiplicity of aldolase in vertebrate organs has been reported (Penhoet et al., 1966: Christen et al., 1966: Foxwell et al., 1966: Anstall et al., 1966: Pietruszko and Baron, 1967: Herskovits et al., 1967). The three distinct aldolases (A,B and C) are present in vertebrate. Aldolase molecule is composed of four subunits (Kawahara and Tanford, 1966: Morse et al., 1967: Penhoet et al., 1967: Rensing et al., 1967). The three molecular hybrid forms constructed from the different subunits of each pair are demonstrated in brain, liver and kidney.

Aldolase isozymes in rat hepatoma were reinvestigated using the antibody for crystalline rat liver or muscle aldolase and by

the electrophoresis using cellulose acetate membrane. This report deals with the presence of the liver- and muscle-types of aldolase and the three hybrid molecules each formed from subunits of aldolase A and B in Morris hepatomas, and suggests that the genes for both aldolase A and B are functioning in a cell of Morris hepatoma.

Materials and methods: Crystalline rat liver aldolase was prepared as previously reported (Matsushima et al., 1968) and crystalline rat muscle aldolase was obtained by the method of Taylor et al.(1948). Aldolase activity was determined by spectrophotometric measurement of the oxidation of $NADH_2$ at 340 mμ using α-glycerophosphate dehydrogenase according to a slightly modified method of Blostein and Rutter (1963).

Morris minimum deviation hepatomas (7316A and 7793) were transplanted intramuscularly in both hind legs of Buffalo rats and trimed carefully. Yoshida ascites hepatoma (AH 130) was transplanted intraperitoneally into Donryu rat and harvested by centrifuging and washing with a cold saline. Rhodamine sarcoma was transplanted subcutaneously in back of Wistar rat and excised carefully. The fresh tissues were homogenized with two volumes of 0.15 M KCl—20 mM Tris—1 mM EDTA (pH 7.4). The homogenates were centrifuged at 105,000x g for 60 min. and the supernatants were used as the enzyme solution.

Anti-γ-globulins were prepared in a hen by intramuscular injection of crystalline rat liver or muscle aldolase with Freund's complete adjuvant and by booster-injection of saline solution of crystalline enzyme into vein. Anti-γ-globulin fractions were obtained from serum by ammonium sulfate fractionation and DEAE-cellulose chromatography (Campbell et al., 1963), and stored at -80°C until used. Inhibition of enzymatic activity by anti-γ-globulin was determined as follows. Enzyme solution mixed with the equivalent amount of anti-aldolase γ-globulin was incubated at 30°C for 2 hrs. and then at 4°C overnight in borate buffer—0.4 M NaCl

(pH 8.4). Supernatants separated from precipitates by centrifugation were determined for enzymatic activity.

Electrophoresis on cellulose acetate membrane (Gelman, 1 × 63/4") was carried out using varonal buffer(pH 8.6, I=0.05) containing 10 mM β-mercaptoethanol at 250 V for 3 hrs. Enzyme solution (3 µl) was placed at the center of the strips. The strips were stained for aldolase activity by a slight modification of the method of Penhoet et al.(1966). Noble agar solution (1 %) contained 5 mM Na_2HAsO_4, 10 mM FDP-tetracyclohexylammonium salt (Boehringer), 1 mM NAD (Sigma), 50 µg/ml glyceraldehyde-3-phosphate dehydrogenase (Boehringer), 25 µg/ml triosephosphate isomerase (Boehringer), 25 µg/ml phenazine methosulfate (Sigma), 500 µg/ml nitroblue tetrazolium chloride (Sigma) and 75 mM Na-pyrophosphate —0.75 mM EDTA (pH 7.5) was used. For a blank reaction, a second strip was stained with the same staining agar without substrate. No band was stained using 5 mM Na_2HAsO_4 in the present experiment, instead of 10 mM in the original method of Penhoet et al. (1966). This result indicates that "nothing dehydrogenase" (Shaw and Koen, 1965) is not stained in our experimental condition.

Results and discussion: Table I shows the results of inhibition of aldolase activity of rat tumors by antibody for liver aldolase or muscle aldolase. Aldolase in Morris hepatoma was inhibited to about the same degree by both anti-aldolase. This suggests that the large portion of aldolase in Morris hepatoma is the hybrid molecules composed of subunit of aldolase A and B, and both aldolase A and B are also presented in small amount. Aldolase in Yoshida hepatoma or Rhodamine sarcoma was considerably inhibited by anti-liver aldolase and completely by anti-muscle aldolase. This indicates the presence of aldolase A and hybrid molecules composed of subunit of aldolase A and B in these tumors.

This observation was confirmed by electrophoresis of the same enzyme solution used for immuno-analysis. Figure 1 shows the isozyme patterns of aldolase in those rat tumors. The three major bands of hybrid molecules composed of subunits of aldolase A and B were celarly observed in both Morris hepatoma, 7316A and 7793. The activity of the middle band of hybrids was higher than other two hybrid bands. The bands of aldolase A and B were also observed

TABLE I. The Inhibition of Aldolase Activity by Antibody for Liver or Muscle Aldolase

	% Inhibition by	
	anti-liver aldolase	anti-muscle aldolase
Liver	100	4
Morris hepatoma 7793	97	84
Yoshida hepatoma AH 130	41	100
Rhodamine sarcoma	47	100
Muscle	10	94
Crystalline liver aldolase	100	0
Crystalline muscle aldolase	0	100

in Morris hepatoma. These results indicate that the genes for both aldolase A and B are functioning in each cell of Morris hepatoma and hybrid molecules are constructed from each subunits of aldolase A and B within a single cell. The presence of the mosaic cell population of different types of cells which contained either aldolase A or B is ruled out. The major band of aldolase A and

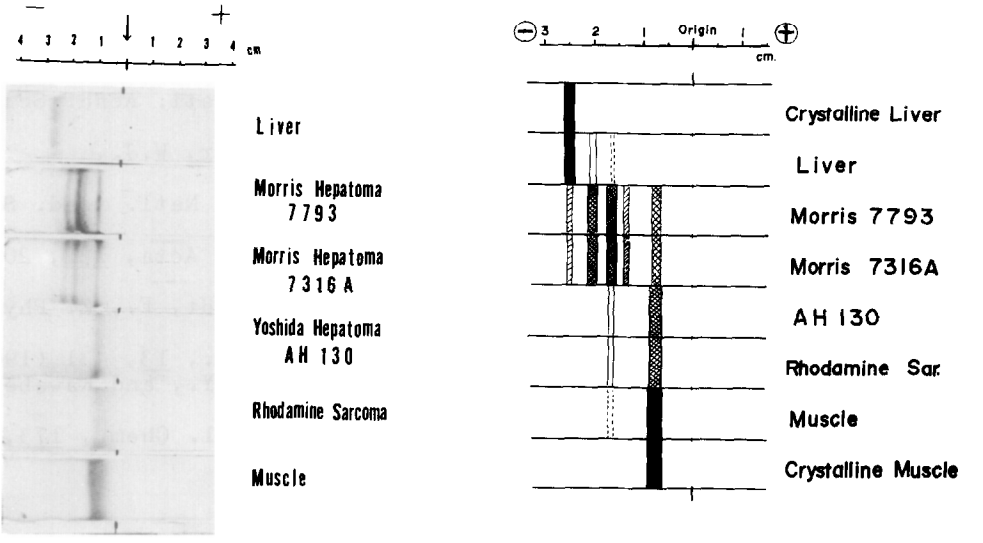

Figure 1. Isozyme patterns of aldolase and their tracings

Electrophoresis on cellulose acetate in veronal buffer pH 8.6 at 250 V for 3 hrs.

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one minor band of hybrid molecule were observed in Yoshida hepatoma and Rhodamine sarcoma.

These results indicate that the molecular species of aldolase different from those in the normal liver appeares in hepatoma, and support the idea of disdifferentiation that the switch-off of gene for aldolase B and switch-on of gene for aldolase A are produced during the hepato-carcinogenesis (Sugimura et al., 1966).

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