RESURGENCE OF TWO FETAL-TYPE OF ALDOLASES (A and C) IN SOME FAST-GROWING HEPATOMAS

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SUMMARY

We have previously demonstrated the resurgence of aldolase "A" (Muscle type) in hepatomas. In this communication we shown that in some solid transplantable hepatomas, this aldolase "A" is hybridized with a third type of aldolase present also in fetal-liver. The kinetic and electrophoretic properties of this third type of aldolase are identical to those found in aldolase "C" (Brain type).

Three molecular forms of Fructose-1-6-diphosphate aldolase (E.C. 4.1.2.7) have been detected in tissues of mammals. Aldolase "A" (Muscle type) acts primarily on Fructose-1-6-diphosphate (F-D-P) and weakly on Fructose-1-Phosphate (F-1-P) ; the aldolase activity ratio (F-D-P/F-1-P) of aldolase "A" is greater than 50. Aldolase "B" (Liver type) has the same activity on the two substrates (aldolase activity ratio about 1 in normal adult liver)(2)(11). The third, more recently discovered, aldolase "C", is found almost exclusively in the brain (9) ; it is more negatively charged than the two other types, and its aldolase activity ratio is intermediate (between 5 and 10).

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The three types of aldolase are true isozymes ; their synthesis is ruled by different genes and may occur in the same cell, as proved by hybrid formation. Aldolase "A", "B" and "C" appear to be tetrameric molecules (7), and hybrids between A and C form five isozymes in the brain.

One of us had reported in 1962, with G.Schapira and J.C.Dreyfus, that hepatoma aldolase is abnormal and that its properties are similar to those of muscle aldolase and even more to those of fetal liver aldolase (13)(14). The aldolase activity ratio in human and experimental (ascitic) hepatoma was shown to be very high (mean 6.25 $\pm$ 1.6 in human hepatoma, instead of 1.0 to 1.1 in normal adult liver). We have suggested that aldolase "A", which predominates in fetal liver, might be derepressed in hepatoma (14). Our subsequent work, with Y.Nordmann, has strenghtened this hypothesis, showing by electrophoretic and immunological methods that the increase of aldolase "A" activity is actually due to an increased amount of the enzyme protein : it is not due to an activation phenomenon (5)(6).

Our findings were confirmed by Matsushima et al.(3). Brox et al. (1) have recently demonstrated that amino acid composition and tryptic finger-prints of aldolase of an ascitic hepatoma were identical to those of muscle aldolase.

We have recently found supplementary, anodic isozymes in human fetal liver, probably type "C" (12) and, according to our working hypothesis, we have looked for similar isozymes in hepatomas.

MATERIAL AND METHODS

Rat hepatomas were solid transplantable tumors previously described (10) : H 175, H 122 and H 189 were slow-growing while H 178 were fast-growing hepatomas. Normal livers were from the same strain, or from carrier animals. Fetal livers were removed at 17-20th day.

Aldolase was determined colorimetrically by a modification of the method of Sibley and Lehninger (15). Horizontal electrophoresis was performed in phosphate citric buffer (pH 7.0) containing 0.01 M β-mercapto-ethanol in starch gel at 3 V/cm for 16 hours at 4° (3). After electrophoresis the aldolase isozymes are localized on the sliced gel, by specific staining based on reduction of tetrazolium salts in a coupled reaction with phosphoglyceraldehyde dehydrogenase (with NAD as coenzyme). Specificity of reaction was tested by omitting the substrate in the mixture.

Specific antiserum against aldolase "B" was prepared in chicken from liver rabbit aldolase. Liver aldolase was prepared according to Morse and Horecker (4). Specificity and purity of antisera were tested by double diffusion tests (in agar gel with 8 % NaCl) and also by specific aldolase coloration of precipitate line. Antialdolase anti "B" shows a strict type specificity, but no significant species specificity.

Inhibition experiments were performed by incubating one hour at 37°C , and then 24 hours at 4° tissues extracts with equivalent amounts of antisera ; supernatants after centrifugation were essayed for enzymatic activity, with serum of normal chicken as control.

Partial purification of aldolase hepatomas was performed according to Penhoet et al. (7)(8) for isolation of aldolase "C".

RESULTS

Table I shows the mean aldolase activities of hepatomas H 178 compared to normal and fetal liver. One can see the increase of F-D-P activity which represents total aldolase activity (17.0 $\pm$ 1.05 instead of 7.8 $\pm$ 0.9). More interestingly the aldolase activity ratio is considerably increased (mean 24 instead 1.1) and very similar to the ratio found in brain : 23. On the contrary, we have found

Table I

	Aldolase Activities (in I.U.)		Ratio F-D-P/F-1-P
	F-D-P	F-1-P	
Normal Liver	7.8 ± 0.9	7.0 ± 0.6	1.1
Fetal Liver (17 day to 21th day)	4.2 to 7.8	1.0 to 5.0	1.7 to 6.0
Brain	14.3 ± 1.0	0.6 ± 0.04	23
Slow growing Hepatomas			
H 122	3.6	1.7	2.1
H 175	4.0	3.08	1.3
H 189	12.1	1.2	1.68
Fast growing Hepatoma			
H 178	17.0 ± 1.05	0.70 ± 0.03	24

that aldolase activity ratio in slow-growing hepatomas is between 1.3 and 2.1.

Figure 1 shows the electrophoretic pattern of aldolase isozymes H 178, compared to normal rat liver, fetal liver, brain and muscle aldolases. Figure 2 shows the pattern of aldolase isozymes in some slow-growing hepatomas.

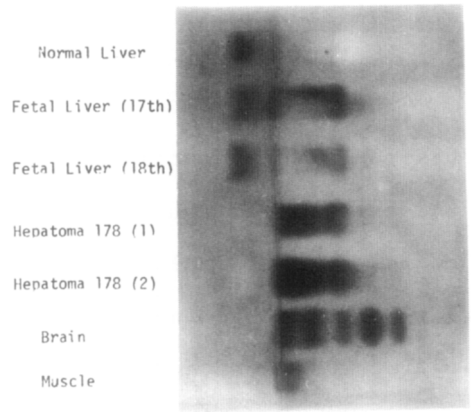

Figure 1 : Aldolase isozymes of fast-growing hepatoma.

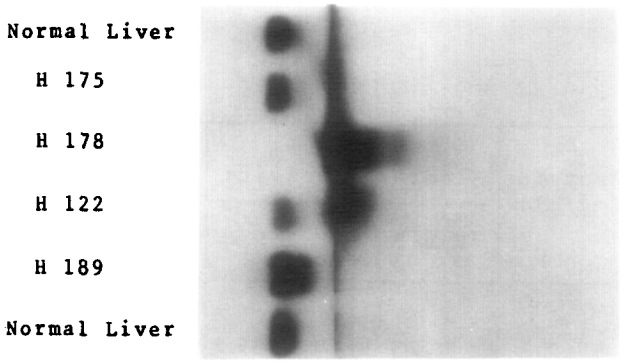

Figure 2 : Aldolase isozyme in slow-growing hepatomas.

It can be seen that hepatomas fall into two categories: slowly growing hepatomas (H 122, H 178 and H 189) display a pattern similar to that of normal liver, with the presence of some aldolase "A" in addition to that of aldolase "B". By contrast, aldolase "B" is lacking in fast-growing hepatoma 178. Anodic isozymes are visible, which are very similar to brain isozyme (hybrids of A and C aldolases). Bands A_3C_1 and A_2C_2 are heavily stained (fig.1) but fainter bands corresponding to A_1C_3 and even pure C_4 isozyme are apparent.

This pattern can be compared to that of fetal liver (12)(fig.1), which shows hybrids A and C in addition to the band of aldolase "B".

In preliminary experiments, we have begun to purify hepatoma aldolase. We have obtained a peak, the order of elution (by NaCl gradient) of which is identical to that of aldolase "C". We have studied its aldolase activity ratio, and the action of antiserum antialdolase "B". Table II shows our results.

Table II shows that this peak contains an aldolase with relative activity towards F-1-P greater than the total extract and which is not at all inhibited by antiserum antialdolase "B". We recall that a relatively high activity towards F-1-P could be due to the pre-

Table II

Fraction	Protein (mg/ml)	Specific Aldolase Activity		Aldolase Activity ratio	Percentage inhibition by antialdolase B
		F-D-P	F-1-P		
Total Extract	56,2	0,23	0,0089	25,8	4
Peak "3"	0,05	3,2	0,32	10	0

sence, with aldolase "A", of some aldolase "B". It is clear from our data that this low aldolase activity ratio is due to the presence of another type of aldolase.

DISCUSSION

The data here presented confirm our previous results : aldolase "B" is lacking or almost lacking in some fast-growing hepatomas, while aldolase "A" is increased. Moreover, we give evidence for a third type of aldolase, characterized by its anodic migration, both in fetal and in cancerous liver. We believe that this aldolase strikingly similar to the brain aldolase is a hybrid of aldolase "A" (Muscle type) and aldolase "C" (Brain type), although, at the present time, we are not yet able to get immunological evidence other than negative for its nature. Similarly, the anodic bands are similar to the isozymes we find in fetal liver. Consequently we advance the hypothesis of the derepression, in some fast-growing hepatomas of two types of aldolases, "A" and "C" (present in fetal liver and repressed in adult liver), at the expense of the normal adult aldolase.

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This manuscript was to be published when appeared the paper of Sugimura et al. (Biochem. Biophys. Res. Comm., 39, 626 (1970)) who find results similar to ours.