THE PRESENCE OF ALDOLASE C IN RAT HEPATOMA

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SUMMARY

Aldolase A and hybrid molecules between aldolases A and C in a strain of Yoshida ascites hepatoma, AH143A of rat were found by electrophoresis on a cellulose acetate membrane. Results of column chromatography and inhibition by anti-aldolase A confirmed the presence of A3C hybrid molecule in this hepatoma. The significance of the aldolase C peptide in the hepatoma is discussed in relation to dis-differentiation.

The formation of the muscle type aldolase (aldolase A) in experimental rat hepatomas, instead of the normal liver type aldolase (aldolase B), has been reported from several laboratories (1,2,3,4,5,6). The aldolase in Novikoff hepatoma was identified as normal muscle aldolase from its amino acid composition and from tryptic finger prints (7,8). Previously, we reported finding aldolase A, aldolase B and their molecular hybrids in slowly growing Morris hepatomas, indicating that the genes for aldolases A and B are both active in single cells of these hepatomas (9). The presence of similar hybrid molecules in human hepatomas was also reported (10).

This paper reports the presence of the brain type of aldolase (aldolase C) in a certain strain of Yoshida ascites hepatoma of rat. Aldolase C has been reported to exist in rat brain and nerve tissue but not in rat liver (11).

MATERIALS AND METHODS

Chemicals ---- Fructose 1,6-diphosphate tetracyclohexylammonium salt, glyceraldehyde 3-phosphate dehydrogenase and a mixture of glycerol 1-phosphate dehydrogenase and triosephosphate isomerase were purchased from Boehringer Mannheim Japan, K. K., Tokyo. NAD, $NADH_2$, nitro blue tetrazolium and phenazine methosulfate were obtained

from Sigma Chemical Co., St. Louis, Mo., and crystalline aldolase A was prepared from muscle by the method of Taylor (12).

Anti-γ-globulin ---- An emulsion of crystalline aldolase A in Freund's complete adjuvant was injected intramuscularly into a hen. The gamma globulin fraction was separated by ammonium sulfate fractionation and DEAE-cellulose column chromatography (13).

Hepatoma cells ---- Yoshida ascites hepatoma cells were inoculated intraperitoneally and one week later, ascites cells were harvested and washed with cold saline.

Aldolase assay ---- The activity of aldolase was determined spectrophotometrically at 340 mμ by the method of Blostein and Rutter (14) in the presence of glycerol 1-phosphate dehydrogenase and triosephosphate isomerase.

Immunological assay ---- Aldolase solution was mixed with an appropriate amount of anti-aldolase A in 8 % NaCl - 0.05 M borate buffer (pH 8.4) and kept at 30°C for 2 hr and then at 4°C overnight. The mixture was centrifuged at 3,000 rpm for 30 min and activity in the supernatant fraction was assayed.

Electrophoresis ---- Electrophoresis was carried out on a cellulose acetate membrane (Gelman, 1 x 6 3/4") in veronal buffer (pH 8.5, I = 0.05) containing 10 mM β-mercaptoethanol at 250 V for 3 hr. Membranes were stained for aldolase activity by the method of Penhoet et al. (11) with a slight modification.

Separation of A - C hybrids ---- A total of 14.3 g of brain was obtained from 10 normal male Donryu strain rats and 12.3 g of AH143A cells were collected from 8 male rats of the same strain after inoculation with hepatoma cells. The brains were homogenized in an equal volume of 20 mM Tris-HCl buffer (pH 7.6) in a Potter-Elvehjem type homogenizer. Ascites hepatoma cells were homogenized in an equal volume of the same buffer using a Vir-Tis 45 type homogenizer. In both cases the homogenate was centrifuged at 18,000 x g for 30 min and the fraction of the supernatant precipitating between 0.35 and 0.60 saturation of ammonium sulfate was obtained. The precipitates were dissolved in 2 ml of 10 mM Tris-HCl buffer (pH 8.6) containing 1 mM EDTA and 0.5 mM FDP, dialysed against the same buffer, and applied to columns of DEAE-cellulose equilibrated with 10 mM Tris-HCl buffer (pH 8.6) - 1 mM EDTA - 0.5 mM FDP. The column sizes were 1 x 10 cm and 1.6 x 18 cm for brain and hepatoma cell extracts, respectively. The columns were washed with the same solution and eluted with a linear gradient of 0 to 0.15 M NaCl in the same buffer (15,16).

RESULTS AND DISCUSSION

The isozyme patterns of the aldolases of rat brain and Yoshida ascites hepatomas AH100B and AH143A on cellulose acetate membrane electrophoresis are shown in Fig. 1. AH100B cells contained only the A type of aldolase, as reported previously for the most rapidly growing strains of Yoshida ascites hepatomas, including AH130 (9). Brain contained five bands of aldolase : aldolase A, aldolase C and three molecular hybrids of aldolases A and C represented as A_3C, A_2C_2 and AC_3 (15). The extract of AH143 showed a dominant band of aldolase A, a distinct band corresponding to the A_3C hybrid and a faint band corresponding to the A_2C_2 hybrid. A very faint band was also present

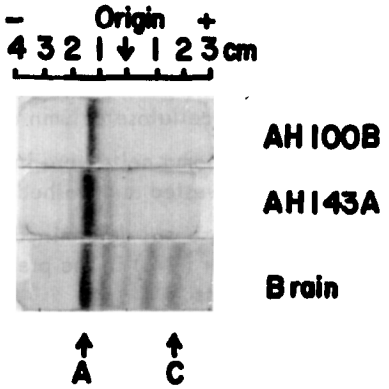

FIG. 1. Aldolase isozyme patterns of Yoshida ascites hepatomas and rat brain.

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further towards the cathode than the band of aldolase A, which may be a hybrid of aldolase A and aldolase B, A_3B, though this has not been verified.

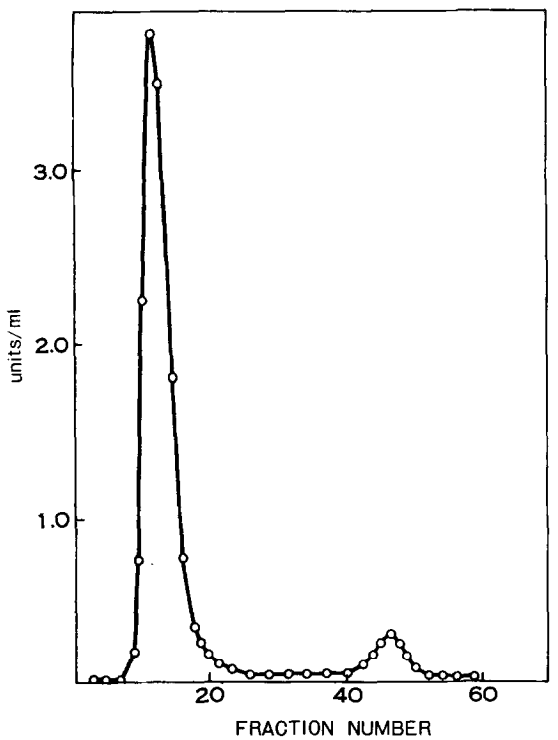

FIG. 2. Elution pattern of AH143A aldolase on DEAE-cellulose column chromatography. The column to which the enzyme solution had been applied was eluted with 10 mM Tris-HCl buffer (pH 8.6) containing 1 mM EDTA and 0.5 mM FDP. After the 40th fraction it was eluted with a linear gradient of 0 to 0.15 M NaCl in the same buffer.

On DEAE-cellulose column chromatography of rat brain aldolase, five distinct peaks (I to V) of aldolase were obtained : aldolase A, three hybrid molecules (A_3C, A_2C_2 and AC_3) and aldolase C, respectively, as reported by Nicholas et al. (16). Fig. 2 shows the elution pattern of the aldolase of AH143A on DEAE-cellulose column chromatography. Two distinct peaks were obtained. The aldolase activities in Peaks I and II migrated similarly to aldolase A and the A_3C hybrid from brain, respectively on electrophoresis on a cellulose acetate membrane.

Table I, shows the percentage inhibition of the aldolase activity by anti-aldolase A in each peak separated with DEAE-cellulose column chromatography. The aldolase activities of the Peak I's of brain and AH143A cells were completely inhibited by anti-aldolase A. Aldolase activities of the Peak II's of brain and AH143A cells were also inhibited to the same extent by anti-aldolase A.

TABLE I. Percentage inhibition by anti-aldolase A of aldolase activity in each peak from a DEAE-cellulose column.

Peak	I	II	III	IV	V
Brain	100	88	43	10	0
AH143A	100	89	—	—	—

These results of electrophoresis, column chromatography and inhibition by anti-aldolase A clearly indicate the presence of the A_3C hybrid in a strain of Yoshida ascites hepatoma, AH143A. Thus the gene for aldolase C functions in these cells.

Twenty strains of Yoshida ascites hepatomas were examined, and some other strains besides AH143A also gave a band corresponding to the A_3C hybrid, as shown in a case of AH66F. Thus the presence of aldolase C in rat hepatomas seems fairly common.

Previously we proposed the term dis-differentiation (3) for phenomena such as the switch off of the gene for aldolase B and switch on of the gene for aldolase A during hepato-carcinogenesis implying a disordered pattern on gene expression in neoplastic cells. The existence in hepatoma cells of aldolase C normally found only in brain and nerve tissue, provides additional support for the concept of dis-differentiation.

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