PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 410–413 (1975)

Immunochemical Pattern of Aspartate Aminotransferase Isozymes in Several Rodents and in Ehrlich Ascites Cells[1] (38549)

LEVY KOPELOVICH
(Introduced by M. Lipkin)

Laboratory of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

The immunochemical analysis of enzymes and their isozymic forms has contributed greatly to the understanding of regulatory mechanisms affecting the structure of proteins and of ontogenetic and evolutionary processes (1). Isozymic profiles have also been used extensively for the elucidation of neoplastic transformation (2, 3). As part of a study of aspartate aminotransferase isozymes (L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1.) (AAT) (4–8), we report in the present work on the enzymic and immunochemical pattern of aspartate aminotransferase isozymes in the liver of several rodents and in Ehrlich ascites cell tumor line from C3H mice (EAC). Study of the immunologic interaction of isofunctional enzymes isolated from different species might indicate the degree of similarity of different regions in the protein molecules (1). The occurrence of two immunologically distinct AAT isozymes that exist in different cellular compartments has been previously described (9). This may represent a regulatory phenomenon and, possibly, a reflection of the site at which these two isozymes are synthesized.

Materials and Methods. Animals and their treatment. The animals examined were adult males, weighing 150–200 g, and consisted of the following species: BUF/Sim rat; C3H/HeJ mouse; Beii (Syr) hamster and MON/Tum gerbil. They were fed Purina Laboratory Chow and water *ad libitum*. Animals were decapitated and their livers excised and washed free of blood. The livers were weighed, minced and homogenized in 9 vol of 5 m*M* Tris buffer, 5 m*M* EDTA, pH 7.4, with a Potter–Elvehjem homogenizer. The homogenates were sonicated for 30 sec at 4 mA in a 60-W, 20 kc/sec MSE Ultrasonic disintegrator. Following an overnight freeze at −20°, these homogenates were centrifuged at 20,000 *g* for 20 min and the resulting supernatant was used for all subsequent AAT assays.

Ehrlich ascites cells were obtained as described by Green and Dobrjansky (10). The cells were washed three consecutive times by suspension and centrifugation at 800 *g* for 5 min in 5 m*M* Tris buffer, 5 m*M* EDTA, pH 7.4, then homogenized in 4 vol of the same buffer with a tight-fitting glass homogenizer. This was followed by sonication for 60 sec at 4 mA, an overnight freeze at −20° and centrifugation at 20,000 *g* for 20 min. The resulting supernatant was used for all subsequent AAT assays.

Enzyme assay. The activity of AAT isozymes was determined as described previously (4), except that 0.1 *M* Tris buffer, pH 7.4, was used instead of barbital buffer. Activity was expressed as μmoles/g/min and as μmoles/ml packed cells/min in the liver and EAC respectively.

Immunochemical procedures. Specific antisera directed against the rat liver anionic isozyme and against the cationic isozyme were prepared in rabbits as described elsewhere (11). Immunochemical analysis of the isozymes was carried out by the double immunodiffusion (Ouchterlony) technique in an agar matrix (Hyland) and by immunoelectrophoresis on microscopic slides coated with 4 ml of 2% agar. Electrophoresis was carried out in barbital buffer (i = 0.05), pH 8.2, at 6 mA per slide. After 4 hr at 4°, slides were removed and a strip of Whatman № 4 filter paper, 1 mm wide, soaked with the appropriate antiserum, was placed on the agar. Precipitin lines were clearly visible after slides had been allowed to develop for 2 days in a humidified chamber at room temperature.

The tissue content of each isozyme was

[1] This work was supported in part by Grant No. CA-08748 from the National Cancer Institute, National Institutes of Health, and Grant No. T-431L from the American Cancer Society.

TABLE I. ACTIVITY PATTERN OF AAT ISOZYMES IN THE LIVER OF SEVERAL RODENTS AND IN EAC.

Animal	Tissue	Activity of AAT isozymes[a]		
		Cationic	Anionic[b]	Total[c]
Rat	liver	218.7 ± 9.6	42.1 ± 2.3 (16)	260.1 ± 15.7
Mouse	liver	436.3 ± 10.1	84.6 ± 1.7 (16)	518.5 ± 11.6
Hamster	liver	310.4 ± 21.2	49.0 ± 4.6 (14)	356.0 ± 25.1
Gerbil	liver	417.7 ± 19.3	123.9 ± 9.7 (23)	540.4 ± 31.9
Mouse	EAC	43.5 ± 1.5	8.3 ± 0.9 (16)	51.4 ± 4.6

[a] Results are presented as the mean of three or more experiments on separate animals and their standard errors. Activity is expressed as μmoles/g/min and as μmoles/ml/min for the livers and EAC respectively.

[b] Figures in parenthesis denote activity of anionic isozyme as % of total activity of AAT isozymes.

[c] Determination of total activity of AAT isozymes was independent of that of the cationic and anionic isozymes.

assayed with appropriately diluted supernatants incubated with either the anticationic or the antianionic antiserum in amounts which had been determined to give complete inactivation of the homologous isozyme. After 90 min at 37°, followed by 2 hr at 4°, the antiserum-tissue extract mixture was centrifuged and the residual AAT activity in the supernatant was determined.

Results and Discussion. Table I shows the activity levels of AAT isozymes in the livers of the rat, mouse, hamster, gerbil and in the EAC. In all rodents and in the EAC, the separate activities of the anionic and cationic isozymes, measured immunochemically, agree closely with the total activity of the AAT isozymes. Except for the gerbil, the activity of the anionic isozyme expressed as a fraction of the activity of total AAT isozymes was about the same in all species investigated (Table I). In the gerbil, this value was about 23% of the total activity of both isozymes (Table I). High levels of the anionic isozyme have also been reported in various other tissues and in several hepatomas (12).

The observation that the activity of AAT isozymes in the livers of the various species and in the EAC was inhibited by antisera directed against rat liver AAT isozymes led us to investigate the degree of immunological cross-reactivity of each AAT isozyme from the various rodents. A double immunodiffusion precipitin test showed that, except for the gerbil, there was a pattern of identity of AAT isozymes in the presence of either the antianionic or the anticationic antisera (Fig. 1). Interspecies cross-reactivity of several enzymes has been previously reported (1, 13). In the case of the gerbil, a pattern of partial identity was obtained with both the antianionic and the anticationic antiserum regardless of isozyme source in the adjacent wells (Fig. 1). Similar results have been obtained by the use of immunoelectrophoresis (Fig. 1). The gerbil anionic isozyme has migrated further toward the anode relative to the anionic isozymes from all other sources. In the case of the gerbil cationic isozyme, its migration toward the cathode was only slightly slower relative to the other cationic isozymes. Thus, as judged by the activity and the precipitin pattern of AAT isozymes (Table I, Fig. 1), the rat and the mouse are phylogenetically closely related to the hamster, but not to the gerbil.

It is of interest that the relative content of the anionic and cationic isozymes in the tumor cells grown in mice was proportionately similar to that observed in livers of the mouse, the rat and the hamster (Table I). The AAT isozymes in the EAC also showed a line of identity with those obtained from the livers of these rodents (Fig. 1). These cells grow in mice but not in the rat or the hamster (unpublished observations). The immunologic pattern of the AAT isozymes in the EAC grown ip in mice remained the same for 7 days, at which time the mice expired due to the growth of EAC in them (Fig. 1).

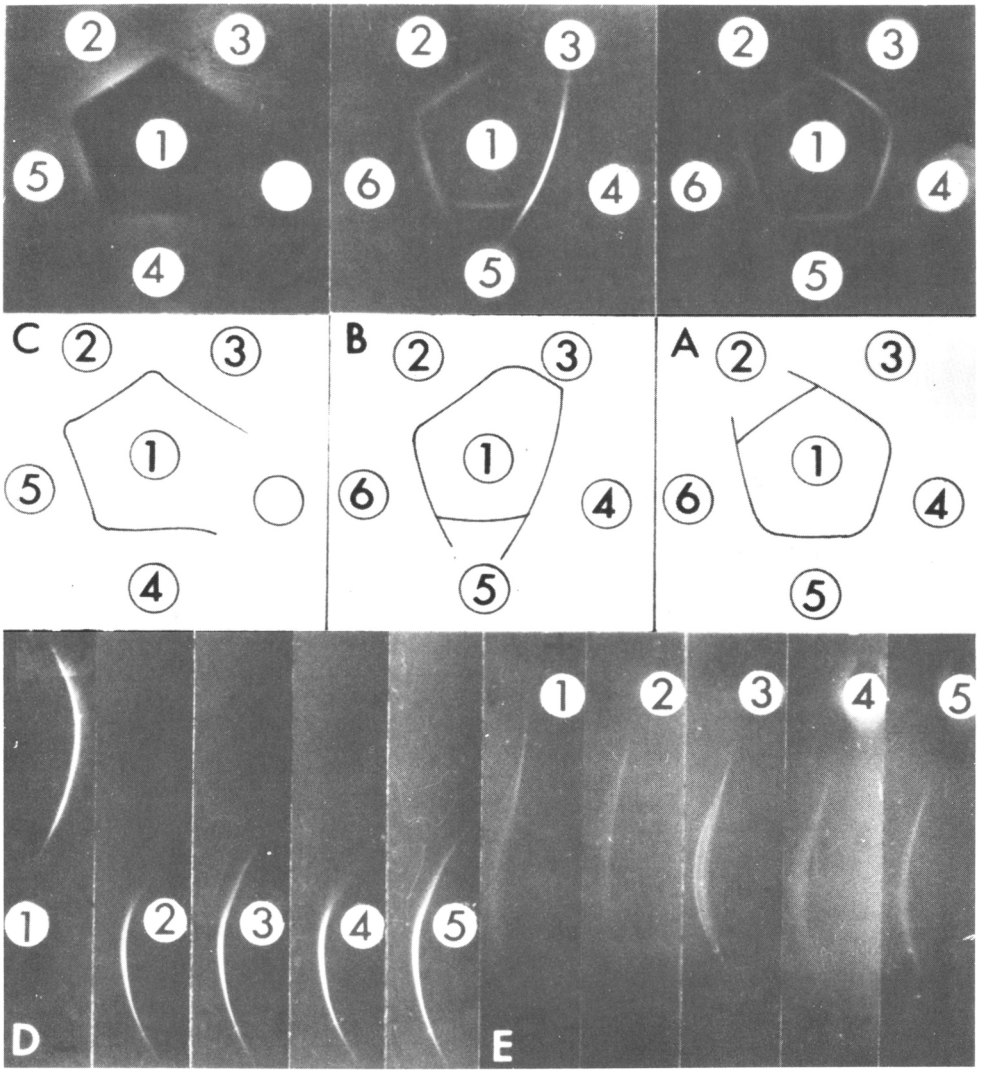

FIG. 1. Double immunodiffusion and immunoelectrophoretic precipitin patterns of AAT isozymes in rodents liver and EAC. A (1) Anticationic antiserum; (2) gerbil; (3) rat; (4) hamster; (5) EAC; (6) mouse. B (1) Antianionic antiserum; (2) hamster; (3) EAC; (4) rat; (5) gerbil; (6) mouse. C Immunologic pattern of AAT in EAC grown ip in the mouse; (1) antianionic antiserum; (2) 0 days; (3) 3 days; (4) 5 days; (5) 7 days. D (1) Gerbil; (2) rat; (3) hamster; (4) EAC; (5) mouse; all in the presence of antianionic antiserum. E (1) Gerbil; (2) rat; (3) hamster; (4) EAC; (5) mouse; all in the presence of anticationic antiserum.

While the immunochemical pattern of the gerbil isozymes is not identical with that of the other rodents tested, there was total inactivation of their catalytic activity by the respective antisera prepared against the rat antigens. These results may suggest that immunochemical recognition is not absolute. Rather, it reflects the extent of antiserum–antigen interaction. It would appear, therefore, that although the gerbil isozymes show a pattern of partial identity with the antisera directed against the rat antigens, the fraction of the molecule that contains the catalytic site of both isozymes has been recognized by yet other components of these antisera. This may indicate that antigenic determinants at the catalytic site of each of the liver AAT isozymes obtained from all species of the muridae family, and possibly beyond, are similar and least likely to change through-

out the evolutionary process. Heterogeneity of antibodies is assumed to be due, in large measure, to the presence of multiple specificity determinants on the antigen (1). Katsiris *et al.* demonstrated (14) the existence of distinct antibody populations specific for the intact AAT molecule and its various tryptic fragments. In this respect, the primary structure at the catalytic site of the AAT isozymes, although different for each isozyme, has been shown to be similar in species far removed from one another (9). This is in contrast to the rest of the molecule which is presumably more likely to undergo extensive changes that parallel differences occurring between individual species.

Summary. Antisera against rat liver aspartate aminotransferase (EC 2.6.1.1) isozymes were used to study the activity and immunologic pattern of these isozymes in the livers of the rat, mouse, hamster, gerbil and in Ehrlich ascites cells. A double immunodiffusion precipitin test and immunoelectrophoresis showed that, except for the gerbil, there was a pattern of identity of AAT isozymes in the presence of either the antianionic or the anticationic antisera. Although gerbil AAT isozymes are immunochemically different from those of the other rodents studied, they were inactivated by the respective antiserum in a manner similar to that observed with the other species. This may suggest that antigenic determinants at the catalytic site of each of the liver aspartate aminotransferase isozymes are least likely to change throughout the evolutionary process.

I wish to thank Miss Frances Grainger for her excellent technical assistance.

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Received September 9, 1974. P.S.E.B.M. 1975, Vol. 140.