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PROPERTIES OF LIVER CYTOPLASMIC ASPARTATE AMINOTRANSFERASE OF RATS OF VARIOUS AGES

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Summary. The activity, induction pattern and kinetic properties of cytoplasmic aspartate aminotransferase (c-AsAT) of the liver of rats of various ages were studied. The activity of this enzyme increases gradually till adulthood and remains constant thereafter with increasing age of the rat. Adrenalectomy decreases and hydrocortisone increases the activity of c-AsAT throughout the life span of the rat. However, the degree of induction of the enzyme by hydrocortisone decreases with increasing age of the rat. The K_m for L-aspartate and α-ketoglutarate, the Ki for amino-oxyacetic acid with respect to both the substrates and the electrophoretic mobility of the purified enzyme from the liver of young and old rats are not significantly different.

Introduction

Differentiation and development are programmed processes which occur due to seqential activation and repression of the genes causing alterations in the levels of enzyme [1,2]. Two homologous and genetically independent isoenzymes of aspartate aminotransferase (AsAT; L-aspartate : 2-oxoglutarate aminotransferase, EC 2.6.1.1) have been found in animal tissues, one in the cytosol (c-AsAT) and the other in the mitochondrial (m-AsAT) fraction [3,4]. Liver AsAT is responsible for the synthesis of glucose from non-carbohydrate precursors [5,6]. Although both the isoenzymes of AsAT are involved in the process of gluconeogenesis, it is the cytosolic enzyme which is regulated by dietary and hormonal treatments [6,7]. Nakata et al. [8] reported that the specific activity of c-AsAT is higher in the adult as compared to that of the fetal rat liver. Estradiol inhibits the developmental formation of AsAT in the liver and prevents the hydrocortisone-mediated induction of c-AsAT in the liver of adult rats [9]. We report here for the first time the changes that occur in c-AsAT of the liver during development and aging of the rat. The studies include the effects of adrenalectomy and hydrocortisone on the activity of this isoenzyme and also certain kinetic properties of the purified isoenzyme.

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Materials and Methods

Materials. Young (6-week), adult (30-week) and old (90-week) male albino rats of Wistar strain, maintained at 24 ± 2°C were used for the induction studies of c-AsAT. The rats were fed with a freshly prepared standard diet (per cent compositions of protein, fat and carbohydrate are 19, 13 and 59 respectively). Tap water was supplied ad libitum. All the chemicals used were of analytical grade. The biochemicals were purchased from Sigma Chemical Co., USA.

The rats were killed by cervical dislocation. The left lobe of the liver was removed and chilled in ice-cold 0.9% NaCl, and the adhering blood was removed. A 10% (w/v) homogenate of the liver was prepared in ice cold 0.25 M sucrose solution using a Potter Elvehjem homogenizer fitted with a teflon pestle. The homogenate was filtered through a double layered cheese cloth and centrifuged at 700 ×g at 0°C for 15 min. The resulting supernatant was centrifuged at 14,000 ×g for 30 min at 0°C. The supernatant obtained was used for the spectrophotometric assay of the isoenzyme [9,10].

The protein content in the liver was determined [11] and the enzyme activity was expressed as specific activity (units/mg protein). Each set of the data was collected from 4-5 rats of specific age groups. All the data were statistically analyzed [12].

Effect of hormone on c-AsAT. Pilot experiments were undertaken to find out the time and dose dependence of this isoenzyme towards hydrocortisone in rats of various ages. Maximum response of the isoenzyme was obtained 3 days after the hormone administration with a dose of 5 mg/100 g body wt. The rats of three ages were divided into three groups each having 4-5 rats. Group I rats served as the normal. The groups II and III rats were bilaterally adrenalectomized and were given 0.9% NaCl ad libitum instead of water for 11 days. On the 11th day, the group II rats were administered with 1.0 ml of 0.9% NaCl intraperitoneally (i.p.), instead of the hormone and these rats served as the control for the induction studies. The rats belonging to groups III were given with i.p. dose of hydrocortisone (5.0 mg/100 g body wt. suspended in 1.0 ml of 0.9% NaCl) at a fixed time of the day for 3 days. All the rats were killed after 3 hr of the final hormone injection, and their livers were taken out and used for the assay of the isoenzyme.

Purification of c-AsAT. c-AsAT was purified from the liver of young (6-week) and old (90-week) male rats [13]. The livers from 6-8 rats of each group were pooled. The following studies were carried out on the purified isoenzyme of both the ages.

(a) K_m for L-aspartate and α-ketoglutarate and the K_i for amino-oxyacetic acid with respect to its amino acid and keto acid substrates were determined.

(b) Polyacrylamide gel electrophoresis of the purified isoenzyme was carried out by mixing 25 µg of the isoenzyme with bromophenol blue (0.1%) and glycerol (10%). This mixture was layered on the top of the separating gel containing 7.5% acrylamide and 0.2% bisacrylamide [14]. Electrophoresis was carried out at 4°C for 3 hr in 0.02 M Tris-glycine buffer, pH 8.3 by applying a current of 3mA/tube. One of gels was stained for general proteins in 0.5% Coomassie blue (prepared in 7% acetic acid) for 30 min. The second set of gels was processed for specific staining of c-AsAT (personal communications). The staining mixture contained L-aspartic acid (0.015 M), α-ketoglutaric acid (0.0068 M) and Tris (0.1 M). The pH of this mixture was maintained at 7.5. Prior to its use, o-dianisidine tetrazotized (Fast Blue B salt) was added to the staining mixture at a final concentration of 0.01 M and the mixture was stirred vigorously. The stained gels were washed and stored in a solution containing distilled water, methanol, and acetic acid in the ratio of 5 : 3 : 1 (v/v). The stained gels were photographed and were also scanned at 500 nm in a Beckman Model Analytrol.

Results and Discussion

The activities of several enzymes of an organism are age-dependent [2]. The changes in their levels, regulation by effectors and inducibility by certain steroid hormones are also influenced by the age of the organism [15]. Our data show that the activity of c-AsAT of the liver increases by 48% till adulthood (30-week) and remains constant thereafter with advancing age of the rat (Table I). Earlier report [9] shows that the fetal tissues of the rat in general have a low level of AsAT as compared to the adult tissues. It has been reported earlier that the activity of c-AsAT increases in the liver during development and growth of rats [8]. The higher activity of c-AsAT in the liver of adult and old rats may be correlated with a higher degree of transamination during these phases in the life-span of the rat. Since hepatic c-AsAT participates in the process of gluconeogenesis [5], higher level of this isoenzyme in the liver of old rat may contribute to the higher concentration of blood glucose in old age [16,17].

It has been reported that the removal of the hormone secreting organ from an animal causes a change in the levels of many enzymes in different tissues [15]. Our investigations show that adrenalectomy decreases significantly the activity of c-AsAT in the liver of rats of all the ages. Administration of hydrocortisone to adrenalectomized rats increases significantly the activity of this isoenzyme in the liver of rats of all the ages (Table I). However, the degree of induction is highest in the liver of young rat and decreases with increasing age. This decrease in the inducibility of the isoenzyme by

TABLE I. EFFECTS OF ADRENALECTOMY (A/D) AND HYDROCORTISONE (HC) ON THE ACTIVITY (units/mg protein) $\times 10^2$ OF CYTOPLASMIC ASPARTATE AMINOTRANSFERASE OF THE LIVER OF MALE RATS OF VARIOUS AGES

Treatments	6-week			30-week			90-week		
	Mean	S.D.	p	Mean	S.D.	p	Mean	S.D.	p
Normal	51.78	± 0.57		76.91	± 0.30		78.47	± 0.97	
A/D	39.55 (-24%)	± 1.32	<0.001	58.05 (-25%)	± 1.49	<0.001	51.81 (-34%)	± 1.12	<0.001
A/D + HC	70.86 (+79%)	± 1.24	<0.001	96.85 (+66%)	± 2.33	<0.001	76.85 (+48%)	± 2.33	<0.001

The data were collected from 4-5 rats of each age group: Standard deviation (S.D.) and the levels of significance ($p < 0.05$) are given. +, increase; -, decrease.

hydrocortisone as a function of age of the rat may be due to a gradual decrease in the level of corticosteroid receptors [18,19] and/or certain regulatory changes occur in the genome which may decrease its responsiveness towards the hormone receptor complex.

The degree of purification of c-AsAT of the liver of 6- and 90-week old rats, achieved was 51- and 48-fold, respectively. The data on the kinetic properties of the purified isoenzyme of the liver of rats of both the ages show that there is no significant difference in the affinity of the isoenzyme towards L-aspartate and α-ketoglutarate (Table II). Furthermore, this isoenzyme is competitively inhibited by amino-oxyacetic acid (AOAA) with respect to L-aspartate and non-competitively with respect to α-ketoglutarate. The K_i values of this isoenzyme for AOAA of both the young and old rats are similar.

Polyacrylamide gel electrophoresis of the purified c-AsAT of the liver of young and old rats shows the presence of one major and one or two minor bands in both the cases when the gels were stained for general proteins (Fig. 1). This shows that the isoenzyme is not purified to homogeneity. However, when the gels were stained specifically for this isoenzyme, they show the presence of a single band for both the ages of the rat (Fig. 1). Further, this band corresponds to the major band obtained after staining for the general proteins. The mobility of this isoenzyme for both the ages is similar. These observations were further clarified through the densitometric scannings of these gels (Fig. 2). The above studies show that the net charge in the isoenzyme molecule of the liver does not alter as a function of age of the rat. Earlier studies from this laboratory also show that the kinetic properties of crystalline alanine aminotransferase [20], tyrosine aminotransferase [21], and c-$NADP^+$-linked isocitrate dehydrogenase [22] of the liver, and acetylcholine esterase [23] and pyruvate kinase [24] of the brain do not change as a function of age of the rat.

TABLE II. K_m AND K_i VALUES OF PURIFIED CYTOPLASMIC ASPARTATE AMINOTRANSFERASE OF THE LIVER OF YOUNG (6-) AND OLD (90-WEEK) NORMAL MALE RATS

Age (week)	Substrates	K_m (mM)	K_i (mM) AOAA
6	L-Aspartate	2.40	0.0018
	α-Ketoglutarate	0.11	0.0120
90	L-Aspartate	2.00	0.0020
	α-Ketoglutarate	0.10	0.0100

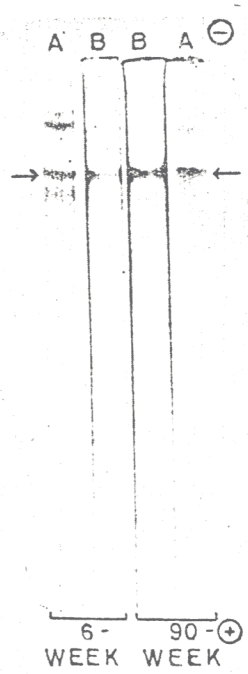

Fig. 1. Polyacrylamide gel electrophoresis of purified c-AsAT from the liver of young (6-) and old (90-week) normal male rats. A-General protein stain (Coomassie blue); B-specific stain.

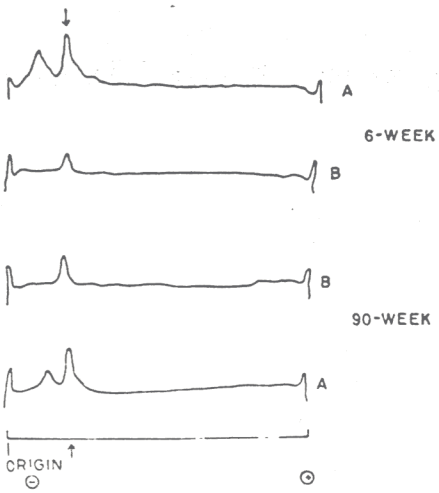

Fig. 2. Densitometric scans of purified c-AsAT from the liver of young (6-) and old (90-week) normal male rats. A-General protein stain; B-specific stain.

The present findings provide firm support to the view that the isoenzyme [illegible] in old age is structurally similar to that synthesized in the young age. Hence, the gene(s) coding for this isoenzyme do not undergo any structural alterations during aging of rats. Therefore, the alterations in the levels and differential induction of this isoenzyme that occur as a function of age may be due to the changes in the template activity of the corresponding gene(s) which are brought about by various modulators [15].

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