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The Kinetics of Synthesis and Degradation of Aspartate Aminotransferase Isozymes in Rat Peripheral Red Blood Cells During Cytodifferentiation[1] (37840)

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Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) has been characterized and purified from various mammalian tissues. It consists of two electrophoretically distinct isozymes: a cationic, mitochondrial isozyme and an anionic, supernatant isozyme. As part of a study of the regulation of rat liver aspartate aminotransferase (AAT), we demonstrated (1–4) that erythrose 4-phosphate, glyceraldehyde 3-phosphate, and glycolaldehyde phosphate inhibit both the cationic and anionic isozymes. These compounds may be involved in the regulation of key metabolic pathways *in vivo* by modifying the activity of AAT isozymes (1–4). This short-term regulation would complement the long-term changes in the isozymes' level that occur as a result of dietary and hormonal alterations (5–8). It is also possible to effect an increase in the level of AAT isozymes in peripheral red blood cells by inducing reticulocytosis (9).

In this report we have followed quantitatively the levels of AAT isozymes in rat peripheral red blood cells during the induction and recovery from phenylhydrazine-induced reticulocytosis. Study of red blood cells during cytodifferentiation provides a unique system which, for the first time, permitted a comparison of the isozymes' turnover rates *in vivo*.

Materials and Methods. Tissue preparation and analytical methods. Materials were obtained from sources previously described (1). Female buffalo rats (Simonsen), weighing 150–200 g, were fed a diet of Purina Laboratory Chow and water *ad lib.* Rats were made reticulocytotic by sc injections with phenylhydrazine (PH) (4 mg/100 g) on Days 0, 1, and 3 (10). At intervals, blood samples were taken in heparin by a direct cardiac puncture. These were centrifuged for 10 min at 600*g* to remove the plasma and the buffy coat. The packed RBC were washed twice with 0.9% saline, then lysed in 10 vol of double-distilled water, and sonicated 15 sec at 4 mA in a 60-A 20-kHz MSE ultrasonic disintegrator. The lysates were centrifuged for 20 min at 20,000*g*, and the supernatants were used for all subsequent aspartate aminotransferase (AAT) assays. The activity of the AAT isozymes was determined as described previously (1) except that 0.1 *M* Tris buffer, pH 7.4, was used instead of barbital buffer. All enzyme units were expressed as μmoles/ml RBC/min.

Immunochemical procedures. For the preparation of specific antisera, 1 mg of each purified rat liver isozyme (1) was emulsified in 2.5 ml of complete Freund's adjuvant (Difco) and injected subcutaneously into the two hindfoot pads and neck of female (New Zealand) rabbits. At 4, 5, and 6 weeks following the single treatment, blood was collected, allowed to clot, and the supernatants containing the antiserum were pooled. Ouchterlony double-diffusion precip-

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itin analysis, on an agar matrix (Hyland), showed that each antiserum formed a single precipitin line with its homologous isozyme and did not cross-react with the heterologous isozyme. Appropriately diluted lysates were incubated with either the antianionic or the anticationic antiserum in amounts which had been determined to result in complete inactivation of the homologous isozyme. After 90 min at 37°, followed by 2 hr at 4°, the mixtures were centrifuged, and the residual AAT activity in the supernatant was determined.

Computations. The rate constants for synthesis (K_s) and degradation (K_d) were determined as described by Segal and Kim (11) and Schimke and Doyle (12). The rate of change in the enzyme level is expressed as the algebraic sum of the zero-order rate of synthesis and the first-order rate of breakdown, $d(E)/dt = K_s - K_d(E)$, where (E) is the enzyme level at any time, t. This equation when integrated and rearranged yields $\ln(E - E_o) = \ln(E'_o - E_o) - K_d t$ for the phase of decline in enzyme activity, where E_o denotes the steady-state level of the enzyme under basal conditions, and E'_o denotes the elevated steady-state level of the enzyme following PH treatment. The values of K_d were calculated from the slopes of plots of $\ln(E - E_o)$ against time using the least-squares method. Values of K_s were calculated by substituting the experimentally determined values for K_d and E_o into the equation $K_s = K_d(E_o)$ where $d(E)/dt = 0$. The half-life for enzyme degradation was calculated from the following expression: $t_{1/2} = 0.693/K_d$. The various assumptions involved in the use of the methods described herein were delineated by Schimke and Doyle (12).

Results and Discussion. Figure 1 shows the time course of changes in the activity of AAT isozymes and of the hematocrit and reticulocyte count in PH-treated rats. The hematocrit fell to its lowest value, about 26%, on Day 3 and attained a value equal to that found in untreated animals, about 50%, on Day 7 (Fig. 1A). The reticulocyte count increased progressively to a value of about 50% on Day 5, attaining a value of essentially 100% on Day 7. Thereafter, there

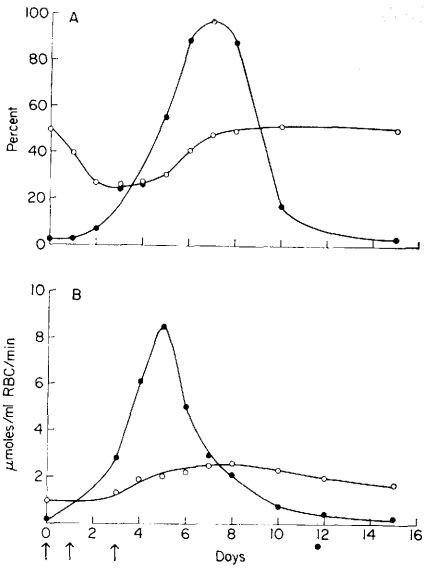

FIG. 1. Effect of PH on hematocrit, reticulocyte count and AAT isozyme activity. (A) Hematocrit, open circles, and reticulocyte count, closed circles. (B) Activity of the anionic isozyme, open circles, and the cationic isozyme, closed circles. Arrows denote days at which PH was injected.

was a decline in the count to a value of about 3% which is similar to that observed in untreated animals (Fig. 1A). The activity pattern of AAT isozymes changed from predominantly anionic in untreated rats to predominantly cationic on the 5th day following PH treatment. Thereafter, there was a progressive change towards a value similar to the ratio of the two isozymes that was observed in untreated rats (Fig. 1B). Total AAT activity was maximal on Day 5, about 10 μmoles/ml RBC/min, which is about 10-fold higher than the control value (Fig. 1B). RNA content was also maximal on Day 5, about 4.5 mg/ml RBC. The increase in total AAT activity on Day 5 was due primarily to a 47-fold increase in the activity of the cationic isozyme. The activity of the anionic isozyme became maximal on the 8th day, about 3 times its control value (Fig. 1B). This was followed by a continuous decline of the activities of both isozymes from their respective maxima to control levels.

On the assumption that reticulocytes in untreated rats contained the same amount of cationic isozyme as that present on the 5th day after initiation of PH treatment, we in-

ferred that the value of 0.18 μmoles/ml RBC/min for the cationic isozyme in untreated rats (Fig. 1) is due to the presence of 2.5% reticulocytes. This value is in good agreement with the microscopic determination of reticulocytes present in untreated rats (Fig. 1A). Mature erythrocytes, therefore, unlike all other tissues thus far investigated (1–8), contain only the anionic isozyme. The metabolic significance of this observation, with regard to the inhibition of AAT isozymes by glyceraldehyde 3-phosphate in particular (1–4), remains to be established.

The use of antiserum directed against each of the AAT isozymes in reticulocytotic rats has enabled us to compare, for the first time, the rate constants of synthesis and degradation and the turnover rates for both isozymes. The slope, K_d, of plots of $\ln(E - E_o)$ against time was about six-fold higher for the cationic than for the anionic isozyme (Fig. 2, Table I). The calculated rate constants for synthesis, K_s, were similar for the two isozymes (Table I). The calculated turnover rates, $t_{1/2}$, show that the cationic isozyme is about six-fold less stable than the anionic isozyme (Table I). It would appear that the shorter half-life of the cationic isozyme is related to its rapid rate of degradation.

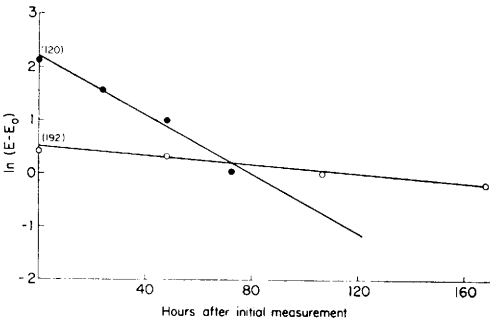

FIG. 2. Logarithmic plot of the decrease in AAT isozymes toward basal levels after their elevation by PH treatment. Open circles denote anionic isozyme, and closed circles denote cationic isozyme. Data were taken from Fig. 1. Numbers in parentheses indicate hours after treatment at which initial measurements were taken.

Since the cationic isozyme is a mitochondrial enzyme, it is possible that the rate constant for its degradation in reticulocytes may reflect the rate of elimination of the mitochondrial fraction during the maturation process (13). In this respect, the K_d of mitochondrial DNA in the maturing reticulocytes was 0.03 (hr^{-1}) with a $t_{1/2}$ of about 20 hr. These values are similar to those found for the cationic isozyme. Whether synthesis of the cationic isozyme, i.e., transcription and translation during RBC cytodifferentiation, occurs in the mitochondrial fraction or is carried out on the cytoribosomes whereupon the cationic isozyme is then transported to the mitochondria is currently under investigation.

TABLE I. The Rates of Synthesis and Degradation of AAT Isozymes in Phenylhydrazine-Treated Rats.

Isozyme	E_o[a] (units/hr)	K_d[b] (hr^{-1})	$t^c_{1/2}$ (hr)	Calculated K_s (units/hr)
Cationic	0.18	0.028	24.6	0.0050
Anionic	0.96	0.0044	154.9	0.0043

[a] E_o is the basal state level for the isozymes in untreated rats.

[b] K_d is the experimental value for the rate constants of enzyme degradation from data presented in Figs. 1 and 2.

[c] $t_{1/2} = \frac{0.693}{K_d}$; $K_s = K_d\,(E_o)$.

An increase in the cationic isozyme could possibly be used as a sensitive measure in the diagnosis of diseases associated with an abnormal increase in the population of young red blood cells.

Summary. The kinetics of synthesis and degradation of aspartate aminotransferase (EC 2.6.1.1) isozymes were investigated in peripheral red blood cells of rats made reticulocytotic with phenylhydrazine. Immunochemical studies showed that reticulocytes contain predominantly the cationic isozyme, whereas mature erythrocytes contain only the anionic isozyme. Following initiation of phenylhydrazine treatment, the cationic isozyme increased 47-fold above its control level on Day 5, and the anionic isozyme increased threefold above its control level on the 8th day. The half-lives were 24.6 and 154.9 hr for the cationic and anionic isozyme, respectively. The faster turnover of the cationic isozyme appears to

be related to its rapid rate of degradation.

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