Effect of Treatment with Pyridoxine on Aspartate Aminotransferase Activities in Pyridoxine-Deficient Rat Tissues

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Summary In rat liver, 90% of the aspartate aminotransferase is present as the holoenzyme. In pyridoxine deficiency, the ratio of holoenzyme activity to total activity is markedly reduced, but after pyridoxine injection it was found to rapidly increase, although the total enzyme activity remained low for a few days. The activities of aspartate aminotransferase isozymes in pyridoxine-deficient rat tissues and the effect of pyridoxine treatment on their activities were examined. The intestinal enzyme activities of pyridoxine-deficient rats were readily reconstituted in the presence of pyridoxal phosphate in vitro, but the enzyme activities in liver and muscle in the deficient rats required several days for complete recovery, suggesting that active enzyme was synthesized de novo in these tissues.

Key Words aspartate aminotransferase, Bε deficiency, inactive enzyme, reconstitution of Asp AT, pyridoxine injection

Various degrees of decrease in activity of pyridoxal phosphate-dependent enzymes have been reported in pyridoxine-deficient animals (1-3), and various mechanisms for this decrease in enzyme activity have been reported. Khairallah and Pitot (4) reported that serine dehydratase [EC 4.2.1.13] in rat liver was stabilized by pyridoxal administration. Kominami and Katunuma (5) also reported that intestinal ornithine aminotransferase [EC 2.6.1.13] has a shorter half-life in pyridoxine-deficient rats than in normal ones. On the contrary, Lee et al. (6) demonstrated a decrease in the synthesis of tyrosine aminotransferase [EC 2.6.1.5], and no change in either the synthesis or degradation of alanine aminotransferase [EC 2.6.1.2] in pyridoxine-deficient rat liver. In our laboratory, cytosolic aspartate aminotransferase [EC 2.6.1.1] has been studied and we have suggested the existence of an abnormal molecule that has little or no enzymatic activity but which is immunologically active as well as the normal molecule (7). Recently we also found the occurrence of an inactive molecule similar to that found in cytosol in the
mitochondrial fraction of pyridoxine-deficient rat liver.\textsuperscript{2}

In the present work we studied the physiological properties of these inactive molecules in pyridoxine-deficient rat liver and certain other tissues.

\section*{MATERIALS AND METHODS}

\textit{Animals.} Male Wistar rats weighing 50–60 g were used. The rats were given 70\% casein diet with or without added pyridoxine \textit{ad libitum} for 4 weeks. The composition of the diet has been described previously\textsuperscript{(7)}. From week 4 on experimental diets, pyridoxine-deficient rats were injected i.p. with pyridoxine hydrochloride in saline daily until slaughter. Control rats were injected with saline.

\textit{Antiserum.} Anti-aspartate aminotransferase of liver cytosol was prepared as described previously\textsuperscript{(7)}.

\textit{Preparation of enzyme solution.} Rat liver was homogenized in a Potter homogenizer in 4 volumes of 0.05M potassium phosphate buffer, pH 7.4, with or without pyridoxal phosphate (10\textsuperscript{-4} M). The homogenate was sonicated at 10 kc for 10 min and then centrifuged at 100,000 \(\times\) g for 1 hr, the supernatant subsequently being used. The small intestine was removed, washed with saline and homogenized in a polytron (Kinematica) in 4 volumes of the same buffer containing pyrodoxal phosphate. The homogenate was centrifuged at 10,000 \(\times\) g for 20 min and the supernatant was used. Muscle extract was prepared in the same way as intestinal extract except that the muscle was not washed with saline.

\textit{Chemicals.} Malate dehydrogenase was purchased from Oriental Yeast Co. Pyridoxal phosphate, NADH and aspartate were obtained from Kyowa Hakko Kogyo Co. Other chemicals were secured from Nakarai Chemicals Co. or Wako Pure Chemicals Co.

\textit{Assay methods.} Aspartate aminotransferase activity was measured by a modification of the method of Karmen\textsuperscript{(8)} as described previously\textsuperscript{(7)}. For determination of mitochondrial enzyme activity, enzyme solution was incubated with excess anti-serum in the presence of 10\textsuperscript{-4} M pyridoxal phosphate (pH 7.4) at 37°C for 30 min and then at 4°C overnight, the supernatant subsequently being used. Cytosolic enzyme activity was calculated by subtracting mitochondrial activity from the total activity which was determined after addition of non-immune serum instead of the anti-serum used for the mitochondrial enzyme. One unit of activity was defined as the amount of the enzyme required for catalyzing the formation of 1 \(\mu\)mol of product in 1 hr at room temperature (22°C).

\section*{RESULTS}

\textit{Alanine aminotransferase activities in rat tissues}

The effect of injection of pyridoxine into pyridoxine-deficient rats on alanine

\textsuperscript{2} Unpublished data by Shibuya and Okada which will appear elsewhere.

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Table 1. Effect of pyridoxine administration on alanine aminotransferase activity in pyridoxine-deficient rats.

| Enzyme activity units/g tissue | Liver       | Intestine   | Muscle      |
|-------------------------------|-------------|-------------|-------------|
| Control                       | 946.6 ± 110.1 | 235.2 ± 64.8 | 44.9 ± 10.3 |
| Deficient                     | 231.6 ± 70.2 | 89.6 ± 41.9  | 9.0 ± 3.0   |
|                               | (24.4)      | (38.0)      | (20.0)      |
| Deficient + PIN<sup>a</sup> (1 day) | 675.5 ± 124.8 | not determined | not determined |
|                               | (71.3)      |             |             |
| Deficient + PIN<sup>b</sup> (3 days) | 1,169 ± 224.6 | 337.1 ± 19.9  | 65.6 ± 19.5 |
|                               | (123.5)     | (143.3)     | (146.1)     |

Rats on experimental diets were treated with pyridoxine hydrochloride once (a) or 3 times (b) at a dose of 10 mg/100 g body weight. Values are means of 4 rats ± SD. Figures in parentheses are percentages of the control.

Table 2. Effect of pyridoxine treatment on hepatic aspartate aminotransferase in pyridoxine-deficient rats.

Enzyme activities were measured 4 hr or 1 day after pyridoxine injection (10 mg/100 g).

| Enzyme activity U/g liver | Aminotransferase activities in the tissues was examined (Table 1). Enzyme activities were markedly decreased in pyridoxine-deficient rat tissues, but the activity in the liver was almost restored to the control level within one day by injection of pyridoxine. Daily injection of pyridoxine completely restored the activities in all tissues tested within 3 days and even resulted in slightly higher activities than in the controls.

Effect of injection of pyridoxine on hepatic enzyme activity

As shown in Table 2, aspartate aminotransferase is mostly present as holoenzyme in normal rats. The amount of holoenzyme was greatly decreased in pyridoxine deficiency, but the enzyme activity partly recovered within 4 hr of the
Table 3. Effect of pyridoxine treatment on hepatic aspartate aminotransferase in pyridoxine-deficient rats.

Pyridoxine-deficient rats were treated with 10 mg/100 g body weight of pyridoxine hydrochloride once and then with 100 µg/100 g daily until slaughter. Activities shown under Total (1) were measured directly without any preincubation. In other cases samples were incubated in medium containing 10⁻⁴ M PLP at 37°C for 30 min and then kept at 4°C overnight before assay. Figures in parentheses show percentages of the control value. Other conditions were as described in the text.

|                | Total (1)   | Total (2)   | Mitochondria | Cytosol   |
|----------------|-------------|-------------|--------------|-----------|
| Control        | 3,996 ± 215 | 4,890 ± 349 | 3,433 ± 292  | 1,456 ± 384 |
| Deficient      | 1,330 ± 468 | 2,691 ± 668 | 2,026 ± 555  | 666 ± 239  |
| (33.5)         | (55.0)      | (58.9)      | (45.6)       |           |
| Deficient + PIN (3 days) | 2,344 ± 624 | 2,717 ± 580 | 2,094 ± 413  | 622 ± 219  |
| (59.0)         | (55.5)      | (60.9)      | (42.7)       |           |
| Deficient + PIN (6 days) | 3,537 ± 253 | 4,564 ± 362 | 2,593 ± 272  | 1,972 ± 225 |
| (89.2)         | (93.3)      | (75.5)      | (135.3)      |           |

Table 4. Effect of pyridoxine treatment on aspartate aminotransferase activities in the small intestine of pyridoxine-deficient rats.

Figures in parentheses are percentages of the control value. Other conditions were as for Table 3.

|                | Total (1)   | Total (2)   | Mitochondria | Cytosol   |
|----------------|-------------|-------------|--------------|-----------|
| Control        | 202.6 ± 27.7| 342.8 ± 33.8| 116.5 ± 14.3 | 226.2 ± 27.4 |
| Deficient      | 70.1 ± 4.6  | 283.9 ± 19.3| 89.0 ± 11.2  | 194.8 ± 23.3 |
| (34.6)         | (82.8)      | (76.4)      | (86.1)       |           |
| Deficient + PIN (3 days) | 270.8 ± 24.6| 341.8 ± 22.4| 103.9 ± 19.3 | 222.9 ± 13.1 |
| (133.6)        | (99.7)      | (89.2)      | (98.5)       |           |
| Deficient + PIN (6 days) | 304.0 ± 23.5| 374.7 ± 30.1| 136.0 ± 13.6 | 238.7 ± 23.3 |
| (152.0)        | (109.3)     | (116.6)     | (105.5)      |           |

injection of pyridoxine, indicating the existence of active apoenzyme in the deficient rat liver. The enzyme activity in pyridoxine-deficient rat liver was restored to about 50% of the control level within 24 hr of pyridoxine injection. The total enzyme activity and enzyme activities of the mitochondria and cytosol of liver were increased by incubation at 37°C for 30 min and then at 4°C overnight in the presence of pyridoxal phosphate (Table 3). After daily injection of pyridoxine for 6 days, the cytosolic enzyme activity in liver was completely restored but the mitochondrial enzyme activity was still lower than that of the controls.

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Table 5. Effect of pyridoxine treatment on aspartate aminotransferase activities in skeletal muscle of pyridoxine-deficient rats. Figures in parentheses are percentages of the control value. Other conditions were as for Table 3.

|                  | Total (1)     | Total (2)     | Mitochondria (units/g tissue) | Cytosol       |
|------------------|---------------|---------------|-------------------------------|---------------|
| Control          | 1,451 ± 378   | 1,662 ± 239   | 910 ± 149                     | 752 ± 101     |
| Deficient        | 508 ± 89      | 922 ± 147     | 535 ± 76                      | 387 ± 77      |
|                  | (34.8)        | (55.4)        | (58.8)                        | (51.4)        |
| Deficient + PIN  | 1,035 ± 130   | 1,230 ± 111   | 568 ± 89                      | 662 ± 146     |
| (3 days)         | (71.3)        | (73.9)        | (62.4)                        | (88.0)        |
| Deficient + PIN  | 1,027 ± 126   | 1,175 ± 123   | 556 ± 76                      | 619 ± 83      |
| (6 days)         | (70.0)        | (70.6)        | (61.0)                        | (82.3)        |

**Effect of pyridoxine injection on the enzyme activities in other tissues**

The effect of injection of pyridoxine into deficient rats on intestinal enzyme activity was studied (Table 4). The activity in pyridoxine-deficient rats was one-third that of the controls, but the activity almost completely recovered on incubation of the tissue extract with pyridoxal phosphate *in vitro*. On the contrary, the activity in skeletal muscle was not restored by pyridoxine administration even after its daily injection for 6 days (Table 5). On daily injection of pyridoxine, cytosolic enzyme activity was restored to near the control level within 3 days, while mitochondrial enzyme activity was not affected within 6 days at least.

**DISCUSSION**

The effect of pyridoxine treatment on alanine and aspartate aminotransferase activities was studied in various tissues of rats given pyridoxine-deficient diet for 4 weeks. Perry et al. (9) reported reconstitution of alanine and tyrosine aminotransferase *in vitro*, and *in vivo* after pyridoxine injection into deficient rats. Apotyrosine aminotransferase readily combined with pyridoxal phosphate *in vivo* and *in vitro*, whereas the inactive form of alanine aminotransferase was not readily converted into its active form, although almost complete reconstitution was achieved in 60 min *in vitro* and 8 hr *in vivo*. We determined alanine aminotransferase activity in various tissues of pyridoxine-deficient rats with or without treatment with pyridoxine (Table 1), and our data are essentially consistent with those of Perry et al. (9).

Most of the properties of aspartate aminotransferase are similar to those of alanine aminotransferase, the activity decreasing greatly in pyridoxine-deficient rat liver (Table 2). The aspartate aminotransferase activities in all the rat tissues tested were increased by incubation with $10^{-4}$ M pyridoxal phosphate, suggesting the
existence of an inactive form that is readily converted to the active. The content of this inactive form seems to be very high in tissues of pyridoxine-deficient rats, though there is also some in the tissues of control rats as previously described (7).

Injection of pyridoxine had different effects on the aspartate aminotransferase activities in different tissues; in the small intestine of deficient rats aspartate aminotransferase activity was readily restored by treatment with pyridoxine, whereas the activities in liver and muscle were not, and the mitochondrial enzyme activities in the latter tissues had not completely recovered even after daily treatment with pyridoxine for 6 days. From the present data we suggest that the enzymatically inactive form that increases in pyridoxine-deficient rat liver cytosol is partly converted to the active form, and partly degraded. In the mitochondrial fraction, as in liver cytosol, synthesis of active molecules also seems to be necessary for the recovery of the enzyme activities in pyridoxine-deficient rat liver and muscle. Probable forms of aspartate aminotransferase molecules in pyridoxine-deficient and control rat liver are schematically drawn in Fig. 1.

It has been shown that the turnover of immunologically identical enzyme molecules is regulated appropriately in each tissue. Different half-lives of ornithine transaminase in liver and kidney have been reported (10). A difference in the half-lives of mitochondrial aspartate aminotransferase in liver and muscle was also suggested from the data obtained.

![Fig. 1. Multiple forms of aspartate aminotransferase in rat liver.](image-url)
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