GLUTAMATE OXIDATION IN LIVER MITOCHONDRIA OF RATS FED ON PYRIDOXINE-DEFICIENT DIET

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Two pathways are known to exist for glutamate oxidation in liver mitochondria. One is by way of glutamate dehydrogenase [EC 1.4.1.3.] reaction and the other by aspartate transaminase [EC 2.6.1.1.] reaction. Although both enzyme activities are high in mitochondrial fraction, it is known that glutamate is preferentially oxidized through the transaminase pathway in isolated intact mitochondria (1-5). Thus glutamate added to the mitochondrial system is stoichiometrically converted to aspartate and the amino group of aspartate formed is excreted in urine as an amino nitrogen of urea molecule. On the other hand, glutamate dehydrogenase reaction is far inclined to the formation of glutamate as judged by its equilibrium constant. In the present work, we studied the pathway of glutamate oxidation in the liver mitochondria of rats subjected to a severe pyridoxine deficiency. The mitochondrial respiration was set in the state 3 where the respiration is most active. This condition should be the most favourable condition to oxidize glutamate by means of glutamate dehydrogenase (6, 7).

Wistar strain rats weighing about 50 g were fed on a 70% casein diet for about one month. Dietary compositions and other feeding conditions were described previously (8). The preparation of liver mitochondria and the composition of reaction medium followed the method of HAGIHARA et al. (9). Aspartate transaminase and glutamate dehydrogenase activities were determined by the method of KATUNUMA et al. (10) and STRECKER (11), respectively. As shown in Table 1, aspartate transaminase activity was decreased in the pyridoxine deficient group to 42% of the normal group. On the other hand, glutamate dehydrogenase activity was somewhat higher in the deficient group in terms of specific activity. But the significance of this finding should be viewed with caution because the amount of mitochondria which reflects the total activity of glutamate dehydrogenase is not quantitated in both groups. TAKAMI et al. (12) have reported that glutamate dehydrogenase activity was not affected by pyridoxine deficiency, although their feeding conditions for rats were different from ours. Glutamate oxidation in isolated mitochondria was studied in state 3 in the presence of glucose and hexokinase. After deproteinization, glutamate and aspartate were
Table 1. Mitochondrial enzyme activity in rat liver. Values in parentheses are number of rat. Reaction media contained 5 μmoles α-ketoglutarate, 20 μmoles L-aspartic acid in 2 ml 0.05 M Tris-HCl buffer (pH 7.5) for the transaminase activity, and 0.2 ml, 0.5 M glutamic acid, 0.3 μmoles NAD in 3 ml 0.05 M potassium phosphate buffer (pH 7.6) for glutamate dehydrogenase.

<table>
<thead>
<tr>
<th></th>
<th>Asp transaminase (μmoles/mg/hr)</th>
<th>Glu dehydrogenase (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>10.68±0.83</td>
<td>18.5±1.7</td>
</tr>
<tr>
<td>Deficient (10)</td>
<td>4.48±0.29</td>
<td>24.5±1.7</td>
</tr>
<tr>
<td>Per cent of normal</td>
<td>42</td>
<td>132</td>
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</tbody>
</table>

Table 2. Glutamate oxidation in isolated liver mitochondria. Reaction was carried out in a Warburg flask at 30°C for 20 min in 0.25 M mannitol containing 0.01 M phosphate (pH 7.4), 0.01 M Tris-HCl (pH 7.4), 0.01 M KCl, 0.2 mM EDTA, 2 mM MgCl₂, 500 μmoles glucose, 14 mg hexokinase (Sigma Chemical Co.), 10 μmoles glutamate as substrate and mitochondrial preparation.

<table>
<thead>
<tr>
<th></th>
<th>Glu remained (μmoles/vessel)</th>
<th>Asp formed (μmoles/vessel)</th>
<th>NH₃ liberated (μmoles/vessel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>6.68±0.36</td>
<td>2.58±0.21</td>
<td>0.24±0.00</td>
</tr>
<tr>
<td>Deficient (10)</td>
<td>6.66±0.43</td>
<td>2.83±0.20</td>
<td>0.29±0.01</td>
</tr>
</tbody>
</table>

analyzed according to the method described by Krebs et al. (1). Ammonia liberated was measured by the indophenol method (13) after diffusion using the Seligson apparatus (14). The result obtained is shown in Table 2. Glutamate disappeared was almost completely recovered as aspartate in both groups. No significant amount of free ammonia was observed even in the mitochondria prepared from the pyridoxine deficient rat. The capacity of mitochondria to consume glutamate via transaminase pathway was not decreased in pyridoxine deficient rats in spite of considerable decrease in aspartate transaminase activity. The result suggests that aspartate transaminase is not the rate-limiting step in the glutamate oxidation in rat liver mitochondria.

Recently we found that the urinary excretion of aspartate was increased in the pyridoxine deficient rats (15), while the excretion of urea was decreased in the deficient animals. The free ammonia level in urine was also decreased in the deficient rats. Taking these observations together, we may suggest the following; in pyridoxine-deficient rats, glutamate is converted to aspartate at the normal rate but the ammonia production is slowed down due to the impaired amino acid metabolism, and the result is that aspartate is not converted to urea and is excreted as such in urine.

REFERENCES
GLUTAMATE OXIDATION IN PYRIDOXINE DEFICIENT RAT