DIFFERENCES BETWEEN THE TRANSAMINASES IN MITOCHONDRIA AND SOLUBLE FRACTION
I. GLUTAMIC-PYRUVIC TRANSAMINASE

NOBUHIKO KATUNUMA¹, KAZUKO MIKUMO, MAKOTO MATSUDA²
AND MITSUKO OKADA*

Department of Metabolism, Institute for Protein Research, Osaka University, Osaka
and *Department of Enzyme Chemistry, Institute for Enzyme Research,
Tokushima University School of Medicine, Tokushima

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The purification procedure, some properties and the mode of action of glutamic-pyruvic transaminase (GPT) from pig heart muscle have been reported in the previous papers (1, 2). The presence of GPT and glutamic-oxaloacetic transaminase (GOT) activities in the mitochondrial fraction has been reported by Rosewell (3, 4), who qualitatively demonstrated the formation of glutamic acid from α-ketoglutaric acid by paper chromatography using the mitochondrial suspension. The authors purified GPT and GOT from pig heart and liver mitochondria. The former was isolated as an approximately single protein and the latter as the enzyme 20 times as active as the original mitochondrion suspension. Using those purified enzymes, the authors found that the characteristic GPT and GOT located in mitochondria had the properties quite different from those in the soluble fraction and both of them could be separated from those in the soluble fraction by calcium phosphate gel column chromatography and starch zone electrophoresis (2, 5, 6). The significance of their localization in mitochondria from the viewpoint of coupling with tricarboxylic acid (TCA) cycle was elucidated in another paper (7).

The purification procedure and some differences in properties of GPT and GOT in mitochondria and those in the soluble fraction will be reported in the following papers.

MATERIALS AND METHODS

1. Materials and Purification of the Enzymes

GPT in the soluble fraction (GPTs) and that in the mitochondrial fraction (GPTm) were extracted from pig heart or rat liver and purified by the method of Katunuma (2).

¹ Present address: Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima.
² Present address: Department of Biochemistry, Jikei-University School of Medicine, Shiba, Tokyo.
2. Assay of Enzyme Activity

The enzyme preparation was incubated with 10 μmoles of α-ketoglutaric acid and 5 μmoles of L-alanine in 0.1 M pH 8.0 phosphate buffer at 38° for 10 minutes. After addition of 5 N HCl the pyruvate formed was determined by the modified method of Freedmann and Haugen. For the purpose of determining the optimal pH, phosphate (up to pH 8.0), veronal (up to pH 9.5) and carbonate-bicarbonate (above pH 9.5) buffers were used.

3. Determination of Protein

Two methods were used for determination of protein. The biuret reaction was used for the crude preparations of the enzymes and Kalckar's method, determining the absorbances at 280 and 260 nm, was applied for the purified enzymes.

4. Radioactive Isotopic Reagents

Specific activities of L-alanine-1-C¹⁴ and L-glutamic acid-C (U) used corresponded to 0.1 mc per 2.1 mg and 0.1 mc per 4.27 mg respectively.

5. Determination of Radioactivity

For the determinations of the radioactivity the gas flow type counter (Nuclea, Chicago) was used.

6. Determination of Michaelis Constants of the Elementary Reactions

Michaelis constants of the following elementary reactions were determined by the Lineweaver and Burk equation.

\[
\begin{align*}
C¹⁴-\text{Alanine} + \text{Pyruvate} & \rightleftharpoons C¹⁴-\text{Pyruvate} + \text{Alanine} \\
C¹⁴-\text{Glutamate} + \alpha-\text{Ketoglutarate} & \rightleftharpoons \alpha-\text{Ketoglutarate} + \text{Glutamate}
\end{align*}
\]

Each radioactive substrate having various concentrations (0.1, to 1.0 μc/10 μmoles) was added to the incubation medium containing 20 μmoles of pyruvate or α-ketoglutarate. After addition of 5 N HCl at the end of the reactions, 2,4-dinitrophenyl hydrazine solution was added, the mixture was washed with ethyl acetate for removal of the hydrazones of the keto acids, and was extracted with 10% aqueous Na₂CO₃ solution. The alkaline phase was extracted with a small volume of ethyl acetate again and an aliquot of the ethyl acetate phase was used for the determination of the hydrazone derivatives of the labeled pyruvate or α-ketoglutarate.

7. Column Chromatography

Two volumes of calcium phosphate gel freshly prepared was mixed with one volume of cellulose powder. The mixture was then suspended in deionized water and poured onto a column, 2 cm in diameter and 10—20 cm in height. The enzyme solution dialyzed against deionized water for 24 hours was applied to the column. Elution was made by stepwise increase in the concentration and pH of phosphate buffer. Gradient elution system was also employed.

8. Horizontal Starch Zone Electrophoresis

Toyo Roshi Block type apparatus was used. The starch used was washed 3 times with saline, then with a small volume of 0.05 M pH 9.2 veronal buffer and the buffered starch was then poured onto the blocked plate. The enzyme
solution dialyzed against 0.05 M veronal buffer overnight was applied to the center of the plate and electrophoresis was carried out under condition of 7.5 mA, 300 volt or 2 mA, 400 volt for 18 hours at 4°. Thereafter the enzymes were eluted with 0.1 M pH 8.0 phosphate buffer from each block and the activities were determined.

RESULTS AND DISCUSSION

1. Destruction of Mitochondria

In order to solubilize the enzyme from mitochondria several techniques were tried, i.e., exposure to sonic oscillation (at 10 kc for 10—15 minutes), treatment with deoxycholate, freezing and thawing, and grinding with glass powder. The soluble enzymes having about the same specific activity were obtained by these different methods.

2. Separation of GPTs and GPTm by Calcium Phosphate Gel Column Chromatography

Fig. 1 shows the results of chromatography of GPTS and GPTM prepared separately from pig heart or rat liver by Katunuma's method (2). GPTS was eluted with 0.02 M and GPTM with 0.06 M buffers. They probably have some different electric charges and structures. In rat liver GPTS and GPTM were found to exist in the ratio of 100 to 15. The specific activity of the GPT in the supernatant after centrifugation at 100,000×g for 60 min was 0.5 and that in mitochondria 0.24. Gradient elution system can be used to separate GPTs from GPTM (Figs. 2 and 3).

Zone electrophoresis can not be used on the purpose of separation. As described in the following paper, GOTs and GOTm were separated well from each other by this technique.
3. Elementary Reactions of GPT

The following reactions took place in the purified enzyme more strongly than the original GPT reaction.

\[
\begin{align*}
\text{C}^{14}\text{-Alanine} + \text{Pyruvate} & \rightarrow \text{C}^{14}\text{-Pyruvate} + \text{Alanine} \\
\text{C}^{14}\text{-Glutamate} + \alpha\text{-Ketoglutarate} & \rightarrow \text{C}^{14}\alpha\text{-Ketoglutarate} + \text{Glutamate}
\end{align*}
\]

There are no significant differences in Michaelis constants between the crude and purified GPTs and GPTM reactions (Table I).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
<th>Crude</th>
<th>Purified</th>
<th>Intact</th>
<th>Solubilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine + \alpha\text{-Ketoglutarate} \rightarrow Pyruvate + Glutamate</td>
<td>GPT\text{s}</td>
<td>3.6-3.8 \times 10^{-2}</td>
<td>4.7-5.0 \times 10^{-2}</td>
<td>3.7-4.0 \times 10^{-2}</td>
<td></td>
</tr>
<tr>
<td>C^{14}\text{-Alanine} + \text{Pyruvate} \rightarrow C^{14}\text{-Pyruvate} + \text{Alanine}</td>
<td>GPT\text{M}</td>
<td>1.0-1.1 \times 10^{-2}</td>
<td>1.2-1.3 \times 10^{-2}</td>
<td>1.1-1.3 \times 10^{-2}</td>
<td></td>
</tr>
<tr>
<td>C^{14}\text{-Glutamate} + \alpha\text{-Ketoglutarate} \rightarrow C^{14}\alpha\text{-Ketoglutarate} + \text{Glutamate}</td>
<td>GPT\text{s}</td>
<td>1.3-1.4 \times 10^{-2}</td>
<td>1.5-1.7 \times 10^{-2}</td>
<td>1.9-2.0 \times 10^{-2}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPT\text{M}</td>
<td>1.5-1.7 \times 10^{-2}</td>
<td>1.9-2.0 \times 10^{-2}</td>
<td>1.5-1.7 \times 10^{-2}</td>
<td></td>
</tr>
</tbody>
</table>

4. Differences in Optimal pH of GPTs and GPTM

GPTs and GPTM have clearly different optimal pH values (Fig. 4). The former is 9.7, as known as the optimal pH of GPT and the latter is 8.5. These enzymes can, therefore, be distinguished from their optimal pH values.
To determine the composition of each fraction from their mixtures, the following equations were derived. Their ratio will be calculated from the equations when the enzyme activities of a mixture at pH 8.5 and 9.7 are measured.

Let \( y \) the activity of the purified GPTS at pH 8.5, \( s \) that at pH 9.7; and \( m \) the activity of the purified GPTM at pH 8.5, \( w \) that at pH 9.7, then \( s/y = k \) and \( w/m = K \) are always constant. If the activities of a mixture at pH 8.5 and 9.7, \( a \) and \( b \) respectively, are determined, the following equations are obtained, \( w + s = b \) and \( y + m = a \).

Thus the following two equations can be derived.

\[
\begin{align*}
    s &= \frac{b - ak}{1 - \frac{K}{k}} \\
    m &= a - \frac{s}{k}
\end{align*}
\]

Such a method is useful for the diagnosis of liver and heart diseases, because it is important to know the amounts of GPT and GOT in the serum in these cases.

SUMMARY

1. Sonication, deoxycholate treatment, freezing and thawing and mechanical grinding techniques were tried to solubilize the glutamic-pyruvic transaminase (GPT) from mitochondria and the enzymes having the same activities were obtained.

2. Glutamic-pyruvic transaminases in soluble fraction and mitochondria (GPTS and GPTM) were eluted with 0.02 M phosphate buffer, pH 6.8, and 0.06 M phosphate buffer, pH 6.8, respectively, in calcium phosphate gel column chromatography by stepwise and gradient eluting systems. In zone electrophoresis, they moved to the cathode and could not be separated from each other. These enzymes also have quite different optimal pH values.

3. Michaelis constants of GPT and their elementary reactions were determined and found to be almost equal in both crude and purified GPTS and GPTM reactions.

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REFERENCES


