ENZYMATIC TRANSAMIDINATION FROM CANAVANINE TO GLYCINE BY HOG KIDNEY EXTRACTS

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(Received for publication, May 31, 1956)

Borsook and Dubnoff (1) demonstrated that the amidine moiety of arginine was transferred to glycine to form glycocyamine by slices or cell-free macerates of mammalian kidneys, and designated the enzyme concerned "transamidinase." Walker (2) observed recently that the amidine moiety of canavanine was transferred to ornithine to form arginine by hog kidney extracts*. The present author has found that hog kidney extracts catalyze a transamidination reaction forming glycocyamine from canavanine and glycine as given in the accompanying diagram.

\[
\begin{align*}
\text{NH} & \quad \text{NH}_2 \\
\text{H}_2\text{N}-\text{C-NH-O-CH}_2\text{CH}_2\text{-CH-COOH} + \text{NH}_2\text{-CH}_2\text{-COOH} & \rightarrow \\
\text{NH}_2 & \quad \text{NH} \\
\text{H}_2\text{N-O-CH}_2\text{CH}_2\text{CH}_2\text{COOH} + \text{H}_2\text{N-C-NH-CH}_2\text{-COOH}
\end{align*}
\]

In this paper the general properties of this reaction are presented.

MATERIALS AND METHODS

Materials—L-Canavanine was isolated from jack bean (3), and other reagents used were commercial products. The buffers employed were Walpole's 1/5 M acetate (pH 4.0–5.0), Sörensen's 1/15 M phosphate (pH 5.9–8.0), and Atkins-Pantin's 1/10 M borate (pH 8.4–10.0).

Enzyme Solution—Hog kidney, purchased from a meat-shop, was ground in a mortar with sea sand and water (0.5 ml. per g. of the moist tissue), and was kept in an ice-box overnight under toluene. The mixture was centrifuged, and the supernatant liquid (somewhat turbid) was used as an enzyme solution.

* During the present author was making this paper after the completion of the work presented here, the Walker's report (2) appeared.
Incubation Procedure—Mixtures of canavanine, glycine, buffer, and the enzyme were incubated under toluene at 35°. After incubation, 0.25 volumes of 2 M acetic acid containing 10 per cent sodium chloride was added to each of the reaction mixtures, and then the mixtures were heated for 5 minutes in a boiling water-bath. To remove the coagulated protein, the mixtures were filtered after cooling, and the filtrates were used for the determination of glycocyamine.

Determination of Glycocyamine—Glycocyamine is the only substance in the reaction mixtures which gives a positive test with the Sakaguchi reagent, but, since the large amounts of canavanine and glycine interfere with the determination of glycocyamine by this reagent, it is necessary to separate glycocyamine from these two amino acids. The separation was achieved by paper chromatography.

The chromatograms were developed by the descending technique on filter paper (Toyo-Roshi No. 52, 2 x 40 cm.) with a butanol-acetic acid-water mixture (4:1:1), and were sprayed with the Sakaguchi reagent to detect guanidino groups, or with ninhydrin to detect primary amino groups. Rf values of glycocyamine, glycine, and canavanine were 0.30, 0.18, and 0.13, respectively. Quantitative chromatograms were run usually with a batch of eleven sheets of paper in a tank, one sheet of which was sprayed with the Sakaguchi reagent for locating glycocyamine. From the other paper strips the areas corresponding to the spot of glycocyamine were cut off, and the glycocyamine so separated was extracted by heating the paper with 10 ml. of water in a stoppered tube at 70-80° for 30 minutes. Taking 5 ml. of the supernatant liquid of the extract, glycocyamine was determined by the Sakaguchi's colorimetric method (4).

RESULTS

Formation of Glycocyamine

When canavanine and glycine were incubated with the extract of hog kidney, a substance was formed which gave a red color with the Sakaguchi reagent. This substance had always the same Rf values as an authentic glycocyamine when developed on filter paper with various solvents. Consequently, it seems sure that the substance formed is glycocyamine.

The effect on the reaction of omitting one of the components is shown in Table I. If the enzyme solution was omitted from the reaction mixture, no glycocyamine was formed. A small amount of glycocyamine was found to be present in the extract of kidney. A very slight increase in glycocyamine was observed when glycine alone was incubated with the enzyme. Canavanine without glycine led to a somewhat significant increase in glycocyamine. When both amino acids were added together, the amount of glycocyamine formed was more than
4 times the amount with canavanine alone. From these results it is sure that the enzymatic canavanine-glycine transamidination reaction as described in the introduction occurs. The increase obtained with canavanine alone may indicate the presence of a small amount of free glycine in the kidney extract. Both α- and β- alanines were inactive as acceptors for amidine groups.

Table II shows the relation between time of incubation and the amount of glycocyamine formed.

**TABLE I**

*Formation of Glycocyamine*

<table>
<thead>
<tr>
<th>Canavanine (µM)</th>
<th>Glycine (µM)</th>
<th>Enzyme soln. (ml.)</th>
<th>Glycocyamine found (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>80</td>
<td>1</td>
<td>10.3</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>0</td>
<td>80</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>40</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., temp., 35°, time of incubation 4 hrs.

**TABLE II**

*Rate of Glycocyamine Formation*

<table>
<thead>
<tr>
<th>Time of incubation hrs.</th>
<th>Glycocyamine formed µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>9.9</td>
</tr>
<tr>
<td>24</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., enzyme soln. 1 ml., canavanine 40 µM, glycine 80 µM, temp. 35°.

*Effect of pH on Activity of Transamidinase*

The effect of pH on the glycocyamine formation is shown in Table III. The reaction proceeded at a significant rate between pH 5.9 and 9.0, with an optimum in the neighborhood of pH 7.4 under the conditions employed.
**TABLE III**

*Effect of pH*

<table>
<thead>
<tr>
<th>pH</th>
<th>5.0</th>
<th>5.9</th>
<th>7.0</th>
<th>7.4</th>
<th>8.0</th>
<th>8.5</th>
<th>9.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocyamine formed μM</td>
<td>0</td>
<td>3.9</td>
<td>9.1</td>
<td>11.6</td>
<td>9.8</td>
<td>6.7</td>
<td>4.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer 2 ml., enzyme soln. 1 ml., canavanine 40 μM, glycine 80 μM, temp. 35°, time of incubation 4 hrs.

**Effect of Canavanine Concentration on Glycocyamine Formation**

The effect of the canavanine concentration on the glycocyamine formation is shown in Table IV. The rate of glycocyamine formation increased with increasing canavanine concentration in the range of 0.0005–0.005 M, but remained almost constant at the concentrations higher than 0.005 M. This observation may be explained by the Michaelis' theory.

**TABLE IV**

*Effect of Canavanine Concentration*

<table>
<thead>
<tr>
<th>Canavanine concentration M</th>
<th>0.0005</th>
<th>0.001</th>
<th>0.002</th>
<th>0.005</th>
<th>0.01</th>
<th>0.02</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocyamine formed μM</td>
<td>2.0</td>
<td>4.3</td>
<td>6.5</td>
<td>11.2</td>
<td>9.7</td>
<td>10.2</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., enzyme soln. 1 ml., glycine 0.02 M, temp. 35°, time of incubation 4 hrs.

**Effect of Glycine Concentration on Glycocyamine Formation**

As shown in Table V, the rate of glycocyamine formation increased approximately in proportion to the concentration of glycine in the range of 0.001–0.01 M. However, the rate remained almost constant at the concentrations of glycine higher than 0.02 M.

**Effect of Dialysis on Enzyme Solution**

The activities of the following enzyme solutions are compared each other in Table VI.
**TABLE V**

*Effect of Glycine Concentration*

<table>
<thead>
<tr>
<th>Glycine concentration M</th>
<th>0.001</th>
<th>0.002</th>
<th>0.005</th>
<th>0.01</th>
<th>0.02</th>
<th>0.05</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocyamine formed µM</td>
<td>1.0</td>
<td>2.1</td>
<td>4.6</td>
<td>9.9</td>
<td>13.4</td>
<td>12.1</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., enzyme soln. 1 ml., canavanine 0.01 M, temp. 35°, time of incubation 4 hrs.

(i) An enzyme solution was dialyzed for 2 days in the presence of toluene against running water (Enzyme solution 1).

(ii) To a dialyzed enzyme solution, sufficient manganese sulfate was added to give a concentration of 2 mg. Mn⁺⁺/ml. (Enzyme solution 2).

(iii) An enzyme solution which had not been subjected to dialysis was diluted with the amount of water equivalent to the expansion of volume of Enzyme solution 1 during dialysis (Enzyme solution 3).

**TABLE VI**

*Effect of Dialysis*

<table>
<thead>
<tr>
<th>Enzyme solution</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocyamine formed µM</td>
<td>6.4</td>
<td>3.7</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., enzyme soln. 1 ml., canavanine 40 µM, glycine 80 µM, temp. 35°, time of incubation 4 hrs.

As seen in Table VI, the activity of transamidinase was increased by dialysis, and was decreased by the addition of manganese sulfate, in contrast with canavanase whose activity was enhanced by manganese ions. The increase of the activity of transamidinase by dialysis may be attributed to the removal of inhibitors, probably heavy metal ions, because the activity of the enzyme was increased also by the addition of a metal binder, ethylenediaminetetraacetate.

*Inhibition by p-Chloromercuribenzoate*

The activity of transamidinase was reduced to about 60 per cent
by the addition of $\rho$-chloromercuribenzoate to give a final concentration of 0.001 $M$, but was restored completely by the treatment with an excess amount of glutathione (final concentration 0.01 $M$). These results are shown in Table VII. Because $\rho$-chloromercuribenzoate is known to react specifically with sulfhydryl groups, it is suggested that sulfhydryl groups may be involved in the enzyme action of transamidinase. This suggestion is also supported by the sensitiveness of the enzyme to heavy metal ions described above.

**Table VII**

_Inhibition by $\rho$-Chloromercuribenzoate_

<table>
<thead>
<tr>
<th>Enzyme soln.</th>
<th>Without $\rho$-chloromercuribenzoate</th>
<th>Inhibited by $\rho$-chloromercuribenzoate</th>
<th>Reactivated by glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocyamine formed $\mu$m</td>
<td>10.4</td>
<td>6.1</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Total volume 4 ml., buffer (pH 7.4) 3 ml., enzyme soln. 1 ml., canavanine 40 $\mu$m, glycine 80 $\mu$m, temp. 35°, time of incubation 4 hrs.

**DISCUSSION**

It seems unlikely at present that canavanine is a normal metabolite of higher animals. It is probable, however, that plants containing canavanine are ingested by higher animals. Consequently, it is possible that the canavanine-glycine transamidination reaction occurs in higher animals and canavanine replaces arginine so far as the glycocyamine formation is concerned.

The mechanism of transamidination reaction has not been made clear. However, it was suggested by the results presented here that sulfhydryl groups might be involved in the enzyme action and no cofactor might be required. On the other hand, glycocyamine can be synthesized by the action of S-methylisothiourea on glycine in a weakly alkaline aqueous solution at a room temperature as given in the following diagram:

\[
\begin{align*}
\text{NH} \\
\text{H}_2\text{N}-\text{C-S-CH}_3 + \text{H}_2\text{N-CH}_2\text{-COOH} & \rightarrow \text{H-S-CH}_3 + \text{H}_2\text{N-} \underset{\text{\|}}{\text{C-NH-CH}_2\text{-COOH}} \\
\end{align*}
\]

Therefore, it is reasonable to speculate that an enzyme-amidine intermediate complex of isothioureia-type may be formed, and the mechanism of the reaction may be tentatively represented by the following diagram:
Since arginine-handling enzymes of many organisms cannot distinguish completely between arginine and canavanine, the enzyme catalyzing the transamidination from canavanine may be the same as that catalyzing the transamidination from arginine. Borsook and Dubnoff reported that a cell-free macerate of kidney was less active than an equivalent amount of kidney tissue in the form of slices (1). This observation may be explained by the sensitiveness of the enzyme to heavy metal ions reported in this paper; the destruction of the cell may involve contamination of the enzyme with various metal ions from which the enzyme is presumably separated in the intact cell.

**SUMMARY**

An enzyme from hog kidney was found to catalyze the transfer of the amidine moiety of canavanine to glycine with the formation of glycocyamine. The enzyme was sensitive to heavy metal ions, and was inhibited by \( p \)-chloromercuribenzoate. No cofactor requirement was observed, and the optimal pH was about 7.4. It was suggested that an isothiourea-type enzyme-amidine intermediate complex might be formed.

The author wishes to thank Dr. S. Shibuya and Dr. N. Izumiya for their valuable advice.

**REFERENCES**