ENZYMATIC TRANSAMINATION REACTIONS INVOLVING
ARGININE AND ORNITHINE

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Recent research has resulted in considerable modification of the earlier concept that enzymatic transamination was limited to reactions between alanine, aspartate, glutamate, and their \( \alpha \)-keto analogues. Reactions leading to the reversible amination of the \( \alpha \)-keto analogues of many of the natural amino acids have been described (1–5), and there is also evidence that aldehyde groups may participate in transamination (6–9). It has been reported that incubation of \( \alpha \)-ketoglutarate and arginine with crude liver and kidney preparations resulted in glutamate formation (2). The presence of arginase in these preparations was apparently not investigated, and the participation of arginine itself in transamination remains to be demonstrated. Transamination between pyruvate and ornithine in liver preparations yielding alanine has also been described (10). Although no evidence concerning the fate of the ornithine carbon chain in the liver system was obtained, a transamination reaction between ornithine and \( \alpha \)-keto-glutarate catalyzed by extracts of Neurospora crassa has been found to yield glutamate and glutamic-\( \gamma \)-semialdehyde (7).

The present report describes an investigation of transamination reactions catalyzed by liver preparations between glutamine and the \( \alpha \)-keto analogues of arginine (\( \alpha \)-keto-\( \delta \)-guanidinovaleric acid) and nitroarginine (\( \alpha \)-keto-\( \delta \)-nitroguanidinovaleric acid). In the course of this study, transamination between ornithine and \( \alpha \)-ketoglutarate yielding glutamate and glutamic-\( \gamma \)-semialdehyde was observed. Ornithine was subsequently found to transamine with glyoxylate, \( \alpha \)-ketobutyrate, and a number of other \( \alpha \)-keto acids to yield glutamic-\( \gamma \)-semialdehyde and the corresponding amino acids. This paper also reports studies on the growth response of rats to \( \alpha \)-keto-\( \delta \)-guanidinovaleric acid.

EXPERIMENTAL

The rat liver glutamine transaminase-deamidase preparation previously described was employed (6). L-Glutamine, L-glutamic acid (11, 12), L-arginine (13), L-ornithine (14), and L-lysine (15) were determined with

1 Cultures of the organisms possessing specific decarboxylase activity were obtained through the courtesy of Dr. E. F. Gale.
the respective specific bacterial decarboxylases. Urea was determined with crystalline urease (16). Ascending paper chromatographic studies were carried out as previously described with six solvent mixtures (6, 17). Urea was visualized by spraying the chromatograms with phenol and hypochlorite (18). Microbiological studies were performed as described by Davis and Mingioli (19).\(^2\) The preparation of the \(\alpha\)-keto acids used in this investigation has been reported (17, 20, 21).

**Results**

**Transamination between Glutamine and \(\alpha\)-Keto-\(\delta\)-guanidinovaleric Acid**—Experiments with *Escherichia coli* and with rat liver preparations revealed that transamination between \(\alpha\)-keto-\(\delta\)-guanidinovaleric acid and glutamate (and a number of other amino acids) was exceedingly slight or did not occur. On the other hand, appreciable transamination was observed between this keto acid and glutamine with liver preparations. Transamination between glutamine and \(\alpha\)-keto acids yielding \(\alpha\)-ketoglutarate, ammonia, and the corresponding \(\alpha\)-amino acids has been previously described (1, 6). It was found that incubation of \(\alpha\)-keto-\(\delta\)-guanidinovaleric acid and glutamine with this liver system at pH 8.2 resulted in ammonia formation and glutamine disappearance, but there was no evidence of arginine formation as determined with arginine decarboxylase or by paper chromatography. Since the enzyme preparation possessed arginase activity, the formation of urea and ornithine was investigated, and it was found that urea and ammonia were formed stoichiometrically.\(^3\) However, only traces of ornithine were formed as determined with ornithine decarboxylase and by paper chromatography. Analyses with *Clostridium welchii* decarboxylase and paper chromatographic studies indicated that glutamate was formed, suggesting transamination between ornithine and \(\alpha\)-ketoglutarate. This reaction was specifically studied and was found to be catalyzed by the enzyme preparation as described below.

Although ornithine was not detected as a major product of the glutamine-\(\alpha\)-keto-\(\delta\)-guanidinovaleric acid reaction when studied at pH 8.2, some ornithine was formed when the reaction was carried out at lower pH values. Investigation of the pH dependence of the glutamine-\(\alpha\)-keto-\(\delta\)-guanidinovaleric acid and the ornithine-\(\alpha\)-ketoglutarate reactions led to the findings described in Fig. 1. The optimal pH range for the ornithine-\(\alpha\)-ketoglutarate reaction was 8.2 to 9.0. The reaction proceeded slowly at pH 7.0, and no activity was observed at pH 6.0. Since the glutamine \(\alpha\)-keto-\(\delta\)-guanidinovaleric acid system was active at pH 6.0, the formation of ornithine could readily be demonstrated under these conditions (Table I).

\(^2\) Cultures of the *E. coli* mutants used in this study were generously donated by Dr. B. D. Davis.

\(^3\) Purified arginase did not act upon \(\alpha\)-keto-\(\delta\)-guanidinovaleric acid (17).
Substitution of a nitro group in the guanidino group of α-keto-δ-guanidinovaleric acid resulted in a somewhat less susceptible substrate for transamination. However, when α-keto-δ-nitroguanidinovaleric acid was incubated with glutamine and enzyme, formation of the corresponding amino acid, nitroarginine, was possible, since nitroarginine is not attacked by arginase (Table II).

![Graph showing pH dependence of the glutamine-α-keto-δ-guanidinovaleric acid and ornithine-α-ketoglutarate reactions.](image)

**Table I**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>pH</th>
<th>NH₃</th>
<th>Ornithine</th>
<th>Glutamate</th>
<th>Urea</th>
<th>Glutamic-γ-semialdehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine (10 µM) + α-keto-δ-guanidinovalerate (20 µM)</td>
<td>8.2</td>
<td>8.04</td>
<td>0.50</td>
<td>8.05</td>
<td>8.14</td>
<td>9.0</td>
</tr>
<tr>
<td>Glutamate (10 µM) + α-keto-δ-guanidinovalerate (20 µM)</td>
<td>6.0</td>
<td>8.21</td>
<td>7.62</td>
<td>0.60</td>
<td>7.94</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Ketoglutarate (10 µM) + ornithine (10 µM)</td>
<td>8.2</td>
<td>0</td>
<td>9.40</td>
<td>0.63</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>(20 µM)</td>
<td>6.0</td>
<td>0</td>
<td>9.81</td>
<td>0.52</td>
<td>0.20</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*The reaction mixtures contained substrates as indicated and 50 mg. of enzyme preparation in 1.0 cc. of 0.05 M Veronal buffer (pH 8.2) or 0.1 M potassium phosphate buffer (pH 6.0); incubated for 2 hours at 37°.*

**Transamination between Ornithine and α-Keto Acids**—Attempts to demonstrate the formation of α-keto-δ-aminovaleric acid as a product of transamination between ornithine and α-ketoglutarate and between glutamine and α-keto-δ-guanidinovaleric acid were not successful. On the other hand, the formation of glutamic-γ-semialdehyde (or its cyclized form, Δ¹-pyrroline-5-carboxylic acid) as a product of these reactions (at pH 8.2) was suggested by the development of a yellow color on addition of α-aminobenzaldehyde to the deproteinized reaction mixtures. Paper chromatography (17) of such mixtures indicated that the yellow compound (presum-
ably the dihydroquinazolinium derivative formed by condensation of o-aminobenzaldehyde with Δ1-pyrroline-5-carboxylic acid (22-24)) exhibited the same mobility as the derivative prepared from an authentic sample of Δ1-pyrroline-5-carboxylic acid.4

Vogel and Davis (22) demonstrated that glutamic-γ-semialdehyde was converted by spontaneous ring closure to Δ1-pyrroline-5-carboxylic acid, and that this compound was a precursor of proline in E. coli. By use of the mutant strains of E. coli developed and studied by these workers, it was found that the deproteinized reaction mixtures of transaminase experi-

### Table II

**Transamination between Glutamine or Glutamate and Several α-Keto Acids**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>NH₃ Transamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine + α-keto-δ-guanidinovalerate</td>
<td>5.78 5.50†</td>
</tr>
<tr>
<td>Glutamate + &quot;</td>
<td>0.20†</td>
</tr>
<tr>
<td>Glutamine + α-keto-δ-nitroguanidinovalerate</td>
<td>3.22 2.91§§</td>
</tr>
<tr>
<td>Glutamate + &quot;</td>
<td>0.51§§</td>
</tr>
<tr>
<td>Glutamine + α-keto-δ-N-carbobenzoxyvalerate</td>
<td>8.02 8.82§§</td>
</tr>
<tr>
<td>Glutamate + &quot;</td>
<td>0.90§§</td>
</tr>
<tr>
<td>Glutamine + α-keto-ε-N-carbobenzoxycapeproate</td>
<td>8.87 9.21§§</td>
</tr>
<tr>
<td>Glutamate + &quot;</td>
<td>1.10§§</td>
</tr>
</tbody>
</table>

* The reaction mixtures contained 10 μM of L-glutamine, or L-glutamate, 20 μM of keto acid, and 40 mg. of enzyme preparation in 1.5 cc. of 0.05 M Veronal buffer at pH 8.2; incubated for 2 hours at 37°.
† Based on urea formation.
§ Based on disappearance of glutamine or glutamate.
§§ The formation of the corresponding amino acid was observed chromatographically.

ments (at pH 8.2) between glutamine and α-keto-δ-guanidinovaleric acid and between ornithine and α-ketoglutarate supported the growth of E. coli, mutant 55-25 (which responds to glutamic-γ-semialdehyde or proline), but did not promote growth of mutant 55-1 (which responds only to proline). The formation of glutamic-γ-semialdehyde was quantitatively determined by assay with mutant 55-25.

The α-ketoglutarate-ornithine reaction proceeded to approximately 90 per cent completion (Fig. 2). Attempts were made to demonstrate the reverse reaction by incubating equimolar quantities of glutamate and glutamic-γ-semialdehyde at the same concentrations as those of α-keto-glutarate and ornithine in the experiment described in Table I. Under

4 Prepared from γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde (22) generously pro-
vided by Dr. H. J. Vogel.
these conditions, no ornithine was formed as determined with ornithine decarboxylase, and only traces of this amino acid were found by chromatography or by microbiological assay. Similar findings with the *Neurospora* system are recorded by Fincham (7). However, with higher concentrations of glutamic-γ-semialdehyde and glutamate, slight but definite formation of ornithine was observed. Thus, when 100 μM each of these substrates were incubated with 20 mg. of enzyme preparation in 0.4 cc. of 0.1 M Veronal buffer (pH 8.2) for 2 hours at 37°, 1.8 μM of ornithine were formed (by assay with *E. coli*, mutant 160-37). Less than 0.9 μM of ornithine was formed when glutamate was replaced by glutamine, alanine, or aspartate. The failure of appreciable transamination between glutamate and glutamic-γ-semialdehyde may be due to conversion of glutamic-γ-semialdehyde (or its cyclized form) to other products.

In view of the wide specificity of certain transaminase systems, it was of interest to determine whether α-keto acids other than α-ketoglutarate or pyruvate were capable of transaminating with ornithine. In these experiments the reaction was followed by determinations of glutamic-γ-semialdehyde, and by observation of the formation of new amino acid. The results, given in Table III, indicate that ornithine transaminated with pyruvate, α-ketoglutarate, α-ketobutyrate, glyoxylate, α-keto-γ-methylbutyrate, phenyl pyruvate, p-hydroxyphenylpyruvate, α-ketoisocaproate, α-ketoisovalerate, and d-α-keto-β-methylvalerate. Although formation of the corresponding amino acid was demonstrated in each case, only the first four of these keto acids transaminated at an appreciable rate.

Effect of α-Keto-δ-guanidinovaleric Acid on Growth of Weanling Rats—Thirty-nine Sprague-Dawley, male rats, 21 days old, weighing 26.4 to 46.8 gm., were obtained from ten litters. The animals were given water and diet *ad libitum*, and were maintained in individual cages in a constant
### Table III

**Transamination between Ornithine and α-Keto Acids***

<table>
<thead>
<tr>
<th>α-Keto acid</th>
<th>Transamination†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvic</td>
<td>21.0</td>
</tr>
<tr>
<td>α-Ketobutyric</td>
<td>6.1</td>
</tr>
<tr>
<td>α-Ketoglutaric</td>
<td>6.5</td>
</tr>
<tr>
<td>Glyoxylic</td>
<td>12.9</td>
</tr>
<tr>
<td>α-Ketoisocaproic</td>
<td>1.7</td>
</tr>
<tr>
<td>Phenylpyruvic</td>
<td>2.9</td>
</tr>
<tr>
<td>p-Hydroxyphenylpyruvic</td>
<td>1.8</td>
</tr>
<tr>
<td>α-Ketoisovaleric</td>
<td>1.3</td>
</tr>
<tr>
<td>d-α-Keto-β-methylvaleric</td>
<td>0.6</td>
</tr>
<tr>
<td>α Keta γ methiolbutyric</td>
<td>3.1</td>
</tr>
<tr>
<td>Indolepyruvic</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* The reaction mixtures contained 25 μM of L-ornithine, 25 μM of α-keto acid, and 25 mg. of enzyme preparation in 0.4 cc. of 0.05 M potassium phosphate buffer (pH 7.5); incubated for 2 hours at 37°.

† Micromoles of glutamic-γ-semialdehyde formed; the formation of the corresponding amino acid was observed by paper chromatography.

### Table IV

**Growth Response of Rats to Arginine and α-Keto-δ-guanidinovaleric Acid***

<table>
<thead>
<tr>
<th>Litter No.</th>
<th>Weight gain, gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>183</td>
<td>20.7</td>
</tr>
<tr>
<td>59</td>
<td>34.9</td>
</tr>
<tr>
<td>142</td>
<td>41.5</td>
</tr>
<tr>
<td>149</td>
<td>27.1</td>
</tr>
<tr>
<td>140</td>
<td>28.9</td>
</tr>
<tr>
<td>57</td>
<td>30.1</td>
</tr>
<tr>
<td>78</td>
<td>33.5</td>
</tr>
<tr>
<td>122</td>
<td>39.3</td>
</tr>
<tr>
<td>169</td>
<td>30.4</td>
</tr>
<tr>
<td>128</td>
<td>21.9</td>
</tr>
</tbody>
</table>

Average: 31.3 20.0 19.6

* The experimental details are given in the text.
The diet described by Borman et al. (25) was used as described, except that folic acid and vitamin B₁₂, at concentrations of 2 mg. and 30 μg per kilo of diet, respectively, were substituted for Wilson’s liver powder. The rats of each litter were divided into three groups. Group I received a complete diet containing arginine, Group II was supplied a ration in which α-keto-δ-guanidinovaleric acid was substituted for arginine at an equimolar level, and Group III was given a diet lacking in arginine. The animals were weighed on alternate days and fresh diet was supplied daily. The results, given in Table IV, indicate that, for each litter, the α-keto analogue of arginine did not replace arginine in stimulating growth. The acceleration of the growth rate of weanling rats by arginine noted by Borman et al. (25) was confirmed. The present results do not demonstrate a significant growth-promoting effect of α-keto-δ-guanidinovaleric acid under conditions similar to those employed in studies which showed a growth response to the α-keto analogues of leucine, isoleucine, valine, and phenylalanine (26).

DISCUSSION

The evidence indicates that α-keto-δ-guanidinovaleric acid is capable of transamination in the rat liver glutamine transaminase system at pH values between 6.0 and 9.5. Arginine formation was not observed owing to the presence of arginase; however, nitroarginine was formed when its keto analogue was incubated in this system. When the glutamine-α-keto-δ-guanidinovaleric acid reaction was studied at pH 6.0, stoichiometric formation of ammonia, urea, and ornithine was demonstrated. At pH 6.0, the α-ketoglutarate-ornithine reaction did not proceed to a significant extent. The experimental findings suggest the following sequence of enzymatic events:

1. Glutamine + α-keto-δ-guanidinovaleric acid → arginine + α-ketoglutarate + NH₃
2. Arginine → urea + ornithine
3. Ornithine + α-ketoglutarate → glutamate + glutamic-γ-semialdehyde

The failure of α-keto-δ-guanidinovaleric acid to promote the growth of rats suggests that amination of the keto acid does not occur at a rate compatible with optimal growth, or that the keto acid undergoes other reactions in vivo.

The ornithine-α-keto acid reaction is unique in that the δ-amino group rather than the α-amino group transaminates. The conversion of lysine by α-Keto-δ-guanidinovaleric acid did not support the growth of E. coli mutants (Davis) 45A-25, 39A-27, 160-37, and 39A-23. All of these responded to L-arginine under the same conditions.
to α-amino adipic acid may also involve an ω-amino group transamination reaction, leading to the corresponding ω-aldehyde (cf. Borsook et al. (27)). When the ω-amino group is substituted as in δ-chloroacetyl-L-ornithine or ω-keto-δ-N-carbobenzoxyvalerate, transamination involving the α-amino group could be demonstrated (28). ω-N-substituted lysine has also been found to transaminate with ω-ketoglutarate (28), and, in the present and previous (6) studies, ω-N-substituted ω-keto analogues of lysine were also active. The ω-keto analogues of lysine and ornithine, in contrast to their ω-N-carbobenzoxy derivatives (Table II), were not active in the glutamine transaminase system. However, the possibility exists that these compounds may transaminate in other systems as previously discussed (17). It is also possible that they undergo ω-amino group transamination, or decarboxylation to the corresponding ω-amino acids. Further investigation of the metabolism of these keto acids is in progress.

The fate of glutamic-γ-semialdehyde in animal tissues has not been specifically examined, although conversion to glutamate and proline as previously considered (29) appears probable. In E. coli, conversion of glutamic-γ-semialdehyde to proline apparently occurs (22) and more recently it has been found that α-N-acetylglycaminic-γ-semialdehyde is a precursor of ornithine in this organism. α-N-Acetylglycaminic-γ-semialdehyde is converted by transamination to α-acetyloranithine, followed by hydrolysis of the latter compound to ornithine (9).

The ornithine-α-keto acid reaction differs from the glutamine-α-keto acid reaction in that α-keto-β-methylvalerate and α-ketoisovalerate transaminated with ornithine but not with glutamine. In addition, with the enzyme system employed, transamination involving ornithine was considerably less rapid than that with glutamine for most of the α-keto acids. Although it appears probable that different enzymes catalyze the two reactions, conclusive evidence of their separate identity will depend upon separation of the activities concerned.

Several transamination reactions involving aldehydes have been reported. These include reactions between glyoxylate and glutamine, glutamate, asparagine, and aspartate (6), transamination between succinic semialdehyde and glutamate (8), and the reactions noted above involving glutamic-γ-semialdehyde and its α-N-acetyl derivative. The transamination reaction between glyoxylate and ornithine (Table III) is unique in that an aldehyde is both a reactant and a product.

\[
\begin{align*}
\text{CHO} & \quad (\text{CH}_3)_2\text{NH}_2 \\
\text{COOH} & \quad \text{CHNH}_2 \\
\text{CHO} + (\text{CH}_3)_2\text{NH}_2 & \rightarrow \text{CH}_2\text{NH}_2 + \text{COOH} \\
\text{COOH} & \quad \text{COOH} \\
\end{align*}
\]
The author wishes to acknowledge the valuable assistance of Miss Phyllis E. Fraser and Miss Sarah V. Tice.

SUMMARY

1. Incubation of α-keto-δ-guanidinovaleric acid, glutamine, and a rat liver fraction at pH 8.2 resulted in formation of equimolar quantities of urea, ammonia, glutamate, and glutamic-γ-semialdehyde. At pH 6.0 the products included urea, ornithine, and ammonia; only traces of glutamate and glutamic-γ-semialdehyde were formed. The evidence is compatible with a mechanism involving transamination of α-keto-δ-guanidinovaleric acid and glutamine, yielding arginine, ammonia, and α-ketoglutarate. This is followed by hydrolysis of arginine to ornithine and urea, and a transamination reaction between α-ketoglutarate and ornithine, yielding glutamate and glutamic-γ-semialdehyde. The latter reaction does not occur to a significant extent at pH 6.0. The formation of arginine could not be demonstrated in this system owing to the presence of arginase activity; however, nitroarginine was formed from its α-keto analogue under these conditions.

2. Transamination between ornithine and several α-keto acids yielding the corresponding amino acids and glutamic-γ-semialdehyde was demonstrated. The reaction between α-ketoglutarate and ornithine proceeded to approximately 90 per cent completion, and this reaction was found to go considerably less rapidly in the direction of ornithine formation.

3. The transamination reaction between glyoxylate and ornithine resulting in glycine and glutamic-γ-semialdehyde formation is unique in that an aldehyde is both a reactant and a product.

4. The effect of α-keto-δ-guanidinovaleric acid in promoting the growth of weanling rats was investigated. The keto acid did not replace arginine in accelerating growth, suggesting that amination does not occur at a rate compatible with optimal growth, or that the keto acid undergoes other reactions in vivo.

BIBLIOGRAPHY

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