DEAMINATION OF SERINE

I. CATALYTIC DEAMINATION OF SERINE AND CYSTEINE BY PYRIDOXAL AND METAL SALTS*

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In an investigation of transamination reactions between pyridoxal and amino acids at 100° (2) serine was found to behave anomalously, apparently being deaminated to pyruvic acid with little change in pyridoxal concentration. Although serine is known to be deaminated by hot concentrated alkalies (3) and acids (4), such a non-enzymatic reaction in the pH range near neutrality has not been reported. This reaction has now been studied in some detail and has been shown to require both pyridoxal and a metal salt for its catalysis. Cysteine undergoes a similar reaction to yield pyruvate, ammonia, and hydrogen sulfide.

Methods

Analytical methods for keto acids, pyridoxal, and pyridoxal plus pyridoxamine and the sources of most chemicals have been described (2). The keto acid assay was standardized against sodium pyruvate and against solutions of sodium hydroxypyruvate.

Ammonia was determined by placing a 2 ml. sample containing 0.5 to 2.5 μM of ammonia in an 18 × 125 mm. culture tube. 1 ml. of 10 per cent NaOH was added and the ammonia collected by aerating for 45 minutes with washed air into 3 ml. of 0.05 n HCl. The collected ammonia was determined colorimetrically with the modified Nessler’s reagent of Johnson (5). Results were reproducible within ±1 per cent.

Chromatography of Keto Acid 2,4-Dinitrophenylhydrazones—The procedure followed was essentially that of Cavallini et al. (6). Keto acids were allowed to react with 0.1 per cent 2,4-dinitrophenylhydrazine in 2 n HCl, avoiding a large excess of reagent. The hydrazones were extracted into ethyl acetate and spotted onto sheets of Whatman No. 1 paper on which had previously been placed 0.01 ml. of 0.2 M phosphate buffer (pH 7.2) at the site of each sample spot. The buffer served to neutralize any HCl carried into the ethyl acetate and minimized spreading of the sample spots. The upper layer of the system n-butanol 50, water 40, ethanol 10 (parts by volume) was the usual solvent. The paper was equilibrated

* A preliminary report has appeared (1).

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with solvent vapors for 1 to 2 hours before it was lowered into the solvent. Development was continued until the solvent front climbed about 30 cm.

With all α-keto acids except ketogluatamic acid, two spots with distinctly different $R_F$ values and different absorption spectra in alkaline solution were obtained. When a fresh ethyl acetate solution of the crystalline dinitrophenylhydrazone of pyruvic acid was placed on paper and chromatographed, only the slower moving spot was obtained; however, the faster spot appeared if the solution was allowed to stand before chromatographing. The crystalline 2,4-dinitrophenylhydrazone of hydroxy-
pyruvic acid\(^1\) gave only the faster spot. In every case, elution of either of the two spots with buffer, followed by acidification, ethyl acetate extraction, and rechromatographing, resulted in the appearance of both the fast and slow moving components. This ready interconversion of the two components suggests that they are syn and anti isomers of the hydrazones. The fact that the crystalline derivatives contain only one form, whereas samples of hydrazones prepared as described above (or by similar procedures commonly used in preparing samples for chromatography) contain both, may lead to confusion and possibly explains some of the “unknown” spots reported in the literature (7, 8).

$R_F$ values were somewhat variable from one day to another. The following values are averages for the two spots obtained from each keto acid. The first value ($R_F$) given is for the major component of the pair: glyoxylic acid, 0.44, 0.61; pyruvic acid, 0.51, 0.67; hydroxypyruvic acid, 0.50, 0.43; α-ketobutyric acid, 0.59, 0.68.

Absorption spectra in neutral buffered solutions and in alkaline solutions were determined on some sample spots after elution with neutral phosphate buffer and proved useful in the identification of the hydrazones.\(^2\) For example, the hydrazones from hydroxypyruvate and pyruvate give spots at $R_F$ 0.50 and 0.51, but the spectra of these in alkaline solution are unmistakably different.

Amino acids were identified by chromatography by the ascending technique on Whatman No. 1 filter paper with water-saturated phenol as the developing solvent. The spots were located with ninhydrin.

Hydroxypyruvic acid was prepared from recrystallized bromopyruvic acid (9). Periodate uptake by the solutions of hydroxypyruvate (10) was 87 per cent of theory. The molarity of such solutions was assumed to be 87 per cent of that calculated from the weight of bromopyruvic acid hydrolyzed.

Reactions were carried out at 100° in aqueous solutions sealed in small

\(^1\) We are indebted to Dr. David B. Sprinson for this sample.

\(^2\) We are grateful to Dr. Norman Radin for suggesting this procedure.
glass tubes, as previously described (2). Unless otherwise specified, 0.1 M acetate buffers (acetic acid and sodium acetate) were used.

Results

Catalysis of Serine Deamination by Pyridoxal and Metal Salts—Both pyridoxal and alum are necessary for rapid keto acid production from serine (Table I). The keto acid produced far exceeds the pyridoxal loss; hence transamination cannot account for the observed results. In separate experiments, the keto acid produced was identified as pyruvic acid by paper chromatography and spectral characterization of the dinitrophenylhydrazones. No hydroxypyruvate could be detected in the reaction mixtures.

TABLE I

Catalysis of Keto Acid Production from Serine by Pyridoxal and Alum

The samples were buffered at pH 5.0 with acetic acid (0.2 M)-ammonia buffer prepared from redistilled acid and ammonia and heated for 10 minutes at 100°.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td></td>
</tr>
<tr>
<td>mmo l.</td>
<td>mmo l.</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>Pyridoxamine 10</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>5.6</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* NH₄Al(SO₄)₂·12H₂O.

Table II shows the dependence of the pyruvate production on the concentrations of the reactants. As shown later, reaction rates are sensitive to salt concentration; the reactions of Table II proceeded more slowly than those of Table I, primarily because a less concentrated buffer was used.

The catalytic effects of various metal salts are presented in Table III. It is significant that the same salts which are catalytically effective in transamination between pyridoxal and glutamic acid (2) are effective here.

The course of the reaction with time for two different serine concentrations is shown in Fig. 1. Pyruvate and ammonia concentrations agree within experimental error except on prolonged heating, when some de-

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3 Some discrepancies exist during the early part of the reaction. The higher ammonia values are probably due to a small amount of deamination continuing at room temperature during the procedure for ammonia determination.
composition of pyruvate may occur. The initial fairly rapid decrease in pyridoxal concentration, which was accompanied by no significant decrease in total vitamin B₆, indicates that about 10 per cent of the serine under-

TABLE II

Effect of Reactant Concentrations on Rate of Pyruvate Production

The samples were buffered at pH 5.0 with 0.1 m acetate buffer and heated at 100° for 10 minutes.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Serine mm per l.</th>
<th>Pyridoxal mm per l.</th>
<th>Alum* mm per l.</th>
<th>Pyruvate formed mm per l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>1</td>
<td>2.28</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.4</td>
<td>2.27</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.2</td>
<td>1.97</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.1</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.05</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>1</td>
<td>4.80</td>
<td></td>
</tr>
</tbody>
</table>

* KAl(SO₄)₂·12H₂O.

TABLE III

Catalysis of Pyruvate Production from Serine by Various Metal Salts

10 mm serine, 4 mm pyridoxal, and 0.1 mm metal salt were heated in 0.1 m acetate buffer, pH 5.0, for 10 minutes at 100°.

<table>
<thead>
<tr>
<th>Metal ion*</th>
<th>Pyruvate formed mm per l.</th>
<th>Metal ion*</th>
<th>Pyruvate formed mm per l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.19</td>
<td>Iron(III)</td>
<td>1.12</td>
</tr>
<tr>
<td>Aluminum</td>
<td>1.52</td>
<td>Magnesium</td>
<td>0.15</td>
</tr>
<tr>
<td>Cobalt(II)</td>
<td>0.20</td>
<td>Manganese</td>
<td>0.23</td>
</tr>
<tr>
<td>Copper(II)</td>
<td>1.07</td>
<td>Nickel(II)</td>
<td>0.20</td>
</tr>
<tr>
<td>Iron(II)</td>
<td>1.23</td>
<td>Zinc</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Added as sulfates or as metal ammonium sulfates.

went transamination to hydroxypyruvate. The latter, as shown later, decomposes under these conditions and could not be detected in the reaction mixture. In 4 hours, only about 75 per cent of the theoretical pyruvate and ammonia production occurred, although production of these substances had become very slow. Chromatographically detectable amounts of serine were still present at this time.
Fig. 1. Pyruvate and ammonia production from serine as a function of time. The solid symbols are for 20 mM serine, open symbols for 10 mM serine. Pyridoxal was 4 mM, alum 1 mM. 0.1 M acetate buffer, pH 5.0, was used. ○, ○, pyruvic acid; △, △, ammonia; ■, ■, pyridoxal.

Fig. 2. The effect of pH on the production of pyruvate from serine. Serine 10 mM, pyridoxal 4 mM, and metal salt 1 mM. Reaction at 100° for 10 minutes. ○, ●, alum; △, ▲, copper sulfate; ○, △, unbuffered solutions; ●, ▲, buffered solutions (0.1 M acetate up to pH 5.6, 0.1 M phosphate, pH 5.6 to 8.3, 0.02 M borate, pH 9 to 10).
Failure of the reaction to proceed to completion is not due to attainment of an equilibrium, since no serine synthesis could be detected when pyruvate, ammonium sulfate, pyridoxal, and alum were heated together under similar conditions. It appeared due rather to a greatly decreased rate of reaction, greater than could reasonably be expected from the changes in

\[ \text{FIG. 3. Pyruvate and ammonia production from cysteine in the presence of pyridoxal and alum. Pyridoxal and cysteine were 10 mM, alum 1 mM. 0.1 M acetate buffer, pH 5.0, 100°. ○, pyruvic acid; △, ammonia; □, pyridoxal.} \]

\[ \text{FIG. 4. The reaction of pyridoxamine and hydroxypyruvate. The open symbols represent the reaction of 10 mM pyridoxamine, 10 mM hydroxypyruvate, and 1 mM alum; the solid symbols show the decomposition of hydroxypyruvate when heated alone with 1 mM alum. Reaction at 100° and pH 5, 0.1 M acetate buffer. ●, ○, keto acid calculated as hydroxypyruvate; △, pyridoxal; □, ammonia.} \]
reactant concentrations alone, and possibly due to inactivation of the metal catalyst in some manner. A somewhat similar rapid falling off of reaction rates was noted in non-enzymatic transamination experiments (2).

A minor ninhydrin-positive compound with an Rₚ value corresponding to alanine was produced. A small amount of alanine would be expected from transamination between pyruvate and the small amount of pyridoxamine present. Another minor component of lower Rₚ was formed but was not identified.

The pH dependence of the reaction rate is shown in Fig. 2. The optimum near pH 4 is quite similar to that for transamination (2). Copper sulfate catalyzes the reaction at alkaline pH values as well. The concentration of buffer has a pronounced effect on the rate, much greater than that noted in transamination reactions (2). Throughout the pH range pyridoxal losses were no greater than those observed at pH 5. Serine and pyruvate are stable up to pH 10 under the conditions used here when pyridoxal is omitted.

Pyridoxal phosphate and 5-desoxypyridoxal⁴ were even better catalysts for the reaction than pyridoxal, while 3-methoxypyridoxal⁴ was without effect.

Deamination of Cysteine—Fig. 3 shows that pyruvate (identified as described earlier) and ammonia are produced in equal amounts from cysteine, pyridoxal, and alum. A complication exists owing to the rapid formation of a thiazolidine compound between pyridoxal and cysteine (11). The incompleteness of the deamination reaction and the decrease in pyridoxal concentration with time are attributed primarily to this cause. Hydrogen sulfide liberation was not measured, but its odor was very noticeable when the reaction tubes were opened.

Reaction of Hydroxypyruvate and Pyridoxamine—This reaction was investigated in an attempt to shed some light on the mechanism of the deamination reaction. Fig. 4 shows the course of keto acid disappearance and pyridoxal and ammonia production. Hydroxypyruvate when heated alone decomposes rapidly, giving no keto acids detectable by chromatography. These data indicate that about 15 per cent of the reactants are transaminated to pyridoxal and serine. The latter was detectable in small amounts on chromatograms after 5 minutes. The observed slow ammonia evolution is probably due to deamination of the serine produced and to a slow oxidative deamination of the pyridoxamine. However, no pyruvate or alanine (which should arise by transamination) was detected. Possibly pyruvate was destroyed by reacting with hydroxypyruvate or products of its decomposition.

⁴ Dr. Karl Folkers kindly supplied these compounds.
DISCUSSION

Serine and cysteine have been shown to be catalytically deaminated by pyridoxal and metal salts to yield pyruvic acid and, in the case of cysteine, hydrogen sulfide. The similarities of pH optima and catalytic metal salts in these deaminations and in transamination reactions (2) suggested that the first step in the deamination of serine might involve a transamination reaction to yield the Schiff base of pyridoxamine and hydroxypyruvic acid, which could then undergo a dehydration, rearrangement, and hydrolysis to the observed products. Experimentally, however, hydroxypyruvate and pyridoxamine yield ammonia only slowly. Nevertheless, the similarities between non-enzymatic transamination and deamination reactions suggest a common feature in the mechanisms. This could be the formation of an intermediate reactive Schiff base, stabilized by chelation with the catalytic metal. By shift of the double bond and migration of the α-hydrogen, transamination can occur, or by splitting out of water (or H₂S) the Schiff base of amino acrylic acid could be formed and undergo spontaneous hydrolysis to free amino acrylic acid, and thence to ammonia and pyruvic acid.

The catalytic role of pyridoxal in these reactions suggests that pyridoxal phosphate may be the coenzyme for enzymatic dehydration of serine as well as for desulfhydration of cysteine (1, 12).

SUMMARY

1. Serine is deaminated by pyridoxal and aluminum, copper, or iron salts at 100° to yield pyruvic acid and ammonia.
2. pH optima for the reaction are at about 4 and above 9. Rates are highly dependent on the concentration of buffer salts and on the catalytic metal ion used.
3. Cysteine undergoes a similar reaction to yield pyruvate, ammonia, and hydrogen sulfide.
4. Hydroxypyruvic acid and pyridoxamine transaminate at pH 5, 100°, to yield serine and pyridoxal. The reaction is partially obscured, however, by the rapid decomposition of hydroxypyruvate under these conditions.
5. A possible mechanism for the catalytic deamination of serine and cysteine is discussed briefly.
BIBLIOGRAPHY

DEAMINATION OF SERINE: I.
CATALYTIC DEAMINATION OF SERINE AND CYSTEINE BY
PYRIDOXAL AND METAL SALTS
David E. Metzler and Esmond E. Snell