STUDIES ON THE MECHANISM OF DEAMINATION OF SERINE AND THREONINE IN BIOLOGICAL SYSTEMS*

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(Received for publication, August 27, 1943)

The biological fate of serine is of particular interest, since this hydroxy-amino acid may be considered to form, in certain respects, a connecting link between the metabolism of proteins and that of phospholipids. In a recent brief communication from this laboratory (1) the mechanism of the deamination of serine by Bacterium coli was discussed. The present paper extends the experiments to the essential amino acid threonine and offers a description of the experimental results obtained with both serine and threonine and the following bacterial species: Bacterium coli, Clostridium welchii, Pseudomonas pyocyanea, Proteus OX-19. A few orienting experiments with mammalian tissue are likewise included, but their detailed presentation will be reserved for a later publication.

With resting suspensions of Bacterium coli, which were very active in the deamination of both d- and l-serine (2), dl-O-ethylserine, l-phosphoserine, and phosphatidyl serine from beef brain were not deaminated; dl-O-methylserine was attacked very slowly but, in contrast to serine, under aerobic conditions only. The inhibition of the deamination of serine on replacement of the hydroxylic hydrogen atom suggests that the first step in the reaction involves the removal of the elements of water.

\[
\text{HOCH}_2\text{-CH\cdot-COOH} \quad \text{-H}_2\text{O} \quad \text{CH}_2\text{-\cdot-COOH} \\
\text{NH}_2 \\ \quad \text{CH}_2\text{-\cdot-COOH} \quad \text{+H}_2\text{O} \quad \text{CH}_2\text{-CO\cdot-COOH + NH}_3
\]

Threonine would similarly be deaminated to α-ketobutyric acid.

It is obvious that if this formulation is correct, the fundamental equation for the oxidative deamination of amino acids (3–6), viz. \(\text{R\cdotCH(NH}_2\text{)\cdotCOOH + \frac{1}{2}\text{O}_2 = R\cdotCO\cdotCOOH + NH}_3\), would, in this special case, have to be replaced by \(\text{R\cdotCH(OH)\cdotCH(NH}_2\text{)\cdotCOOH = R\cdotCH}_2\text{\cdotCO\cdotCOOH + NH}_3\).

It is possible under suitable conditions, with all the bacterial species examined, to isolate pyruvic acid and α-ketobutyric acid in considerable quantities.

* This work has been supported by a grant from the John and Mary R. Markle Foundation.
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amounts in the form of their 2,4-dinitrophenylhydrazones as the deamination products of serine and threonine respectively. The postulate that the pathway of breakdown of these hydroxyamino acids differs from that of the other amino acids is further strengthened by the finding (2, 7), confirmed in this laboratory, that serine is deaminated by Bacterium coli under aerobic and anaerobic conditions, whereas alanine is attacked only aerobically.¹ The experiments here discussed have, furthermore, generally shown that both serine and threonine are deaminated anaerobically by all bacterial species examined. Both d- and l-serine were attacked, a finding already reported for Proteus (9) and Pseudomonas pyocyanea (10) by Bernheim and his collaborators.

A discussion of the transformations which the hydroxyamino acids undergo in the animal organism will be deferred to a later occasion. It will suffice to point out here that in the course of the present study cell-free extracts from rat, mouse, and rabbit livers were obtained which, especially after the addition of Mg²⁺ to the thoroughly dialyzed solutions, attacked serine with liberation of appreciable amounts of ammonia under anaerobic conditions. Like the bacterial preparations discussed above, these extracts failed to deaminate the O-ethers of serine. The anaerobic formation of pyruvic acid from serine by a rat liver extract was demonstrated by the isolation of the corresponding 2,4-dinitrophenylhydrazone. In contrast, kidney slices have been found not to deaminate serine under anaerobic conditions (5).

The formation of α-keto acids from serine and threonine is analogous to the conversion of serine to pyruvic acid by the action of hot acidic (11, 12) or alkaline (13) solutions or of acetic anhydride (14). The possible intermediate rôle of α-aminoacrylic acid in the production of pyruvic acid from serine has been repeatedly discussed by Bergmann and his collaborators (15, 16) on the basis of in vitro experiments with glycy!serine and alanylserine.² The findings on the breakdown of serine in biological systems here presented are in harmony with the views of Brand and his collaborators (19) and of Binkley and du Vigneaud (20) on the rôle of serine (or aminoacrylic acid) and its peptides as biological precursors of cysteine by way of cystathionine.

The conception of a dehydration as the primary step of attack on serine and threonine places the enzyme or enzymes performing this task in the class of dehydrases.³ The most thoroughly studied representative of this group

¹ A review of the entire field of the bacterial breakdown of amino acids will be found in an article by Gale (8).

² Compare also the discussions by Dakin (17) and Nicolet (18) of α,β-unsaturated α-amino acids as hypothetical biological intermediates.

³ This term is not to be confused with “dehydrogenases” (cf. (21)).
is enolase, which catalyzes the conversion of 2-phosphoglyceric acid into phosphopyruvic acid (22, 23). This enzyme is known to occur in Bac-
terium coli (24). We plan to compare the properties of the serine and
threonine dehydrases of bacteria and animal tissues with those of other
dehydrases and to define more sharply the substrate specificities of the
enzymes in question.4

EXPERIMENTAL

Methods

Substrates—Phosphatidyl serine was prepared from beef brain (26).
The preparation used had the following analytical figures: P 3.57, N 1.69,
amino N 1.67, amino acid N 1.37, iodine value 72.

A sample of l-phosphoserine was obtained through the courtesy of Dr.
F. Lipmann of the Massachusetts General Hospital, Boston. dl-O-Methyl-
serine was prepared from the corresponding N-benzoyl derivative kindly
supplied by Dr. H. E. Carter of the University of Illinois, Urbana. The
authors are indebted to Dr. V. du Vigneaud for the dl-O-ethylserine employed
in these experiments. dl-Serine and dl-threonine were Merck products.

Bacterial Suspensions—Bacterium coli, Pseudomonas pyocyanea, and
Proteus OX-19 were grown in Roux bottles at 37° on a medium composed of
2 per cent of Bacto-tryptone (Difco), 3 per cent of agar, 0.5 per cent of
sodium chloride, and 0.5 per cent of yeast concentrate. For the anaerobic
cultivation of Clostridium welchii a similar medium, but with the omission of
agar, was employed. The microorganisms were, after 20 to 24 hours
growth, collected in cold physiological saline, sedimented in a refrigerated
angle centrifuge, and washed three times with ice-cold saline.

Analytical Procedures—For the determination of ammonia by the
Nessler procedure, an equal volume of a solution of 12.4 gm. of H₂BO₃
in 110 cc. of N NaOH (27) was used to make the solutions alkaline and 1
drop of n-decanol was added, the alkaline solutions were aerated, and the
ammonia collected in 20 cc. of water containing 2 drops of 0.1 N HCl.
After the addition of the Nessler reagent, the solutions were brought to a
volume of 25 cc. and the color intensities estimated by means of a Klett-
Summerson photoelectric colorimeter. The values were ascertained from a
standard curve obtained in the same manner.

The oxygen consumption was determined in the customary manner in
Warburg vessels.

4 The enzymatic degradation of cysteine with the formation of H₂S, ammonia,
and pyruvic acid (25) exhibits many similarities with the reactions discussed here.
The reversibility of the dehydration of ß-hydroxy-α-amino acids, a characteristic of
other dehydrases, has not been tested, as the corresponding unsaturated amino acids
are not available.
A 0.6 to 0.8 per cent solution of 2,4-dinitrophenylhydrazine in 2 N HCl was employed for the precipitation of the keto acid hydrazones from the deproteinized solutions (23). Details will be found later in the paper.

**Deamination by Bacteria**

**Attack on Various Substrates**—Representative data on the aerobic and anaerobic deamination of a number of substrates by several bacterial species are summarized in Table I. It will be seen that with *Bacterium coli* dl-

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Ammonia nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aerobic</td>
</tr>
<tr>
<td><em>Bacterium coli</em> (1 hr.)</td>
<td><em>dl</em>-Serine</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td><em>l</em>-Serine</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-Threonine</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td><em>l</em>-Phosphoserine</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Phosphatidyl serine</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-O-Methylserine</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-O-Ethylserine</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-Alanine</td>
<td>114</td>
</tr>
<tr>
<td><em>Pseudomonas pyocyanea</em> (90 min.)</td>
<td><em>dl</em>-Serine</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-Threonine</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-Alanine</td>
<td>124</td>
</tr>
<tr>
<td><em>Proteus OX-19</em> (90 min.)</td>
<td><em>dl</em>-Serine</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td><em>dl</em>-Threonine</td>
<td>117</td>
</tr>
</tbody>
</table>

Serine was deaminated as rapidly as was the *l* isomer. With the exception of O-methylserine which was attacked very slowly, but only under aerobic conditions, none of the O-substituted compounds was deaminated. *dl*-Alanine was attacked only under aerobic conditions, whereas the hydroxy-amino acids were deaminated anaerobically also, although at a slower rate.

Table II offers a comparison between oxygen consumption and ammonia production with serine and alanine as substrates. If pyruvic acid is the deamination product of both amino acids, the oxidative deamination of
alanine as compared with the breakdown of serine should manifestly require the uptake of 1 additional oxygen atom. A comparison of the \( O_2 : NH_3 \) ratios for serine and alanine given in Table II shows this to be approximately the case.

**Isolation of Keto Acids As Deamination Products**—Between 3 and 4 gm. of wet bacteria were suspended in water to give a total volume of 25 cc., and the suspension was shaken with 5 cc. of toluene for 5 to 10 minutes. Most of the toluene could be removed at this point, but this proved unnecessary. The bacterial suspension was added to a solution of the amino acid in 25 cc. of 0.1 M phosphate buffer of pH 7.5 and the mixture shaken in the presence of air or, in the case of *Clostridium welchii*, of nitrogen for 2½ hours at 38°. After centrifugation 6 to 10 cc. of 30 per cent trichloroacetic acid were added to the supernatant. The addition to the filtered solution of a 0.6 to 0.8 per cent solution of 2,4-dinitrophenylhydrazine in 2 N HCl produced the immediate precipitation of the hydrazone which, after being stored in the refrigerator overnight, was filtered off and twice recrystallized for analysis from ethyl acetate (in the case of the pyruvic acid hydrazone) or ethyl acetate-ligroin (for the \( \alpha \)-ketobutyric acid hydrazone). The melting points of the crude products were only 2–3° lower than those of the purified samples. When \( dl \)-alanine was similarly treated in the presence of *Bacterium coli*, no hydrazone was precipitated. Since *Pseudomonas pyocyanea* decomposes \( \alpha \)-ketobutyric acid only slowly, its hydrazone could be isolated from the deamination of threonine regardless of whether these bacteria had been previously treated with toluene or not. The results obtained with serine and threonine are presented in Tables III and IV respectively.

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**Table II**

Relationship between Oxygen Consumption and Ammonia Production with Serine and Alanine As Substrates

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Time</th>
<th>Oxygen consumed</th>
<th>NH(_3)-N produced</th>
<th>( O_2: NH_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacterium coli</em></td>
<td>( dl )-Serine</td>
<td>50</td>
<td>100</td>
<td>4.5</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>( dl )-Alanine</td>
<td>50</td>
<td>179</td>
<td>8.0</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>( dl )-Serine</td>
<td>90</td>
<td>248</td>
<td>11</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>( dl )-Alanine</td>
<td>90</td>
<td>331</td>
<td>15</td>
<td>127</td>
</tr>
<tr>
<td><em>Pseudomonas pyocyanea</em></td>
<td>( dl )-Serine</td>
<td>90</td>
<td>149</td>
<td>6.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>( dl )-Alanine</td>
<td>90</td>
<td>303</td>
<td>13.5</td>
<td>124</td>
</tr>
</tbody>
</table>

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Deamination by Cell-Free Liver Extracts

Preparation of Extracts—Freshly obtained rat or mouse livers were chilled, homogenized in a small tissue grinder, and extracted with 2 volumes of cold saline. The supernatants, after centrifugation of the mixtures for 30 minutes at 5000 R.P.M. in a refrigerated angle centrifuge, were dialyzed overnight against running tap water and then for 24 hours against several volumes of cold saline.

### Table III

**Pyruvic Acid from dl-Serine**

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate used</th>
<th>Yield</th>
<th>M.p.*</th>
<th>Analysis†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>°C</td>
<td>C</td>
</tr>
<tr>
<td>Bacterium coli</td>
<td>500</td>
<td>227</td>
<td>215</td>
<td>40.3</td>
</tr>
<tr>
<td>Pseudomonas pyocyanea</td>
<td>500</td>
<td>112</td>
<td>216</td>
<td>40.3</td>
</tr>
<tr>
<td>Proteus OX-19</td>
<td>500</td>
<td>255</td>
<td>215</td>
<td>40.3</td>
</tr>
<tr>
<td>Clostridium welchii</td>
<td>550</td>
<td>193</td>
<td>216</td>
<td>40.6</td>
</tr>
</tbody>
</table>

* No depression of the melting point was observed on admixture of an authentic specimen of pyruvic acid 2,4-dinitrophenylhydrazone.
† Calculated for pyruvic acid 2,4-dinitrophenylhydrazone (268), C 40.3, H 3.0, N 20.9.

### Table IV

**α-Ketobutyric Acid from dl-Threonine**

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate used</th>
<th>Yield</th>
<th>M.p.*</th>
<th>Analysis†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>°C</td>
<td>C</td>
</tr>
<tr>
<td>Bacterium coli</td>
<td>500</td>
<td>340</td>
<td>195</td>
<td>42.6</td>
</tr>
<tr>
<td>Pseudomonas pyocyanea (with toluene)</td>
<td>500</td>
<td>456</td>
<td>195</td>
<td>42.5</td>
</tr>
<tr>
<td>Pseudomonas pyocyanea (without toluene)</td>
<td>500</td>
<td>354</td>
<td>195</td>
<td>42.8</td>
</tr>
<tr>
<td>Proteus OX-19</td>
<td>400</td>
<td>158</td>
<td>195</td>
<td>42.6</td>
</tr>
</tbody>
</table>

* Mixed melting point with a sample kindly furnished by Dr. H. Waelsch of this College (cf. (29)), 195°.
† Calculated for α-ketobutyric acid 2,4-dinitrophenylhydrazone (282), C 42.6, H 3.6, N 19.8.
changes of large volumes of ice-cold distilled water. The centrifugation of the dialyzed solutions resulted in clear supernatants containing approximately 10 mg. of dissolved material per cc. These extracts of rat or mouse liver were almost inactive in the deamination of \textit{dl}-serine in evacuated vessels, unless Mg\textsuperscript{++} was added.

A similarly prepared extract of rabbit liver in which 1 volume of saline was used for the extraction did not require the activation by magnesium ions; but it is possible that the dialysis was less complete in this case. A selection of data on deamination by such extracts is presented in Table V.

It should be mentioned that quite often completely inactive dialysates (even after addition of Mg\textsuperscript{++}) were encountered. The reasons for these frequent failures are not yet clear. It is probable that the nutritional state of the animals, the duration and manner of the dialysis, and the presence in the dialysates of impurities (e.g. glycogen) in varying amounts are responsible. These questions are being studied at present.

\textit{Formation of Pyruvic Acid from Serine by Rat Liver}—13 gm. of rat liver were ground with sand and extracted with 2 volumes of cold saline. The supernatant, after centrifugation of the mixture at 5000 \textit{R.P.M.} in the cold, was treated with 5 cc. of toluene and incubated at 38\textdegree C in a nitrogen atmosphere with a solution of 150 mg. of \textit{dl}-serine in 25 cc. of 0.1 M phosphate buffer of pH 7.6 for 16 hours. The filtrate, treated as described before, yielded 30 mg. of \textit{pyruvic acid 2,4-dinitrophenylhydrazone}, which after crystallization from ethyl acetate melted with decomposition at 216\textdegree C.

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Tissue} & \textbf{Substrate} & \textbf{Addition} & \textbf{Ammonia nitrogen} & \textbf{Extent of deamination} \\
\hline
\textit{Rat liver} & \textit{dl}-Serine & \textit{MgCl\textsubscript{2}} & 11 & 61 \textper cent \\
& " & " & 8 & 0 \textper cent \\
\textit{Mouse liver} & \textit{dl}-O-Methylserine & \textit{MgCl\textsubscript{2}} & 110 & 61 \textper cent \\
& " & " & 11 & 0 \textper cent \\
\textit{Rabbit} & \textit{dl}-Serine & \textit{MgCl\textsubscript{2}} & 170 & 95 \textper cent \\
& " & " & 9 & 0 \textper cent \\
\hline
\end{tabular}
\caption{Deamination of \textit{dl}-Serine by Liver Extracts}
\end{table}
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C₉H₁₈O₄N₄ (268). Calculated, C 40.3, H 3.0; found, C 40.5, H 3.2

The authors are grateful to Dr. H. E. Carter, Dr. V. du Vigneaud, Dr. F. Lipmann, and Dr. H. Waelsch for preparations used in the course of this work, to Dr. T. Rosebury of this College for help and advice with respect to the bacterial preparations, and to Mr. W. Saschek for the microanalyses reported here.

SUMMARY

The deamination of serine by Bacterium coli is inhibited by replacement of the hydroxyl hydrogen atom. Pyruvic and α-ketobutyric acids have been identified as end-products of the deamination of serine and threonine respectively by bacteria (Bacterium coli, Pseudomonas pyocyanea, Proteus OX-19, Clostridium welchii). The anaerobic deamination of serine by cell-free extracts from mouse, rat, and rabbit livers has been demonstrated to proceed in a similar manner.

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