The Indirect Role of Biotin in the Synthesis of Ornithine Transcarbamylase

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A number of years ago studies with bacteria indicated a role of biotin in the carboxylation reaction forming a 4 carbon unit essential for aspartate biosynthesis (1, 2). These studies led to extensive work on the role of biotin in the incorporation of bicarbonate into various metabolites. A role of biotin in transcarbamylase reactions was demonstrated when biotin-deficient cells of Streptococcus lactis 8039 and Lactobacillus arabinosus 17-5 were found to have appreciably less ornithine transcarbamylase and aspartate transcarbamylase than biotin-sufficient cells (3, 4). Ornithine transcarbamylase activity can be restored to biotin-deficient cells of Streptococcus lactis upon incubation for a short period of time in the presence of biotin in an amino acid medium (5). However, acid hydrolysates of highly purified preparations of the enzyme do not contain significant amounts of biotin (6), and, therefore, the role of biotin appeared to be an indirect one associated with the synthesis of the enzyme. The lack of a biotin effect on the synthesis of carbamate kinase in Streptococcus lactis (7) eliminated the possibility of an effect resulting from decreased synthesis of the substrate, carbamyl phosphate.

In the present investigation, an enzymatic digest of purified ornithine transcarbamylase was found to replace biotin in stimulating the production of the enzyme; however, enzymatic digests of casein are also effective. A study of the nature of the peptides replacing biotin indicated that derivatives of aspartic acid, particularly peptides of asparagine, replace biotin by supplying a source of 4 carbon units, the biosynthesis of which is dependent upon a carboxylation reaction involving biotin.

EXPERIMENTAL PROCEDURE

Materials and Methods

Materials—The enzymatic digest of casein (N-Z-Case) was obtained from Sheffield Chemical Company. The peptides were obtained from Nutritional Biochemicals Corporation and Mann Research Laboratories, Inc. dl-Homobiotin was obtained from Hoffmann-LaRoche, Inc. Highly purified ornithine transcarbamylase (specific activity, 80,000) was prepared as previously described (6).

Methods—Biotin-deficient cells of S. lactis 8039 were obtained by growing the cells on a previously described amino acid medium (9) which was modified to contain per ml: 0.2 μg of pantothenic acid, 0.1 mg of l-glutamic acid, and 0.01 mg of biotin. The cells were harvested after 20 to 24 hours of incubation at 30° and were washed twice with 0.85% sodium chloride solution. The yield of cells was approximately 75 mg (dry weight) of cells per liter of medium. The dry weight of cells was determined by the use of a turbidimeter and a standard curve of milligrams (dry weight) of cells versus galvanometer deflection.

Reactivation of biotin-deficient cells of S. lactis was carried out in a previously described medium (5) which was modified to contain per ml: 0.2 μg of pantothenic acid, 0.1 mg of l-glutamic acid, and 0.01 mg of biotin. The concentration of cells was approximately 0.5 mg (dry weight) per ml. After 2 hours of incubation at 30°, 0.05 ml of toluene per ml of cell suspension was added with shaking, and the incubation continued at 37° for an additional 15 minutes. The cells were harvested by centrifugation and resuspended in 0.04 M Tris buffer, pH 8.5.

For L. arabinosus 17-5, the growth medium was further modified by the omission of arginine and uracil, and the biotin concentration was 0.02 μg per ml. Reactivation of biotin-deficient cells of L. arabinosus was carried out at pH 7 in medium of the same composition as that in which the cells were grown.

Ornithine transcarbamylase activity was determined as previously described (6, 10). The results are expressed as enzyme units (micromoles of citrulline formed per mg (dry weight) of cells per hour) or as per cent of control.

RESULTS AND DISCUSSION

To investigate the possibilities that biotin could be bound in ornithine transcarbamylase in such a manner that it is destroyed on acid hydrolysis or that biotin is involved in the formation of a reactive group in the enzyme, a tryptic digest of purified ornithine transcarbamylase from S. lactis was made and tested for its ability to replace the biotin requirement for the synthesis of the enzyme. The digest of the enzyme was prepared by incubating ornithine transcarbamylase, 5 mg of protein, in 0.04 M Tris buffer, pH 8.5, with 0.1 mg of crystalline trypsin (50% magnesium sulfate) for 6 hours at 25°. The mixture was heated at 90° for 20 minutes to stop the reaction. The digest was assayed for biotin (11), and no appreciable amount could be detected. The digest was then tested for its ability to replace the biotin requirement for enzyme synthesis. As seen in Table I, the digest replaces the biotin requirement for the synthesis of ornithine transcarbamylase. A heated preparation of ornithine transcarbamylase without enzymatic hydrolysis does not replace the biotin requirement, nor does a heated preparation of the trypsin. Additional evidence that the factor liberated by tryptic digestion is not biotin is shown by the results obtained with homobiotin.
TABLE I
Replacement of biotin requirement for synthesis of ornithine transcarbamylase by tryptic digest of ornithine transcarbamylase

<table>
<thead>
<tr>
<th>Supplements</th>
<th>mg/ml Homobiotin</th>
<th>Ornithine transcarbamylase activity</th>
<th>μg/ml</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>32</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Biotin, μg/ml</td>
<td>0.5</td>
<td>80</td>
<td>1.0</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>196</td>
<td>2.0</td>
<td>52</td>
</tr>
<tr>
<td>Tryptic digest of ornithine transcarbamylase, mg/ml</td>
<td>0.2</td>
<td>68</td>
<td>0.5</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>172</td>
<td>1.0</td>
<td>200</td>
</tr>
</tbody>
</table>

* The peptide mixture contained the indicated amounts of each of the following peptides: L-alanyl-L-phenylalanine, β-alanyl-L-histidine, glutathione, and the glycy peptide of DL-isoleucine, L-leucine, DL-methionine, DL-threonine, DL-serine, L-tyrosine, DL-valine, L-tryptophane, L-proline, L-glutamic acid, L-lysine, and L-asparagine.

† The control cells reactivated in the presence of 2 μg of biotin per ml contained 196 units of ornithine transcarbamylase.

TABLE II
Replacement of biotin requirement by tryptic digests and synthetic peptide mixture

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Ornithine transcarbamylase activity</th>
<th>μg/ml</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptic digest of ornithine transcarbamylase</td>
<td>mg/ml</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The peptide mixture contained the indicated amounts of each of the following peptides: L-alanyl-L-phenylalanine, β-alanyl-L-histidine, glutathione, and the glycy peptide of DL-isoleucine, L-leucine, DL-methionine, DL-threonine, DL-serine, L-tyrosine, DL-valine, L-tryptophane, L-proline, L-glutamic acid, L-lysine, and L-asparagine.

† The control cells reactivated in the presence of 2 μg of biotin per ml contained 196 units of ornithine transcarbamylase.

TABLE III
Replacement of biotin requirement by synthetic peptides

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Ornithine transcarbamylase activity</th>
<th>μg/ml</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Without glycy-L-asparagine</td>
<td>33</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Glycy-L-asparagine</td>
<td>75</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>23</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>300</td>
<td>25</td>
<td>01</td>
<td>01</td>
</tr>
</tbody>
</table>

* The control cells reactivated in the presence of 10 μg of biotin per ml contained 328 units.

Homobiotin prevents the utilization of biotin but does not alter the effectiveness of the tryptic digest.

Further investigation, however, showed that enzymatic digests of other naturally occurring materials or a mixture of synthetic peptides are effective in replacing the biotin requirement. These results are given in Table II. A mixture containing 0.1 mg of each of the peptides listed in Table II completely replaces the biotin requirement. When the peptides in the mixture were tested individually, only glycylasparagine was found to be active (Table III). Concentrations of 1 μmole or more per ml of glycylasparagine allow synthesis of ornithine transcarbamylase up to 90% of that obtained in the presence of biotin. Peptides of α-alanine or methionine, although inactive alone, when added with glycylasparagine allow synthesis equal to that obtained with biotin.

As seen in Table IV, a high concentration of exogenous aspartic acid replaces the biotin requirement to some extent but is far less effective than asparagine, glycylasparagine, or glutamylasparagine. It is of interest to note that the peptides are not only active at lower exogenous concentrations but also replace the biotin requirement to a greater extent. This is in accord with many previous reports which have shown that peptides are often more effectively utilized by whole cells than the corresponding amino acid. Reactivation of the biotin-deficient cells in the presence of biotin and asparagine or an asparagine peptide does not increase the enzyme activity above that obtained with biotin alone. In some experiments the cells were harvested before treatment with toluene, disrupted by sonic oscillation, and the cell-free extracts assayed for ornithine transcarbamylase. No appreciable differences were found in the ornithine transcarbamylase activity of toluene-treated and sonically disrupted cells.

The results obtained with S. bovis indicate that the role of biotin in the synthesis of ornithine transcarbamylase is concerned with the production of an active 4 carbon unit which is necessary for the synthesis of this enzyme. This conclusion is fortified by results obtained in a study of ornithine transcarbamylase synthesis by biotin-deficient cells of L. arabinosus 17-5. These results differ only in the relative effectiveness of asparagine and asparagine peptides in replacing the biotin requirement for enzyme synthesis. L-Asparagine (0.3 to 3 μmoles per ml) allows enzyme synthesis up to 90% of that obtained with biotin. Although L-glutamyl-L-asparagine is effective at a lower exogenous con-
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concentration (0.03 to 0.3 μmole per ml), the extent of enzyme synthesis is approximately 60% of that obtained with biotin. Glycyl-L-asparagine and L-aspartic acid replace the biotin requirement to a lesser degree.

Blanchard et al. (12) had previously shown that biotin is required for the synthesis of the adaptive malic enzyme in *L. arabinosus*. Partially purified preparations of the malic enzyme do not contain significant amounts of biotin (13) and are not inhibited by avidin (14). Recent studies2 with *L. arabinosus* have shown that the biotin requirement for malic enzyme synthesis can be replaced by asparagine peptides.

It appears, therefore, that the function of biotin in the synthesis of at least two enzymes, which are affected by a biotin deficiency but which do not contain biotin, is in the biogenesis of a 4 carbon unit. This 4 carbon unit can be synthesized through a carboxylation reaction by the organism or supplied exogenously by derivatives of aspartic acid. Since the original reports (1, 2) of a role of biotin in the carboxylation reaction, biotin has been found to be involved as a component of enzymes which carboxylate propionate (14) and pyruvate (15), and which carry out the transfer reaction between methylmalonate and pyruvate (16). The nature of the reaction involving biotin in the synthesis of a 4 carbon unit in *S. lactis* and *L. arabinosus* is still unknown.

**SUMMARY**

An enzymatic digest of purified ornithine transcarbamylase replaces the biotin requirement for ornithine transcarbamylase synthesis in *Streptococcus lactis* 8039. However, enzymatic digests of casein and a mixture of synthetic peptides are also effective. Of the peptides tested individually, only derivatives of aspartic acid (in particular, asparagine and peptides of asparagine) were found to replace the biotin requirement. Similar results were obtained in studying the synthesis of ornithine transcarbamylase in *Lactobacillus arabinosus* 17-5. It appears, therefore, that the function of biotin is in the synthesis of a 4 carbon unit. This 4 carbon unit can be either synthesized by the organism through a reaction involving biotin or supplied exogenously by derivatives of aspartic acid.

**REFERENCES**


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The Indirect Role of Biotin in the Synthesis of Ornithine Transcarbamylase
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