THE SYNTHESIS OF COENZYME A FROM PANTETHEINE: PREPARATION AND PROPERTIES OF PANTETHEINE KINASE*

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It had previously been shown (1–3) that extracts of pigeon liver could convert pantetheine and ATP to CoA in low yield. It was found, however, that fresh extracts of pigeon liver, from which CoA has been removed with Dowex 1 rather than by aging, effected a much more vigorous synthesis of CoA. Since the aging procedure apparently inactivated the enzyme responsible for the conversion of pantetheine to phosphopantetheine, purification of this enzyme was carried out starting with freshly prepared extracts.

EXPERIMENTAL

Materials—Synthetic pantetheine and the three phosphopantetheines used in these studies were kindly supplied by Dr. J. Baddiley (4–6). ATP was obtained as the disodium salt from the Pabst Laboratories. CoA was prepared in this laboratory by the method of Gregory et al. (7). Phosphopantetheine was prepared enzymatically by treating CoA with purified CoA pyrophosphatase (1). Prostate phosphomonoesterase was kindly supplied by Dr. G. Schmidt. Protamine sulfate was obtained from the Nutritional Biochemicals Corporation, and through the courtesy of Sharp and Dohme, Inc. Calcium phosphate gel was prepared by the method of Keilin and Hartree (8). Dowex 1 (200 to 400 mesh) was obtained from The Dow Chemical Company and washed with HCl as previously described (9).

Methods—CoA was determined by measuring sulfanilamide acetylation

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1 The following abbreviations will be used: coenzyme A = CoA; adenosinetriphosphate = ATP; adenosinediphosphate = ADP.
with aged crude pigeon liver extracts (10). Phosphopantetheine was also measured by this procedure, since it is readily converted to CoA by pigeon liver extracts and ATP. Pantothenic acid was measured microbiologically with Lactobacillus arabinosus as previously described (1), except that the medium of Craig and Snell was employed (11). Pantetheine was determined as a function of the growth of Lactobacillus helveticus H-802 with the same medium; protein by the turbidimetric method of Bucher (12).

**Fractionation of Enzymes**—Acetone powder of pigeon liver (10) was extracted for 30 minutes with 10 volumes of ice-cold 0.02 M KHCO₃, and the residue was removed by centrifugation at 5°C. 200 ml. of the extract, containing 6.5 mg. of N per ml., were treated with 10 ml. of 2 per cent protamine sulfate (added dropwise, with stirring); the precipitate was centrifuged at 5°C and discarded. The supernatant solution was treated with an additional 20 ml. of 2 per cent protamine sulfate. This second supernatant fraction was entirely inactive in converting pantetheine to CoA but was as active as the original extract in catalyzing the synthesis of CoA from phosphopantetheine. It therefore seemed that a separation of the initial step from the succeeding steps in the synthesis of CoA from pantetheine had been achieved.

The enzyme catalyzing the phosphorylation of pantetheine was further purified as follows: the gummy precipitate from the second protamine treatment was suspended in 50 ml. of cold 0.02 M phosphate buffer, pH 7.0, stirred for several minutes, and centrifuged.

The supernatant fraction was treated with 75 ml. of calcium phosphate gel (dry weight, 29 mg. per ml.) which adsorbed almost all the activity. Elution was achieved with a single treatment with 25 ml. of cold 0.2 M K₂HPO₄. The eluate was dialyzed for 12 hours against 0.04 M KCl at 5°C. The procedure gave a 16-fold purification with 50 per cent recovery of the activity of the original extract.

**Assay of Enzyme Activity**—Pantetheine is readily phosphorylated by ATP in the presence of pantetheine kinase. The reaction is followed by converting the reaction product to CoA through a second incubation with the supernatant fraction from the second protamine treatment. Since this latter fraction also contains the enzyme system for acetylating sulfanilamide, the CoA formed can be simultaneously measured. As shown in Fig. 1, the phosphorylation of pantetheine proceeds in a nearly linear fashion until most of the pantetheine has reacted, and, in the presence of an excess of ATP, continues until all the pantetheine is phosphorylated.

**Properties of Enzyme**—As has been indicated, the enzyme is largely inactivated after 4 hours at 25°C. It is completely inactivated by heating to 50°C for 10 minutes, and the partly purified preparation very slowly loses activity when stored in the frozen state at −10°C.

*A culture of this organism was kindly provided by Dr. E. E. Snell.*
The reaction does not proceed in the absence of divalent cations. Mn$^{++}$ is about one-fourth more effective than Mg$^{++}$; both are optimal at 0.01 M. Ca$^{++}$ is about one-half as effective as Mn$^{++}$, while Co$^{++}$, Cu$^{++}$, and Fe$^{++}$ are inactive. Omission of phosphate ions from the incubation mixture reduces the rate by one-half or more.

Identification of Product—Since the rate of synthesis was slow (about 1 μM per hour per mg. of protein nitrogen), it was not possible to accumulate sufficient product to allow isolation. Consequently, indirect methods were employed to identify the product as phosphopantetheine.
Phosphopantetheine is converted to pantetheine by the action of prostate phosphomonoesterase, and the latter compound cannot serve as a CoA precursor with the pigeon liver supernatant fraction. After treatment of the pantetheine kinase product with phosphomonoesterase, the ability to serve as a precursor of CoA was similarly lost.

Previous observations have shown that phosphopantetheine derived from CoA by treatment with CoA pyrophosphatase is about 3 times as active in stimulating the growth of Acetobacter suboxydans as is CoA (13, 14). This is also true of the enzymatically synthesized material.

**Table I**

<table>
<thead>
<tr>
<th>Enzymatic Conversion of Various Phosphopantetheines to CoA by Purified Pigeon Liver Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Per cent converted to CoA</strong></td>
</tr>
<tr>
<td>Pantetheine ............................................. 0</td>
</tr>
<tr>
<td>2'-Phosphopantetheine .............................. 0</td>
</tr>
<tr>
<td>Cyclic phosphopantetheine .......................... 0</td>
</tr>
<tr>
<td>dL-4'-Phosphopantetheine ........................... 39</td>
</tr>
<tr>
<td>d(+)-4'-Phosphopantetheine ......................... 82</td>
</tr>
<tr>
<td>Pantetheine kinase product ........................ 90</td>
</tr>
<tr>
<td>CoA pyrophosphatase product ....................... 85</td>
</tr>
</tbody>
</table>

Each tube contained aged protamine supernatant fraction, 10 μM of cysteine, 10 μM of ATP, 5 μM of Mg++, 0.1 M PO₄, pH 7.2, and 0.1 μM of the test compound in a final volume of 1.0 ml. The tubes were incubated for 1 hour at 37°. The pantetheine kinase product was prepared by preincubating pantetheine and ATP with the purified enzyme. The CoA pyrophosphatase product was prepared by incubating CoA with snake venom CoA pyrophosphatase (1).

While these studies were in progress, Baddiley and Thain (15) found that mild acid or alkaline hydrolysis of CoA gave rise to a product indistinguishable by paper chromatography from pantothenic acid 4'-phosphate. This suggested that the product of pantetheine kinase was 4'-phosphopantetheine. Subsequently, Baddiley et al. (6) synthesized various phosphate esters of pantetheine and these compounds were compared with the product of pantetheine kinase and with that of CoA pyrophosphatase. From the results of this experiment, shown in Table I, it is clear that only the d (+) isomer of 4'-phosphopantetheine is converted to CoA. This experiment thus identifies the product of pantetheine kinase as 4'-phosphopantetheine. The reaction may be formulated as shown in the accompanying scheme. The enzymes responsible for the remaining steps in CoA synthesis will be discussed in the following communication (16).
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\[
\text{HO-CH}_2-C-\text{CHOH-C-NH-CH}_2-\text{CH}_2-C-\text{NH-CH}_2-\text{CH}_2-SH + \]
\[\text{ATP} \xrightarrow{\text{pantetheine kinase}} \]
\[\text{pantetheine} \]
\[\text{OH} \]
\[\text{CH}_3 \]

\[\text{O} = \text{P-O-CH}_2-C-\text{CHOH-C-NH-CH}_2-\text{CH}_2-C-\text{NH-CH}_2-\text{CH}_2-SH + \text{ADP} \]
\[\text{OH} \]
\[\text{CH}_3 \]

\[\text{(4'-phosphopantetheine)} \]

SUMMARY

The enzyme catalyzing the phosphorylation of pantetheine has been separated from the succeeding steps in CoA synthesis and partially purified. Several characteristics of "pantetheine kinase" have been studied. The product of the reaction between ATP and pantetheine has been shown to be 4'-phosphopantetheine.

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BIBLIOGRAPHY

THE SYNTHESIS OF COENZYME A
FROM PANTETHEINE: PREPARATION
AND PROPERTIES OF PANTETHEINE
KINASE
Leon Levintow and G. David Novelli


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