BIOSYNTHESIS OF COENZYME A FROM PHOSPHOPANTE- 
THEINE AND OF PANTETHEINE FROM PANTOTHENATE

BY M. B. HOAGLAND† AND G. DAVID NOVELLI‡
(From the Biochemical Research Laboratory, Massachusetts General Hospital, 
and the Department of Biological Chemistry, Harvard Medical 
School, Boston, Massachusetts)
(Received for publication, July 14, 1953)

The enzymatic degradation of CoA, giving rise to various fragments 
which are resynthesized to CoA by pigeon liver extracts, has been reported 
in a previous communication (1). Fractionation resulted in the isolation 
of the enzyme, pantetheine kinase, which converts pantetheine and ATP 
to 4'-phosphopantetheine (2).

The present communication will describe the preparation and purification 
of the enzymes involved in the conversion of 4'- phosphopantetheine to CoA.

EXPERIMENTAL

Materials—Pantetheine and 4'-phosphopantetheine were synthetic pro-
ducts generously supplied by Dr. J. Baddiley (3). Dephospho CoA was 
prepared from CoA with the specific adenosine-3'-phosphate nucleotidase 
from barley (4) which was kindly supplied by Dr. Nathan Kaplan. Acetyl 
phosphate was prepared by the method of Stadtman and Lipmann (5). 
For other reagents see the preceding paper.

Methods—CoA was determined by the phosphotransacetylase assay (6); 
CoA standardized by sulfanilamide acetylation (7), acetyl phosphate by 
the hydroxamic acid method of Lipmann and Tuttle (8), protein by the 
turbidimetric procedure of Bucher (9), and inorganic phosphate and pyro-
phosphate by the time-color method of Lipmann, Flynn, and Jones2 were 
used as standards of reference.

* This work was supported in part by a research grant from the Office of Naval 
Research, contract No. NONR 606.
† American Cancer Society Scholar.
‡ Present address, Department of Microbiology, School of Medicine, Western 
Reserve University, Cleveland, Ohio.
1 The following abbreviations will be used: Coenzyme A = CoA; adenosinetriphos-
phate = ATP; adenosinediphosphate = ADP; inorganic pyrophosphate = P-P; di-
phosphopyridine nucleotide = DPN; 4'-phosphopantetheine = p-pantetheine; tris-
(hydroxymethyl)aminomethane = Tris; trichloroacetic acid = TCA.
Results

Localization in Cell Fractions of Enzymatic Steps in Conversion of Pantetheine to CoA—Sonicly disrupted, Dowex-treated mitochondrial, microsomal, and nuclear fractions, prepared according to Schneider and Hogenboom (10) from pigeon liver, were found to contain relatively little CoA-synthesizing activity compared to a liver supernatant fraction free of these particles. Mitochondria contained all three enzymes in amounts large enough to make a clear cut statement about localization of the enzymes in particles impossible.

The supernatant fractions of rat and hog liver were found to be about as active as those from pigeon liver in the conversion of pantetheine to CoA. Because of its ready availability, the hog liver supernatant fraction was used as a source of the enzymes for purification. The enzyme catalyzing the formation of dephospho CoA from phosphopantetheine and ATP has been named enzyme "C" (C = dephospho CoA pyrophosphorylase), while the enzyme transforming dephospho CoA to CoA has been called "dephospho CoA kinase (K,)."

Assay for K, was followed by measuring the transformation of dephospho CoA to CoA under the following conditions: 0.2 µM of dephospho CoA (about 60 units), 1.0 µM of ATP, 5.0 µM of Mg++, 10.0 µM of cysteine, 40 µM of glycylglycine buffer, pH 7.4, and enzyme sufficient to make 10 to 20 units of CoA. The incubation was at 37° for 30 to 60 minutes in a final volume of 1.0 ml. The reaction was stopped by boiling for 2 minutes, and CoA was measured by the phosphotransacetylase assay on an aliquot of the supernatant fraction.

Assay for Dephospho CoA Pyrophosphorylase—in the cruder preparations both enzymes C and K, were present and any dephospho CoA formed from phosphopantetheine was converted to CoA; therefore, the assay conditions given above for K, were employed, except that the ATP concentration was increased to 5 to 10 µM and the substrate was phosphopantetheine.

However, in the more highly purified enzyme preparations, the activity of C was also determined directly by measuring the formation of inorganic pyrophosphate, the other product of the reaction. 2 µM of phosphopantetheine, 5.0 µM of ATP, 2.0 µM of Mg++, 10 µM of cysteine, and 40 µM of glycylglycine buffer, pH 7.4, were incubated with enzyme sufficient to make 1 to 2 µM of inorganic pyrophosphate in 1.0 hour at 37° in a final volume of 1.0 ml. The reaction was stopped with 0.2 ml. of 25 per cent TCA, and 0.4 ml. of the supernatant fraction was taken for pyrophosphate determination.

The activity of both K, and C is expressed in terms of units of product (determined as CoA) synthesized per 30 minutes per mg. of protein under the specified conditions.

Purification of C and K, from Hog Liver Supernatant Fraction—Fresh hog
liver (usually 3 kilos at a time) was ground and blended in a Waring blender for 45 seconds with an equal volume of 0.15 M KCl. This homogenate was filtered through cheese-cloth and centrifuged in the preparative Spinco at 40,000 × g for 1.5 hours to remove microsomes.

The supernatant fraction was diluted with 2 volumes of water and treated with 0.06 volume of 2 per cent protamine sulfate and the resulting precipitate collected in a Sharples centrifuge at 50,000 × g and discarded. The supernatant fraction was adjusted to pH 8.0 with Tris and brought to 38 per cent saturation with solid ammonium sulfate. The precipitate was collected, dissolved in water (to make about 50 mg. of protein per ml.), and treated with 2.5 to 5 volumes of calcium phosphate gel (36 to 40 mg. per ml. of dry weight) to remove impurities. The supernatant fraction was re-

| TABLE I |
| Formation of Inorganic Pyrophosphate during Synthesis of CoA from Phosphopantetheine |

<table>
<thead>
<tr>
<th>Enzyme + ATP</th>
<th>CoA</th>
<th>P-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>&quot; + p-pantetheine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot; + ATP + p-pantetheine</td>
<td>0.67</td>
<td>0.90</td>
</tr>
</tbody>
</table>

0.83 mg. of protein was incubated for 60 minutes at 37° with 2 μM of Mg++, 10 μM of cysteine, 40 μM of glycylglycine, pH 7.4, in a volume of 1 ml., and 2 μM of p-pantetheine and 5 μM of ATP were added as indicated. CoA was determined by phosphotransacetylase assay (6) on a small aliquot of the reaction mixture after boiling it for 2 minutes, and P-P was determined on an aliquot of the TCA filtrate of the remaining reaction mixture (see foot-note 2).

adjusted to pH 8, the solution brought to 70 per cent ammonium sulfate saturation, and the precipitate collected and dissolved in water. All operations were carried out as close to 0° as possible. These procedures gave an over-all purification of 100 (15 per cent recovery) for C and 125 (30 per cent recovery) for K_H from the original homogenate.

K_i, or pantetheine kinase, is completely adsorbed on the gel. The use of 5 volumes of gel, although not giving as high a specific activity as 2.5 volumes, yielded preparations relatively more rich in C and was free of ATPase. The enzyme preparations are quite stable when kept frozen.

Nature of Reactions Catalyzed by C and K_r—The low ATPase and pyrophosphatase activity of the gel preparations, and the fact that the relatively high activity permitted synthesis of CoA in micromolar quantities, allowed the direct measurement of the expected formation of inorganic pyrophosphate. The experiment illustrated in Table I shows that pyrophosphate is formed in fairly good equivalence with the CoA synthesized.
Reversibility of Condensation Reaction—In general, condensation reactions between ATP and mononucleotides are expected to be reversible. Kornberg and Pricer demonstrated that the condensation of nicotinamide mononucleotide with ATP yielding DPN and pyrophosphate is freely reversible (11). We therefore tried to determine whether CoA synthesis likewise is reversible. Since C and KII were present in the preparation, the following dismutation could occur.

1. Dephospho CoA + P-P ⇌ ATP + phosphopantetheine
2. Dephospho CoA + ATP → CoA + ADP + phosphopantetheine

Sum, 2 dephospho CoA + P-P → CoA + ADP + phosphopantetheine

In Reaction 1 ATP would be formed by a pyrophosphorolysis of dephospho CoA, and in Reaction 2 the newly formed ATP would be used by KII.

**Table II**

<table>
<thead>
<tr>
<th>Dismutation of Dephospho CoA with KII, C, and Inorganic Pyrophosphate</th>
<th>CoA (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme + P-P + dephospho CoA + P-P + dephospho CoA</td>
<td>0.5</td>
</tr>
<tr>
<td>Enzyme + P-P + dephospho CoA</td>
<td>0</td>
</tr>
<tr>
<td>P-P + dephospho CoA</td>
<td>0</td>
</tr>
<tr>
<td>“ + dephospho CoA</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme + P-P + dephospho CoA</td>
<td></td>
</tr>
</tbody>
</table>

3.2 mg. of protein were incubated for 65 minutes at 37° with 2 μM of Mg++, 10 μM of cysteine, 100 μM of Tris buffer, pH 8.0, in a volume of 1 ml. P-P (2 μM) or dephospho CoA (1.34 μM) was added as indicated. The reaction was stopped by boiling for 2 minutes and a suitably diluted aliquot was taken for CoA determination by phototransacetylase.

to convert dephospho CoA to CoA. The data of Table II show that, when 1.34 μM of dephospho CoA are incubated with 2.0 μM of pyrophosphate, 0.5 μM of CoA is formed. This value approximates the theoretical for complete reversibility which would have been 0.67 μM of CoA.

Characteristics of Enzymes—The pH optimum for dephospho CoA pyrophosphorylase appears to be near pH 7.5, while that for KII is nearer pH 9.0. Mg²⁺ is required by both enzymes. The optimal concentration for the condensation is about 2 × 10⁻³ M. Both enzymes require the substrate to be in the reduced form. Cysteine or H₂S was found to be an effective reducing agent.

Synthesis of Pantetheine from Pantothenic Acid—Freshly prepared rat liver supernatant fraction was found to be as active in converting pantothenic acid to pantetheine as it was in the subsequent steps in CoA synthesis, while fresh pigeon liver supernatant fractions or acetone powder was inert.

The supernatant fraction was prepared by homogenizing for 30 seconds
with a Potter-Elvehjem homogenizer in 2 to 3 volumes of KCl and centrifuging in the Spinco at 140,000 r.p.m. for 1 hour. It was dialyzed against isotonic KCl for 3 to 4 hours at 0° and treated with Dowex 1 to remove CoA.

**Assay Procedure**—0.4 ml. of the Dowex-treated supernatant solution was incubated with 5 μM of Mg++, 10 μM of ATP, 50 μM of Tris, pH 7.7, 0.05 to 0.5 μM of pantothenate (calcium salt), and 0.1 to 0.5 μM of cysteine in a volume of 1.0 ml. at 37° for 60 minutes. The reaction was stopped by boiling for 2 minutes. After centrifuging the protein, pantetheine was measured in a 0.5 ml. aliquot by converting it to CoA with the purified pantetheine to CoA-synthesizing system of hog liver supernatant fraction. The second incubation contained 0.2 ml. of the 0–38 ammonium sulfate fraction from hog liver supernatant fraction, 10 μM of ATP, 5 μM of Mg++, 10 μM of cysteine, 100 μM of Tris, pH 8.0, and a 0.5 aliquot of the first incubation supernatant fraction in a volume of 1.0 ml. Incubation was at 37° for 60 minutes. CoA was determined by the phosphotransacetylase assay on a properly diluted aliquot.

**Results**—After freezing, activity dropped slowly over several weeks. With fresh preparations the synthesis of pantetheine requires only the addition of pantothenate, but upon dialysis the system was found also to require cysteine. This compound produced a 7-fold increase in synthesis after dialysis. The requirements for ATP, pantothenate, cysteine, and Mg++ are shown in Table III. Cystine is inactive. The fact that the system has a specific requirement for cysteine as such and not a general reducing requirement is illustrated by the fact that the following compounds failed to replace cysteine: homocysteine, thioacetate, thioglycolate, mercaptosuccinate, β-mercaptoethylamine, and H2S. Glutathione was nearly as effective as cysteine, presumably because of liberation of cysteine due to hydrolysis. It should be noted that β-mercaptoethylamine is inactive.

### Table III

<table>
<thead>
<tr>
<th>ATP, Pantothenate, and Cysteine Requirement for Pantetheine Synthesis</th>
<th>Units CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>10.4</td>
</tr>
<tr>
<td>Without ATP</td>
<td>0</td>
</tr>
<tr>
<td>&quot; pantothenate</td>
<td>0</td>
</tr>
<tr>
<td>&quot; cysteine</td>
<td>1.7</td>
</tr>
<tr>
<td>With cystine replacing cysteine</td>
<td>1.5</td>
</tr>
</tbody>
</table>

0.4 ml. of rat supernatant fraction, Dowex-treated and dialyzed 4 hours, was incubated with 10 μM of ATP, 0.05 μM of pantothenate, 0.05 μM of cystine (or cysteine), 5 μM of Mg++, 50 μM of Tris buffer, pH 7.7, in a volume of 1 ml. at 37° for 30 minutes. Pantetheine was converted to CoA as described in the text.
These data suggested that the synthesis of pantetheine proceeded by an initial condensation of pantothenate with cysteine in the presence of ATP to form pantothenylcysteine which is subsequently decarboxylated to pantetheine. In the meantime Brown and Snell (12) reported evidence suggesting pantothenylcysteine as a precursor for pantetheine in bacteria. They also observed that Acetobacter suboxydans was able to decarboxylate pantothenylcysteine to pantetheine.

We have recently been able to test synthetic pantothenylcysteine, for which we are indebted to Dr. J. Baddiley. The data of Table IV demonstrate that pantothenylcysteine forms pantetheine in the absence of ATP. The greater yield of pantetheine from pantothenylcysteine in the presence of ATP is due to the protective effect of ATP against the pantetheine-splitting enzyme. These data then confirm the observations of Brown and Snell (12), suggesting pantothenylcysteine as a precursor of pantetheine.

### TABLE IV

**Pantothenylcystine Replacement of ATP-Pantothenate-Cysteine System**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Units CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme + ATP + pantothenate + cysteine</td>
<td>10.4</td>
</tr>
<tr>
<td>&quot; + pantothenylcysteine (+H$_2$S)</td>
<td>5.2</td>
</tr>
<tr>
<td>&quot; + ATP + pantothenylcysteine</td>
<td>10.4</td>
</tr>
</tbody>
</table>

The incubation system was identical with that described in Table III. 0.05 μM of pantothenylcysteine and 0.5 μM of H$_2$S were used as indicated.

and Snell (12), suggesting pantothenylcysteine as a precursor of pantetheine.

### SUMMARY

The biosynthesis of CoA in mammalian tissues has now been largely elucidated and appears to follow the scheme:

1. Pantothenate + cysteine + ATP → pantothenylcysteine
2. Pantothenylcysteine → pantetheine
3. Pantetheine + ATP → 4'-phosphopantetheine
4. 4'-Phosphopantetheine + ATP → dephospho CoA
5. Dephospho CoA + ATP → CoA

Mg$^{++}$ is necessary for the reactions and the substrates must be in their reduced forms. The enzymes catalyzing reactions (1) and (2) have been found in fresh rat liver supernatant fraction and the cysteine requirement appears to be specific. Reaction (3) appears to be localized in the supernatant fraction of liver; reactions (4) and (5) are in both mitochondria
and supernatant fraction. The enzymes catalyzing reactions (4) and (5) have been purified some 100-fold from hog liver, and reaction (4) has been shown to be a reversible condensation of ATP and phosphopantetheine with the elimination of inorganic pyrophosphate.

We wish to thank Dr. Fritz Lipmann for his encouragement, advice, and continued interest during this investigation.

BIBLIOGRAPHY

BIOSYNTHESIS OF COENZYME A FROM PHOSPHOPANTETHEINE AND OF PANTETHEINE FROM PANTOTHENATE

M. B. Hoagland and G. David Novelli


Access the most updated version of this article at http://www.jbc.org/content/207/2/767.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/207/2/767.citation.full.html#ref-list-1