suggest that a two-step mechanism involving an acyl enzyme intermediate is the general reaction path for the hydrolysis of both specific and nonspecific substrates by \( \alpha \)-chymotrypsin.

REFERENCES


Benzoate Derivatives as Intermediates in the Biosynthesis of Coenzyme Q in the Rat*  

ROBERT E. OLSON, RONALD BENTLEY, A. S. AIYAR,† G. HONORIN DIALLAMEN, PHILIP H. GOLDF,‡ VIRGINIA G. RAMSEY, AND C. M. SPRINGER

From the Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, Pittsburgh 13, Pennsylvania

(Received for publication, June 26, 1963)

In the rat, the benzoquinone moiety of coenzyme Q is known to be derived from the phenylalanine acids (1, 2). The detailed intermediary metabolism of phenylalanine leading to the formation of the quinone moiety, however, is unknown. The work reported here indicates that the side chains of phenylalanine and tyrosine are totally lost in the biosynthesis of coenzyme Q, and that certain benzoate derivatives may function as intermediates in the biosynthesis. Further, formate has been established as the source of the ring bound methyl group as well as the methoxyl methyl groups. These unexpected findings have led to some novel concepts of amino acid metabolism.

The details of experiments both in vivo and in vitro have been previously described (3). A number of possible coenzyme Q precursors were administered intraperitoneally to 150-g rats; the animals were killed after 3 hours, and the total body coenzyme Q was then isolated and crystallized with carrier to constant specific activity. As further evidence of radiochemical purity, diacetyl coenzyme Q, hydroquinone was prepared by reductive acetylation and was also crystallized to constant specific activity. As shown in Table I. The pure diacetate was then degraded according to Bentley et al. (2) to give 3',6'-diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (m.p. 137°C) as a ring fragment and the bis-dinitrophenylhydrazone of levulinaldehyde as a side chain fragment. The relative incorporation of precursor radioactivity into the ring fragment and the side chain were calculated on the assumption that the terminal isopropylidene group had the same relative specific activity as the levulinaldehyde.

The incorporation of label into coenzyme Q, from all precursors studied is of the same order of magnitude, ranging from 0.005% with formate-C\textsuperscript{14} to 0.022% with L-tyrosine-U-C\textsuperscript{14} Acetate, L-tyrosine-C\textsuperscript{14}, and DL-phenylalanine-C\textsuperscript{14} label predominantly the polyisoprene side chain, whereas L-tyrosine-U-C\textsuperscript{14}, L-phenylalanine-U-C\textsuperscript{14}, ring-labeled benzoate-C\textsuperscript{14}, and formate-C\textsuperscript{14} label predominantly the diacetoxyphenylacetic acid fragment. The increased incorporation of label from tyrosine-U-C\textsuperscript{14} as compared with phenylalanine-U-C\textsuperscript{14} into coenzyme Q suggests that benzoate was probably not on the main pathway of coenzyme Q biosynthesis. A possible sequence for ring formation is therefore as follows.

Phenylalanine \( \rightarrow \) tyrosine \( \rightarrow \) p-hydroxybenzoate \( \rightarrow \) coenzyme Q\textsubscript{9} nucleus \( \rightarrow \) benzoate

To gain additional information about the role of p-hydroxybenzoate, "swamping" experiments were conducted in vivo with liver slices. Slices of rat liver from normal albino rats (180 to 200 g) were incubated for 3 hours at 38°C in pH 7.4 bicarbonate buffer with 95% O\textsubscript{2}-5% CO\textsubscript{2} as the gas phase. After incubation, the coenzyme Q\textsubscript{9} was isolated from the nonsaponifiable fraction by a methylation procedure. The data are presented in Table I. The pure diacetate was then degraded to determine the extent to which formate carbon entered into coenzyme Q\textsubscript{9} labeled with formate-C\textsuperscript{14} was further degraded to determine the extent to which formate carbon entered coenzyme Q\textsubscript{9}.

Although C-methylations of benzenoid molecules have not heretofore been demonstrated in animal tissues, the above data suggested to us that such a methylation might occur in coenzyme Q biosynthesis. The diacetoxyphenylacetic acid fragment obtained from coenzyme Q\textsubscript{9} labeled with formate-C\textsuperscript{14} was further degraded to determine the extent to which formate carbon entered coenzyme Q\textsubscript{9}.

* This research was supported in part by Research Grant AM-03737 from the National Institute of Arthritis and Metabolic Diseases and General Support Grant 1 S01 FR05151 from the General Research Support Branch, Division of Research Facilities and Resources, United States Public Health Service.  
† Postdoctoral Fellow, Biochemistry Training Grant TI GM 564, United States Public Health Service.  
‡ Predoctoral Fellow, Biochemistry Training Grant TI GM 564, United States Public Health Service.
Incorporation of C\textsuperscript{14} label from selected coenzyme Q\textsubscript{9} precursors into coenzyme Q\textsubscript{9} in intact normal and vitamin A-deficient rats

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Animal status\textsuperscript{a}</th>
<th>No. of experiments</th>
<th>Dose</th>
<th>Incorporation into coenzyme Q\textsubscript{9}</th>
<th>Specific activities</th>
<th>C\textsuperscript{14} distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Coenzyme Q\textsubscript{9}</td>
<td>Disaccharide of coenzyme Q\textsubscript{9} hydroquinone</td>
</tr>
<tr>
<td>Acetate-1-C\textsuperscript{14}</td>
<td>D</td>
<td>1</td>
<td>1.0</td>
<td>0.0150</td>
<td>4277</td>
<td>5008</td>
</tr>
<tr>
<td>Acetate-2-C\textsuperscript{14}</td>
<td>D</td>
<td>1</td>
<td>1.0</td>
<td>0.0060</td>
<td>2606</td>
<td>2242</td>
</tr>
<tr>
<td>L-Phenylalanine-U-C\textsuperscript{14}</td>
<td>N</td>
<td>1</td>
<td>2.0</td>
<td>0.0061</td>
<td>2432</td>
<td>2417</td>
</tr>
<tr>
<td>DL-Phenylalanine-3 C\textsuperscript{14}</td>
<td>D</td>
<td>1</td>
<td>1.0</td>
<td>0.0220</td>
<td>7918</td>
<td>9079</td>
</tr>
<tr>
<td>DL-Tyrosine-U-C\textsuperscript{14}</td>
<td>D</td>
<td>1</td>
<td>2.0</td>
<td>0.0052</td>
<td>2989</td>
<td>2057</td>
</tr>
<tr>
<td>Formate-C\textsuperscript{14}</td>
<td>D/N</td>
<td>2</td>
<td>2.0</td>
<td>0.0039</td>
<td>1932</td>
<td>1882</td>
</tr>
<tr>
<td>Ring-labeled benzoate-C\textsuperscript{14}</td>
<td>N</td>
<td>1</td>
<td>0.5</td>
<td>0.0064</td>
<td>1243</td>
<td>1181</td>
</tr>
<tr>
<td>Benzoate 7-C\textsuperscript{14}</td>
<td>N</td>
<td>1</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Benzoquinone-U-C\textsuperscript{14}</td>
<td>N</td>
<td>1</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} In cases marked D, animals were fed vitamin A-deficient diets for 3 to 6 weeks in order to increase the incorporation of precursor into hepatic coenzyme Q\textsubscript{9} (4). N indicates normal animals.

\textsuperscript{b} Ring fragment = 3',6'-diacetoxy-4',5'-dimethoxo-2'-methylphenylacetic acid.

\textsuperscript{c} Side chain = levulinic aldehyde, bis-2,4-dinitrophenylhydrazone.

\textsuperscript{d} All precursor activities were in the range of 2 to 20 mc per mmole.

\textsuperscript{e} Benzoate-7-C\textsuperscript{14} = carboxyl-labeled benzoate.

\textsuperscript{f} Dashes indicate that activities were not determined.

It appears from these studies, both in vivo and in vitro, and from previous work from this laboratory (1-3), that phenylalanine and tyrosine are the distal precursors of the benzoquinone moiety of coenzyme Q\textsubscript{9} in the rat. Wiss, Gloor, and Weber (4, 5) found that label from phenylalanine-U-C\textsuperscript{14} and tyrosine-U-C\textsuperscript{14} was poorly incorporated in vivo into rat liver coenzyme Q\textsubscript{9}, but no attempt was made to degrade the labeled product. Our experiments show that the incorporation of activity from the phenylalanino acids into the ring fragment is of the same order of magnitude as that of given precursors of the polyisoprene side chain such as acetate. They also demonstrate that the C\textsubscript{3} side chain of the phenylalino acids is available only for the biosynthesis of the polyisoprene side chain of coenzyme Q\textsubscript{9}. The \( \beta \) carbons of tyrosine and phenylalanine, as well as the carboxyl of benzoate, are lost in the synthesis of the benzoquinone ring and its attached methyl group. Although homogentisate may be a likely intermediate for that portion of the phenylalino acid carbon entering the mevalonate pathway, our results are not complete.

| Table II |

Incorporation of given precursors into coenzyme Q\textsubscript{9} in rat liver slices and effect of suspected intermediates in “swamping” experiments

Specific activities for substrates were: acetate-1-C\textsuperscript{14}, 2.0 mc per mmole; DL-mevalonate-2-C\textsuperscript{14}, 0.5 mc per mmole; L-tyrosine-U-C\textsuperscript{14}, 2.1 mc per mmole; benzoquinone-U-C\textsuperscript{14}, 2.4 mc per mmole; phenol-U-C\textsuperscript{14}, 1.6 mc per mmole. Six to eight grams of slices were used per run in 40 ml of medium.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cold intermediate</th>
<th>Specific radioactivity of coenzyme Q\textsubscript{9}</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate-1-C\textsuperscript{14}</td>
<td>Mevalonate</td>
<td>3,191</td>
<td>96</td>
</tr>
<tr>
<td>Acetate-1-C\textsuperscript{14}</td>
<td>Mevalonate</td>
<td>1,119</td>
<td>96</td>
</tr>
<tr>
<td>DL-Mevalonate-2-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>7,376</td>
<td>5</td>
</tr>
<tr>
<td>DL-Mevalonate-2-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>7,063</td>
<td>5</td>
</tr>
<tr>
<td>L-Tyrosine-U-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>15,283</td>
<td>96</td>
</tr>
<tr>
<td>L-Tyrosine-U-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>11,814</td>
<td>96</td>
</tr>
<tr>
<td>Benzoquinone-U-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phenol-U-C\textsuperscript{14}</td>
<td>p-Hydroxybenzoate</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE III

Results of degradation of C\textsuperscript{14}-labeled substituted phenylacetic acid (ring fragment of coenzyme Q\textsubscript{9} diacette) obtained from formate-1-C\textsuperscript{14}

Specific activities are corrected for dilutions with carrier from the original CoQ\textsubscript{9} isolated.
consistent with the hypothesis (3) postulating homogenates and toluquinone as intermediates in the aromatic ring. It seems likely that the pathway to the benzoquinone ring is by a stepwise degradation of the phenylamino acid side chain to the benzoate level, with the ultimate loss of this carboxy group. The carboxy group may be essential as a "handle" for the oxidative reactions leading to hydroxylation, and possibly alkylolation. The degradation of phenylalanine to benzoic acid (6) and of tyrosine to p-hydroxybenzoic acid (7) and the conversion of benzoate to p-hydroxybenzoate (8) have been clearly established in mammals. p-Hydroxybenzaldehyde, which could readily be oxidized to p-hydroxybenzoic acid, has been reported as a natural product in human liver (9) and as an intermediate in dhurrin biosynthesis in plants (10). The aromatic nucleus, lacking the carboxyl group, is apparently not used for coenzyme Q biosynthesis since benzoquinone and phenol are inactive as coenzyme Q precursors, both in vivo and in vitro, to the extent tested (see Tables I and II).

Our evidence that both methoxyl and ring methyl groups of coenzyme Q may be derived from formate is of considerable interest. It explains earlier work (4, 11) in which it was not possible to recover in the methoxyl groups all of the radioactivity found in the coenzyme Q molecule biosynthesized from formate-C14. Although S-adenosylmethionine is a likely donor for the O-methylation reaction (12), the mode of C-methylation of the benzene ring is unknown and represents a new type of reaction in physiological chemistry. Additional studies are in progress to elucidate these interesting pathways.

Acknowledgments—The authors wish to acknowledge the work of Dr. M. Riegl, who supervised the preparation of animals for these studies, and to thank Mr. Don Lee and Miss H. S. Tsao for able technical assistance. Thanks are also due Dr. K. Folkers and M. Isler of Hoffmann-La Roche, Basle, Switzerland, for ample supplies of coenzyme Q9 and coenzyme Q10.

REFERENCES


Fabry's Disease: Classification as a Sphingolipidosis and Partial Characterization of a Novel Glycolipid*

CHARLES C. SWEETLEY AND BERNARD KLIONSKY

From the Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, and the Department of Pathology, Magee-Womens Hospital, University of Pittsburgh School of Medicine, Pittsburgh 13, Pennsylvania

(Received for publication, July 5, 1963)

Fabry's disease (angiokeratoma corporis diffusum), originally considered a dermatological disorder, is now recognized as a sex-linked systemic lipidosis in which deposits of an unknown lipid occur primarily in renal glomeruli and tubules and in blood vessels throughout the body (1). Microscopic study of kidney tissue from these patients reveals a distinctive ballooning of the glomerular epithelium. The lipid deposits can be demonstrated with fat stains or as doubly refractile crystals in polarized light. Several reports (2) have appeared in which the authors referred to Kuhnau's studies of lipids from heart and kidney from two patients with Fabry's disease. The lipid was purported to be a daminophosphatide related to sphingomyelin but with unusual solubility properties. Although no descriptive chemical data were given in these reports, Fabry's disease was classified tentatively as a sphingolipidosis.

We have carried out an examination of kidney lipids from a 28-year-old man† who died of renal failure and whose clinical symptoms were the classical ones of Fabry's disease.* Formamide-hyde-preserved tissue was extracted with chloroform-methanol (2:1, v/v). Examination of the crude extract of total lipids by thin layer chromatography revealed a pair of polar lipids which were not observed in extracts of normal preserved kidney. The total concentration of these two lipids was estimated to be about 20 mg per g of wet tissue. Evaporation of the extract gave a cuprous white precipitate which, on thin layer chromatography, was found to be largely the more polar of the two lipids. This substance, designated as GL 3, was purified by gradient chromatography on silicic acid columns, from which it was eluted with about 20% methanol in chloroform. The product was washed with diethyl ether and with acetone, in which it was highly insoluble. Finally, a solution of the lipid in chloroform-methanol (4:1, v/v) was percolated through a bed of diatomaceous earth

* This investigation was supported in part by Research Grants A-4307 and H-0901 from the National Institutes of Health, United States Public Health Service.
† Magee-Womens Hospital Unit No. 172713.
Benzoate Derivatives as Intermediates in the Biosynthesis of Coenzyme Q in the Rat


Access the most updated version of this article at http://www.jbc.org/content/238/9/PC3146.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/238/9/PC3146.citation.full.html#ref-list-1