The Conversion of \( p \)-Hydroxybenzaldehyde to the Benzoquinone Ring of Ubiquinone in *Rhodospirillum rubrum*

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The pathways involved in the biosynthesis of the benzoquinone ring of the ubiquinones have not been established. Recognition of the inability of mammals to synthesize aromatic rings de novo has led to the supposition that, in mammals, the benzoquinone ring of the ubiquinones arises from dietary aromatic amino acids. In support of this theory, Bentley et al. (1) administered phenylalanine-U-\( ^{14} \)C to the rat and recovered 0.002 to 0.003% of the radioactivity in the quinone ring of liver rings de novo has led to the supposition that, in mammals, the benzoquinone ring of the ubiquinones have not been established. Recognition of the inability of mammals to synthesize aromatic rings de novo has led to the supposition that, in mammals, the benzoquinone ring of the ubiquinones arises from dietary aromatic amino acids. In support of this theory, Bentley et al. (1) administered phenylalanine-U-\( ^{14} \)C to the rat and recovered 0.002 to 0.003% of the radioactivity in the quinone ring of liver.

Rudney and Sugimura (3) found that homogentisic acid was an intermediate in the conversion of phenylalanine to coenzyme \( Q_0 \). However, Wiss, Gloor, and Weber (2) have concluded that tyrosine is not a precursor of coenzyme \( Q_0 \) in the rat.

In microorganisms, where several pathways for the synthesis of aromatic rings are available, no evidence has been reported that the aromatic amino acids serve as precursors of the ubiquinones. Thus, Rudney and Sugimura (3) administered phenylalanine-U-\( ^{14} \)C to coenzyme \( Q_0 \) in yeast. Braun, Dewey, and Kidder (4) administered phenylalanine-U-\( ^{14} \)C to *Tetrahymena pyriformis*, a microorganism auxotrophic for phenylalanine and tyrosine. On the basis of specific activity data, they rejected the possibility of direct conversion of the phenylalanine ring into the benzoquinone portion of coenzyme \( Q_0 \).

Some representative experiments described here explore the roles of several aromatic compounds in coenzyme \( Q_0 \) biosynthesis in the photosynthetic microorganism *Rhodospirillum rubrum*. Part of the incorporation of \( ^{14} \)C into coenzyme \( Q_0 \) when *R. rubrum* is grown with tyrosine-U-\( ^{14} \)C has been traced to an impurity in the commercial tyrosine preparation. The impurity has been identified as \( p \)-hydroxybenzaldehyde. Evidence is offered that this substance is readily transformed into the benzoquinone ring of coenzyme \( Q_0 \) and that, in this transformation, the aldehyde carbon is lost.

If tyrosine gives rise to coenzyme \( Q_0 \) by way of homogentisic acid, the \( \beta \) carbon of the tyrosine side chain might be expected to become the ring methyl group of coenzyme \( Q_0 \) (5). To test this, *R. rubrum* was grown in the presence of \( \alpha \)-tyrosine-\( ^{14} \)C (1.63 mmoles, 4.0 \( \times \) 10\(^6\) c.p.m. per mmole). The growth medium was that of Kohmiller and Gost (6), and coenzyme \( Q_0 \) was isolated (7) when the cell yields from 1 liter of medium were approximately 500 mg, dry weight. Although the cells took up about 40% of the radioactivity in these experiments, only 0.00013% of the \( ^{14} \)C added appeared in coenzyme \( Q_0 \) (2.85 mmoles, less than 2.8 c.p.m. per mmole). In marked contrast with this negligible incorporation, administration of uniformly labeled \( \alpha \)-tyrosine-\( ^{14} \)C (9.82 mmoles, 2.56 \( \times \) 10\(^6\) c.p.m. per mmole) under the same conditions resulted in the incorporation of 0.058% of the radioactivity into coenzyme \( Q_0 \) (2.97 mmoles, 4.0 \( \times \) 10\(^6\) c.p.m. per mmole).

Since dilution of the administered tyrosine-U-\( ^{14} \)C with unlabeled tyrosine failed to decrease the specific activity of the coenzyme \( Q_0 \) recovered, it appeared likely that part of the observed incorporation was due to the presence of another compound in the radioactive tyrosine preparation. Paper chromatography of the tyrosine-U-\( ^{14} \)C revealed the presence of small amounts of several radioactive compounds, one of which proved active as a precursor of coenzyme \( Q_0 \). This material was indistinguishable from authentic \( p \)-hydroxybenzaldehyde in three paper chromatographic systems. It was easily obtained by addition of carrier \( p \)-hydroxybenzaldehyde to the radioactive tyrosine, extraction with ether, chromatography on Celite, and crystallization from water or toluene to constant specific activity. The specific activity of the coenzyme \( Q_0 \) formed by cells grown with this material was correspondingly decreased if the sample administered was diluted with additional unlabeled \( p \)-hydroxybenzaldehyde.

\( p \)-Hydroxybenzaldehyde of higher specific activity was prepared by enzymatic transamination of tyrosine-\( ^{14} \)C to \( p \)-hydroxyphenylpyruvate (with \( \alpha \)-ketoglutarate-tyrosine transaminase purified from rat liver as described by Kenney (8)) and treatment of the reaction mixture with NaOH. This procedure utilizes the observation of Doy (9) that at pH 11, \( p \)-hydroxyphenylpyruvate decomposes rapidly to form \( p \)-hydroxybenzaldehyde. The product was purified by ether extraction, Celite chromatography, and crystallization to constant specific activity. By starting with the appropriately labeled tyrosine, \( p \)-hydroxybenzaldehyde-\( ^{14} \)C and \( p \)-hydroxybenzaldehyde-carbonyl-\( ^{14} \)C were prepared.

When *R. rubrum* was grown in the presence of \( p \)-hydroxybenzaldehyde-\( ^{14} \)C (16.1 mmoles, 7.45 \( \times \) 10\(^6\) c.p.m. per mmole), 5% of the \( ^{14} \)C administered was taken up by the cells. Of the \( ^{14} \)C taken up, 97% could be extracted, along with coenzyme \( Q_0 \) (7), by three extractions with ether-ethanol (3:1). This extract was dried and redissolved in 25 ml of petroleum ether. Five extractions of the petroleum ether with 5-ml portions of 95% methanol-5% water removed chlorophyll (7), but the petroleum ether fraction retained 83% of the \( ^{14} \)C taken up by the cells. When the petroleum ether residue was chromatographed on alumina (7), essentially all of the \( ^{14} \)C was eluted in two peaks. Coenzyme \( Q_0 \) (1.98 mmoles, 1.07 \( \times \) 10\(^6\) c.p.m. per mmole) from the first of these peaks (eluted with 7% ether in petroleum ether) contained 52% of the total radioactivity found in the cells. In one experiment, the specific activity of the coenzyme \( Q_0 \) after recrystallization to constant specific activity was as high as 70% of that of the administered \( p \)-hydroxybenzaldehyde. The second peak, eluted by 16% ether in petroleum ether, contained approximately 40% of the total radioactivity. The identity of the radioactive component of the second peak is under investigation.

4 The sample of tyrosine-U-\( ^{14} \)C was from Schwartz BioResearch, Inc., Lot 0208. The levels of discrete impurities were stated to be below 0.5%.

5 Since submitting this manuscript the authors have identified the second peak as rhodoquinone, first described by Glover and...
Administration of p-hydroxybenzaldehyde carbonyl C\textsuperscript{14} (6.28 μmoles, 1.42 X 10\textsuperscript{4} c.p.m. per μmole) to R. rubrum resulted in a markedly different distribution of radioactivity in the cells, although the percentage uptake was approximately the same (8.5%). Most of the C\textsuperscript{14} found in the cells was not extracted into ether-ethanol (3 : 1), and the petroleum ether fraction contained only 2.37% of the counts taken up. The coenzyme Q\textsubscript{9} did not contain significant radioactivity. Apparently, in the conversion of p-hydroxybenzaldehyde to coenzyme Q\textsubscript{9}, the side chain is lost.

The evidence that the p-hydroxybenzaldehyde ring can give rise to coenzyme Q\textsubscript{9} in direct manner was strengthened by degradation of the radioactive coenzyme Q\textsubscript{9} by the method of Bentley et al. (1). In a degradation carried out on coenzyme Q\textsubscript{9} isolated from R. rubrum grown with p-hydroxybenzaldehyde-U-Cl\textsubscript{4} as described above, the 3', 6'-diacetoxy-4', 5'-dimethoxy-2'-methylphenylacetic acid, derived from the coenzyme Q\textsubscript{9} benzoquinone ring, was found to account for 86.5% of the total C\textsuperscript{14} in the coenzyme Q\textsubscript{9}. Thus coenzyme Q\textsubscript{9} having a specific activity of 24.2 c.p.m. per pmole after the addition of carrier afforded the ring derivative with an activity of 20.9 c.p.m. per pmole. The coenzyme Q\textsubscript{9} side chain contained no detectable C\textsuperscript{14}.

Although a relatively small amount of p-hydroxybenzaldehyde is taken up by the cells, these data suggest that once inside the cell p-hydroxybenzaldehyde is converted fairly directly to coenzyme Q\textsubscript{9}. The data appear to exclude tyrosine as an intermediate in the conversion of p-hydroxybenzaldehyde to coenzyme Q\textsubscript{9}. In further confirmation of this concept, the addition of unlabeled tyrosine to cells growing with p-hydroxybenzaldehyde-U-C\textsuperscript{14} does not decrease the specific activity of the coenzyme Q\textsubscript{9} isolated. Phenol-U-C\textsuperscript{14} did not prove to be an effective precursor of coenzyme Q\textsubscript{9}, although the observed loss of the aldehyde carbon from p-hydroxybenzaldehyde suggests that the formation of coenzyme Q\textsubscript{9} involves a 6 carbon aromatic intermediate, which is subsequently hydroxylated, O-methylated, and C-methylated.\textsuperscript{4}

Iwasaki, Iwasaki, and Gest (12) have found that p-hydroxybenzaldehyde and p-hydroxybenzoic acid are among several aromatic compounds excreted into the medium when R. rubrum is grown with methionine-\textsuperscript{14}C\textsubscript{6}. These considerations and the finding that labeled p-hydroxybenzaldehyde is converted to coenzyme Q\textsubscript{9} without apparent dilution by tyrosine suggest that in R. rubrum the coenzyme Q\textsubscript{9} benzoquinone nucleus is not formed directly from aromatic amino acids, but is derived from aromatic compounds which arise earlier in the shikimic acid pathway. The possibility must also be considered, however, that aromatic compounds similar to p-hydroxybenzaldehyde may arise via the acetate pathway (15).

REFERENCES


On the Question of an Acyl-enzyme Intermediate in the Chymotrypsin-catalyzed Hydrolysis of Hydroxamic Acids*

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That the reactions of esters of hippuric acid catalyzed by chymotrypsin pass through an acyl-enzyme intermediate has been demonstrated by the fact that in 0.1 M NH\textsubscript{2}OH solution the same fractions of 10 esters of hippuric acid were converted to hippurylhydroxamic acid (1). These reactions are depicted...
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