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Biochemical and Genetic Studies on Ubiquinone Biosynthesis in *Escherichia coli* K-12: 4-Hydroxybenzoate Octaprenyltransferase

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Three ubiquinone-deficient mutants of *Escherichia coli* unable to convert 4-hydroxybenzoate into 3-octaprenyl-4-hydroxybenzoate were isolated and examined. The results of genetic analysis suggest that each of the mutants carries a mutation in a gene designated *ubiA* which can be represented at minute 79 on the *E. coli* chromosome map. The conversion of 4-hydroxybenzoate into 3-octaprenyl-4-hydroxybenzoate, catalyzed by 4-hydroxybenzoate octaprenyltransferase, was studied with a strain of *E. coli* that is blocked in the common pathway of aromatic biosynthesis and consequently accumulates the precursor of the side chain of ubiquinone. Both the side-chain precursor and 4-hydroxybenzoate octaprenyltransferase were shown to be membrane-bound. The enzyme required Mg^{2+} for optimal activity. The *ubiA*$^-$ mutants were found to lack 4-hydroxybenozate octaprenyltransferase activity, which suggested that the *ubiA* gene is the structural gene coding for this enzyme.

The genetics and biochemistry of ubiquinone biosynthesis have been approached in this laboratory through the isolation and examination of mutants of *Escherichia coli* K-12 unable to form ubiquinone. Five classes of ubiquinone mutants have been examined in detail and five ubiquinone precursors, each having an octaprenyl side chain, have been isolated and chemically characterized (2, 14, 19). Genetic analysis of these mutants suggested that strains within each class carry mutations in a single gene, and the five *ubi* genes have been located on the chromosome (1, 2, 14, 19). The first three reactions specific to ubiquinone biosynthesis (Fig. 1) have been demonstrated with cell extracts (2, 4, 5). The present paper describes biochemical and genetic studies with a further class of ubiquinone mutants which are affected in the conversion of 4-hydroxybenzoate to 3-octaprenyl-4-hydroxybenzoate.

MATERIALS AND METHODS

Bacterial strains. All of the strains used were derived from *E. coli* K-12 and are described in Table 1. The ubiquinone-deficient strains were maintained on nutrient agar supplemented with 0.5% glucose and were checked before use for the presence of revertants which, in contrast to the mutants, are able to grow on succinate as sole source of carbon.

Media. The minimal medium used and the concentrations of supplements have been described previously (14). The medium used for the growth of the *aroB*$^-$ strains was supplemented with phenylalanine (0.2 mM), tyrosine (0.2 mM), tryptophan (0.2 mM), 4-aminobenzoate (1 μM), 2,3-dihydroxybenzoate (10 μM), and shikimate (4 μM).

Isolation and characterization of ubiquinone-deficient mutants. The methods used for the isolation of the ubiquinone-deficient mutants and for the extraction and chromatography of the quinones were as described previously (1, 2).

Detection of 4-hydroxybenzoate in culture supernatant fluids. Cultures, in glucose-minimal medium containing 10^{-2} M sodium citrate to repress the synthesis of enterochelin and its precursor 2,3-dihydroxybenzoate (18), were grown to stationary phase in shaken flasks. A sample of 20 ml of culture supernatant fluid was acidified to pH 1.5 with HCl and was extracted once with 80 ml of diethylether. The ether extract was dried over anhydrous $MgSO_4$, concentrated by rotary evaporation, and applied to a sheet of Merck thin-layer cellulose along with 4-hydroxybenzoate as marker. The sheet was developed with isopropanol-ammonia-water (200:10:20, v/v) as solvent. The plate was then dried and sprayed with diazotized *p*-nitraniline (13). 4-Hydroxybenzoate runs with an R_F of 0.1 in the solvent used and gives a characteristic intense red color after spraying.

Chemicals. 4-Hydroxy-U-^{14}C-benzoate was prepared (8) from L-[U-^{14}C]-tyrosine (specific activity, 468 μCi/μmole) obtained from the Radiochemical Centre (Amersham, England). Its radiochemical purity was confirmed by chromatography in two different solvents.

Preparation of cell extracts. Cultures were

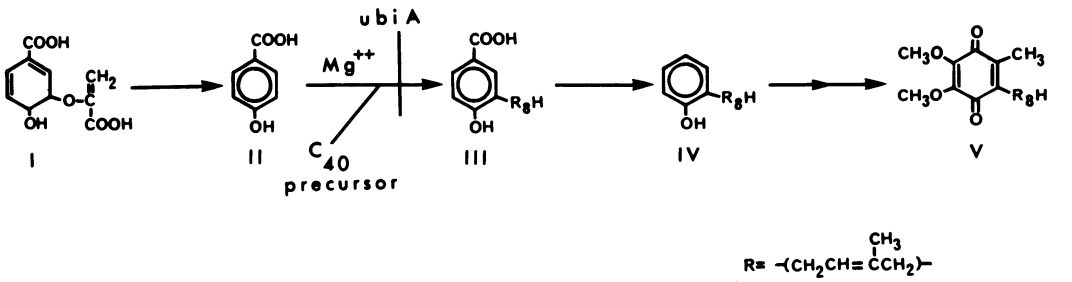

FIG. 1. *Scheme showing the first three reactions in ubiquinone biosynthesis in E. coli and the reaction affected by mutations in the ubiA gene. (I) Chorismic acid; (II) 4-hydroxybenzoic acid; (III) 3-octaprenyl-4-hydroxybenzoic acid; (IV) 2-octaprenylphenol; (V) ubiquinone-8.*

TABLE 1. *Strains of E. coli K-12 used*

Strain	Sex	*ubiA*	Other relevant genetic loci[a]	Other information
AB2826 str^R	F$^-$	+	*aroB351, str^R*	Streptomycin-resistant derivative of AB2826 (10)
AB2830	F$^-$	+	*aroC$^-$*	Pittard and Wallace (10)
AB3282	F$^-$	+	*his-4, proA2, argE3, ilvC7, leu351, trp$^-$, str-704*	
AB3285	F$^-$	351	*trp$^-$*	Cox et al. (1)
AB3311	Hfr	+	*metB$^-$*	Hfr Reeves 1
AB3321	Hfr	+		metB$^+$ transductant of AB3311
AT2246	Hfr	+	*leu-13, thr-13*	Hfr P10 (12)
AN84	Hfr	419	*metB$^-$*	Isolated after NTG[b] treatment of AB3311
AN144	Hfr	420	*metB$^-$*	Isolated after NTG treatment of AB3311
AN157	Hfr	419	*metB$^-$, aroB351, str^R*	Derived from AN84 by transduction with P1 grown on AB2826 *str^R*
AN164	Hfr	+	*metB$^-$, aroB351, str^R*	Derived from AB3311 by transduction with P1 grown on AB2826 *str^R*
AN165	Hfr	420	*metB$^-$, aroB351, str^R*	Derived from AN144 by transduction with P1 grown on AB2826 *str^R*
AN168	F$^-$	419	*argH$^-$*	Derived from PA505MPE11 by transduction with P1 grown on AN84
AN169	F$^-$	420	*argH$^-$*	Derived from PA505MPE11 by transduction with P1 grown on AN144
AN179	F$^-$	419	*metB$^-$, his-4, proA2, leu-351, trp$^-$, str-704*	Recombinant from AN84 × AB3282
AN186	F$^-$	351	*argH$^-$*	Derived from PA505MPE11 by transduction with P1 grown on AB3285
PA505MPE11	F$^-$		*metA$^-$, argH$^-$*	M. Schwartz[c]
0205	F$^-$		*leu$^-$, thr$^-$, proA$^-$, purA$^-$*	P. G. De Haan[c]

[a] Genetic nomenclature is that used by Taylor (15).
[b] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
[c] Source of strain.

grown into late exponential phase in glucose-minimal medium containing the appropriate supplements. The cells were centrifuged and washed in 0.05 M potassium phosphate buffer (*p*H 7.0), and were resuspended in 2 ml of the same buffer for each gram (wet weight) of cells. The cells were disrupted at 20,000 psi in a Sorvall Ribi Cell Fractionator. The supernatant fluid, after centrifugation at 5,000 × *g* for 20 min, was used as the cell extract. The centrifugation of the culture and subsequent operations were all carried out at 4 C.

Estimation of protein. Protein concentrations were determined by the method of Lowry et al. (6) with bovine serum albumin as standard.

Assay for 4-hydroxybenzoate octaprenyltransferase. The assay mixture contained 0.1 ml of enzyme preparation (about 3.5 mg of protein in the case of cell extracts), ^{14}C-4-hydroxybenzoate (10,000 counts/min), unlabeled 4-hydroxybenzoate (0.5 to 4 nmoles as stated), and potassium phosphate buffer (25 μmoles, *p*H 7.0) in a total volume of 0.5 ml. Each assay was done in duplicate, and the tubes were incubated at 37 C for 15 min unless otherwise stated. The reaction was stopped by the addition of acidified acetone (2.4 ml of acetone plus 0.1 ml of 5 N HCl). The tubes were heated at 60 C for 3 min and then centrifuged. The pellet was washed twice with 2.5 ml of acetone, and the washings were combined

with the supernatant fluid. The acetone was evaporated off on a steam bath, and 0.5 ml of water was added to the aqueous residue. The solution was then extracted twice with 5 ml of pentane, and the combined pentane extract was evaporated to dryness in a scintillation vial. A 10-ml amount of scintillant fluid (5) was added, and the solution was counted by use of a Packard model 3320 Tri-Carb Liquid Scintillation Spectrometer.

Genetic mapping. The technique used for interrupted mating experiments was based on that described by Taylor and Thoman (16), and transduction experiments with the generalized transducing phage P1kc were performed as described by Pittard (9).

Preparation of strains carrying an aroB⁻ allele. A P1 lysate was prepared from strain AB2826 *str*^{R} and was used to infect streptomycin-sensitive strains into which the *aroB*⁻ allele was to be introduced. The transduced recipient cells were grown for three generations in nutrient broth containing 10 mM sodium citrate, to allow phenotypic expression, and then were plated onto a glucose-nutrient agar medium containing streptomycin (200 μg/ml) and shikimate (2×10^{-4} M). Streptomycin-resistant transductants were then screened to detect those which were *aroB*⁻ and therefore required the aromatic end products for growth. About 6% of these transductants were found to be *aroB*⁻.

RESULTS

Ubiquinone-deficient mutants were obtained, after treatment of strain AB3311 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, from among those strains able to grow on glucose but unable to grow on succinate as sole carbon source (1, 2). Biochemical and genetic characterization of five classes of mutants, each accumulating a different ubiquinone precursor with an octaprenyl side chain, has been reported previously (2, 14, 19). Another class of mutants which can form 4-hydroxybenzoate, but which form no detectable ubiquinone precursors possessing the octaprenyl side chain, has now been examined. This class consists of three mutants, two of which (AN84 and AN144) were derived from strain AB3311; the third mutant, AB3285, was isolated previously (1).

Examination of strains AN84 and AN144 for accumulation of ubiquinone precursors. Lipid extracts from the cells from three 1-liter cultures of strains AN84 and AN144 were chromatographed on silica gel plates with chloroform-light petroleum (70:30, v/v) as solvent. In both cases, menaquinone was present (R_F, 0.7) but ubiquinone was not detected.

A lipid extract of the parent strain (AB3311) was also chromatographed, and the plates from the two mutants were compared with that from the parent strain before and after spraying with diazotized *p*-nitraniline. The mutants did not accumulate any of the five ubiquinone precursors already characterized, and no compounds were detected that were not present in the parent strain. The levels of menaquinone were estimated spectrophotometrically (7), and both of the mutants formed about three times the level of menaquinone formed by the parent strain.

Since 4-hydroxybenzoate is the only known intermediate specific to ubiquinone biosynthesis which does not possess the octaprenyl side chain (Fig. 1), the mutants were tested for the ability to form 4-hydroxybenzoate. This compound is formed in excess, even by strains which form normal levels of ubiquinone, and can be detected in culture supernatant fluids by chromatography. Comparison of the culture supernatant fluids of the two mutants with that of the parent strain indicated that the levels of 4-hydroxybenzoate present in supernatant fluids from the mutants were slightly higher than in that of the parent strain. Since the mutants did not appear to accumulate any ubiquinone precursors possessing an octaprenyl side chain but could form 4-hydroxybenzoate normally, it appeared that they were blocked in the conversion of 4-hydroxybenzoate to the next known intermediate in ubiquinone biosynthesis, 3-octaprenyl-4-hydroxybenzoate.

Growth response to 4-hydroxybenzoate. Like other ubiquinone-deficient mutants, both AN84 and AN144 are unable to grow on succinate as sole carbon source. However, it was found that strain AN144 could grow on succinate if relatively high concentrations of 4-hydroxybenzoate (10^{-4} M final concentration) were included in the growth medium. Strain AN84 did not respond to 4-hydroxybenzoate under these conditions. This observation suggested that the enzyme catalyzing the conversion of 4-hydroxybenzoate to 3-octaprenyl-4-hydroxybenzoate in strain AN144 had been altered by mutation such that it had a considerably reduced affinity for 4-hydroxybenzoate. Cells of strain AN144 grown on a glucose medium supplemented with 10^{-4} M 4-hydroxybenzoate contained 20% of the normal level of ubiquinone, whereas strain AN84, grown under the same conditions, formed no detectable ubiquinone.

The growth response of strain AN144 to 10^{-4} M 4-hydroxybenzoate is similar to the behavior of another ubiquinone-deficient mutant (AB3285) which was reported previously (1). Genetic analysis of strains AN84, AN144, and AB3285 (see below) suggested that they all car-

ried mutations in the same gene (designated *ubiA*). The effect of 4-hydroxybenzoate on the growth of the *ubiA*$^-$ transductants derived from the above three strains and of AB2830, which is not affected in ubiquinone biosynthesis but cannot form 4-hydroxybenzoate, owing to a block in the common pathway of aromatic biosynthesis, is shown in Fig. 2. It is clear that the multiple aromatic auxotroph (AB2830) responds to much lower concentrations of 4-hydroxybenzoate than do the transductants AN169 and AN186 (derived from AN144 and AB3285, respectively), and that AN168 (derived from AN84) does not respond at all.

Preliminary studies on 4-hydroxybenzoate octaprenyltransferase. The above observations suggested that each of the *ubiA*$^-$ mutants was affected in the conversion of 4-hydroxybenzoate into 3-octaprenyl-4-hydroxybenzoate. The trivial name 4-hydroxybenzoate octaprenyltransferase will be used for the enzyme catalyzing this conversion, although the structure of the precursor of the octaprenyl side chain has not yet been established with certainty.

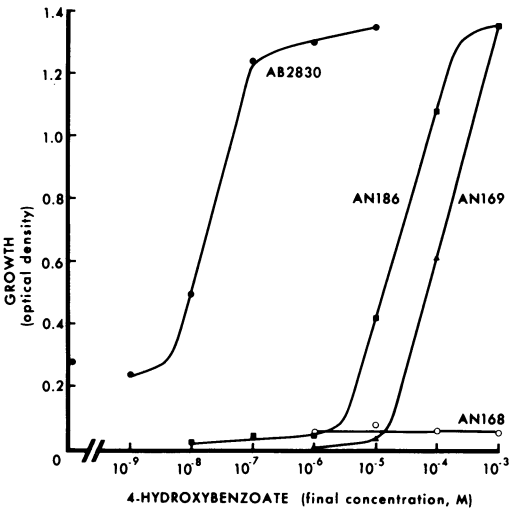

FIG. 2. *Growth responses of the multiple aromatic auxotroph AB2830 and the ubiA*$^-$ *strains AN168, AN169, and AN186 to 4-hydroxybenzoate. The minimal medium contained succinate (20 mM) as carbon source and was supplemented with the following growth factors: L-tyrosine (0.2 mM), L-tryptophan (0.2 mM), L-phenylalanine (0.2 mM), L-arginine (0.7 mM), 4-aminobenzoate (10 μM), 2,3-dihydroxybenzoate (0.1 mM), thiamine (0.02 μM). Inocula, washed once in distilled water, were from 24-hr nutrient agar slopes and were added to give initial populations of about 10^6 cells per ml. Growth was measured by use of a Spekker colorimeter with a neutral density filter after the following periods of incubation in shaken tubes at 37 C: AB2830, 19 hr; AN168, 25 hr; AN169, 25 hr; AN186, 25 hr.*

It has been shown previously (5) that 4-hydroxybenzoate can be converted to a mixture of 3-octaprenyl-4-hydroxybenzoate and its decarboxylation product by cell extracts of strain AB2830, which is blocked in the common pathway of aromatic biosynthesis, but not by cell extracts from a wild-type strain. This suggested that the conversion was dependent on the accumulation of a precursor of the side chain of ubiquinone in the cells of the mutant strain which is unable to synthesize the precursors of the quinone rings of ubiquinone and menaquinone. These cells were found to accumulate the all-*trans*-octaprenol farnesylfarnesylgeraniol, which it was suggested was a break-down product of the true side-chain precursor.

Preliminary studies on 4-hydroxybenzoate octaprenyl transferase were performed with cell extracts from strain AN164, which carries an *aroB*$^-$ allele. The effect of the concentration of 4-hydroxybenzoate on the amount of prenylated compounds formed over a period of 1 hr was tested (Fig. 3). The maximal conversion was obtained with 5 nmoles of added unlabeled 4-hydroxybenzoate, but in most experiments smaller amounts were used to retain sensitivity while conserving radioactive 4-hydroxybenzoate. The rate of reaction was examined with 2 nmoles of added 4-hydroxybenzoate. It was found to decrease markedly after the first 10 min (Fig. 4), and the conversion ceased after 30 min. The reason for this rapid decline in rate is not known, although it is probably not due to inactivation of 4-hydroxybenzoate octaprenyltransferase or to degradation of the side-chain precursor. Thus, preincu-

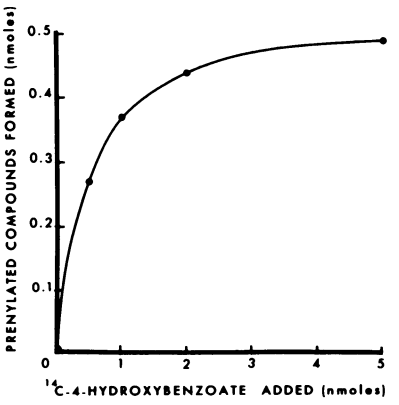

FIG. 3. *Effect of substrate concentration on the prenylation of 4-hydroxybenzoate. The assays were carried out as described in Materials and Methods except that the incubation period was 1 hr.*

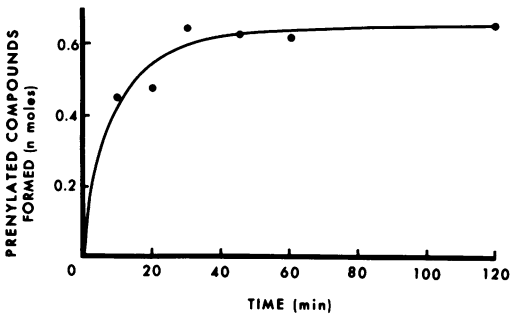

FIG. 4. *Effect of incubation time on the prenylation of 4-hydroxybenzoate. The assays were carried out as described in Materials and Methods except that 4 nmoles of 4-hydroxybenzoate was used per assay.*

bation of the cell extract for 2 hr at 37 C prior to assay, in the absence of 4-hydroxybenzoate, resulted in a decrease of only 15% of prenylated compounds formed compared with a control in which the cell extract was not preincubated. Attempts to increase the amount of 4-hydroxybenzoate converted, by the addition of yeast extract, magnesium ions plus adenosine triphosphate, coenzyme A, or dithiothreitol to the reaction mixture, were unsuccessful. Ethylenediaminetetraacetic acid (10 mM) inhibited activity 98% when added to the normal assay system, suggesting the possible involvement of a metal ion.

Sedimentation properties and magnesium requirement of 4-hydroxybenzoate octaprenyltransferase. The supernatant fraction obtained after ultracentrifugation of a cell extract from strain AN164 for 3 hr at 150,000 × *g* contained no 4-hydroxybenzoate octaprenyltransferase activity, and the washed pellet had about 25% of the original activity. The original activity was regained on mixing the pellet and the supernatant fluid (Table 2). The supernatant factor could be replaced by magnesium ions (Table 2). Cobalt and nickel ions were also partly effective in restoring the activity, manganese ions were less so, and the other metal ions tested either had no effect or were inhibitory (Table 2). Whereas all of the 4-hydroxybenzoate octaprenyltransferase activity was sedimented at 150,000 × *g* for 3 hr, centrifugation at 40,000 × *g* for 1 hr resulted in 25% of the activity being sedimented and 100,000 × *g* for 1 hr caused about 70% sedimentation. Since the washed 150,000 × *g* pellet plus magnesium ions catalyzed the isoprenylation of 4-hydroxybenzoate as efficiently as the original cell extract, it appears that both the enzyme and the precursor of the side chain are membrane-bound.

Estimation of 4-hydroxybenzoate octaprenyltransferase activity in the ubiA⁻ strains. To test for the presence of 4-hydroxybenzoate octaprenyltransferase activity in the *ubiA*⁻ strains AN84 and AN144, *aroB*⁻ derivatives of these mutants were prepared. Cell extracts from either of these derivatives (AN157 and AN165) showed no activity (Table 3) but did contain the side-chain precursor as shown by their ability to complement with cell extracts of the wild-type strain AB3311 (Table 3). Examination of cell extracts from the various strains by chromatography (5) indicated that the *ubiA*⁻, *aroB*⁻ strains accumulated the octaprenol farnesylfarnesyl geraniol, whereas the *ubiA*⁻, *aroB*⁺ strains did not. Although the enzymatic activity observed in the complementation tests was low compared with the activity from the *ubi*⁺, *aroB*⁻ strain AN164, the rate of reaction in the former case was linear over 2 hr. The slow rate probably re-

TABLE 2. *Sedimentation of 4-hydroxybenzoate octaprenyltransferase activity: effect of metal ions on enzymatic activity in washed membranes*

Enzyme prepn[a]	Metal added[b]	4-Hydroxybenzoate octaprenyltransferase[c]
Cell extract	None	230
Supernatant (150,000 × *g*)	None	<1
Pellet (150,000 × *g*)	None	60
Pellet plus supernatant	None	150
Pellet (150,000 × *g*)	$MgSO_4$[d]	190
	$CoSO_4$	170
	$NiSO_4$	100
	$MnSO_4$	84
	$FeSO_4$	51
	$CaCl_2$	23
	$ZnSO_4$	21
	$CdCl_2$	17
	$CuSO_4$	6
Cell extract	$MgSO_4$	210

[a] A 10-ml amount of cell extract (35 mg of protein/ml) was centrifuged at 150,000 × *g* for 3 hr. The pellet was resuspended in 10 ml of 0.05 M potassium phosphate buffer (pH 7.0) and centrifuged again under the same conditions. The resulting pellet was then suspended in 10 ml of the same buffer, the final protein concentration being 8 mg/ml, and 0.1 ml of the suspension was used per assay.

[b] The metals were added to the basal system at a concentration of 1 mM.

[c] The assay was carried out as described in Materials and Methods except that 1 nmole of 4-hydroxybenzoate was added per test. Activity is expressed as picomoles of product formed in 15 min.

[d] $MgSO_4$ at 10 mM gave an activity of 250.

TABLE 3. *4-Hydroxybenzoate octaprenyltransferase activity in ubiA⁺ and ubiA⁻ strains: enzymatic complementation tests*

Strain	4-Hydroxybenzoate octaprenyltransferase[a]
AN164 (*ubiA⁺*, *aroB⁻*)	258
AN157 (*ubiA419*, *aroB⁻*)	<0.5
AN165 (*ubiA420*, *aroB⁻*)	<0.5
AB3311 (*ubiA⁺*, *aroB⁺*)	<0.5
AB3311 (*ubiA⁺*, *aroB⁺*) plus AN157 (*ubiA419*, *aroB⁻*)	7
AB3311 (*ubiA⁺*, *aroB⁺*) plus AN165 (*ubiA420*, *aroB⁻*)	23
AN157 (*ubiA419*, *aroB⁻*) plus AN165 (*ubiA420*, *aroB⁻*)	<0.5
AN186 (*ubiA351*, *aroB⁺*) plus AN157 (*ubiA419*, *aroB⁻*)	<0.5
AN186 (*ubiA351*, *aroB⁺*) plus AN165 (*ubiA420*, *aroB⁻*)	<0.5

[a] The assay was carried out as described in Materials and Methods except that 0.2 ml of cell extract (7 mg of protein) and 0.5 nmole of 4-hydroxybenzoate were used. Activity is expressed as picomoles of product formed in 30 min.

flects a difficulty in performing complementation tests when enzyme and substrate are membrane-bound and from different cells.

Complementation tests between cell extracts of strains AN157 (*ubiA419*, *aroB⁻*) and AN165 (*ubiA420*, *aroB⁻*) were negative, as were complementation tests between strain AN186 (*ubiA351*, *aroB⁺*) and either of the above strains. These results indicate that all three *ubiA⁻* mutants are deficient in 4-hydroxybenzoate octaprenyltransferase activity.

Mapping of the ubiA gene. The gene in strain AN84 that carries the mutation preventing ubiquinone synthesis was designated *ubiA*. The *ubiA419* allele was transferred to a female by crossing the Hfr male AN84 with strain AB3282 and selecting for *ilv⁺* recombinants. One of the *ilv⁺* recombinants (AN179), which could not grow on succinate as sole carbon source, was shown to be unable to form ubiquinone, indicating that it had received the *ubiA⁻* allele. Time of entry experiments were then carried out with strain AN179 (F⁻, *ubiA⁻*, *metB⁻*, *str*^{R}) as recipient and the Hfr males AB3321 and AT2246 as donors, selecting for *metB⁺* and *ubiA⁺* recombinants. Strain AB3321 transfers its chromosome with a clockwise order of gene transfer from minute 74 as represented on the chromosome map. In crosses with strain AB3321, times of entry of *ubiA⁺* relative to *metB⁺* were obtained which placed the *ubiA* gene at about 2.5 min clockwise from *metB* on the chromosome map. This suggested that the *ubiA* gene was located at about minute 79.5. Strain AT2246 did not transfer the *ubiA* gene as an early marker, although normal numbers of *metB⁺* recombinants were obtained. Strain AT2246 (Hfr P10) has an anticlockwise order of gene transfer, and, because the earliest known marker transferred by strain AT2246 is *malB* (minute 79; (12), the *ubiA* gene is clockwise to *malB* on the map.

To verify the position of the *ubiA* gene, attempts were made to cotransduce it with *argH*, *metA*, or *purA*. The *ubiA* gene was found to be cotransducible with *metA* at 9% but not with *argH* or *purA* (Table 4). The cotransduction data, together with the results of the time of entry experiments, suggest that the *ubiA* gene can be represented at minute 79 on the chromosome map as shown in Fig. 5. The genes affected in strains AN144 and AB3285 were also shown to be cotransducible with *metA* at about the same frequency (Table 4), suggesting that the mutations in each of these strains are also carried in the *ubiA* gene.

DISCUSSION

The above results indicate that the enzyme 4-hydroxybenzoate octaprenyltransferase, which catalyzes the second reaction specific to ubiquinone biosynthesis in *E. coli*, is a membrane-bound enzyme requiring magnesium ions, which couples 4-hydroxybenzoate to a membrane-bound side-chain precursor. Other workers, using an alternative approach in which a cell extract of *Micrococcus lysodeikticus* supplemented with isopentenyl pyrophosphate was used as the source of side-chain precursor(s), obtained evidence for the presence of enzymes converting 4-hydroxybenzoate into 3-polyprenyl-4-hydroxybenzoate in cell extracts of *Rhodospirillum rubrum* (11) and in rat tissues (17).

Unlike 4-hydroxybenzoate octaprenyltransferase, the enzyme (4-hydroxybenzoate synthetase) which catalyses the first reaction of the ubiquinone biosynthetic pathway is not membrane-bound (J. Lawrence, *unpublished data*). Since both 3-octaprenyl-4-hydroxybenzoate and ubiquinone are membrane-bound (3), it seems likely that all of the reactions of ubiquinone biosynthesis, except the first, involve membrane-bound intermediates.

In addition to ubiquinone, *E. coli* forms menaquinone (vitamin K_2), which also contains a C_{40} isoprenoid side chain. The imme-

TABLE 4. *Tests for cotransduction between the ubiA gene and the argH, metA, or purA gene*

P1 grown on strain	Recipient strain	Marker selected	No. of transductants carrying the unselected marker
AN84 (*ubiA419*, *argH*$^+$, *metA*$^+$)	PA505MPE11 (*ubiA*$^+$, *argH*$^-$, *metA*$^-$)	*argH*$^+$	0/80 *ubiA*$^-$
		metA$^+$	16/80 *argH*$^+$ (20%), 7/80 *ubiA*$^-$ (9%)
AN84 (*ubiA419*, *purA*$^+$)	0205 (*ubiA*$^+$, *purA*$^-$)	*purA*$^+$	0/80 *ubiA*$^-$
AN144 (*ubiA420*, *metA*$^+$)	PA505MPE11 (*ubiA*$^+$, *argH*$^-$, *metA*$^-$)	*argH*$^+$	0/40 *ubiA*$^-$
		metA$^+$	3/40 *ubiA*$^-$ (8%)
AB3285 (*ubiA351*, *metA*$^+$)	PA505MPE11 (*ubiA*$^+$, *argH*$^-$, *metA*$^-$)	*argH*$^+$	0/40 *ubiA*$^-$
		metA$^+$	10/80 *ubiA*$^-$ (13%)

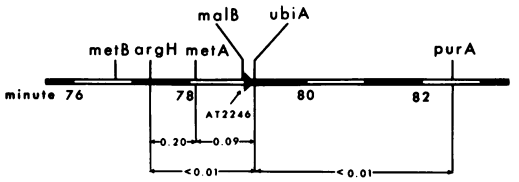

FIG. 5. *Genetic map showing the approximate position of the ubiA gene and the cotransduction frequencies between it and adjacent genes. The locations of the adjacent genes are based on the genetic map compiled by Taylor (15). The point of origin of the Hfr strain AT2246 (12) is also shown.*

diate precursor of the side chains of both quinones is likely to be an octaprenylpyrophosphate, but this has not yet been established, although the corresponding dephosphorylated compound, farnesylfarnesylgeraniol, is found in cell extracts containing the active side-chain precursor (5). The experiments with the *ubiA*$^-$, *aroB*$^-$ strains indicate that mutations in the *ubiA* gene affect 4-hydroxybenzoate octaprenyltransferase but not the formation of the side chain. The *ubiA*$^-$, *aroB*$^+$ strains formed about three times the normal levels of menaquinone and did not accumulate the octaprenol. These results suggest that in the latter strains the side-chain precursor which is normally used for ubiquinone biosynthesis was available as a source of side chain for menaquinone biosynthesis.

Two of the three *ubiA*$^-$ strains, even though they were unaffected in 4-hydroxybenzoate synthesis, gave growth responses to relatively high levels of 4-hydroxybenzoate. These levels are several orders of magnitude greater than the minimal levels of 4-hydroxybenzoate required to support the growth of a multiple aromatic auxotroph under conditions in which ubiquinone is required for growth. The *ubiA*$^-$ strains which respond to 4-hydroxybenzoate form 20% of the normal level of ubiquinone when supplied with 10^{-4} M 4-hydroxybenzoate, and presumably have altered 4-hydroxybenzoate octaprenyltransferases which have a greatly reduced affinity for 4-hydroxybenzoate. These observations suggest that the *ubiA* gene is probably the structural gene for 4-hydroxybenzoate octaprenyltransferase rather than a regulatory gene affecting ubiquinone biosynthesis. Further evidence on this point awaits the development of quantitative assays for the other enzymes concerned in ubiquinone biosynthesis.

The *ubiA* gene was previously located on the chromosome map by interrupted mating experiments at about minute 83 (1), but the present work indicates that the true location is at minute 79 according to the map of Taylor (15). Thus, six genes concerned with ubiquinone biosynthesis have now been located. Three of these (*ubiB*, *ubiD*, and *ubiE*) are closely linked and are represented at minute 75 on the map, whereas the *ubiA*, *ubiF*, and *ubiG* genes are represented at minutes 79, 16, and 42, respectively.

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