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Alternative Hydroxylases for the Aerobic and Anaerobic Biosynthesis of Ubiquinone in *Escherichia coli*†

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ABSTRACT: The synthesis of ubiquinone under anaerobic conditions was examined in a variety of strains of *Escherichia coli* K12. All were shown to synthesize appreciable quantities of ubiquinone 8 when grown anaerobically on glycerol in the presence of fumarate. Under these conditions, ubiquinone 8 was in most cases the principal quinone formed, and levels in the range 50–70% of those obtained aerobically were observed. Studies with mutants blocked in the various reactions of the aerobic pathway for ubiquinone 8 synthesis established that under anaerobic conditions three alternative hydroxylation reactions not involving molecular oxygen are used to derive the C-4, -5, and -6 oxygens of ubiquinone 8. Thus, mutants blocked in either of the three hydroxylation reactions of the aerobic pathway (*ubiB*, *ubiH*, or *ubiF*) are each able to synthesize ubiquinone 8 anaerobically, whereas mutants lacking the octaprenyltransferase (*ubiA*), carboxy-lyase (*ubiD*), or methyltransferases (*ubiE* or *ubiG*) of the aerobic pathway remain blocked anaerobically. The demonstration that *E. coli* possesses a special mechanism for the anaerobic biosynthesis of ubiquinone suggests that this quinone may play an important role in anaerobic metabolism.

The effects of aerobic or anaerobic conditions of growth on the relative levels of ubiquinone and menaquinone synthesized by members of the *Enterobacteriacea* have been examined by several groups of workers (Lester and Crane, 1959; Kashket and Brodie, 1960; Bishop et al., 1962; Polglase et al., 1966; Whistance and Threlfall, 1968; El Hachimi et al., 1974). Although the results of these studies are to some extent contradictory, most reports indicate that ubiquinone is formed in much higher levels aerobically than anaerobically, whereas the menaquinones predominate under anaerobic conditions.

We have recently reported a role for ubiquinone 8 in the anaerobic electron-transport chain to nitrate in *E. coli* K12 (Wallace and Young, 1977b). The strains used in this study formed 10–20% of the aerobic level of ubiquinone 8 when grown anaerobically with nitrate. Since molecular oxygen is utilized for the three aromatic hydroxylations in the aerobic biosynthesis of ubiquinone 8 (see Alexander and Young, 1978), the above result suggests that *E. coli* must possess special mechanisms for the synthesis of ubiquinone 8 anaerobically.

The following study shows that appreciable quantities of ubiquinone 8 (50–70% of aerobic levels) are synthesized by a wide variety of strains of *E. coli* K12 when grown anaerobically in the presence of fumarate. Using mutants blocked in the various reactions of the aerobic pathway (Young et al., 1973), it is shown that three alternative hydroxylases are utilized under anaerobic conditions.

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Experimental Section

Bacterial Strains. The strains used were all derivatives of *E. coli* K12 and are described in Table I.

Media. The mineral salts medium has been described previously (Stroobant et al., 1972). Supplements were added as sterile solutions to give the following final concentrations: glycerol, 50 mM; sodium fumarate, 50 mM; D-glucose, 30 mM; potassium bicarbonate, 20 mM; casamino acids, 0.1%; L-methionine, 0.15 mM; L-isoleucine, 0.3 mM; L-valine, 0.3 mM; L-histidine hydrochloride 0.2 mM; L-tryptophan, 0.1 mM; L-leucine, 0.3 mM; L-threonine, 0.7 mM; uracil, 0.2 mM; and thiamin hydrochloride, 3 μM.

Growth of Cells. Cells were grown either aerobically or anaerobically in 10-L volumes at 37 °C. For aerobic cultures, cells were grown in glass fermentors with aeration and stirring and were harvested in late logarithmic phase. For anaerobic cultures, cells were grown in glass bottles fitted with inlet and outlet tubes. The bottles containing the minimal medium were autoclaved, filled to the neck with sterile supplements and additional medium, allowed to cool under oxygen-free nitrogen prior to inoculation, and maintained under oxygen-free nitrogen during growth. Under these conditions, there is no growth detectable with glycerol if fumarate is omitted. Strains were purified before use by streaking for single colonies on brain–heart infusion plates. Inocula for large-scale cultures were obtained from purified strains grown aerobically on brain–heart infusion plates. Aerobic subcultures of anaerobic growths were inoculated with cells from 10 mL of the anaerobic culture. All cultures were checked for purity on brain–heart infusion, glucose, and succinate minimal medium plates to ensure that no contamination or reversion had occurred. Ubiquinone-deficient strains do not grow with succinate as the sole carbon source and give relatively small colonies on glucose or brain–heart infusion plates, under aerobic conditions (Gibson and Young, 1978). Cultures were cooled to 0–4 °C before harvesting cells.

Nitrogen Gas. Oxygen-free nitrogen gas was obtained from Commonwealth Industrial Gases and was purified of any residual traces of oxygen by bubbling through Fieser's solution (Fieser, 1924). Hydrogen/carbon dioxide (95:5) was obtained from the same source and used in the presence of a "cold" catalyst (Newton et al., 1971).

Extraction of Lipids. Harvested cells were extracted by the Soxhlet procedure (Young et al., 1973). Ten grams (wet weight) of cells was extracted with 150 mL of acetone for 4 h. The acetone extract was evaporated to dryness on a steam bath and the residue extracted with 3 × 50 mL of light petroleum (60–80 °C). In the case of strain AN63 which accumulates 3-octaprenyl-4-hydroxybenzoic acid, the residue from the acetone extract was acidified with 2 N HCl to a pH of approximately 1.5 before extraction with 3 × 50 mL of light petroleum (60–80 °C).

Thin-Layer Chromatography. Lipid extracts were chromatographed on silica gel GF_{254} plates (0.5 mm) run in chloroform/light petroleum (60–80 °C) (7:3, v/v). In the case of strain AN63, a portion of the lipid extract was run in chloroform/methanol (9:1, v/v) in order to isolate 3-octaprenyl-4-hydroxybenzoic acid. Acidified silica gel G plates containing 0.7% of concentrated sulfuric acid were used to isolate 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone from strain AN151 (Gibson and Young, 1978). Quinones were detected visibly by their yellow color or under ultraviolet light, and the phenolic compounds were located by spraying a narrow side band with diazotized *p*-nitroaniline spray (Bray et al., 1950). Silica gel F_{254} thin-layer plates (0.2 mm) were used for subsequent analytical chromatography of isolated isoprenoid compounds.

TABLE I: Strains of *E. coli* K12 Used.

strain	sex	relevant genetic loci[a]	
AN387	F⁻		*rpsL*
AN385	F⁻	*ubiA420*	*rpsL*
AN595	F⁻		*ilv, his, trp, rpsL*
IY61	F⁻	*ubiB437*	*ilv, his, trp, rpsL*
AB3311[b]	Hfr		*metB*
AN63[c]	Hfr	*ubiD410*	*metB*
AN70[c]	Hfr	*ubiE401*	*metB*
AN78[c]	Hfr	*ubiF411*	*metB*
AN151[c]	Hfr	*ubiG423*	*metB*
AN160[c]	Hfr	*ubiH429*	*metB*
AN147	F⁻		*ura*
AN146	F⁻	*ubiF411*	*ura*
AB2332	Hfr		*metB*
AB2154	Hfr		*metE, thr-1, leu-6, rpsL*

[a] Genetic nomenclature is that used by Bachmann et al. (1976). [b] Hfr Reeves 1. [c] Isolated after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment of AB3311 (Cox et al., 1969; Young et al., 1971, 1973; Stroobant et al., 1972).

Estimation of Isoprenoid Compounds. Isoprenoid products of biosynthesis were separated by thin-layer chromatography and eluted with ethanol, with the exception of 3-octaprenyl-4-hydroxybenzoic acid which was eluted with diethyl ether. Chromatography also results in the oxidation of any quinols, and all quinonoid compounds were therefore isolated in the fully oxidized form as quinones. In addition, duplicate chromatographic separations were performed routinely on portions of lipid extracts in which any quinonoid compounds had already been fully oxidized with ferric chloride. In these cases, the lipid extract (75 mL) was treated with 20 mL of an ethanolic solution of ferric chloride (5 mg of $FeCl_3.6H_2O$/mL), left to stand for 10 min at room temperature in the dark, and washed with water (3 × 25 mL) prior to chromatography (Wallace and Young, 1977b).

Isolated quinones and phenols were characterized by comparison with authentic samples by thin-layer chromatography in chloroform/light petroleum (60–80 °C) (7:3, v/v) and ethyl acetate/hexane (25:75, v/v) and by their absorption spectra in ethanol (Gibson and Young, 1978; Wallace and Young, 1977a,b; Leppik, 1973; Crane and Barr, 1971; Dunphy and Brodie, 1971). The concentrations of 3-octaprenyl-4-hydroxybenzoic acid (Σ 9380), 2-octaprenylphenol (Σ 2630) and 2-octaprenyl-6-methoxyphenol (Σ 2500) were estimated from their absorbance at 257, 274, and 274 nm, respectively (Leppik, 1973; Young et al., 1973). The concentrations of 2-octaprenyl-6-methoxy-1,4-benzoquinone ($\Delta\Sigma$ 9500), 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone ($\Delta\Sigma$ 11 300), 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinone ($\Delta\Sigma$ 4700), and ubiquinone 8 ($\Delta\Sigma$ 12 700) were determined by measuring the reduction in absorbance at 264, 270, 275, and 275 nm, respectively, after the addition of solid sodium borohydride (Wallace and Young, 1977a; Crane and Barr, 1971).

Demethylmenaquinone 8 levels were determined by measuring the increase in absorbance at 560 nm immediately after the addition of KOH to a final concentration of 0.2 M (Baum and Dolin, 1965). A molar extinction coefficient of $\Delta\Sigma$ 8300 was used, which was calculated from the increase in absorbance observed (unpublished results) at 560 nm due to the addition of KOH to known concentrations of chromatographically purified biosynthetic demethylmenaquinone 8 (Campbell

TABLE II: Isoprenoid Compounds Synthesized by Strains of *E. coli* K12 in the Presence or Absence of Atmospheric Oxygen.

growth conditions[a]	strain	product levels[b]			
		ubiquinone 8	2-octaprenylphenol	demethylmenaquinone 8	menaquinone 8
aerobic	AN387	236	<10	37	13
	AB3311	196	34	43	14
	AB2332	165	<10	30	10
anaerobic + fumarate	AN387	130	100	78	44
	AB3311	143	92	53	141
	AB2332	103	64	11	99
	AN147	177	96	20	81
	AB2154	133	109	45	79
	AN595	93	111	45	129

[a] Glycerol used as carbon source (see Experimental Section). [b] Nanomoles per gram wet weight of cells.

and Bentley, 1969) and chemically synthesized demethylmenaquinone 1 (the latter compound was kindly supplied by Dr. D. I. Magrath). To determine the concentration of menaquinone 8, the increase in absorbance at 245 nm was measured after the addition of solid sodium borohydride to a sample containing 10 mM ammonium acetate buffer (pH 5.0) (Lester et al., 1964), the increase in absorbance due to demethylmenaquinone 8 ($\Delta\Sigma$ 19 800) was subtracted, and the concentration of menaquinone 8 ($\Delta\Sigma$ 25 800) was calculated (Dunphy and Brodie, 1971).

Spectroscopy. Mass spectra were measured using an AEI MS9 double-focus mass spectrometer with a direct-insertion probe. The spectrometer was operated at 70 eV with an electron current of 100 μA, an accelerating voltage of 8 kV, and an ion source temperature of 210 °C. Ultraviolet spectra were measured on a Cary 14 spectrophotometer.

Results

Biosynthesis of Ubiquinone under Anaerobic Conditions. A preliminary study of quinone levels in a variety of strains of *E. coli* K12 showed that all synthesized appreciable quantities of ubiquinone 8 when grown anaerobically on glycerol–fumarate medium (Table II). Under these conditions, ubiquinone 8 was in many cases the principal quinone formed, and levels in the range 50–70% of those obtained under aerobic conditions were observed (Table II).

In general agreement with previous work (El Hachimi et al., 1974; Wallace and Young, 1977b), a series of control experiments indicated that ubiquinone 8 is the predominant quinone formed aerobically and total menaquinone levels were found to be low aerobically and high anaerobically (Table II).

Characterization of Anaerobically Synthesized Ubiquinone. The ubiquinone biosynthesized anaerobically was indistinguishable from authentic ubiquinone 8 (from aerobic growth) in terms of its chromatographic mobility in two different solvents and its ultraviolet (Crane and Barr, 1971) and mass spectra (Muraca et al., 1967; Morimoto et al., 1967; Alexander and Young, 1978). In the mass spectrum, the peak at *m/e* 726, assigned to the molecular ion, was found to have an accurate mass of 726.5595 ($C_{49}H_{74}O_4$ requires 726.5587).

Anaerobic Accumulation of 2-Octaprenylphenol. The lipid extracts of the *ubi*$^+$ cells grown anaerobically in the presence of fumarate were assayed (see Experimental Section) for the presence of any of the intermediates of the aerobic pathway for ubiquinone biosynthesis (Figure 1). In all the strains examined, high levels of 2-octaprenylphenol (III) were found to be accumulated under anaerobic conditions (Table II). In contrast, this compound did not accumulate in significant quantities in aerobically grown cells (Table II).

Anaerobic Pathway for Ubiquinone Biosynthesis. The synthesis of high levels of ubiquinone 8 in anaerobically grown wild-type strains of *E. coli* K12 requires an exclusively anaerobic pathway, distinct from the aerobic process that involves the incorporation of three atoms of molecular oxygen (Young et al., 1973; Alexander and Young, 1978). The nature of the intermediates and enzymes of the anaerobic pathway was examined using mutant strains of *E. coli* blocked in the various reactions of the aerobic pathway (Figure 1, Table I). These strains were grown anaerobically with fumarate, and their isoprenoid products were isolated and characterized (see Experimental Section).

Strains AN385 (*ubiA*), AN63 (*ubiD*), and AN70 (*ubiE*) remained deficient in ubiquinone 8 (X) when grown in the absence of air (Table III). The *ubiA* mutant is blocked during aerobic growth in the prenylation of 4-hydroxybenzoic acid (I) (Young et al., 1972). No ubiquinone intermediates were detected in cells of the *ubiA* strain grown anaerobically (Table III). The *ubiD* and *ubiE* mutants lack the carboxy-lyase and 2-octaprenyl-6-methoxy-1,4-benzoquinol methyltransferase enzymes, respectively, of the aerobic pathway (Cox et al., 1969; Young et al., 1971). The accumulation of 3-octaprenyl-4-hydroxybenzoic acid (II) by the *ubiD* strain and of 2-octaprenyl-6-methoxy-1,4-benzoquinol (VI) by the *ubiE* strain was observed in anaerobically grown cells (Table III). From these observations it follows that the octaprenyltransferase, carboxy-lyase, and 2-octaprenyl-6-methoxy-1,4-benzoquinol methyltransferase enzymes are common to both pathways and that 4-hydroxybenzoic acid (I), 3-octaprenyl-4-hydroxybenzoic acid (II), and 2-octaprenyl-6-methoxy-1,4-benzoquinol (VI) are also involved as intermediates in the anaerobic biosynthesis of ubiquinone 8 (X). Accordingly, the *ubiA* and *ubiD* mutants did not synthesize any 2-octaprenylphenol (III) anaerobically (Table III). This is in contrast to the *ubiE*, *ubiF*, *ubiG*, and *ubiH* strains which accumulated 2-octaprenylphenol (III) at levels comparable with those found in the isogenic wild-type strain AB3311 (Table III), implicating 2-octaprenylphenol (III) as an intermediate in the anaerobic biosynthesis.

The *ubiG* mutant studied, strain AN151, aerobically accumulates 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol (VIII), being affected in the corresponding *O*-methyltransferase (Table III) (Stroobant et al., 1972). This mutant is partially leaky and also forms about 10% of the wild-type level of ubiquinone 8 (X) that is found aerobically in the isogenic *ubi*$^+$ strain AB3311 (Table III). Essentially analogous results were obtained with strain AN151 in the absence of molecular oxygen (Table III), indicating that both 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol

(VIII) and its *O*-methyltransferase are involved in the anaerobic biosynthesis of ubiquinone 8 (X).

The *ubiB, ubiH,* and *ubiF* genes code for the three monooxygenase enzymes that catalyze the hydroxylation reactions involved in the aerobic biosynthesis of ubiquinone 8 (Cox et al., 1969; Young et al., 1971, 1973; Alexander and Young, 1978). When *ubiB, ubiH,* and *ubiF* mutants were grown anaerobically they were in each case able to synthesize appreciable quantities of ubiquinone 8 (Table III), providing clear evidence that alternative hydroxylases are involved in the anaerobic pathway. Strains AN160 (*ubiH*) and AN78 (*ubiF*) formed levels of ubiquinone 8 comparable to that found anaerobically in the isogenic *ubi*$^+$ strain AB3311 (Table III). In the case of the *ubiB* mutant (strain IY61), the level of ubiquinone 8 formed was about 30% of that found in the corresponding *ubi*$^+$ strain AN595 (Table III).

The significance of the anaerobic synthesis of ubiquinone 8 by the *ubiB, ubiH,* and *ubiF* mutants was confirmed by the completely tight blocks that were reimposed on the corresponding hydroxylation steps when these strains were grown aerobically with inocula subcultured from the anaerobic experiments (Table III). Under these conditions, no ubiquinone 8 (X) was detected, and the *ubiB, ubiH,* and *ubiF* mutants accumulated 2-octaprenylphenol (III), 2-octaprenyl-6-methoxyphenol (V), and 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol (VII), respectively. The additional accumulation of 2-octaprenylphenol (III) by strain AN160 is a characteristic feature of this mutation under aerobic conditions (Young et al., 1973).

The anaerobic synthesis of high levels of ubiquinone 8 by the *ubiF* mutant was observed in strains of different genetic background (AN78 and AN146) and under alternative conditions of anaerobic growth, i.e., with the glycerol–fumarate system or with glucose as sole carbon source (Table III).

Discussion

The above results show that high levels of ubiquinone 8 are synthesized anaerobically by *E. coli* K12 strains in the presence of fumarate. Studies on the anaerobic pathway indicate that mutants blocked in either of the three hydroxylation reactions of the aerobic pathway (*ubiB, ubiH,* or *ubiF*) are able to synthesize ubiquinone 8 anaerobically, whereas mutants lacking the octaprenyltransferase (*ubiA*), carboxy-lyase (*ubiD*), or methyltransferases (*ubiE* or *ubiG*) of the aerobic pathway remain blocked anaerobically (Figure 1). In addition, it was established that 4-hydroxybenzoate (I), 3-octaprenyl-4-hydroxybenzoate (II), 2-octaprenylphenol (III), 2-octaprenyl-6-methoxy-1,4-benzoquinol (VI), and 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol (VIII) are obligatory intermediates of the anaerobic pathway (Figure 1).

The *ubiB, ubiH,* and *ubiF* mutants are tightly blocked in the aerobic hydroxylations, and the anaerobic synthesis of ubiquinone 8 by these mutants is in each case significant, with wild-type levels being formed by strains AN160 (*ubiH*) and AN78 (*ubiF*). The relatively low yield of ubiquinone 8 (X) and the particularly high concentration of 2-octaprenylphenol (III) found in anaerobically grown cells of strain IY61 (*ubiB*) imply that this strain is not only defective in the 2-octaprenylphenol monooxygenase but may also be affected in a subsequent enzyme of the pathway, such as the closely associated 2-octaprenyl-6-hydroxyphenol *O*-methyltransferase. 2-Octaprenylphenol (III) rather than 2-octaprenyl-6-hydroxyphenol (IV) would be expected to accumulate, since the latter compound (IV) does not appear to occur as a free intermediate in *E. coli*

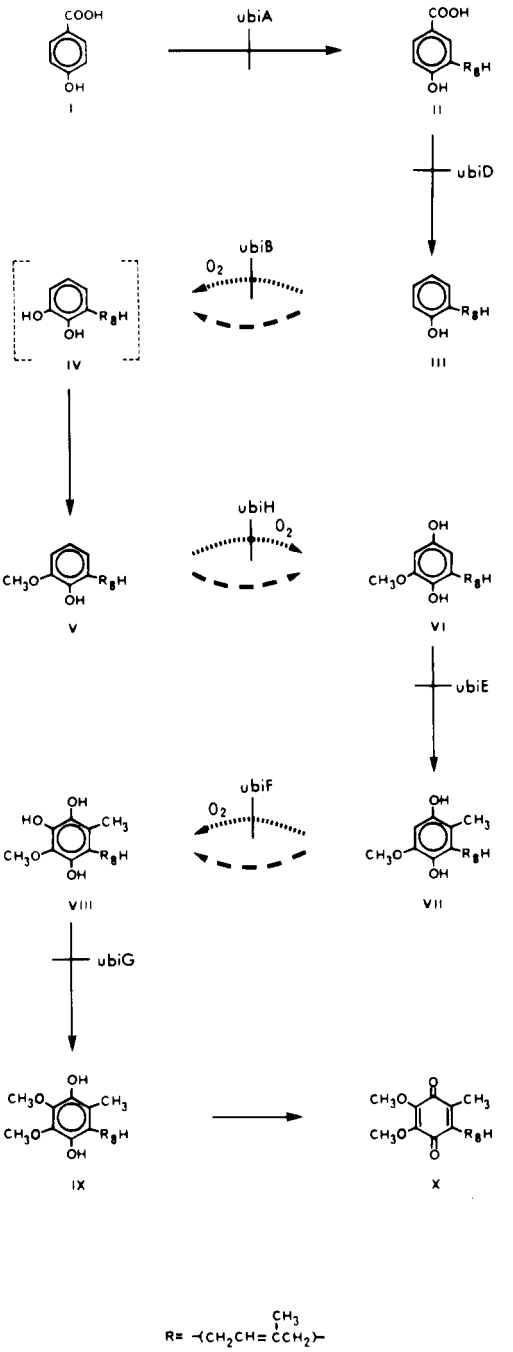

FIGURE 1: The pathway for ubiquinone biosynthesis in *E. coli*, showing the duplication of the aerobic hydroxylation steps under anaerobic conditions. The reactions affected by mutations in the various *ubi* genes are indicated. Reactions catalyzed by the same enzyme aerobically or anaerobically are shown with solid arrows. Steps involving alternative enzymes in the presence or absence of molecular oxygen are illustrated with dotted arrows: I, 4-hydroxybenzoic acid; II, 3-octaprenyl-4-hydroxybenzoic acid; III, 2-octaprenylphenol; IV, 2-octaprenyl-6-hydroxyphenol (hypothetical intermediate) (Young et al., 1973); V, 2-octaprenyl-6-methoxyphenol; VI, 2-octaprenyl-6-methoxy-1,4-benzoquinol; VII, 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol; VIII, 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol; IX, ubiquinol 8; X, ubiquinone 8.

(Young et al., 1973), despite indirect evidence for its involvement in the reaction sequence (Young et al., 1973; Alexander and Young, 1978; Goewert et al., 1977).

It is concluded that under anaerobic conditions three alternative hydroxylation reactions not involving molecular oxygen are used to derive the C-4, -5, and -6 oxygens of ubi-

TABLE III: Isoprenoid Compounds Accumulated by *E. coli* Mutants Blocked in the Aerobic Pathway for Ubiquinone Biosynthesis.

	growth conditions[a] and products[b]					
	anaerobic[d] + fumarate			aerobic		
strain[c]	ubiquinone 8	2-octaprenylphenol	others[e]	ubiquinone 8	2-octaprenylphenol	others[e]
AN385 (*ubiA420*)	nd	nd	nd			
AN387 (*ubi*$^+$)	130	100	nd			
IY61 (*ubiB437*)	27	253	(MMQ-8) 12[f]	nd	453	nd
AN595 (*ubi*$^+$)	93	111	nd			
AN63 (*ubiD410*)	nd	nd	(HB-8) 301			
AN70 (*ubiE401*)	nd	141	(MQ-8) 63			
AN78 (*ubiF411*)	167	102	nd	nd	nd	(MMQ-8) 358
AN151 (*ubiG423*)	13	75	(MMHQ-8) 89	20	42	(MMHQ-8) 140
AN160 (*ubiH429*)	114	102	nd	nd	257[h]	(MP-8)[h]
AB3311 (*ubi*$^+$)	143	92	nd	196	34	nd
AN146 (*ubiF411*)	108	132	nd	nd	nd	(MMQ-8) 400
AN147 (*ubi*$^+$)	177	96	nd			
AN78 (*ubiF411*)	58[g]	87[g]	nd[g]			

[a] Glycerol as carbon source. [b] Nanomoles per gram wet weight of cells, menaquinone levels not included; nd, not detectable: <2 for quinones and <10 for octaprenylphenols. [c] Arranged in isogenic groups (see Table I). [d] Nitrogen gas phase. [e] MMQ-8, 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol (VII); HB-8, 3-octaprenyl-4-hydroxybenzoic acid (II); MQ-8, 2-octaprenyl-6-methoxy-1,4-benzoquinol (VI); MMHQ-8 2-octaprenyl-3-methyl-5-hydroxy-6-methoxy-1,4-benzoquinol (VIII); MP-8 2-octaprenyl-6-methoxyphenol (V). [f] The mass spectrum of this compound was consistent with it being MMQ-8 or 6-demethoxyubiquinone 8; it was not further characterized. [g] Levels obtained for anaerobic growth under hydrogen/carbon dioxide (95:5), with glucose as carbon source and no added fumarate. [h] MP-8 was detectable with diazotized *p*-nitraniline spray (Bray et al., 1950); the amount present was below the limits of sensitivity of the spectrophotometric assay (<10) (see Experimental Section). *UbiH* mutants are blocked in the aerobic hydroxylation of MP-8 (V) and characteristically accumulate low levels of MP-8 (V) and high levels of 2-octaprenylphenol (III) under aerobic conditions (Young et al., 1973).

quinone 8. These steps are presumably catalyzed by novel anaerobic hydroxylation enzymes that duplicate the monoxygenases of the aerobic pathway. The reaction mechanisms of the anaerobic hydroxylases are at present unknown but may be similar to the mechanisms proposed for the anaerobic hydroxylations of nicotine (Hochstein and Rittenberg, 1959; Hochstein and Dalton, 1965), nicotinic acid (Hunt et al., 1958; Hirschberg and Ensign, 1971), picolinic acid (Dagley and Johnson, 1963; Tate and Ensign, 1970), 2-furoylcoenzyme A (Kitcher et al., 1972) and *p*-cresol (Hopper, 1976; Hopper and Taylor, 1977). The remaining features of the anaerobic pathway are identical to those found aerobically: i.e., biosynthetic intermediates, reaction sequence, octaprenyltransferase, carboxy-lyase, and methyltransferase enzymes. The results complement ^{18}O-labeling studies which have established the incorporation of molecular oxygen in each of the hydroxylation steps during aerobic growth (Alexander and Young, 1978). In view of the accumulation of 2-octaprenylphenol (III) consistently found in cells grown in the absence of atmospheric oxygen, it would appear that the first hydroxylation reaction of the anaerobic pathway is a rate-limiting step. It seems likely that other facultative anaerobes which synthesize ubiquinone anaerobically in the presence of fumarate (Höllander and Mannheim, 1975; Kröger, 1977) will use a similar mechanism.

The demonstration of a separate mechanism for the anaerobic synthesis of ubiquinone 8 in *E. coli* suggests that ubiquinone 8 may play an important role in anaerobic metabolism. Most anaerobes and anaerobically grown facultative anaerobes are reported to use menaquinones as electron-transport carriers (Thauer et al., 1977), although a number of studies indicate that ubiquinone 8 is also present in several anaerobically grown bacteria (Thauer et al., 1977; Kröger, 1977), including *Proteus rettgeri* (Kröger et al., 1971), *Rhodospirillum rubrum* (Parson and Rudney, 1965), and some species of *Haemophilus* (Höllander and Mannheim, 1975). It has been shown in *E. coli* (Wallace and Young, 1977b) and *Proteus rettgeri* (Kröger et al., 1971) that both ubiquinone and menaquinone may participate in anaerobic electron transport to nitrate, presumably according to the quinone preferences of the respiratory dehydrogenases. In the present study, *E. coli* grown anaerobically in the presence of fumarate formed much higher levels of ubiquinone 8 than have been reported (Wallace and Young, 1977b) for cells grown anaerobically with nitrate as electron acceptor, even though the anaerobic oxidation of glycerol 3-phosphate is apparently linked to fumarate reduction by menaquinone (Lambden and Guest, 1976; Guest, 1977; Kröger, 1977). In addition to their involvement in the electron-transport pathways to nitrate and fumarate, quinones have also been implicated in the anaerobic oxidation of protoporphyrinogen (Jacobs and Jacobs, 1977) and in anaerobic flagella formation (Hertz and Bar-Tana, 1977) in *E. coli*. The extent of the involvement of ubiquinone in anaerobic quinone function remains to be elucidated.

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