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Function of Ubiquinone in *Escherichia coli*: a Mutant Strain Forming a Low Level of Ubiquinone

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A ubiquinone-deficient mutant of *Escherichia coli* K-12 forming 20% of the normal amount of ubiquinone was compared with a normal strain. This lowered concentration of ubiquinone is still four times the concentration of cytochrome b_1. The mutant strain grew more slowly than the normal strain on a minimal medium with glucose as sole source of carbon and gave a lower aerobic growth yield than the normal strain. The reduced nicotinamide adenine dinucleotide (NADH) oxidase rate in membranes from the mutant strain was 40% of the oxidase rate in membranes from the normal strain, and the percentage reduction of cytochrome b_1 in the aerobic steady state, with NADH as substrate, was increased in membranes from the mutant strain. It is concluded that ubiquinone is required for maximum oxidase activity at the relatively high concentration (27 times that of cytochrome b_1) found in normal cells. The results are discussed in relation to a scheme previously advanced for ubiquinone function in *E. coli*.

In membrane systems containing ubiquinone, the quinone is present at a greater molar concentration than other electron transport components such as flavines and cytochromes. The mitochondria from animal cells contain ubiquinone at a concentration six- to eightfold in excess of the other components, whereas in yeast and bacteria it can be up to 36 times in excess (8). If all components were tightly bound and not capable of diffusion and if ubiquinone functioned at one site as a carrier in sequence with the cytochromes, it would be expected that a decrease in the amount of ubiquinone present would not have a significant effect on the rate of electron transport until the concentration fell below those of the other carriers. For example, in membrane preparations of *Haemophilus parainfluenzae* which contains a large molar excess of cytochrome *c* over the other cytochromes, the oxidase activity does not decrease until a large amount of the cytochrome *c* is removed by washing (12). However, Ernster et al. (5), using pentane-extracted lyophilized beef heart mitochondria, showed that the percentage loss of ubiquinone paralleled the degree of inactivation of both the reduced nicotinamide adenine dinucleotide (NADH) and succinate oxidases, even though there was still more ubiquinone than cytochromes present on a molar basis. This finding was said to indicate that ubiquinone operates according to "saturation kinetics," acting as a "hydrogen-collecting pool" (8).

The solvent extraction technique of depleting membranes of ubiquinone is not ideal in that it is relatively nonspecific and may not only damage the membranes but is also known to extract other materials important for oxidase activity (1). A more specific method of ubiquinone depletion is the use of ubiquinone-deficient mutants, and a strain of *Escherichia coli* completely lacking ubiquinone has been used in a study of the function of this quinone (3). The present report presents the results of a study of the effects of the partial depletion of ubiquinone on the growth and metabolism of *E. coli*, in which a strain forming about 20% of the normal level of ubiquinone was used.

MATERIALS AND METHODS

Chemicals. Piericidin A was kindly provided by S. Tamura, Department of Agricultural Chemistry, University of Tokyo, Japan. Ubiquinone (Q-3) was kindly provided by O. Isler of F. Hoffman-La Roche

& Co., Basle, Switzerland. Chemicals generally were of the highest purity available commercially and were not purified further.

Organisms. The strains of *E. coli* K-12 used were AB2154, normal with respect to ubiquinone content, and a transductant derived from AB2154, strain AN66, which carries a mutation in the *ubiD* gene resulting in a low level of ubiquinone (4). The genotypes of these strains are: strain AB2154, MetE$^-$, Leu$^-$, Thr$^-$; and strain AN66, Leu$^-$, Thr$^-$, Ubi D$^-$.

Media and growth of organisms. The minimal medium used was double-strength medium 56 (10). To the sterilized mineral salts base were added L-leucine, L-threonine, and L-methionine each at a final concentration of 0.2 mM and thiamine at a final concentration of 0.02 μM. Glucose was added as a sterile solution either in excess at a final concentration of 30 mM or at limiting concentrations as indicated.

Growth yield determinations. Aerobic growth yields were determined as described previously (3). For the determination of anaerobic growth yields, cells were grown in a volume of 5 ml in 7.5-ml Pyrex screw-cap rubber-sealed test tubes. After inoculation of each tube with 0.1 ml of a suspension of cells (about 10^6 cells/ml) from a nutrient agar slope, the caps were loosely fitted on the tubes which were then placed in an anaerobic culture jar containing a platinum catalyst. The jar was partially evacuated, flushed with hydrogen, sealed, and then incubated overnight at 37 C. The jar was then opened, and the screw caps were tightened. The turbidity was measured with a Klett-Summerson colorimeter, and incubation at 37 C was continued until growth was complete. A reading of 200 Klett units is equivalent to about 0.4 mg dry weight of cells per ml of culture.

Determinations of glucose and lactic acid. Glucose concentrations were determined as described by Slein (11), and lactate concentrations were determined as described by Barker (2).

Preparation and fractionation of cell extracts. Cell extracts were prepared by using a Sorvall Ribi cell fractionator, and membrane preparations were obtained by $(NH_4)_2SO_4$ fractionation as described previously (3).

Protein determinations. Proteins were determined by using the Folin phenol reagent (9) with bovine serum albumin as a standard.

Measurement of oxygen uptake. Oxygen uptake at 25 C was measured polarographically as described previously (3).

Determination of ubiquinone. The ubiquinone contents of cells were determined by continuously extracting 2 to 7 g wet weight of cells with acetone for 3 hr. The acetone was then removed by evaporation, and the residue was extracted with a small volume of light petroleum (bp 60 to 80 C). The light petroleum extract was then chromatographed on silica gel thin-layer plates in chloroform-light petroleum (7:3, v/v). The yellow ubiquinone band was eluted into diethyl ether, and the concentration of ubiquinone was determined from the absorbance at 272 nm (ϵ = 14,950).

Difference spectra. Difference spectra were recorded in an Aminco-Chance dual wavelength spectrophotometer as described previously (3).

Degree of ubiquinone reduction. The determination of the degree of reduction of ubiquinone in the aerobic steady state was made by the rapid solvent extraction technique of Hoffman et al. (7) as described previously (3).

Determination of steady-state oxidation-reduction levels. The kinetics of reduction of cytochrome b_1 in the membrane fractions were determined with an Aminco-Chance dual wavelength spectrophotometer, by using the wavelength pair used previously (3).

RESULTS

Concentrations of membrane components. The ubiquinone concentration in strain AN66 was 19% of that of the normal strain, whereas there was little variation between the two strains in the concentrations of the other membrane components (Table 1). The concentration of ubiquinone in strain AN66 is still four times that of cytochrome b_1. The concentrations of the respiratory components in the normal strain AB2154 are higher than those reported previously for a related strain, AN62 (3). This is probably due to the different concentrations of minimal medium 56 used in the present experiments.

Growth curves and growth yields. When the bacteria were growing on a medium with glucose as sole source of carbon, the mean generation time of strain AN66 was 2.5 hr, compared with 1.25 hr for the normal strain AB2154 growing under the same conditions.

TABLE 1. *Concentrations of some membrane components in strains AB2154 and AN66*[a]

Component	Concn of component (nmoles/mg of protein) in	
	Strain AB2154 (Ubi D$^+$)	Strain AN66 (Ubi D$^-$)
Total flavine	0.67	0.75
Cytochrome b_1	0.32	0.42
Cytochrome a_2	0.08	0.09
Cytochrome o	0.23	0.22
Cytochrome a_1[b]	+	+
Ubiquinone (Q-8)	8.6	1.6

[a] Flavines and cytochromes were determined by direct spectrophotometric examination of suspensions of the membranes. Ubiquinone was first extracted and partially purified before spectrophotometric determination. Details of methods are given in the text.

[b] Cytochrome a_1 was present but the quantities were too low for determination.

When growth yields for the normal and ubiquinone-deficient strains were determined, diauxic growth curves were obtained with the latter strain (Fig. 1). The second phase of growth with strain AN66 was apparently due to the metabolism of lactate accumulated (Fig. 1) during the comparatively inefficient aerobic growth on glucose.

The growth yields obtained for strains AN66 and AB2154 aerobically or anaerobically with various concentrations of limiting glucose are shown in Fig. 2. Values for growth yields for strain AN66 were determined by taking the optical density at the point of inflection of the diauxic curves. There was no difference between the two strains in their anaerobic growth yields; aerobically, however, strain AN66 grew much less efficiently than strain AB2154. A meaningful comparison of the aerobic growth yields of strain AN66 and the normal strain is not feasible except at the lower glucose concentrations because the growth of strain AB2154 becomes less efficient with increasing turbidity under the conditions of aeration used.

NADH oxidase activities. The NADH oxidase activity of membranes from strain AN66 was 40% of the NADH oxidase activity of membranes from strain AB2154 (Table 2). However, the NADH oxidase activities of membrane preparations from the mutant and normal strains were similar in the presence of added ubiquinone (Q-3). As expected, piericidin A inhibited the NADH oxidase activity of membranes from both strains (Table 2).

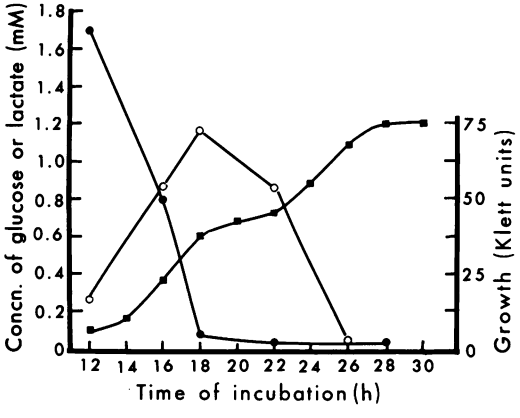

FIG. 1. *Aerobic metabolism of glucose by strain AN66 (Ubi D⁻). Symbols: (■) growth; (●) glucose concentration; (○) lactate concentration. Times were measured from the start of observable growth. Conditions for aerobic growth were as indicated in the text.*

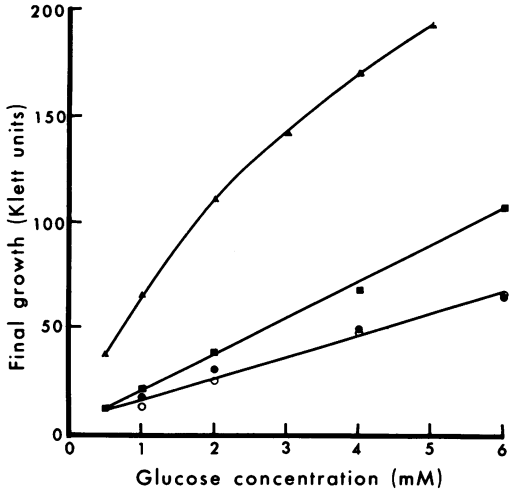

FIG. 2. *Growth yields (turbidity) of strains AB2154 (Ubi D⁺) and AN66 (Ubi D⁻) grown on limiting concentrations of glucose under various conditions. Cultures were aerated by shaking, and anaerobic cultures were incubated as described in the text. Symbols: (▲) strain AB2154, aerobic; (■) strain AN66, aerobic; (●) strain AB2154, anaerobic; (○) strain AN66, anaerobic.*

TABLE 2. *NADH oxidase activities in membranes from strain AB2154 and AN66*[a]

Addition and final concn	O_2 uptake (ng-atoms per min per mg of protein) by membranes from	
	Strain AB2154 (Ubi D⁺)	Strain AN66 (Ubi D⁻)
None	865	344
Ubiquinone (Q-3) (48 μM)	1,000	1,085
Piericidin A (48 μM)	69	21

[a] Rates of oxygen uptake with NADH as substrate were measured by using an oxygen electrode in a final volume of 2.5 ml at 25 C as described by Cox et al. (3). All values for oxidase activities given by Cox et al. (3) should be multiplied by 2.5 to correct for an arithmetical error.

Percentage reduction of cytochrome b_1 in the aerobic steady state. The percentage reduction in the aerobic steady state of cytochrome b_1 with NADH as substrate was increased in strain AN66 as compared with strain AB2154 (Table 3). The degree of increase was less than that obtained for strain AN59, a strain completely lacking ubiquinone. The effects of the addition of ubiquinone (Q-3) and the inhibitor piericidin A were similar to those obtained previously (3).

TABLE 3. *Percentage of cytochrome b_1 reduced in the aerobic steady state in membranes from strains AB2154 and AN66*[a]

Membrane from	Addition and final concn	Steady state percentage reduction of cytochrome b_1
AB2154 (Ubi D$^+$)		13
AB2154 (Ubi D$^+$)	Piericidin A (67 μM)	22
AN66 (Ubi D$^-$)		18
AN66 (Ubi D$^-$)	Piericidin A (67 μM)	21
AN66 (Ubi D$^-$)	Ubiquinone (Q-3) (40 μM)	13

[a] Steady-state oxidation-reduction levels were determined with NADH as substrate by using a dual-wavelength spectrophotometer as described in the text.

The percentage reduction of ubiquinone in the absence of added substrate, determined by the rapid solvent extraction technique, could be determined for the normal strain AB2154 only and agreed with the value (50%) reported earlier for the related strain AN62 (3). In strain AN66, where the level of ubiquinone was low, there was too much absorption in the 270 nm region of the ultraviolet spectrum by both menaquinone and the accumulated ubiquinone intermediate, 3-octaprenyl-4-hydroxybenzoate, to allow accurate determination of the percentage of ubiquinone reduced. The radical attributed to ubisemiquinone (6) was, however, observed in membrane preparations from strain AN66 (J. Hamilton, *personal communication*).

DISCUSSION

The above results indicate that, for efficient aerobic electron transport, ubiquinone is required in considerable molar excess over other components of the electron transport system. In strain AN66 the concentration of ubiquinone was only 19% of that of the normal strain, but this concentration still represented a fourfold molar excess over the other components. The comparison of the aerobic metabolism of the normal and mutant strains showed that for the mutant the mean generation time was twice normal, the aerobic growth yield was about 25% normal, and the NADH oxidase rate was 40% of that obtained for the normal strain. Anaerobic growth appeared to be unaffected.

This type of data is difficult to interpret on a quantitative basis. Growth rates on glucose as carbon source are not necessarily a measure of efficiency of growth. Aerobic growth yields provide a better estimate of efficiency of growth but, in a mutant strain with partially impaired respiration, it is difficult to determine the contribution from anaerobic energy-yielding reactions.

A comparison of the NADH oxidase rates of normal and mutant strains would appear to provide the best basis for assessing the effect of the partial depletion of ubiquinone. A correction still has to be applied for the NADH oxidase activity present in membrane preparations from cells completely lacking ubiquinone (3). Such a correction reduces the apparent residual NADH oxidase activity in membranes of the mutant strain AN66 from 40 to 35% of that in membranes from the normal strain. It is apparent, therefore, that for the maximum NADH oxidase rate ubiquinone is required at a molar concentration considerably greater than those of the other measured components of the respiratory chain. This conclusion is in agreement with that of Ernster et al. (5) who, by using a lyophilization and pentane-extraction technique, showed a close correlation between the percentage of ubiquinone extracted from mitochondria and the percentage loss of NADH oxidase activity.

In a scheme for ubiquinone function in *E. coli*, it has been proposed (3) that the quinone is involved at two sites in the electron transport system. It has been further proposed that ubiquinone is complexed to an electron carrier, possibly nonheme iron. Four molecules of ubiquinone at most, per molecule of cytochrome b_1, can be accounted for by this scheme. The limiting ubiquinone concentration is much greater than four times the concentration of cytochrome b_1, indicating that "saturation kinetics" must be operating, with the further implication that ubiquinone is readily diffusible, if only within limited areas of the membrane. In this regard, a comparison of the aerobic steady states of cytochrome b_1 shown in membranes from the normal strain, the partially ubiquinone-deficient mutant, and the completely ubiquinone-deficient mutant (3) indicates that both proposed sites of ubiquinone function are equally affected by the partial depletion of the quinone. The requirement for relatively high concentration of ubiquinone and its apparent mobility may mean that the formation of the proposed nonheme iron-ubiquinone complex follows saturation kinetics with respect to ubiquinone concentration. Fur-

ther, ubiquinone may be involved, as a mobile substrate in one form or another, in an additional step(s) between the electron transport chain and adenosine triphosphate formation.

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