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Role of Coenzyme Q in the Mitochondrial Respiratory Chain. Reconstitution of Activity in Coenzyme Q Deficient Mutants of Yeast†

Gregory G. Brown‡ and Diana S. Beattie*

ABSTRACT: The reduction of cytochrome *c* by the reduced form of the 6-decyl analogue of coenzyme Q follows first-order kinetics with respect to cytochrome *c* and increases in a linear manner with added mitochondrial protein. The activity is completely sensitive to antimycin A in whole cell extracts of yeast as well as in isolated mitochondria and fractionates with markers for the mitochondrial electron-transport chain. The presence of both cytochrome *b* and c_1 in an approximately 2:1 ratio appears essential for enzymatic activity. Reduced coenzyme Q:cytochrome *c* reductase obeys Michaelis–Menten kinetics when assayed in mitochondria obtained from a yeast strain lacking coenzyme Q. Both reduced nicotinamide adenine dinucleotide and succinate:cytochrome *c* reductase activities were not detectable in six coenzyme Q deficient strains tested, but were restored after addition of the oxidized form of the coenzyme Q analogue. No marked difference in the concentration of the analogue required to restore the two activities was observed.

The mitochondrial electron-transfer chain is considered to be composed of discrete lipid–protein enzyme complexes, each of which catalyzes a distinct part of the overall oxidation reaction (Hatefi et al., 1962, 1975). This concept has been useful in the study of mitochondrial energetics, as it has allowed the identification and purification of many proteins directly involved in electron transfer. The role of coenzyme Q in this scheme is to act as a mobile electron carrier between the primary flavoprotein dehydrogenase complexes (complexes I and II) and the cytochrome *b*–cytochrome c_1 complex (complex III).

While it has long been accepted that coenzyme Q acts as a mediator of electron transfer between the NADH[1] dehydrogenase complex and complex III (Rieske, 1976), the role of coenzyme Q in the transfer of electrons from the succinate dehydrogenase complex has been the subject of some disagreement (Albracht et al., 1971). Furthermore, a functional heterogeneity and compartmentation of coenzyme Q within the mitochondria has been suggested to explain the results obtained in reconstitution experiments with coenzyme Q depleted mitochondria and submitochondrial particles (Lenaz et al., 1968; Gutman et al., 1971a). For example, maximum succinate oxidase activity can be achieved at lower concentrations of coenzyme Q than can NADH oxidase activity. In addition, the two oxidase activities differ with respect to the stimulatory and inhibitory effects of analogues and homologues of coenzyme Q (Lenaz et al., 1968, 1975; Jeng et al., 1968; Castelli et al., 1971). Kröger and Klingenberg (1973), however, have maintained that no such compartmentation exists because the coenzyme Q pool appears kinetically homogeneous.

The recent development by Folker's group of stable, low-molecular-weight analogues of coenzyme Q has provided a new tool to probe coenzyme Q function (Wan et al., 1975). We have previously used one of these analogues, 2,3-dimethoxy-5-methyl-6-*n*-decyl-1,4-benzoquinone (DB) in which a straight chain aliphatic group of ten carbons is substituted for the isoprenoid side chain, to assay complex III formation in yeast undergoing respiratory adaptation (Brown and Beattie, submitted for publication). In the present study, a detailed investigation of the catalytic activity of complex III using DB as substrate was undertaken. The analogue was also used to reconstitute NADH and succinate:cytochrome *c* reductase activities in a series of coenzyme Q deficient mutants of yeast. The results obtained indicate that reduced DB can be used to monitor accurately the catalytic activity of the cytochrome *b*–cytochrome c_1 complex. The antimycin A sensitive reduction of cytochrome *c* by both NADH and succinate requires coenzyme Q with a similar concentration dependence. No evidence for the "functional heterogeneity" of coenzyme Q has been obtained.

Materials and Methods

Strains of Yeast: Saccharomyces cerevisiae. D273-10B, prototrophic (α) and coenzyme Q deficient mutants derived

† From the Department of Biochemistry, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029. *Received March 21, 1977.* This work was supported, in part, by Grants HD-04007 from the National Institutes of Health and PCM 76-21599 from the National Science Foundation.

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[1] Abbreviations used are: DB, 2,3-dimethoxy-5-methyl-6-*n*-decyl-1,4-benzoquinone; NADH, reduced nicotinamide adenine dinucleotide; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

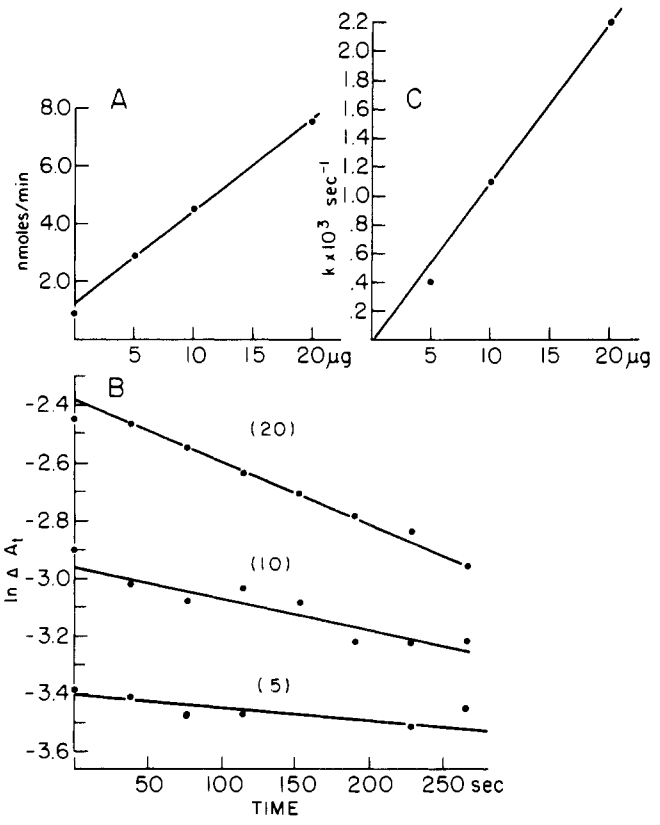

FIGURE 1: Kinetics of the reduction of cytochrome *c* by DBH_2. (A) Initial rates of cytochrome *c* reduction obtained with increasing amounts of added mitochondrial protein. (B) Analysis of the absorbance vs. time curves used to obtain the data in A by the Guggenheim method. The amounts of added mitochondrial protein in micrograms are indicated by parentheses: Δt = 38 s. (C) First-order rate constants, determined from slopes of the lines in B, vs. added mitochondrial protein. Mitochondria from strain D273-10B were used.

from it (Tzagoloff et al., 1975) were obtained from Dr. A. Tzagoloff; 585-11C($\rho_1{}^0$a, Lys) was obtained from Dr. R. Needleman, and was used as a petite tester. For purification studies, commercial (Budweiser) yeast was used.

Cell Growth, Breakage, and Fractionation. Cells were grown routinely at 30 °C in semisynthetic medium (Schatz and Kovac, 1974), with galactose as carbon source. Cells were aerated by vigorous shaking (200 rpm) on a New Brunswick shaker. After stationary phase was reached, cells were harvested by centrifugation for 5 min at 5000*g*, and washed once with distilled water. Cells were broken with glass beads, and cell-free extracts and mitochondria were prepared as described previously (Kim et al., 1973). In purification studies, 8 lb of compressed commercial yeast was frozen in liquid nitrogen and broken with a Waring blender, and purification steps were performed as described by Lin and Beattie (1976).

Estimation of Reversion and Petite Production. The percentage of revertants in a culture of respiratory-deficient strains was determined by plating approximately 200 cells on solid YPD medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar), incubating the plates at 30 °C for 2 days, and then replating them onto plates containing solid YPGE medium (1% yeast extract, 2% peptone, 3% ethanol, 3% glycerol, 2% agar). Colonies capable of growth on YPGE were scored as respiratory competent revertants. The percentage of ρ^- cells in the population was estimated from the number of small colonies after growth for 2 days on YPD (Tzagoloff et al., 1975). The technique was checked by crossing cells from both large and small colonies with a petite tester (ρ^0) strain of opposite mating types. In general, only cells from the larger colonies gave rise to diploids capable of growth on YPGE.

Enzymatic Assays. Cytochrome *c* reductase assays were performed in a 1-mL cuvette of 1-cm path length containing 600 μg of cytochrome *c*, 2 mM EDTA, 5 mM sodium azide, 1 mg of bovine serum albumin, and 25 mM sodium phosphate buffer, pH 7.6. Sodium succinate and NADH were added to give final concentrations of 10 or 0.5 mM, respectively. Antimycin A was added to give a final concentration of 5 μg/mL, except where indicated. DBH_2 was prepared by reduction of DB by the method of Rieske (1976). It was added as a 5 mg/mL solution to give a final concentration of 25 μg/mL. Reduced DB and antimycin A were added as solutions in ethanol. In studies of the reconstitution of NADH and succinate:cytochrome *c* reductase in coenzyme Q deficient mutants, DB in the oxidized form was added as a 1 mg/mL solution in ethanol.

The rate of cytochrome *c* reduction was monitored by following the increase in absorption at 550 nm. An extinction coefficient of 18.5 μM^{-1} cm^{-1} was used to calculate the rate in terms of nmol of cytochrome *c* reduced per minute.

Cytochrome oxidase was determined as described previously (Wharton and Tzagoloff, 1967). Difference spectra of various preparations were obtained by scanning dithionite reduced vs. ferricyanide oxidized samples from 500 to 630 nm in a Cary Model 15 double-beam spectrophotometer. The specific cytochrome content of the preparations was calculated from their spectrum by a variation of the method of Claisse and Pajot (1975), as described previously (Brown and Beattie, 1977, submitted for publication). Difference spectra in the ubiquinone region (240–320 nm) were obtained by the method of Kröger and Klingenberg (1966).

Chemicals. Yeast extract and "bacto" peptone were obtained from Difco. Mannitol, glycerol, dextrose, $FeCl_3$, $CaCl_2$, and sodium azide were purchased from Fisher. Galactose (purified), bovine serum albumin, NADH, sodium deoxycholate, sodium cholate, magnesium sulfate, cytochrome *c* (horse heart type VI), sodium succinate, and "Tris" were from Sigma. Enzyme grade ammonium sulfate and sucrose were obtained from Schwarz/Mann. Antimycin A was obtained from Ayrest Laboratories. 6-Decyl-2,3-dimethoxy-5-methyl-1,4-benzoquinone was the generous gift of Dr. Karl Folkers.

Results

Reduction of Cytochrome c by DBH_2. Before investigating the role of coenzyme Q in the respiratory chain using the analogue DB, it was first necessary to show that reduced DB interacts specifically with the cytochrome *b*-cytochrome c_1 complex. When yeast mitochondria are added to a cuvette containing excess DBH_2, cytochrome *c*, azide, and buffer, the rate of cytochrome *c* reduction may be monitored by measuring the increase in absorbance at 550 nm. As seen in Figure 1A, the initial slopes of the absorbance vs. time curves bear a linear relationship to the amount of added mitochondrial proteins. Both the extrapolated and experimental rates of cytochrome *c* reduction in the absence of added mitochondria are comparable.

The analysis of absorbance vs. time curves for various concentrations of mitochondria was further examined by the method of Guggenheim (Jencks, 1969). The logarithm of changes in absorbance ΔA_t, occurring in a relatively short period of time (in this case t = 38 s), is plotted as a function of t, the time after the addition of mitochondria during which ΔA_t occurs (Figure 1B). The straight lines obtained indicate that the reduction of cytochrome *c* follows first-order kinetics

TABLE I: Specific Activity and Antimycin A Sensitivity of Succinate and DBH_2:Cytochrome c Reductase in Cell-Free Extracts and Mitochondria.[a]

	Cell-free extract		Mitochondria		
	nmol/min or $K(min^{-1})$ mg of protein	% inhibition by antimycin A	nmol/min or $K(min^{-1})$ mg of protein	% inhibition by antimycin A	Purification (fold)
DBH_2:Cyt *c* reductase	67.4	100	386	100	5.7
Succinate:Cyt *c* reductase	26.4	93	180	100	6.8
Cytochrome oxidase	6.50		29.6		4.5

[a] Cell-free extracts and mitochondria were prepared from a culture of strain D273-10B. Succinate and DBH_2:cytochrome *c* reductase activities were determined in both fractions in the presence or absence of antimycin A (5 μg/mL). Values for DBH_2:cytochrome *c* reductase are corrected for nonenzymatic reduction.

during the initial part of the reaction over a fourfold range of protein concentrations. The pseudo-first-order rate constants obtained from the slopes of these lines are directly proportional to added mitochondrial protein (Figure 1C).

The initial rates of cytochrome *c* reduction, calculated by multiplying the experimentally measured first-order rate constants by the concentration of cytochrome *c*, are in good agreement with the observed rates. These results indicate that the catalytic activity of the cytochrome *b*–cytochrome c_1 complex may be estimated conveniently by subtracting the nonenzymatic rate of cytochrome *c* reduction from the initial reduction rate obtained in the presence of mitochondria. Alternately, a first-order rate constant for cytochrome *c* reduction can be determined. For most purposes, the measurement of initial velocities is preferable because of its simplicity; however, its accuracy is limited at higher protein concentrations, where measurement of initial velocities may not be feasible.

A major problem encountered with many substances used as substrates to assay the cytochrome *b*–cytochrome c_1 complex is their lack of specificity. Much of the apparent activity measured in whole cell extracts results from the reduction of cytochrome *c* by extra-mitochondrial electron-transport chains. Although corrections for nonmitochondrial activity can be made, these large differences can introduce substantial errors into the estimation of mitochondrial activity. Using the analogue DBH_2 as a substrate circumvents most of these difficulties. The enzymatic activity of the cytochrome *b*–cytochrome c_1 complex measured in whole cell extracts or in isolated mitochondria using DBH_2 as substrate was completely inhibited by antimycin A (Table I). Furthermore, upon fractionation, this activity sediments with cytochrome oxidase and succinate:cytochrome *c* reductase, markers for the mitochondrial respiratory chain. These results suggest that DBH_2 selectively reduces the mitochondrial cytochrome *b*–cytochrome c_1 complex and that reduction of extra-mitochondrial cytochromes (e.g., cytochrome b_5) is negligible.

The relationship between DBH_2:cytochrome *c* reductase activity and mitochondrial cytochrome content was further examined in order to determine to what extent DBH_2:cytochrome *c* reductase reflects the activity of the cytochrome *b*–cytochrome c_1 complex. Various fractions obtained during the purification of cytochrome *b* were examined for DBH_2:cytochrome *c* reductase activity. As seen in Table II, the specific activity of DBH_2:cytochrome *c* reductase more than doubled when submitochondrial particles were prepared from mitochondria and further increased concomitantly with the specific cytochrome content during extraction of submitochondrial particles with bile salts and KCl. The large increase in activity observed upon addition of bile salts may result, in part, from an activation of the enzyme, since treatment of submitochondrial particles with deoxycholate results only in a twofold increase in activity of DBH_2:cytochrome *c* reductase. When this detergent-solubilized preparation was treated with ammonium sulfate, an insoluble fraction was obtained in which the specific content of both cytochromes *b* and c_1 was increased, but DBH_2:cytochrome *c* reductase activity was completely absent. The ratio of cytochrome *b* to c_1 is considerably greater in this fraction than that observed in the cholate-solubilized fraction or in intact mitochondria, suggesting that the cytochrome *b*–cytochrome c_1 complex is no longer intact. The presence of cytochrome c_1 in the fraction may have resulted from the coprecipitation of this protein with cytochrome *b*, an interpretation consistent with the observation that ammonium sulfate in the presence of bile salts cleaves the isolated complex (Rieske, 1976). Furthermore, Katan et al. (1976) have recently reported that the cytochrome *b*–cytochrome c_1 complex of yeast mitochondria is more sensitive to cleavage by ammonium sulfate in the presence of bile salts than the complex from beef heart mitochondria. These authors found it necessary to stabilize the complex by addition of antimycin A during the purification procedure to isolate the complex from yeast mitochondria. These results provide further evidence that DBH_2:cytochrome *c* reductase activity is a reflection of the activity of the cytochrome *b*–cytochrome c_1 complex.

TABLE II: DBH_2:Cytochrome c Reductase Activity and Spectral Properties of Various Fractions Prepared during Purification of Cytochrome *b*.[a]

	$a + a_3$	*b*	c_1	b/c_1	DBH_2:*c* reductase
Mitochondria					270
Submitochondrial particles	0.289	0.224	*b*		636
F_4 (deoxycholate–KCl extraction)	0.902	0.872	0.500	1.74	3316
F_5 (cholate–$AmSO_4$ fraction)	1.10	4.08	0.990	4.12	0
F_6 (*b*–c_1 separation)	0.529	5.01	0		0

[a] Cytochrome *b* was purified from commercial yeast according to the procedure of Lin and Beattie (1976). At various stages during the purification, aliquots were removed for the determination of cytochrome content and DBH_2:cytochrome *c* reductase activity. Cytochrome content is expressed as nmol/mg of $protein^{-1}$; DBH_2:cytochrome *c* reductase as nmol of cytochrome *c* reduced min^{-1} (mg of $protein)^{-1}$. [b] The cytochrome c_1 content of submitochondrial particles could not be accurately estimated because of the presence of cytochrome *c*.

TABLE III: Properties of Presumptive Mutants in Coenzyme Q Biosynthesis.[a]

Strain	Complementation group[b]	% revertant	% petite	NADH:Cyt *c* −DB	NADH:Cyt *c* +DB	Succ:Cyt *c* −DB	Succ:Cyt *c* +DB	DBH_2:Cyt *c*	Cyt oxidase
N9-57	II	0	44	5.8	56.0	1.0	21.8	78.5	2.20
E3-24	V	0	7.5	5.4	62.3	0	24.0	66.3	3.00
E3-71	I	0	110	6.5	51.0	0	11.8	71.7	2.98
E2-247	III	29	12.9	7.6	73.0	5.8	30.1	75.0	3.10
E1-237	IV	0	3.1	5.8	56.0	0	11.4	35.0	4.72
E2-249	V	0	5.1	13.1	64.7	0	13.0	98.7	2.60
D273-10B	wt		<1			180	180	386	29.6

[a] Cells of mutant and wild-type strain were grown to stationary phase on galactose media at which time petite frequency was determined. Mitochondria were prepared and assayed for enzymatic activity as described under Materials and Methods. DB-stimulated rates were >90% inhibited by antimycin A (5 μg/mL) and DBH_2:cytochrome *c* reductase activity was completely inhibited by antimycin A. To reconstitute NADH: and Succ:cytochrome *c* reductases, 10 μg of DB was added to the reaction mixture as an ethanolic solution. [b] From Tzagoloff et al. (1975).

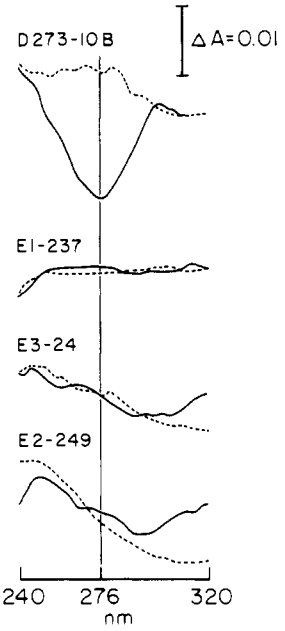

FIGURE 2: Spectra of petroleum ether–methanol extracts of wild-type (D273-10B) and coenzyme Q deficient mitochondria. Mitochondria were extracted by the method of Kröger and Klingenberg (1966). Dried extracts were dissolved in 3 mL of ethanol–cyclohexane (1:4, v/v), and 1-mL aliquots were reduced with 10 μg of $NaBH_4$ and oxidized with 10 μg of $FeCl_3$. Solid lines (—) indicate reduced vs. oxidized samples. Dotted lines (- - -) indicate oxidized vs. oxidized samples. Amounts of mitochondrial protein extracted D273-10B, 10 mg; E1-237, E3-24, E2-249, 2 mg.

Properties of Coenzyme Q Deficient Mutants. The recent isolation of a number of mutants presumed to be deficient in coenzyme Q biosynthesis (Tzagoloff et al., 1975) permitted studies of the effectiveness of DB as a replacement for coenzyme Q in promoting electron transfer from the primary dehydrogenases to the cytochrome *b*–cytochrome c_1 complex. After a preliminary screening of 13 strains, six were selected for further studies on the basis of their relative long-term stability. These six mutants, which fall into five different complementation groups, have a complete, or near complete, absence of both NADH and succinate:cytochrome *c* reductase activity in the absence of DB, with the exception of strain E2-247 (see Table III). This strain has an appreciable succinate:cytochrome *c* reductase activity in the absence of added quinone; however, a high percentage of revertant cells were present in the culture used. The activities of both NADH and succinate:cytochrome *c* reductases were markedly stimulated in all strains upon addition of 10 μg of DB to the assay medium. It should be noted that the DB-stimulated succinate:cytochrome *c* reductase and cytochrome oxidase were also lower in the mutants, probably a result of the inability of respiratory-deficient cells to undergo complete catabolite derepression (Perlman and Mahler, 1974).

The ability of DB to promote electron transfer from NADH or succinate to cytochrome *c* in these mutants is further confirmation that the defect in these mutants is the absence of coenzyme Q and not another component of the respiratory chain. Further direct evidence for this conclusion has been obtained by spectral analysis of petroleum ether–methanol extracts of mitochondria from both wild-type and mutant cells. As seen in Figure 2, the wild-type cells contain spectrally detectable coenzyme Q, while extracts of three mutant mitochondria do not.

Reconstitution of NADH and Succinate:Cytochrome c Reductases by DB. An investigation of the reconstitution of NADH and succinate:cytochrome *c* reductases in the coenzyme Q deficient mutants, using DB, was undertaken in an attempt to clarify the considerable discrepancies in the literature with regard to the role of coenzyme Q in NADH and succinate oxidation (Albracht et al., 1971). The restoration of NADH and succinate:cytochrome *c* reductase activity in the mutant E3-24 with increasing concentrations of DB shows a sigmoidal dependence on added quinone. The NADH-dependent activity reaches a threefold higher maximum velocity than the succinate dependent, and requires a higher concentration of DB to reach half-maximal velocity. It should be mentioned that succinate:cytochrome *c* reductase activity showed a marked lag upon addition of coenzyme Q before attaining a constant velocity. This lag may result from the well-documented activation of succinic dehydrogenase by reduced quinone (Gutman et al., 1971b) as the lag was eliminated by preincubation of mitochondria in the presence of succinate and DB. The rates presented in Figure 3A were obtained after the lag period.

By contrast, the substrate dependence of the DBH_2:cytochrome *c* reductase activity is markedly different. At substrate concentrations varying from 0 to 50 μg/mL, a hyperbolic relationship between substrate concentration and specific activity is obtained. Typical Michaelis–Menten kinetics are observed when the data are analyzed by a double-reciprocal plot (Figure 3B). A comparison of Figure 3A,B indicates that the maximum specific activities of NADH or succinate:cytochrome c reductase are considerably lower than the activities of DBH_2:cytochrome *c* reductase obtained with DBH_2 at less than saturating concentrations. The V_{max} for the latter enzymatic activity, as estimated from the double-reciprocal plot, is 555

nmol min^{-1} (mg of protein)$^{-1}$, a value 4 and 12 times higher than the maximal velocities attainable with NADH and succinate, respectively. Furthermore, DBH_2:cytochrome *c* reductase activity continues to increase at concentrations of the analogue that are considerably higher than those necessary to reconstitute NADH and succinate:cytochrome *c* reductase.

These results may reflect the lower affinity of complex III for the reduced form of the analogue than that of complexes I and II for the oxidized form. This interpretation is complicated by the presence of some oxidized analogue in the preparation of DBH_2 used in this experiment; hence, the actual substrate concentration would be somewhat lower than that indicated in Figure 3B where substrate concentration is expressed in terms of total DB content. Since the addition of the oxidized form of the analogue does not influence DBH_2:cytochrome *c* reductase activity, we assume that its presence does not influence the nature of the substrate dependence.

Discussion

The activity of complex III of the respiratory chain has proven difficult to assay properly because readily available substrates lack specificity. Most substrates reduce extramitochondrial cytochromes or reduce cytochrome *c* nonenzymatically. Previously, coenzyme Q_2, the lower molecular weight homologue of coenzyme Q, was shown to be a satisfactory substrate for complex III, but this compound is rare in nature and unstable (Wan et al., 1975). The synthetic analogue of coenzyme Q, DBH_2, is stable for long periods of time and can be used satisfactorily as a substrate in the assay described in this paper. The nonenzymatic rates of cytochrome *c* reduction are low and can be simply corrected. Furthermore, the reaction is highly specific for the mitochondrial cytochrome *b*-cytochrome c_1 complex, as it is completely sensitive to antimycin A in unfractionated cell extracts and sediments with markers for the electron-transport chain upon fractionation.

Our initial interest in the possible compartmentation of coenzyme Q in the respiratory chain arose during studies of yeast undergoing respiratory adaptation in the presence of chloramphenicol, the specific inhibitor of mitochondrial protein synthesis (Beattie, 1971). The activity of succinate:cytochrome *c* reductase increased in the presence of the inhibitor to a greater extent than either DBH_2 or NADH:cytochrome *c* reductases. Induction of the latter enzymatic activity was almost completely prevented by chloramphenicol. One possible explanation for these results is that the NADH and succinate-dependent reduction of cytochrome *c* has a different dependence of coenzyme Q which also increases during respiratory adaptation (Brown and Beattie, 1977, submitted for publication).

Our experimental approach to this problem was to use the coenzyme Q analogue DB in its oxidized form to reconstitute these activities in mitochondria obtained from a coenzyme Q deficient strain of yeast. Hence, the dependence of these two enzymes on DB could be measured directly. Furthermore, the specific activities obtained could be compared to that of DBH_2:cytochrome *c* reductase. The results obtained in the reconstitution experiments have indicated that both succinate and NADH oxidation are completely dependent on added quinone and that complex III is normally not rate limiting for either NADH or succinate:cytochrome *c* reductase activity. At low concentrations of quinone it is not possible to discern from these experiments whether the reduction or oxidation of DB is rate limiting. A convenient explanation for the sigmoid curves obtained in Figure 3A, however, is to postulate that at low concentrations of DB the oxidation of the reduced form may be rate limiting, while at higher concentrations of DB the primary dehydrogenases with their lower V_{max} might become rate limiting.

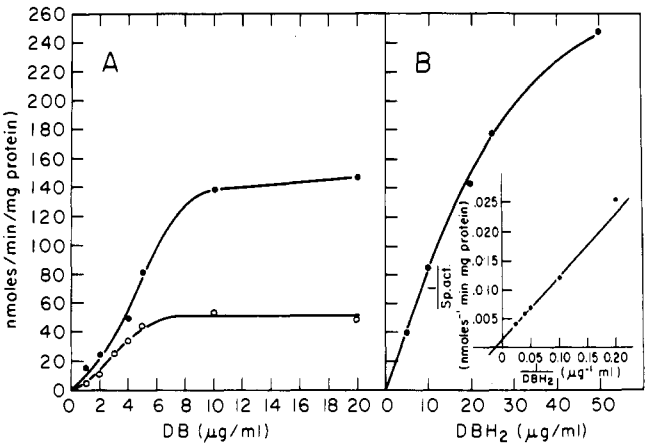

FIGURE 3: Concentration dependence of cytochrome *c* reductase activities on added DB or DBH_2. (A) Specific activity of NADH (●–●) and succinate (○–○) cytochrome *c* reductase in mitochondria of strain E3-24 vs. concentration of DB (μg/mL). Mitochondrial protein (42.5 μg) was added. (B) Specific activity of DBH_2:cytochrome *c* reductase in mitochondria of strain E3-24 vs. increasing concentrations of reduced DB (μg/mL). Insert: double-reciprocal plot of the same. Mitochondrial protein (17 μg) was added.

The results obtained in the reconstitution experiments using coenzyme Q deficient mutants of yeast have allowed us to make several interesting observations pertaining to the role of coenzyme Q in mitochondrial electron transport. Slater's group (Albracht et al., 1971) has postulated the existence of a compound other than coenzyme Q which is capable of restoring respiration to coenzyme Q depleted particles with succinate but not with NADH as substrate. This result was interpreted to mean that coenzyme Q was not essential for succinate oxidase activity. By contrast, the present study indicates that coenzyme Q is absolutely essential for the oxidation of succinate, as succinate:cytochrome *c* reductase activity is absent in all strains free of revertants. Furthermore, since the coenzyme Q deficient strains fall into five of the six known different genetic complementation groups, it is unlikely that an intermediate in coenzyme Q biosynthesis or a compound synthesized from an intermediate can replace the quinone in this function. These experiments do not, however, rule out the possibility that the compound described by Albracht et al. (1971) has coenzyme Q as a biosynthetic precursor.

Previous reconstitution studies have involved the use of pentane extraction of lyophilized mitochondria or submitochondrial particles to remove coenzyme Q (Szarkowska, 1966; Ernster et al., 1969). This procedure has the disadvantage that lyophilization generally causes inactivation of both NADH dehydrogenase and oxidase activities (Gutman et al., 1971a). Studies with particles obtained after lyophilization and extraction have suggested that restoration of succinate oxidation required lower concentrations of coenzyme Q than does NADH oxidation (Gutman et al., 1971a) and that these differences are exaggerated when low-molecular-weight homologues of coenzyme Q are used (Lenaz et al., 1975). These results were interpreted as evidence for the "functional heterogeneity" of the coenzyme Q pool (Lenaz et al., 1975; Gutman et al., 1971a). By contrast, we were unable to find a marked difference in the concentration of DB required to reconstitute NADH and succinate:cytochrome *c* reductase activities, suggesting a common pool of coenzyme Q.

NADH dehydrogenase, or complex I, of *S. cerevisiae* differs from that of most other organisms as it lacks the non-heme-iron

center believed to be involved in site I phosphorylation and is insensitive to rotenone and piericidin A (Ohnishi, 1973). Hence, the site of interaction with coenzyme Q may differ in complex I of these organisms as compared to *S. cerevisiae*, resulting in an increase in their relative affinity for quinone. An alternate explanation may be that the lack of complete reactivation of NADH oxidase observed after lyophilization results in an increased dependence on coenzyme Q. For example, it is possible that inactivated particles compete with active particles for the added quinone, thereby giving rise to spuriously high estimates for the amount of CoQ necessary to reactivate NADH oxidase. Thus, the suggestion of a functional heterogeneity or compartmentation of the coenzyme Q pool depends on the experimental system employed. It is impossible to decide whether the disparity of our results with those of others results from the use of yeast mitochondria or the use of the lyophilization procedure. However, on the basis of kinetic experiments performed with intact particles, Kröger and Klingenberg (1973) suggested that the active coenzyme Q fraction was not compartmentalized. Our reconstitution experiments with intact mitochondria lead us to the same conclusion.

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