BBA 46094

THE PREPARATION AND PROPERTIES OF A SOLUBILIZED RESPIRATORY COMPLEX FROM *ESCHERICHIA COLI*

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(Received November 23rd, 1970)

SUMMARY

1. Treatment of the respiratory particle fraction from *Escherichia coli* with cholate in the presence of ammonium sulfate liberated a stable, soluble, cytochrome-containing moiety ("soluble respiratory complex") of "molecular weight" of about $2 \cdot 10^6$.

2. The soluble respiratory complex contained nonheme iron, ubiquinone, cytochrome b_1, cytochrome *o*, acid-resistant flavin and acid-released flavin in a ratio of 56:18:3.7:0.96:0.7:1.

3. The soluble respiratory complex was devoid of NADH and succinate oxidase activities until ubiquinone isoprenologues were added. Ubiquinone Q-2 was the most effective derivative in restoring oxidase activities.

4. Reactivation of succinate oxidase by Q-2 was produced by a 10-fold lower concentration than that required for NADH oxidase.

5. Addition of Q-2 lowered the aerobic steady state level of cytochrome b_1 reduction in the soluble respiratory complex with succinate but not with NADH as substrate. The inhibitor 2-*n*-heptyl 4-hydroxyquinoline *N*-oxide (HQNO) increased this value with the former but not with the latter substrate. Thus, deficiency of ubiquinone and addition of HQNO give a similar effect on the succinate-reducible cytochrome b_1.

6. It is proposed that in the soluble respiratory complex part at least of the cytochrome b_1 reducible by succinate is either on a side-branch from the main respiratory chain or that Q-2 interacts between succinate-reducible cytochrome b_1 and oxygen.

INTRODUCTION

Soluble preparations of membrane-bound enzyme systems should permit the investigation of properties not easily studied in the intact membrane. Such a membrane-bound system is the electron transport chain of *Escherichia coli*[1].

Detergents have been used to only a limited extent for the isolation of large fragments of the bacterial respiratory chain. ITAGAKI *et al.*[2] and LINNANE AND WRIGLEY[3] both isolated soluble preparations of formate–nitrate reductase from *E. coli*. JONES AND REDFEARN[4] solubilized two fractions from different portions of the respiratory chain of *Azotobacter vinelandii*. In a previous paper[5] we have described the

Abbreviation: HQNO, 2-*n*-heptyl 4-hydroxyquinoline *N*-oxide.

use of deoxycholate to cleave the membrane-originated respiratory particle fraction of *E. coli* into a cytochrome-containing complex *plus* a variety of enzymes including several NADH dehydrogenases (menadione reductases).

This paper describes an extension of our previous work. By use of cholate and ammonium sulfate we have disaggregated the respiratory particle fraction to yield a stable, soluble complex of "molecular weight" of about $2 \cdot 10^6$. This complex ("soluble respiratory complex") contains the components of the respiratory chain. In the presence of added ubiquinone cyanide-sensitive succinate and NADH oxidase activities are reconstituted.

METHODS

Reagents

All chemicals were of Fisher reagent-grade purity. Crystalline bovine plasma albumin, yeast alcohol dehydrogenase, yeast hexokinase, chymotrypsin, bovine liver catalase, and coenzymes were obtained from Calbiochem. Porcine thyroglobin, equine apoferritin, bovine fibrinogen, human γ-globulin, and crystalline human hemoglobin were obtained from Mann Research Laboratories. Crystalline trypsin, and Sepharose 4B and 6B were obtained from Boehringer and Pharmacia, respectively. Sodium cholate was obtained from Nutritional Biochemicals Corporation.

Calcium phosphate gel was prepared by the method of SWINGLE AND TISELIUS[6].

Analytical methods

Protein concentrations were determined by the method of LOWRY *et al.*[7] with crystalline bovine plasma albumin as a standard.

Cytochrome b_1 was measured from the dithionite-reduced *minus* oxidized difference spectrum obtained in a Cary 15 spectrophotometer using the extinction coefficients for the α-band given by DEEB AND HAGER[8] or the Soret band given by ITAGAKI *et al.*[2]. Both extincition coefficients gave identical values for the cytochrome content. Cytochrome *o* was measured as described by BRAGG[9] using the extinction coefficient of $160 \cdot 10^3$ $l \cdot moles^{-1} \cdot cm^{-1}$ for the peak (415 nm) to trough (430 nm) height of the carbon monoxide spectrum. Unless indicated the values given for cytochrome b_1 contain contributions from cytochrome *o*. Where the cytochrome b_1 value has been corrected for cytochrome *o* content this has been done by assuming that the extinction coefficient of cytochrome *o* was the same as that of cytochrome b_1 in the reduced minus oxidized difference spectrum. The concentration of cytochrome *o* determined from the carbon monoxide difference spectrum was then subtracted from that of cytochromes b_1 *plus o* determined from the reduced minus oxidized difference spectrum. The total iron content was assayed after wet ashing the sample with sulfuric acid and hydrogen peroxide by the method of KING *et al.*[10]. Non-heme iron was calculated by substracting heme iron from the total iron.

Total flavin was measured by the fluorimetric procedure of WILSON AND KING[11] using a Baird Atomic Fluorescence Spectrophotometer. The samples were digested with trypsin and chymotrypsin, and subsequently incubated at 37° with 8% trichloroacetic acid to hydrolyse any flavin adenine dinucleotide to the mononucleotide. Release of acid-extractable flavin was achieved by incubation in 10% (w/v) trichloroacetic acid at 0° for 30 min followed by fluorescence measurements as for total flavin.

Acid-resistant flavin was calculated by difference.

Ubiquinone was extracted from the particles and chromatographed on magnasol-celite columns as described by BRAGG AND POLGLASE[12]. The quinone was estimated using the extinction coefficient given by REDFEARN[13].

Lipid phosphorus was measured by extracting lyophilised samples with a chloroform–methanol (2:1, v/v) mixture and estimation of total phosphorus in the lipid extract by the method of SUMNER[14]. The phospholipid content of samples was obtained by multiplying the lipid phosphorus value by a factor of 25.5 (ref. 15).

Molecular weight of the soluble respiratory complex was estimated by gel filtration on a column (2.5 cm × 37 cm) of Sepharose 4B (ref. 16) using 0.05 M Tris–HCl–0.1 M NaCl (pH 8.0) as eluting buffer. Fractions from the column were weighed to determine elution volumes accurately[17].

Organism and fractionation procedures

The origin, culture and preparation of the respiratory (small) particle fraction of *E. coli* have been described previously[5]. All fractionation procedures were carried out at 0–4°. Typical experiments are described.

Preparation of soluble respiratory complex for analysis

Sodium cholate solution (20 %, w/v) was added dropwise to a suspension (15–20 mg protein/ml) of respiratory (small) particle fraction in 0.01 M Tris–HCl buffer (pH 7.4), containing 0.01 M $MgCl_2$, to a final concentration of 0.75 mg of sodium cholate per mg protein. The liquid was stirred for 15 min. Saturated aqueous ammonium sulfate solution (pH 7.4) was then added to a final concentration of 0.33 saturation. After a further 30 min the precipitate which had formed was removed by centrifuging the solution at 115000 × *g* for 30 min. The supernatant, which contained about 50 % of the protein of the respiratory particle fraction, was taken to 0.6 saturation by the addition of solid ammonium sulfate. The liquid was stirred for 15 min and the precipitate recovered by centrifuging at 10000 × *g* for 10 min. For subsequent chromatography the pellet was dissolved in a minimum volume of the buffer with which the chromatography medium was equilibrated.

Purification of the soluble respiratory complex was achieved by chromatography in 0.05 M Tris–HCl–1 M KCl–0.1 % sodium cholate buffer (pH 8.0) on a column of Sepharose 6B (2.5 cm × 40 cm) coupled in series with a column of Sepharose 4B (2.5 cm × 85 cm). The cytochrome b_1-containing fractions with elution volumes from 280 ml to 420 ml (see Fig. 1) were bulked and dialysed *versus* distilled water at 4°. The dialysed solution was lyophilised, redissolved in distilled water, and dialysed again before analyses were made. The soluble respiratory complex so obtained retained its soluble nature for at least a month when this solution was stored at 4°.

Preparation of soluble respiratory complex for enzyme experiments

The procedure was slightly modified from that described above. To minimize the concentration of salt and cholate in the soluble respiratory complex the buffer used for chromatography on Sepharose 4B was replaced by a buffer containing 0.01 % dithiothreitol and 20 % (v/v) glycerol in 0.05 M Tris–HCl (pH 8.0). For cholate treatment the respiratory particle fraction was dissolved in dithiothreitol–glycerol–Tris buffer containing 20 mM sodium succinate. A final modification in the interests of

rapidity of preparation of the enzyme was to use a single column (2.5 cm × 40 cm) of Sepharose 4B. The soluble respiratory complex was thus contaminated by some ribosomal proteins. For this reason the amount of soluble respiratory complex used in the enzyme experiments is expressed in terms of its cytochrome b_1 content.

Purification of respiratory particles

The respiratory particles were purified by chromatography on a column of Sepharose 4B (2.5 cm × 40 cm) equilibrated with 0.3 M NaCl in 0.01 M Tris–HCl buffer (pH 7.4), containing 0.01 M $MgCl_2$. The purified respiratory particles appeared in the void volume fraction from the column. They were sedimented by centrifuging this fraction at 176000 × *g* for 30 min. The particles were suspended at a concentration of about 1.8 mg protein per ml dithiothreitol–glycerol–Tris buffer.

Assay procedures

Oxidase activity was measured polarographically at 22° using a Yellow Springs Instrument Co. Model 55 oxygen monitor connected to a linear recorder. The assay mixture contained 2 ml soluble respiratory complex or respiratory particles (about 1 nmole cytochrome b_1) in dithiothreitol-glycerol–Tris buffer, and 2.5 ml 0.05 M Tris–HCl containing 0.02 M $MgCl_2$. Ubiquinone or inhibitor was added where indicated and preincubation was given for 5 min. The reaction was started by the addition of 50 μl 0.5 M sodium succinate or 0.1 M NADH.

The rate of cytochrome b_1 reduction was measured at 22° by following the change in absorbance at 560 nm relative to 540 nm with an Aminco-Chance dual wavelength spectrophotometer. The reaction mixture contained 2.0 ml soluble respiratory complex or respiratory particles in dithiothreitol–glycerol–Tris buffer and 0.0125 M $MgCl_2$. The substrate was 25 μl 0.5 M sodium succinate or 0.1 M NADH. The reduction occurred too rapidly to permit reliable measurement of the initial rate with the equipment available to us. Therefore, the rate of reduction is expressed as the time in minutes taken for the cytochrome to become half-reduced at the transition from the aerobic steady state level to the anaerobic state level of reduction ($t_{1/2}$).

The aerobic steady state levels of reduction of cytochrome b_1 were measured with the same system as used to measure cytochrome b_1 reductase activity except that the difference spectrum from 580 to 400 nm was continually scanned in a Cary 15 spectrophotometer after addition of substrate. The aerobic steady state levels are expressed in terms of percentage reduction of dithionite-reducible cytochrome b_1.

Succinate dehydrogenase was assayed as described by KING[18] except that the final concentration of phosphate buffer was 3 mM, that albumin was omitted, and that only a single concentration (0.6 mM) of phenazine methosulfate was used.

Stock solutions of ubiquinones were made in ethanol.

RESULTS

Preparation of soluble respiratory complex

Treatment of the respiratory particles with cholate (0.75 mg/mg protein) and ammonium sulfate (0.33 saturation) resulted in solubilization of about 50 % of both cytochrome b_1 and protein to a form which did not sediment under conditions of centrifugation which sedimented the untreated respiratory particles (Table I). If the particles were treated with cholate alone, cytochrome b, was not solubilized.

TABLE I

DISTRIBUTION OF PROTEIN, CYTOCHROME AND SUCCINATE DEHYDROGENASE DURING SOLUBILIZATION OF RESPIRATORY PARTICLES FROM 10 g CELLS

The fractionation procedure, and the assay of cytochrome b_1 and succinate dehydrogenase are described in METHODS.

Fraction	*Protein (mg)*	*Cytochrome b_1 (nmoles)*	*Succinate dehydrogenase (μmoles/min)*
Respiratory particles	329	53	10.3
Cholate/$(NH_4)_2SO_4$ supernate	169	24	3.6
Cholate/$(NH_4)_2SO_4$ residue	147	25	2.4
Soluble respiratory complex fraction	145	22	

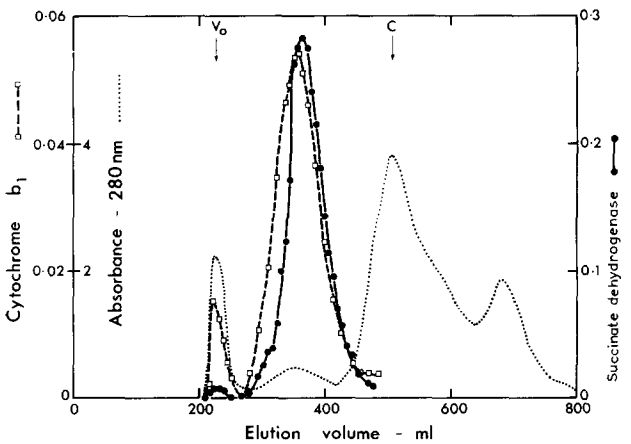

Fig. 1. Separation of cholate-solubilized fraction of respiratory particles on coupled columns of Sepharose 6B and Sepharose 4B. The conditions of chromatography and the enzyme assays are described in METHODS. 460 mg protein was applied to the columns. V_0, void volume; C, elution position of catalase. Cytochrome b_1 is expressed as $A_{428-410\ nm}$ and succinate dehydrogenase in μmoles/min per ml fraction.

Chromatography of the solubilized material on a column of Sepharose 6B coupled in series to a column of Sepharose 4B showed that most of the cytochrome migrated as a single broad peak well separated from the bulk of the contaminating protein (Fig. 1). Succinate dehydrogenase was eluted at the same volume as the cytochrome but these components did not have exactly coincident elution profiles. If 0.01 % dithiothreitol was included in the column buffer the two profiles became almost coincident. The concentration of cytochrome b_1 was nearly constant at 1.33 nmoles per mg protein in the fractions throughout the peak so the material in this region was treated as a single fraction ("soluble respiratory complex").

Molecular size of soluble respiratory complex

An estimate of the molecular size of the soluble respiratory complex was obtained by chromatography on a single column of Sepharose 4B using the method of MARRINK AND GRUBER[16] (Fig. 2). Since the soluble respiratory complex was eluted from the column in a region for which standard proteins were not available the "molec-

ular weight" of approx. $2 \cdot 10^6$ obtained for the cytochrome preak is considered provisional.

Composition of the soluble respiratory complex and respiratory particle fractions

Analysis of the Sepharose-purified respiratory particle and soluble respiratory complex gave the results shown in Table II. Both preparations contained about 30 % phospholipid, together with cytochrome, flavin, ubiquinone, and nonheme iron. A comparison of the ratio of nonheme iron:ubiquinone:cytochrome b_1:cytochrome *o*: acid-resistant flavin:acid-extractable flavin for respiratory particles and soluble respiratory complex gave 63:76:6.8:1.6:1:1 and 56:18:3.7:0.96:0.7:1, respectively.

Effect of ubiquinone on enzyme activity of solubilized respiratory complex

The soluble respiratory complex had no succinate or NADH oxidase activities. Addition of ubiquinone isoprenologues restored NADH and succinate oxidase activi-

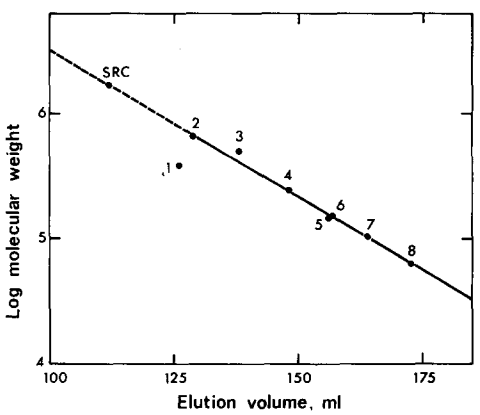

Fig. 2. Calibration curve for determination of the molecular weight of soluble respiratory complex (SRC). The calibration was carried out as described in METHODS. No. 1–8 represent the position of elution of fibrinogen, thyroglobulin, apoferritin, catalase, alcohol dehydrogenase, γ-globulin, hexokinase and hemoglobin, respectively.

TABLE II

COMPOSITION OF PURIFIED RESPIRATORY PARTICLES AND SOLUBLE RESPIRATORY COMPLEX

The analyses were performed as described in the text. The analytical values ± S.D. are from at least three different preparations with the exception of respiratory particles where only two preparations were analysed. The values are quoted as nmoles per mg protein except that phospholipid is expressed as percent of protein + lipid.

Component	*Respiratory particles*	*Soluble complex*
Cytochrome b_1 (total)	0.445 ± 0.005	1.16 ± 0.07
Cytochrome *o*	0.087 ± 0.02	0.24 ± 0.01
Flavin (total)	0.11 ± 0.005	0.42 ± 0.02
Flavin (acid-released)	0.053 ± 0.002	0.25 ± 0.03
Ubiquinone	4.0 ± 0.09	4.5 ± 0.5
Iron (total)	3.8 ± 0.5	15.4 ± 1.2
Phospholipid	27.3	30 ± 3

ties (Table III). Of the quinones tested the most effective was Q-2 when either substrate was used. The major ubiquinone found in *E. coli*, Q-8, showed some activity with both substrates but its biological activity may have been underestimated due to the difficulty of getting it into aqueous solution. Ethanolic solutions of the quinones were employed since the method of ERNSTER *et al.*[19] could not be used as the enzymes were denatured by lyophilization (also see ref. 20).

Since Q-2 was the most effective quinone in restoring oxidase activity it was used in the further studies. In Table IV is shown the effect of concentration of Q-2 on

TABLE III

EFFECT OF UBIQUINONE ISOPRENOLOGUES ON NADH AND SUCCINATE OXIDASES OF SOLUBLE RESPIRATORY COMPLEX

The assay procedure is described in METHODS. Oxidase activity is expressed as natoms O/min per nmole cytochrome b_1.

Quinone	*Concn.* (μM)	*NADH oxidase*	*Succinate oxidase*
None		0	0
Q-0	5.6	51	
	11.1		69
	16.7	82	
	44.4	112	
	72.2	112	
Q-2	8.8	71	483
	26.3	127	
	70.3	250	
	114	265	
Q-8	2.4	10	
	7.3	31	
	12.2		82
	19.6	36	

TABLE IV

EFFECT OF Q-2 ON SUCCINATE AND NADH OXIDASES AND ON RATE OF CYTOCHROME b_1 REDUCTION IN SOLUBLE RESPIRATORY COMPLEX

The assay procedures are described in METHODS. Oxidase activity is expressed as natoms O/min per nmole cytochrome b_1. Rate of cytochrome b_1 reduction is given as time (min) for half-reduction of the cytochrome to the anaerobic state value ($t_{1/2}$) (see METHODS). The soluble respiratory complex contained 0.55 nmole cytochrome b_1/ml in the reductase assay.

Q-2	*NADH*		*Succinate*	
(μM)	*Oxidase*	*Cytochrome reduction* ($t_{1/2}$)	*Oxidase*	*Cytochrome reduction* ($t_{1/2}$)
0	0	2.3	0	1.5
3.6			328	0.16
8.2	214	1.5	510	0.13
12.3			510	
18.4		0.95	443	0.15
26.3	354			
70.3	523			
96.5		0.62		
114	563			

succinate and NADH oxidase, and on the time taken for the cytochrome to become half-reduced ($t_{1/2}$) by these substrates on transition from the aerobic to the anaerobic steady state level of reduction. With succinate both $t_{1/2}$ and oxidase activity reached final values at a similar concentration of Q-2. The same phenomonen was observed with NADH as substrate. However, the concentration of Q-2 required to saturate the succinate systems was 10-fold lower than that for the NADH systems.

Addition of Q-2 to the soluble respiratory complex lowered the aerobic steady

TABLE V

EFFECT OF Q-2 ON THE AEROBIC STEADY STATE LEVEL OF CYTOCHROME b_1 REDUCTION BY SUCCINATE AND NADH IN THE SOLUBLE RESPIRATORY COMPLEX

The assay procedure is described in METHODS. The soluble respiratory complex contained 0.55 nmole cytochrome b_1 per ml.

Q-2 (μM)	*Aerobic steady state (% reduced)*	
	Succinate	*NADH*
0	40	7
3.9	24	
7.7	13	
19.3	12	
40.0		6
96.2	17	

TABLE VI

EFFECT OF INHIBITORS ON OXIDASE ACTIVITIES OF SOLUBLE RESPIRATORY COMPLEX AND PURIFIED RESPIRATORY PARTICLES

The assay procedure is described in METHODS. Oxidase activity is expressed in natoms O/min per nmole cytochrome b_1. The soluble complex and respiratory particles contained 0.25 nmole and 0.65 nmole cytochrome b_1/ml, respectively. The soluble complex system contained 8 μM and 40 μM Q-2 when succinate and NADH were substrates, respectively. For carbon monoxide inhibition the assay system was diluted with an equal volume of CO-saturated assay buffer.

Substrate	*Inhibitor*	*Concn. (mM)*	*Soluble complex*		*Respiratory particles*	
			Oxidase	%	*Oxidase*	%
Succinate	—	—	553	100	355	100
	HQNO	0.0035	277	50		
	HQNO	0.0067	178	32		
	HQNO	0.0150			220	62
	KCN	0.56	183	33		
	KCN	2.38	43	8	25	7
	CO		355	64		
NADH	—	—	301	100	718	100
	HQNO	0.0035	229	76		
	HQNO	0.0067	205	69		
	HQNO	0.0150			138	19
	KCN	0.56	233	78		
	KCN	2.38	87	29	29	4
D-Lactate					54	

state level of cytochrome b_1 reduction with succinate as substrate but had little effect with NADH (Table V). The concentration of Q-2 (about 8 μM) giving the maximum effect on the steady state level was approximately that required for maximum succinate oxidase activity (Table IV).

Under N_2 85 % and 77 % of the total (dithionite-reducible) cytochrome b_1 was reduced in the anaerobic steady state in the Q-2-supplemented soluble respiratory complex by succinate and NADH, respectively.

Succinate and NADH oxidase activities of the respiratory particles and the soluble complex were inhibited by KCN and HQNO (Table VI). With the respiratory particles NADH oxidase seemed to be more sensitive than succinate oxidase to these inhibitors but the reverse was true with the soluble respiratory complex.

TABLE VII

EFFECT OF INHIBITORS ON THE AEROBIC STEADY STATE LEVEL OF CYTOCHROME b_1 REDUCTION IN SOLUBLE RESPIRATORY COMPLEX AND PURIFIED RESPIRATORY PARTICLES

The assay procedure is described in METHODS. The soluble respiratory complex was assayed in the presence of 8 μM Q-2 and 40 μM Q-2 with succinate and NADH, respectively. The soluble complex and respiratory particles contained 0.55 and 0.65 nmole cytochrome b_1 per ml, respectively.

Substrate	*Inhibitor*	*Aerobic steady state (% reduced)*	
		Soluble complex	*Respiratory particles*
Succinate	—	32 *	
	—	17	10
	2.88 mM KCN	77	70
	0.015 mM HQNO	36	12
NADH	—	7 *	
	—	6	7
	2.88 mM KCN	40	52
	0.015 mM HQNO	8	5
D-Lactate	—		4
	2.88 mM KCN		26
	0.015 mM HQNO		4

* Q-2 omitted.

The effect of these inhibitors on the aerobic steady state level of reduction of cytochrome b_1 was examined (Table VII). Cyanide increased the aerobic steady state level of cytochrome reduction with both respiratory particles and ubiquinone-supplemented soluble respiratory complex. It is probable that KCN reacts with cytochrome *o* since this pigment reacts in its reduced form with carbon monoxide. Progressive inhibition by KCN occurred after addition of substrate even when the inhibitor had been preincubated for 10 min with the enzyme. This suggests that KCN had reacted with the reduced form of the cytochrome. With succinate as substrate HQNO markedly increased the aerobic steady state level of cytochrome b_1 reduction in the soluble respiratory complex supplemented with ubiquinone but only had a slight effect on the respiratory particles. This inhibitor did not produce any marked changes when NADH was substrate.

DISCUSSION

The soluble respiratory complex contains all the respiratory carriers detected in the membrane-bound system but their ratios are somewhat altered. The major difference is the much lower ubiquinone content of the soluble complex. In line with this observation is the fact that the NADH and succinate oxidase activities of the soluble respiratory complex are very low until ubiquinone isoprenologues are added.

That the electron transport chain of the soluble respiratory complex may not be identical in all respects to the membrane-bound chain of the respiratory particles is also shown by the oxidase activities. In the ubiquinone-supplemented soluble complex NADH and succinate were oxidized at 301 and 553 nmoles/min per nmole cytochrome b_1 compared to 718 and 355 nmoles/min per nmole cytochrome b_1 for the respiratory particles.

A distinct difference can be seen between the NADH oxidase and the succinate oxidase chains of the soluble respiratory complex in their requirements for Q-2. The NADH oxidase system required a 5 to 10-fold higher concentration of Q-2 to give maximal activity. This difference could be due to the existence of separate respiratory chains for the oxidation of NADH or succinate, but could also be due to the different binding capacities or extent of ubiquinone depletion in the substrate to cytochrome region of the chain. No clear-cut distinction could be made between these possibilities. The greater sensitivity to KCN of succinate compared to NADH oxidase activity of the soluble respiratory complex favours the existence of some separate chains for the two substrates. But at least some respiratory chains must be common since both substrates alone will reduce about 80 % of the total cytochrome b_1.

A further distinction between NADH and succinate oxidases of the soluble respiratory complex was observed in the response of cytochrome b_1 reduction to addition of Q-2. With succinate the aerobic steady state level of cytochrome b_1 reduction was markedly lowered by addition of ubiquinone whereas little difference could be seen with NADH as substrate. These results suggest that Q-2 is required for the oxidation of at least part of the cytochrome b_1 of the succinate oxidase chain but this is not so for the NADH oxidase chain. Similarly, the inhibitor HQNO had only a slight effect on the aerobic steady state level of reduction of cytochrome b_1 by NADH but markedly increased this value with succinate. Thus, with the succinate oxidase chain the effect of HQNO was similar to that produced by ubiquinone deficiency.

We have previously found with respiratory particles that HQNO acts between ubiquinone and cytochrome b_1 in the NADH oxidase chain[9]. A similar placement has been made by JONES[21]. The present results for both the soluble respiratory complex and the respiratory particles do not contradict this result. With succinate the sites of action of Q-2 and HQNO in the soluble respiratory complex appear to be located between oxygen and at least part of the cytochrome b_1. This could mean either that part of the cytochrome b_1 reducible by succinate is on a side-branch or that there is a linear sequence of succinate dehydrogenase, cytochrome b_1, ubiquinone, and cytochrome *o* as has been suggested for an electron transport system solubilized from beef heart mitochondria[22]. COX *et al.*[23] have also suggested that part of the ubiquinone in respiratory particles of *E. coli* is located between cytochrome b_1 and oxygen. However, the effect of HQNO on the aerobic steady state level of reduction of cytochrome b_1 in our preparation of respiratory particles is not sufficiently marked to determine if the suc-

cinate oxidase chain of the intact respiratory particles is similar to that of the soluble respiratory complex or whether the observed sequence in the complex is artifactual.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Medical Research Council of Canada and by the award of a Medical Research Council Postdoctoral Fellowship to R.D.B. We wish to thank Dr. A. F. Wagner of Merck, Sharp and Dohme for a generous gift of Q-0 and Q-2.

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