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ELECTRON TRANSPORT PARTICLES RELEASED UPON LYSIS OF SPHEROPLASTS OF *ESCHERICHIA COLI* B BY BRIJ 58

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SUMMARY

The non-ionic detergent, Brij 58, has been shown to specifically lyse the cytoplasmic membrane of *Escherichia coli*. This communication examines the electron transport system in membrane fractions prepared from such lysates and compares this system to those prepared by mechanical procedures.

1. The particulate fraction contained all of the respiratory carriers demonstrable in whole cells in essentially the same ratios although enriched by 3–5-fold over their concentration in whole cells.

2. Succinate, formate, and NADH but not glutamate, malate, or dihydroorotate were actively oxidized by the particulate fraction.

3. With the exception of the formate oxidase system which appeared to utilize a system bypassing flavoprotein and cytochrome b_1, the other enzymatic activities appeared to function primarily through normal electron transport routes.

4. The NADH oxidase and succinoxidase systems were sensitive to inhibition by 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide and antimycin A. The results suggest that these inhibitors function at the level of cytochrome b_1.

5. All three activities were sensitive to complete inhibition by CN^-.

6. The results obtained from inhibitor studies coupled with the results obtained from examination of steady state, anaerobic, and chemical reduction of the respiratory pigments permitted a scheme for electron flow to be proposed.

INTRODUCTION

The method of cell disintegration not only influences the number and kinds of substrates oxidized[1] but also affects the pathway(s) of electron flow through the respiratory chain[2]. Most studies of bacterial electron transport have been carried out on particulate fractions derived from bacterial suspensions by ballistic or sonic disruption[1]. The present study was designed to examine the electron transport system in membranes prepared by gentle lysis resulting from osmotic shock of spheroplasts.

In the course of these studies we observed that the conversion of lysozyme

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

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spheroplasts to EDTA–lysozyme spheroplasts exposed the cytoplasmic membrane to the environment[3]. This membrane exposure resulted in the susceptibility of these substructures to lysis by the nonionic detergent, Brij 58 (ref. 4). The study described below examines the electron transport machinery of a particulate fraction of *Escherichia coli* B derived from detergent lysis of EDTA–lysozyme spheroplasts. The results indicate that this method of lysis enables the isolation and enrichment of a membrane fraction possessing a functional electron transport system.

MATERIALS AND METHODS

The conditions for growth, spheroplast production and detergent treatment were the same as previously described[3,4]. The procedure for differential centrifugation to obtain subcellular fractions was that of KASHKET AND BRODIE[5].

Cytochrome spectra

Whole cells were aerated 1–2 h at 30° in Tris buffer to decrease endogenous reserves, concentrated by centrifugation, and resuspended in 70% glycerol[7] to 15–20 mg protein per ml. Large and small particle preparations and supernatant fractions were used as prepared or diluted to contain 5–10 mg protein per ml. Difference spectra ($S_2O_4^{2-}$ or substrate-reduced *minus* air oxidized) were determined on a Cary-15 recording spectrophotometer. The CO spectra were determined after bubbling CO through a suspension previously reduced with $S_2O_4^{2-}$. The reference cell was also reduced with $S_2O_4^{2-}$. Concentrations of the electron transport carriers were determined from difference spectra using the following wavelength pairs and extinction coefficients: Flavoprotein 510–465 nm, $E = 11000$ (ref. 8); cytochrome b_1 560–575 nm, $E = 17500$ (ref. 9); and cytochrome a_2 630–615 nm, $E = 8500$ (ref.9).

Polarography

O_2 consumption was measured with a Clark oxygen electrode (Yellow Springs Instruments) polarized with 0.8 V d.c. at 35°. The reaction mixture contained 100 μmoles phosphate buffer (pH 7.5), enzyme, and aerated glass-distilled water to make 1.0 ml. After establishment of an endogeneous rate, substrate (neutralized to pH 7.4) was added to the concentration desired. Rates were recorded as pmoles O_2 consumed per min.

Inhibitors and uncouplers

After determination of the endogenous rate of O_2 consumption polarographically, saturating amounts of substrate were added. When the polarographic trace became linear, the inhibitor or uncoupler was added sequentially at the desired concentrations. Preliminary studies had revealed no differences between sequential and batch additions of inhibitors. The percentage inhibition was calculated from the polarograph traces. In all cases where organic solvents were used the values given were corrected for nonspecific inhibition (or activation).

Steady-state levels of respiratory carriers

The steady-state levels of reduction of the respiratory carriers and the rate of O_2 consumption were determined simultaneously on an Aminco–Chance dual-wave-

length spectrophotometer (American Instrument Co., Silver Spring, Md.) equipped with an Aminco vibrating platinum electrode. The wavelength pairs used were the same as for quantitation of cytochromes from the difference spectra. The levels of reduction were determined during respiration, following anaerobiosis, and after addition of $S_2O_4^{2-}$. Steady-state levels were compared to both enzymatically reducible carrier and total (chemically) reducible carrier. Usually instrument settings of 2 % transmission with a slit of 0.5 mm were used. Measurement of reduced cytochrome a_2 required the 1 % transmission mode.

Oxidative phosphorylation

The method of BRODIE[10] was used to estimate the extent of oxidative phosphorylation. The 100000 × *g* supernatant fluid was either used as such or fractionated by $(NH_4)_2SO_4$ to test for supernatant coupling factors.

Protein determinations

Protein concentration was estimated by either the method of LOWRY *et al.*[11] or the biuret reaction as modified by GORNALL *et al.*[6] with 0.2 % deoxycholate added to decrease the turbidity. Desiccated bovine serum albumin was used as a standard.

Electron microscopy

The techniques for preparation of materials for electron microscopic examination were the same as described previously[3].

Spectrophotometric assays

NADH oxidase was measured by observing the decrease in absorbance at 340 nm with a Beckman DB-G recording spectrophotometer. The reaction mixture contained 100 μmoles phosphate buffer (pH 7.5), 11 μmoles NADH (Calbiochem, Los Angeles, Calif.), and enzyme *plus* glass-distilled water to make 1.0 ml. CN^--insensitive NADH oxidase activity was determined by assaying duplicate samples to which 10 μmoles neutralized KCN were added.

RESULTS

Detergent concentration

The effect of the concentration of Brij 58 on the release and inactivation of NADH oxidase activity (measured spectrophotometrically) from EDTA–lysozyme spheroplasts is shown in Fig. 1. Low concentrations of detergent enhance the activity of NADH oxidase compared to that of the control. As the detergent concentration was increased to 60 μg/mg protein the amount of activity released into the small particle fraction increased. Such concentrations, however, resulted in the loss of activity from the crude lysate. At a detergent concentration of 30 μg/mg protein maximal oxidase activity was released and minimal activity destroyed. Thus, 30 μg/mg protein was selected for all experiments.

Oxidase activities

Cell-free extracts from detergent-treated spheroplasts were able to couple the oxidation of succinate, formate, and NADH to O_2 consumption (Table I).

Substrates such as malate, glutamate, or dihydroorotate were not oxidized, even when concentrated supernatant factors were added to the polarograph vessel. No increase in the rate of oxidation of succinate, formate, or NADH was observed when either dialyzed or lyophilized supernatant fluid was added to the reaction mixture at protein concentrations 4–5-fold greater than that of the particles.

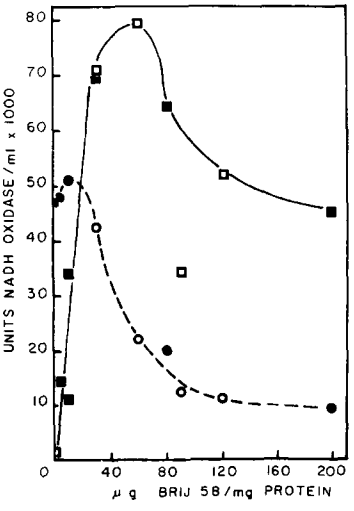

Fig. 1. Effect of Brij 58 concentration on the release and inactivation of NADH oxidase activity. Aliquots of EDTA–lysozyme spheroplast suspensions (12.5 mg protein) were sedimented, resuspended in 15.0 ml Tris–HCl buffer (pH 8.0) containing the desired detergent concentration. After 30 min at 0°, each sample was fractionated as described in MATERIALS AND METHODS and CN^--sensitive NADH oxidase was determined spectrophotometrically. ○, ●, small particle fractions; □, ■, crude lysates; ○, □, Expt. 1; ●, ■, Expt. 2.

TABLE I

AVERAGE SPECIFIC ACTIVITIES AND DISTRIBUTION OF OXIDASE ACTIVITIES IN FRACTIONS FROM DETERGENT-TREATED EDTA–LYSOZYME SPHEROPLASTS

Results are expressed as pmoles O_2 consumed per min per mg protein.

Fraction	*Succinoxidase*		*Formate oxidase*		*NADH oxidase*	
	Specific activity	*Percent of total*	*Specific activity*	*Percent of total*	*Specific activity*	*Percent of total*
Whole cells	25.9		55.2		—	
Crude lysate	38.9	100	55.9	100	66.7	100
Cell-free extract	33.9	27.7*	51.0	38.2	94.8	32.5
Large particles	115.5	26.3	160.5	38.2	241.0	27.1
Small particles	21.5	0.3	20.4	0.3	61.5	1.1
Supernatant fluid	0	0	0	0	0	0

* The remainder of the activity was recoverable in the 15000 × *g* pellet.

Particle fractionation

The average specific activities and distribution of succinoxidase, formate oxidase, and NADH oxidase in various *E. coli* fractions are given in Table I. The

specific activity of the large particle fraction was 2–5 times as large as that of either whole cells or of EDTA–lysozyme spheroplasts. Although only 30–40% of the total activity was found in the cell-free extract, the remainder was recoverable in the 15000 × *g* pellet. While more activity could be released by increasing the detergent concentration, higher concentrations of detergent were avoided to minimize inactivation of oxidase activities (see Fig. 1). Essentially all of the activity found in the cell-free extract was recovered in the large particle fraction. All three activities fractionated in a similar manner.

We were able to demonstrate that particles released by detergent treatment were all sedimentible at 40000 × *g* for 1 h. The amount of activity present in the small particle fraction was dependent upon the method of removal of the 40000 × *g* supernatant fluid. For each activity examined decanting the 40000 × *g* supernatant fluid increased the activity in the small particle fraction by the same increment that the activity associated with the large particle fraction decreased. The oxidase activities of both large and small particles banded at the same place in identical linear sucrose gradients indicating that these activities were associated with similar-sized structures. Thus particles prepared from detergent treatment of EDTA–lysozyme spheroplasts constitute a size distribution that enables complete sedimentation by 40000 × *g* for 1 h. All of the remaining work was carried out on that material sedimenting between 15000 and 40000 × *g*. The 105000 × *g* centrifugation for 5 h was continued to make certain that no particulate material was present in the supernatant fraction.

Cytochrome spectra

The cytochrome spectra of large particles, small particles and supernatant fluid are compared to whole cells in Fig. 2. Absorption maxima at 560, 530 and 430 nm correspond to the α, β, and Soret peaks, respectively, of cytochrome b_1. The cytochrome a_2 peak at 630 nm and the flavoprotein-non-heme iron trough at 465 nm are also evident. These three carriers were the only ones detectable in $S_2O_4^{2-}$-reduced *versus* air oxidized difference spectra. The supernatant fluid of the 105000 × *g* centrifugation was devoid of absorption maxima characteristic of any of these carriers. Neither *c*-type cytochromes nor cytochrome a_1 were detected. Difference spectra obtained form CO–$S_2O_4^{2-}$-reduced *minus* $S_2O_4^{2-}$-reduced samples demonstrated the presence of cytochrome *o* with the characteristic Soret peak at 416 nm and α and β peaks at 536 and 560–570 nm. The ratio of the respiratory pigments of the large particle fraction (1.75:1.00:0.45 flavoprotein-non-heme iron proteins: cytochrome b_1:cytochrome a_2) was essentially the same as that of the whole cells. The large particle fraction were 3–5-fold higher in these carriers than were the whole cell suspensions.

Steady-state levels of the respiratory carriers

The levels of reduction were determined during respiration, following anaerobiosis, and after the addition of $S_2O_4^{2-}$. Steady-state levels were compared to both enzymatically reducible carrier and total (chemically) reducible carrier. Table II lists the levels of reduction of flavoprotein-non-heme iron proteins and cytochromes b_1 and a_2 during steady-state respiration and following anaerobiosis with the three substrates given above as the electron donors. Succinate and formate resulted in

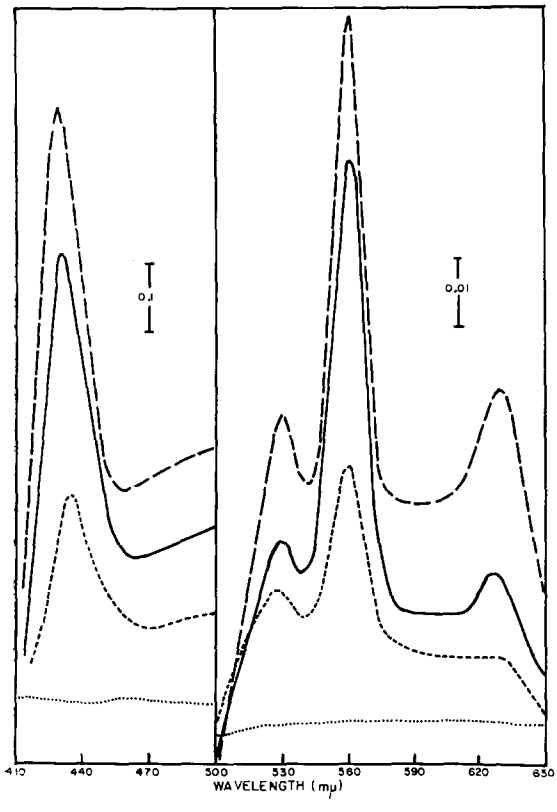

Fig. 2. Cytochrome spectra of whole cells, large particles, small particles, and supernatant fractions. Whole cells, 19.0 mg protein per ml; large particles, 7.7 mg protein per ml; small particles, 10.0 mg protein per ml; and supernatant fluid, 2.0 mg/ml. ——, whole cells; – – – –, large particles; - - - - -, small particles; ·····, supernatant fluid.

TABLE II

THE LEVELS OF REDUCTION OF THE RESPIRATORY PIGMENTS OF LARGE PARTICLES DURING RESPIRATION OF SUCCINATE, FORMATE, AND NADH

Substrate	*Number of determinations*	*Steady-state percent enzymatically reduced*	*Steady-state percent total reduced*	*Anaerobic percent total reduced*
Cytochrome b_1				
Succinate	11	9.7	5.2	53.1
Formate	14	9.1	4.6	52.7
NADH	18	16.5	5.4	28.3
Flavoprotein				
Succinate	8	30.9	13.6	46.0
Formate	6	31.7	9.3	31.9
NADH	7	23.7	6.5	28.3
Cytochrome a_2				
Succinate	2	10.5	10.8	94.3
Formate	3	—	17.5	90.0
NADH	4	26.6	27.7	75.1

the same degree of reduction of cytochrome b_1 although enzymatic reduction of flavoprotein-non-heme iron proteins was greater with succinate as the electron donor. The anaerobic reduction of both flavoprotein and cytochrome b_1 was substantially less for NADH respiration than for either succinate or formate respiration. None of the three substrates was capable of reducing greater than 50–55 % of the total carrier present in the preparations. The three substrates added sequentially were never able to reduce greater than 75–85 % of the total flavoprotein or cytochrome b_1. In addition, regardless of the pair of substrates examined or the sequence of their addition, the amount of reduction of cytochrome b_1 upon anaerobiosis did not equal the sum of the individual levels of reduction. This was especially true when the third substrate was added. The discrepancies between the sequential values and the calculated values suggest that an overlap of the three pathways exists at the level of cytochrome b_1. It was also noted that both succinate and formate were capable of reducing 'bound' NAD^+ following anaerobiosis.

Effects of inhibitors

The effects of five known inhibitors of electron transport on the respiration of succinate, formate, and NADH by large particle preparations are summarized in Table III. The values presented are averages of two separate preparations. All

TABLE III

EFFECT OF RESPIRATORY INHIBITORS ON THE OXIDATION OF SUCCINATE, FORMATE, AND NADH BY LARGE PARTICLES

These values are averages of two separate preparations. One contained 306 μg protein per reaction mixture, the other 386 μg protein per reaction mixture. Rates were measured polarographically. Additions were sequential.

Inhibitor	*Concn.*	*Succin-oxidase*	*Formate oxidase*	*NADH oxidase*
KCN (mM)	2	12.9	100	38.8
	4	32.5	—	81.9
	6	72.0	—	94.7
	8	88.5	—	97.8
	10	95.6	—	99.0
N_3^- (mM)	20	26.7	97.5	8.9
	40	42.0	100	31.9
	60	53.6	—	38.1
	80	64.0	—	53.5
	100	74.0	—	70.8
HOQNO (μg/ml)	2	14.1	−2.0	43.4
	4	21.7	−2.0	52.8
	6	29.0	3.5	66.9
	8	32.6	0.5	68.0
	10	20.3	1.4	63.0
Antimycin A (μg/ml)	2	5.2	−1.1	5.9
	4	16.6	−2.3	10.3
	6	17.3	0.9	21.0
	8	21.2	2.9	25.3
	10	20.3	1.4	25.5
Amytal (mM)	2	4.8	24.1	1.7
	4	6.2	36.7	8.8
	6	12.3	46.8	15.8
	8	13.3	53.7	34.3
	10	33.4	63.2	37.6

three activities were completely inhibited by low concentrations of CN^- and, to a lesser extent, by N_3^-. 2 *n*-Heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) at low concentrations inhibited NADH oxidase activity 60 %, succinoxidase activity 20 %, and stimulated formate oxidase activity slightly. Antimycin A inhibited both succinoxidase and NADH oxidase to the same extent with maximal inhibition (20 %) at a concentration of between 10 and 15 μg/mg protein. As was the case for HOQNO, formate oxidase activity was slightly stimulated by low concentrations of antimycin A and slightly inhibited at higher concentrations. Some inhibition of formate oxidase was noted at 0.02–0.04 M amytal, but only at high concentrations was any effect observed for the other oxidase activities. That amount of inhibition noted after addition of 8–12 μmoles was most likely non-specific as protein precipitation was observed at concentrations above 0.01 M.

Effect of inhibitors during steady-state respiration

The effect of HOQNO on the level of reduction of cytochrome b_1 during the respiration of NADH and succinate was examined in the Aminco–Chance-dual-wavelength spectrophotometer. Although the addition of 9 μg HOQNO per mg protein inhibited NADH oxidation 65 %, the level of reduction of cytochrome b_1 remained unchanged. HOQNO produced the same effect on succinoxidase activity. The level of reduction remained unchanged although O_2 consumption was inhibited by 42 %. Although the inhibition by antimycin A was not as marked as that observed for HOQNO, its effect on the steady-state levels of reduction of cytochrome b_1 was the same as that of HOQNO.

As noted previously, formate oxidase activity was considerably more sensitive to CN^- and N_3^- inhibition than the other oxidase systems. Results obtained in the dual-wavelength spectrophotometer suggested that the pathway of electron flow from formate to O_2 was *via* a different route than the other activities. The particle preparations were capable of very rapid O_2 consumption during succinate and NADH oxidation in the presence of sufficient N_3^- to inhibit formate oxidase 98 %. The effect of N_3^- inhibition on the levels of reduction of the respiratory carriers indicated that the alternate pathway for formate oxidation originated prior to flavoprotein.

Oxidative phosphorylation

Two sets of experiments designed to examine whether or not phosphorylation could be coupled to succinate oxidation were carried out. No conditions were found in which measurable phosphorylation was observed. The addition of dialyzed or $(NH_4)_2SO_4$-fractionated supernatant fluid was without effect. Washing the particle preparation did not result in phosphorylation. The relatively low yields of material (approx. 75 mg protein per preparation) did not allow an exhaustive search for conditions for phosphorylation to be carried out.

DISCUSSION

We have previously reported[4] that Brij 58 only produces lysis of EDTA–lysozyme spheroplasts. Brij 58 treatment of EDTA–lysozyme spheroplasts released the electron transport chain and several oxidase systems into a form which was non-

sedimentible at 15000 × *g* but was sedimentible at 40000 × *g*. The content and ratio of respiratory pigments present in whole cells is enriched 3–5-fold in this large particle fraction. The presence of an enzymatically active small particle fraction was found to be spurious and to result from an imprecise separation of the 40000 × *g* supernatant fluid from the large particle fraction.

Electron microscopic examination of the 'particle' fractions released by detergent lysis revealed a heterogeneous mixture of membrane fragments. Relatively few workers have examined the ultrastructure of their bacterial electron transport particles[12–15]. They invariably report the presence of heterogeneous mixtures of membrane fragments.

Succinate, formate, and NADH were actively oxidized by particles derived from EDTA–lysozyme spheroplasts by the action of Brij 58 (Table I). The oxidases of these substrates were susceptible to inhibitors of terminal respiration (N_3^- and CN^-; see Table III) and utilized O_2 as the terminal electron acceptor, indicating that the particles contained a functional electron transport system. The particles were not capable of oxidation of glutamate, dihydroorotate, or malate. The activity of these particles can be contrasted to that of particles derived from *E. coli* by sonication[16,17]. Particles prepared by extensive sonication[17] oxidized succinate very slowly (1/30 of the rate of detergent-released preparations) and NADH at about 1/2 the rate of detergent-treated preparations. Only 55% inhibition of NADH oxidase was noted upon addition of CN^-. In contrast to the other systems obtained from sonicated *E. coli* described above, that of KASHKET AND BRODIE[5] was completely inhibited by CN^-. Adding supernatant fluid to their particles enhanced succinoxidase activity 4-fold[5]. Adding dialyzed supernatant fluid to particles prepared by detergent treatment did not alter the rate of O_2 consumption with succinate, formate or NADH as the electron donor. Large and small particles prepared by KASHKET AND BRODIE[5] differed in their enzymatic activities and in their quinone content. Fractionation techniques identical to those used by KASHKET AND BRODIE did not yield two kinds of particles. As previously demonstrated, that activity found in the small particle fraction was derived from the large particle fraction.

HOQNO has had variable effectiveness when applied to particles derived from mechanical breakage of bacteria. In their original description of this inhibitor, LIGHTBROWN AND JACKSON[18] reported that reduction of cytochrome b_1 was blocked in cell-free preparations of *E. coli*. Our results support this observation. The three oxidative pathways examined in detergent-treated preparations vary in their sensitivity to HOQNO from slight stimulation (formate oxidase) to 70% inhibition of O_2 consumption (NADH oxidase). It is not known whether this differential effect is due to inherent differences in the pathways (different quinones as described by KASHKET AND BRODIE[20]) or to changes introduced during detergent treatment and subsequent fractionation. Both the NADH oxidase and succinoxidase activities were similarly inhibited by antimycin A (Table III). Since both HOQNO and antimycin A appear to inhibit electron transport prior to cytochrome b_1 in those systems lacking a *c*-type cytochrome and appear to affect different pathways to different degrees, it would be of interest to examine the steady-state levels of quinones under the effect of these inhibitors. In general, all of the carriers which were examined in the dual-wavelength spectrophotometer were reduced during steady-state respiration (*i.e.* when O_2 consumption was linear) (Table II). These values agree quite

well with those reported by ASANO AND BRODIE[21] for particles derived from *Mycobacterium phlei*. Although the respiratory carriers present are quite different in the two organisms, the maximum reducible levels of the carriers during steady-state reduction and following anaerobiosis with single and multiple substrates are similar. The total anaerobically reducible cytochrome *b* from *M. phlei* was 73 % compared to 75–85 % for the *E. coli* cytochrome b_1. All of the chemically reducible carrier present was not available for enzymatic reduction.

Although it is not possible to determine the exact routes of electron transfer or the degree of participation by each respiratory enzyme in particles derived by the action of detergent, the data available do allow some conclusions to be made and suggestions to be proposed. Fig. 3 is a schematic representation of probable routes of electron flow from succinate, formate, and NADH to O_2. Since no quantitative data were available for the levels of cytochrome *o* in the preparations, its concentration is assumed to be the same as that of cytochrome a_2. The two oxidases are assumed to function similarly in the following discussion. A third necessary assumption is that there are two species of oxidase components, one which is sensitive to low concentrations of inhibitors (high sensitivity), and another which requires higher concentrations for maximal inhibition (low sensitivity).

The suggestion that there are two different species of cytochrome oxidase comes primarily from studies of formate respiration. At azide concentrations which inhibited 98 % of the formate oxidase activity, the activity of NADH oxidase and

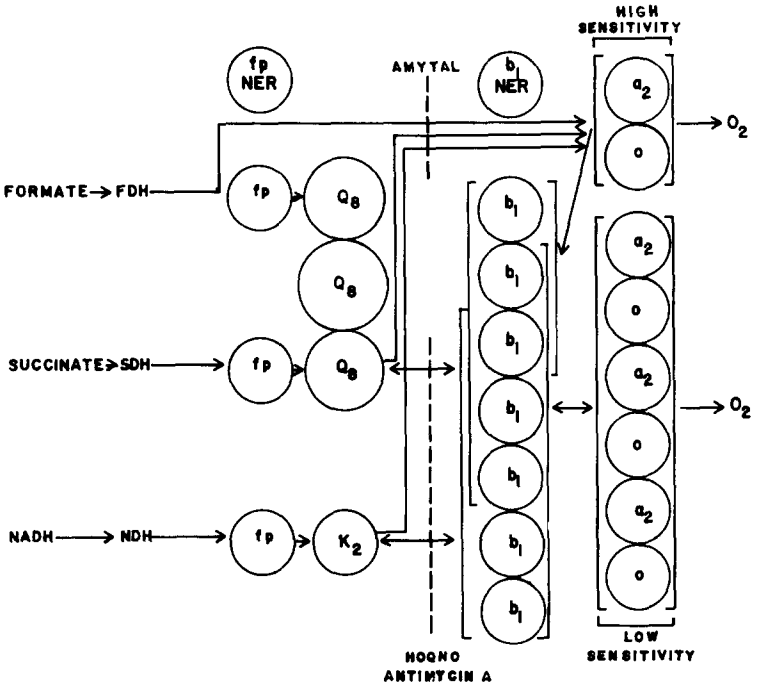

Fig. 3. Schematic representation of electron flow and sites of action of respiratory inhibitors on particles derived by detergent action on EDTA–lysozyme spheroplasts. See text for description. Abbreviations: FDH, formate oxidase; SDH, succinoxidase; NDH, NADH oxidase; fp, flavoproteins; NER, non-enzymatically reducible.

succinoxidase were inhibited only slightly. Formate oxidation was approx. 10-fold more sensitive to CN^- and N_3^- than were either NADH or succinate oxidation (Table III). The complete inhibition of all three pathways by N_3^- and particularly CN^- (Table III) indicates that bypass enzymes, if functioning in the particulate system, ultimately transfer electrons to O_2 through CN^--sensitive cytochrome oxidases.

The formate oxidase system appears to function entirely through a bypass system utilizing the high sensitivity oxidases for electron transfer to O_2. The lack of further reduction of flavoprotein in the presence of an N_3^- block suggests that the bypass originates before the level of flavoprotein. Amytal inhibition is placed on this bypass pathway since only the formate oxidase pathway is sensitive to low concentrations of this inhibitor (Table III).

If the sensitivity to low concentrations of N_3^- and/or CN^- and to low concentrations of amytal can be used as a measure of the degree of functioning of the bypass system, then neither the succinoxidase nor the NADH oxidase pathway utilize this bypass to any substantial degree. Both NADH and succinate oxidation failed to produce cytochrome b_1 reduction in the presence of HOQNO alone or antimycin A alone. Based on this observation which confirms the suggestion by LIGHTBROWN AND JACKSON[18] and BONGERS[19], the site of action of HOQNO and antimycin A has been placed at the level of cytochrome b_1.

The inclusion and placement of quinones in Fig. 3 is based on data from other workers[5]. The sensitivity of the NADH oxidase and succinoxidase systems to inhibitors of cytochrome oxidase as well as to HOQNO and antimycin A suggests that electron flow is as described in Fig. 3. Furthermore, demonstrations of flavoprotein reduction and cytochrome b_1 reduction during steady-state oxidation suggest that these are functional, supporting the argument for the integrity of this pathway.

The non-oinic detergent, Brij 58 has been shown to release the electron transport system in a relatively intact structure. The specificity of this detergent for the cytoplasmic membrane[4] may aid a variety of studies where an assay for the exposure of the cytoplasmic membrane is needed and in studies similar to the one presented here where it is desired to degrade the membrane system to smaller units with minimal destruction of preexisting functions.

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