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Myeloperoxidase-Mediated Inhibition of Microbial Respiration: Damage to *Escherichia coli* Ubiquinol Oxidase†

Robert M. Rakita, Bryce R. Michel, and Henry Rosen*

Department of Medicine, University of Washington, and Swedish Hospital Medical Center, Seattle, Washington 98104

Received October 12, 1988; Revised Manuscript Received December 9, 1988

ABSTRACT: A microbicidal system, mediated by neutrophil myeloperoxidase, inhibits succinate-dependent respiration in *Escherichia coli* at rates that correlate with loss of microbial viability. Succinate dehydrogenase, the initial enzyme of the succinate oxidase respiratory pathway, catalyzes the reduction of ubiquinone to ubiquinol, which is reoxidized by terminal oxidase complexes. The steady-state ratio of ubiquinol to total quinone (ubiquinol + ubiquinone) reflects the balance between dehydrogenase-dependent ubiquinone reduction and terminal oxidase-dependent ubiquinol oxidation. Myeloperoxidase had no effect on total quinone content of *E. coli* but altered the steady-state ratio of ubiquinol to total quinone. The ratio doubled for organisms incubated with the myeloperoxidase system for 10 min, suggesting decreased ubiquinol oxidase activity, which was confirmed by observation of a 50% decrease in oxidation of the ubiquinol analogue 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinol. Despite inhibition of ubiquinol oxidase, overall succinate oxidase activity remained unchanged, suggesting that succinate dehydrogenase activity was preserved and that the dehydrogenase was rate limiting. Microbial viability was unaffected by early changes in ubiquinol oxidase activity. Longer (60 min) exposure of *E. coli* to the myeloperoxidase system resulted in only modest further inhibition of the ubiquinol oxidase, but the ubiquinol to total quinone ratio fell to 0%, reflecting complete loss of succinate dehydrogenase activity. Succinate oxidase activity was abolished, and there was extensive loss of microbial viability. Early myeloperoxidase-mediated injury to ubiquinol oxidase appeared to be compensated for by higher steady-state levels of ubiquinol which sustained electron turnover by mass effect. Later myeloperoxidase-mediated injuries eliminated succinate-dependent ubiquinone reduction, through inhibition of succinate dehydrogenase, with loss of succinate oxidase activity, effects which were associated with, although not clearly causal for, microbicidal activity.

The microbicidal effects of neutrophils and monocytes are mediated, in part, by myeloperoxidase, a granule-bound enzyme that is released into the phagosome and extracellular space during phagocytosis (Klebanoff & Clark, 1978). Myeloperoxidase, together with phagocyte-derived hydrogen peroxide and chloride, generates hypochlorous acid (Harrison & Schultz, 1976) and derivative oxidants (Thomas, 1979) that mediate a potent microbicidal effect. The microbicidal mechanisms of the oxidants are incompletely understood. Multiple sites of microbial injury have been described, most of them located in the microbial cytoplasmic membrane [for review see Hurst and Barrette (1989)]. Among these is the loss of succinate-dependent respiration mediated by the succinate oxidase system (Rosen et al., 1987) which is comprised of several components that vary depending on growth conditions. Under appropriate conditions, the electron-transfer pathway can be schematized as (Lorence et al., 1987; Ingledew & Poole, 1984; Poole & Ingledew, 1987; Pudek & Bragg, 1974)

$$\text{succinate} \xrightarrow{\text{succinate dehydrogenase}} Q_8 \xrightarrow{\text{cytochrome } d \text{ complex}} O_2$$

Myeloperoxidase rapidly inactivates the succinate dehydrogenase component (Rosen et al., 1987). Because of this inactivation, succinate cannot be employed as a probe of the

† These investigations were supported by U.S. Public Health Service Grants AI 25606, AI 07763, and AI 07744 and by a grant from the Cystic Fibrosis Foundation.

* Address correspondence to this author at the Department of Medicine, Swedish Hospital Medical Center, 747 Summit Ave., Seattle, WA 98104.

0006-2960/89/0428-3031$01.50/0

functional integrity of the latter components of the chain, Q_8[1] and cytochrome *d*.

Q_8 is a simple molecule that is relatively resistant to myeloperoxidase-derived oxidants (Albrich et al., 1981; Rosen et al., 1987). Its spectroscopic and chromatographic integrities (Rosen et al., 1987) argue strongly for its functional integrity. The cytochrome *d* complex is, on the other hand, an intricate heterotetramer, made up of two different polypeptide chains containing two protoheme IX groups in different microenvironments and two heme *d* groups (Miller et al., 1988). Spectral integrity of such a complicated structure does not assure functional integrity, and an approach was sought to evaluate the ubiquinol oxidase activity of the terminal oxidase complex. The steady-state level of reduced Q_8 was used to assess the balance of electron flow toward (succinate–ubiquinone reductase activity) and away from (ubiquinol oxidase activity) the quinone. Further, a reduced quinone analogue was used as a substrate to probe the ubiquinol oxidase activity of the cytochrome *d* complex. A defect in ubiquinol oxidase activity was identified and compared to myeloperoxidase-mediated changes in succinate oxidase activity as well as to microbicidal activity.

Experimental Procedures

Special Reagents. Myeloperoxidase was prepared from canine pyometrial pus by the method of Agner (1958) to the end of step 6 and assayed by the guaiacol method (Klebanoff et al., 1984). One unit of enzyme is the amount utilizing 1 μmol of H_2O_2/min at 25 °C. Glucose oxidase (type V from *Aspergillus niger*), phenazine methosulfate, dichlorophenolindophenol, deoxyribonuclease I, and ribonuclease A (type 1-a) were obtained from Sigma Chemical Co., St. Louis, MO. Stock solutions of these reagents were prepared and stored as previously described (Rosen et al., 1987).

Synthesis of 2,3-Dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DB) (Wan & Folkers, 1978). Diundecanoyl peroxide was prepared from undecanoyl chloride by warming 18.6 g of undecanoic acid (100 mmol, Aldrich Chemical Co.) with 8.7 mL of thionyl chloride (120 mmol) for 3 h at 40 °C, with subsequent removal of HCl under vacuum (Cason, 1955). The acyl chloride was dissolved in 175 mL of diethyl ether, mixed with 8.7 mL of 30% hydrogen peroxide, and cooled in an ice bath; 9.7 mL of pyridine (120 mmol) was added dropwise, with stirring, while the temperature of the solution was maintained between 0 and 5 °C, and the mixture was stored overnight at −20 °C. Crystals of diundecanoyl peroxide were recovered by filtration (Silbert & Swern, 1959).

Diundecanoyl peroxide (3.04 g, 8.2 mmol) in 20 mL of glacial acetic acid was added dropwise over 4 h to a solution of 750 mg (4.1 mmol) of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (Q_0, Sigma) in 50 mL of glacial acetic acid stirred under nitrogen at 95 °C. The mixture was stirred for an additional 20 h at 90 °C, and the solvent was removed by vacuum distillation. Residue was dissolved in 15 mL of chloroform and flash chromatographed (Still et al., 1978) on a 3 × 15 cm column of silica gel 60 (Merck No. 9385) equilibrated with chloroform and sequentially eluted with 200 mL of chloroform, 200 mL of chloroform–methanol (1:1), and 200 mL of methanol. Quinone-containing fractions were identified by analytical thin-layer chromatography (silica gel C_{18} reverse-phase plates, Baker No. 7013-0, with methanol as solvent) and concentrated by evaporation at room temperature, and the oily residue was applied to preparative silica gel thin-layer chromatography plates (Merck No. 5766) and developed twice with chloroform. DB was eluted from the plate with absolute ethanol, and homogeneity was determined by analytical reverse-phase thin-layer chromatography, as described above. Yield was 35%, on the basis of Q_0. The product was stored at −20 °C in ethanol, protected from light.

Concentration of DB was estimated spectrophotometrically, assuming E_{275} = 15.1 mM^{-1} cm^{-1} (Morton, 1965). Reduction with sodium borohydride produced a shift in the absorbance maximum to 289 nm with E_{289} = 4.3 mM^{-1} cm^{-1}. Reduced DB (DBH_2) used for determination of ubiquinol oxidase activities was used at stock concentrations of 18.6–20.5 mM.

DBH_2 was prepared by a modification of the method described by Hatefi (1978). A total of 0.3 mL of DB in absolute ethanol was mixed in a glass-stoppered tube with 3.0 mL of ethanol and 3.3 mL of water and reduced with 1–2 mg of sodium borohydride. Upon disappearance of the yellow color of oxidized quinone, 0.5 g of KCl was added, followed by 2 mL of hexane, with vigorous agitation for 30 s. After separation of phases, the upper hexane phase was removed, and the aqueous phase was reextracted with 2 mL of hexane. The combined hexane extracts were evaporated under a stream of nitrogen and dissolved in 0.5 mL of methanol containing 2.5 μL of 1 N HCl to retard oxidation. Oxidized DB, used as a control for ubiquinol oxidase assays, was prepared following the same procedure omitting the sodium borohydride.

Microorganisms. Escherichia coli (ATCC 11775, American Type Culture Collection, Rockville, MD) were maintained on blood agar plates and grown for 5–8 h on a rotating shaker in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD). One milliliter of broth culture was inoculated into 1 L of medium A [7 g of K_2HPO_4, 3 g of KH_2PO_4, 0.5 g of sodium citrate dihydrate, 0.1 g of $MgSO_4 \cdot 7H_2O$, 1 g of $(NH_4)_2SO_4$, 1 g of sodium succinate] (Rosen & Klebanoff, 1982; Davis & Mingioli, 1950) which was magnetically stirred and continuously aerated at 37 °C for 14–20 h. Organisms were harvested in stationary phase by centrifugation, washed twice with 0.1 M Na_2SO_4, and suspended in 0.1 M Na_2SO_4/0.05% gelatin to the required absorbance at 540 nm. Stock suspensions were diluted 10-fold in the final reaction mixture. Viability of *E. coli* was determined by the pour-plate method using trypticase soy agar (Rosen & Klebanoff, 1982).

Myeloperoxidase-Mediated Oxidation of E. coli. Nonenzymatic components indicated in the legends to the figures were prewarmed in 125-mL Erlenmeyer flasks in an oscillating water bath at 37 °C, and *E. coli* were added approximately 1 min prior to initiation of the reaction with myeloperoxidase and glucose oxidase. The final reaction volume was 55 mL. At indicated intervals, samples were removed, and the reaction was stopped by the addition of 0.001 volume of 0.1 M sodium azide, followed by immersion of the sample in an ice bath. The *E. coli* were pelleted by centrifugation at 4 °C for 10 min at 8000*g* and washed once with 30 mM Tris·HCl, pH 8.0.

Bacterial Sonicates. A total of 3.3 × 10^{10} *E. coli* were suspended in 5.0 mL of 30 mM Tris·HCl, pH 8.0, containing 10 μg/mL deoxyribonuclease, 10 μg/mL ribonuclease, and 10 mM $MgSO_4$ and sonicated with the microtip of either a Branson 200 sonicator (Branson Instruments, Inc., Stamford, CT) in pulse mode, 50% duty cycle, power setting of 7, or a Microson ultrasonic cell disruptor MS-25 (Heat Systems Ultrasonics, Inc., Farmingdale, NY) for 5 min with cooling in an ice bath. After 20 min of further incubation, sonicated bacteria were collected by centrifugation at 45000*g* for 30 min at 4 °C.

[1] Abbreviations: Q_n, ubiquinone *n*, where *n* designates the number of isoprene subunits in the long side chain; Q_nH_2, quinol of Q_n; DB, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone; DBH_2, quinol of DB; DCIP, dichlorophenolindophenol.

Quinone Content and Oxidation State. A total of 1.65×10^{11} myeloperoxidase-oxidized *E. coli* were suspended in 0.6 mL of 30 mM Tris·HCl, pH 8.0, and sonicated as described above. A 0.5-mL aliquot of sonicate was incubated with 20 mM sodium succinate for 10 min at 37 °C, and quinones were extracted with methanol–petroleum ether (3:2) as previously described (Rosen et al., 1987). Q_8 and Q_8H_2 were determined by high-pressure liquid chromatography using a Q_6 internal standard (Rosen et al., 1987).

Oxygen Consumption. Bacterial sonicates were suspended in 30 mM Tris·HCl, pH 8.0, to a protein concentration of 0.5–1.0 mg/mL and depleted of endogenous substrate by incubation with 5 mM sodium 2,4-dinitrophenol for 3 h at 37 °C in a rapidly oscillating water bath (Rosen, 1986). O_2 consumption was determined with a Clarke-type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) prior to and following stimulation with 33 mM succinate or with 300–340 μM DBH_2. Results were expressed as nanomoles of O_2 consumed per minute per milligram of protein, on the basis of a concentration of 215 μM O_2 in air-saturated buffer at 37 °C (Dean, 1979).

Succinate–Dichlorophenolindophenol (DCIP) Reductase Assay. E. coli sonicates were suspended in 30 mM Tris·HCl, pH 8.0, at 25 °C to a concentration of approximately 50 μg of protein/mL. Aliquots of 950 μL of *E. coli* membranes, 10 μL of 2 M sodium succinate, and 15 μL of DCIP (4.65 mM, 1.35 mg/mL) were combined in a 1.5-mL disposable spectrophotometer cuvette, and base-line absorbance was determined. DCIP reduction was initiated by the addition of 25 μL of phenazine methosulfate (65 mM, 20 mg/mL), and the initial reduction rate was determined with 21 mM^{-1} cm^{-1} for the extinction coefficient of oxidized DCIP at 600 nm (Hatefi & Stiggall, 1978). Results are expressed as nanomoles of DCIP reduced per minute per milligram of protein.

Proteins. Protein concentrations were determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

Statistics. Results are expressed as the mean ± SE of *n* experiments, and group means were compared with Student's two-tailed *t* test.

Results

Incubation of *E. coli* with myeloperoxidase, glucose, glucose oxidase, and chloride resulted in loss of microbial viability (Figure 1a), which was not observed when chloride was replaced by isosmolar sulfate. The microbicidal effect was not detected after 10-min exposure to the myeloperoxidase system but was significant at 15 min ($p < 0.05$) and was complete at 30 min. At the intervals indicated in Figure 1b, samples of *E. coli* were pelleted, sonicated, and incubated for 10 min at 37 °C with 20 mM sodium succinate. Oxidized and reduced Q_8 were determined by high-pressure liquid chromatography. The total Q_8 content for *E. coli* ranged between 0.7 and 2.1 nmol/mg of protein (1.3 ± 0.6, mean ± SD, $n = 39$) and did not vary systematically with duration of incubation or between the control and complete myeloperoxidase systems. For example, Q_8 content prior to incubation with the complete myeloperoxidase system averaged 1.3 ± 0.3 nmol/mg of protein (mean ± SD, $n = 4$) compared to 1.6 ± 0.03 ($n = 2$) after 60 min of incubation.

At the beginning of each set of incubations, the steady-state fraction of reduced Q_8 ranged between 27 and 41% (33 ± 2%, mean ± SE), and these values did not change significantly in control preparations throughout the 60-min incubation (Figure 1b). For organisms incubated with the complete myeloperoxidase system, the fraction of reduced Q_8 remained stable

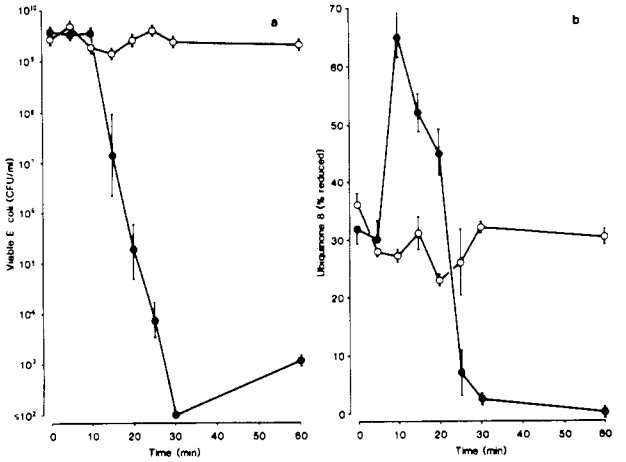

FIGURE 1: Microbial viability and steady-state levels of reduced quinone following incubation with the myeloperoxidase antimicrobial system. The complete myeloperoxidase system (●) contained (per milliliter) 5×10^9 *E. coli*, 0.27 unit of myeloperoxidase, 0.22 unit of glucose oxidase, 0.1 M NaCl, 0.04 M sodium acetate, pH 5.0, 0.01 M glucose, 0.01 M Na_2SO_4, and 0.05 mg of gelatin. In the control system (○), NaCl was replaced by isosmolar Na_2SO_4. At the indicated times, samples were removed for determination of microbial viability (20-μL samples, panel a), and the remainder of the reaction mixture (55 mL) was pelleted, washed, and sonicated. Sonicates were incubated for 10 min at 37 °C with 20 mM succinate, and quinones were extracted and quantitated as described under Experimental Procedures. The steady-state fraction of reduced Q_8 is shown in panel b. Results are the means ± SE of two to four experiments at each time point. CFU: colony forming units.

at 5 min and then doubled to 65 ± 4% at 10 min (Figure 1b) when the organisms were fully viable (Figure 1a). At 15 and 20 min the fraction of reduced Q_8, 52 ± 3% and 45 ± 4%, respectively, declined but remained above the initial value. Thereafter, the fraction of reduced Q_8 fell below the initial value, reaching values of 7 ± 4% at 25 min, 2 ± 1% at 30 min, and 0.3 ± 0.4% at 60 min.

The rise in the steady-state fraction of Q_8 reducible by succinate at 10 min suggested either stimulation of succinate–ubiquinone reductase activity or, more likely, inhibition of ubiquinol oxidase activity. Ubiquinol oxidase activity was determined as ubiquinol-dependent oxygen consumption, with the artificial substrate 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (DB). DB is an alkyl analogue of Q_2 which has been used effectively in quinone-dependent assays of mitochondrial electron transport (Wan et al., 1975). The shorter side chains of Q_2 and DB facilitate the use of these lipids in heterogeneous aqueous–hydrophobic systems.

An illustrative ubiquinol oxidase determination is described in Figure 2. Sonicates were prepared from *E. coli* incubated for 60 min with either the control or complete myeloperoxidase systems as described in the figure legend and were depleted of endogenous substrate by incubation with the uncoupling agent 2,4-dinitrophenol. The stable base line in Figure 2a (control *E. coli*, viability 3.7×10^9 colony forming units/mL) is consistent with effective substrate depletion. Addition of 25 μL of methanol to 1.5 mL of *E. coli* particles produced a small, unexplained, but consistent upward deflection in the tracing. Further addition of 25 μL of 360 mM DB (in methanol) produced only the upward deflection characteristic of methanol alone. Repeating this experiment with 25 μL of 320 mM DBH_2 instead of DB (Figure 2b) indicated that the initial methanol-induced upward deflection was just discernible before it was overwhelmed by the downward deflection of ubiquinol-induced oxygen consumption [165 nmol min^{-1} (mg of protein)$^{-1}$]. Figure 2c reflects succinate oxidase activity

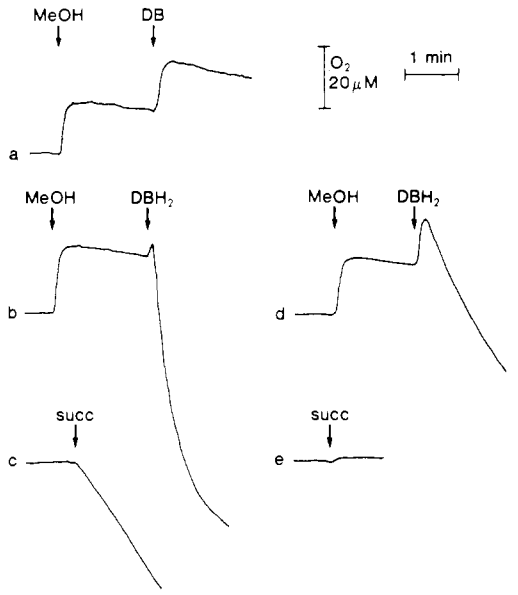

FIGURE 2: Succinate and ubiquinol oxidase activities of electron-transport particles from myeloperoxidase-treated *E. coli*. *E. coli* were incubated for 60 min with the control (tracings a–c) or complete (tracings d and e) myeloperoxidase systems described in Figure 1, except that the glucose oxidase concentration was 0.33 unit/mL. Microbial viability for the complete system was <100 colony forming units/mL, compared to 3.7×10^9 for the control. Bacterial sonicates (1.0–1.1 mg of protein/mL) were prepared and depleted of endogenous substrate as described under Experimental Procedures. Oxygen consumption was determined following the addition of methanol (MeOH), 357 μM oxidized DB in methanol (DB), 318 μM reduced DB in methanol (DBH_2), or 33 mM succinate (succ).

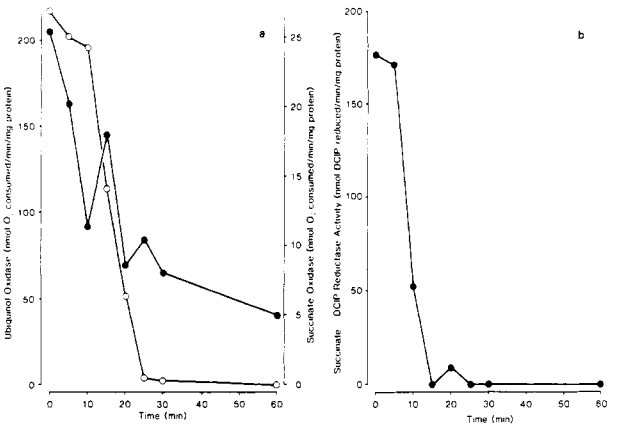

FIGURE 3: Myeloperoxidase-mediated damage to ubiquinol and succinate oxidase activities and succinate–DCIP reductase activity in *E. coli*. At the indicated times, samples of *E. coli* incubated with the complete myeloperoxidase system described in Figure 2 were removed for determination of ubiquinol (●) and succinate (○) oxidase activities (panel a) as well as succinate–DCIP reductase activity (panel b). Results are the means of two to five experiments per data point.

of the preparation. Upon addition of 50 μL of aqueous 1.0 M sodium succinate to electron-transport particles, there was a simple downward deflection of succinate-induced oxygen consumption [23 nmol min^{-1} (mg of protein)$^{-1}$], without any methanol effect.

Tracings d and e of Figure 2 represent ubiquinol oxidase and succinate oxidase activities of sonicates prepared from *E. coli*, which had been incubated with the complete myeloperoxidase system (viability <100 colony forming units/mL). The addition of DBH_2 (Figure 2d) resulted in a methanol effect followed by readily detectable ubiquinol oxidase activity [32 nmol min^{-1} (mg of protein)$^{-1}$, 20% of control]. In contrast, succinate oxidase activity (Figure 2e) was completely absent in this preparation.

The succinate concentration employed in this experiment was saturating; that is, higher concentration of substrate did not further increase the rate of O_2 consumption. Such was not the case with the ubiquinol, where increasing the DBH_2 concentration substantially increased O_2 consumption (data not shown).

Ubiquinol oxidase and succinate oxidase activities were determined for sonicates prepared from *E. coli* incubated for 0–60 min with the complete myeloperoxidase system (Figure 3). Microbial viability and succinate–DCIP reductase activities were also determined. The rate of microbicidal activity was similar to that described in Figure 1 (data not shown).

Loss of ubiquinol oxidase activity was apparent within 10 min of incubation with the myeloperoxidase system, with rates of O_2 consumption 45 ± 10% (n = 3) of the initial value. Thereafter, inactivation of ubiquinol oxidase proceeded at a slower rate, so that at 60 min of incubation 20 ± 2% (n = 5) of initial activity remained. As previously described (Rosen et al., 1987), succinate oxidase was inactivated with a different kinetic pattern. Activity remained nearly constant through the first 10 min of incubation (91 ± 38% of initial value) after which it fell rapidly to reach nearly undetectable levels by 25 min. The decline in ubiquinol oxidase activity and preservation of succinate oxidase activity at 10 min corresponded to the doubling in steady-state levels of reduced Q_8 (Figure 1b).

In contrast to succinate oxidase activity, which was largely unaffected at 10 min, succinate–DCIP reductase activity, commonly taken as a measure of succinate dehydrogenase activity, fell to 30 ± 16% of the initial value and was nearly undetectable thereafter.

Discussion

Electron flow in the *E. coli* succinate oxidase pathway is sequential from succinate through succinate dehydrogenase, Q_8, one of two terminal cytochrome complexes, designated *d* and *o*, and, finally, to oxygen (Lorence et al., 1987; Ingledew & Poole, 1984; Poole & Ingledew, 1987; Pudek & Bragg, 1974). Under growth conditions employed in this study, the cytochrome *o* complex is at low to undetectable levels, and the electron-transport chain contains the cytochrome *d* complex as the exclusive terminal oxidase (Pudek & Bragg, 1974).

Figure 4 uses the analogy of water flow to describe electron flow through the *E. coli* succinate oxidase system before and after modification by myeloperoxidase-derived oxidants. Succinate availability corresponds to the pressure head in the system. Succinate dehydrogenase corresponds to the flow valve on the spigot. The size of the bucket represents the size of the total ubiquinone pool, which remains constant [this paper and Rosen et al. (1987)]. The fluid level in the bucket represents the fraction of ubiquinone that is in the reduced form. The outflow tube represents the cytochrome *d* terminal oxidase, and the rate of outflow corresponds to the rate of oxygen consumption.

For sonicates obtained from *E. coli* prior to incubation with the myeloperoxidase system (Figure 4a), electron flow through succinate dehydrogenase is maximal. The balance between ubiquinone reduction (flow into the bucket) and ubiquinone oxidation (flow out of the bucket) is such that the reduced ubiquinone pool is 30% of the total, corresponding to the zero time observation in Figure 1b.

Following 10 min of incubation with the myeloperoxidase system (Figure 4b), we assume that succinate dehydrogenase activity is unchanged. The 50% reduction in ubiquinol oxidase activity, noted in Figure 3a, 10-min time period, is reflected

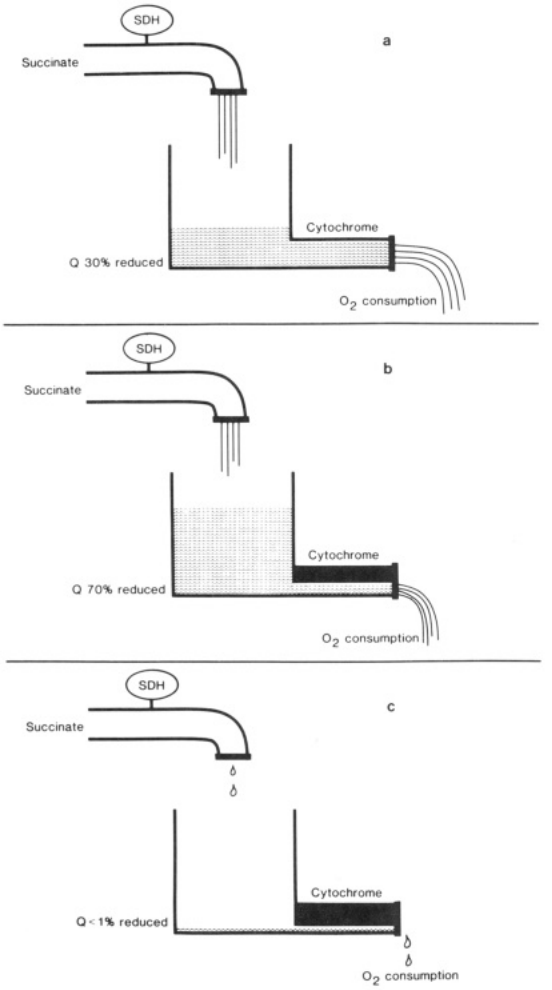

FIGURE 4: Schematic of myeloperoxidase-mediated changes in the *E. coli* succinate oxidase. For details, see text.

by the diminished aperture of the effluent tube. As a consequence, the balance between ubiquinone reduction and oxidation shifts, with a resultant doubling in the amount of steady-state reduced ubiquinone, consistent with the observation at 10 min in Figure 1b. The accumulation of reduced ubiquinone compensates for the decreased activity of the cytochrome *d*–ubiquinol oxidase, with the result that the net flow of electrons to oxygen is unchanged, reflected in the figure by an unchanged outflow of water from the bucket and consistent with the observation in Figure 3a that succinate oxidase activity is minimally decreased at 10 min.

After 60 min of incubation with the myeloperoxidase systems (Figure 4c), succinate dehydrogenase has been completely inactivated, as reflected by the closed spigot. Thus, there is no further reduction of ubiquinone. Meanwhile, the cytochrome *d*–ubiquinol oxidase retains 20% of its original activity, as described in Figure 3a, and oxidizes all residual ubiquinol to ubiquinone. As a consequence, the ubiquinone pool is entirely in the oxidized state, as described in Figure 1b, and oxygen consumption stops, as described in Figure 3a.

The scheme described in Figure 4 assumes that the rate-limiting step for the succinate oxidase system is the dehydrogenase component. The basis for this view is 2-fold. The rate of succinate-driven respiration at saturating levels of succinate is one-eighth that of ubiquinol-driven respiration, even when ubiquinol levels are nonsaturating (Figure 3a). Further, at saturating levels of succinate, the steady-state fractions of reduced Q_8 and cytochrome *d* are 30% (Figure 1b) and 20% (Rosen et al., 1987), respectively, suggesting that neither ubiquinol nor cytochrome oxidation is rate limiting. Similar observations and conclusions have been made by Lorence et al. (1987).

The idea that the succinate dehydrogenase remains fully active after 10 min of exposure to the myeloperoxidase system is consistent with the finding that overall succinate oxidase activity is intact (Figure 3a). It is at variance, however, with the finding that succinate–DCIP reductase activity is diminished by 70% (Figure 3b). These differences are unreconciled at present and await further investigations for resolution. It may be that succinate–DCIP reductase activity represents a parallel pathway of electron flow through the succinate dehydrogenase which is more susceptible to the oxidizing action of myeloperoxidase but does not contribute directly to electron transport through the oxidase system. The precise mechanism of succinate dehydrogenase inactivation by the myeloperoxidase system has been not established. While prior studies have demonstrated rapid, generalized destruction of microbial iron–sulfur centers by myeloperoxidase-derived oxidants (Rosen & Klebanoff, 1985), the effects of myeloperoxidase on the specific iron–sulfur centers of *E. coli* succinate dehydrogenase remain to be characterized.

The cytochrome *d* terminal oxidase of the succinate oxidase system is coupled to proton translocation across the membrane (Koland et al., 1984) and therefore contributes to the protonmotive force believed to drive metabolic functions (Rottenberg, 1986). The terminal oxidase contains two copies of each of two different polypeptide chains. Each of one identical pair of peptides contains a heme *d*, while the other pair of peptides is each associated with a protoheme IX (Miller et al., 1988). The heme *d* groups appear, by spectroscopic criteria, to be in identical environments, while the two protoheme IX groups, although associated with identical peptide chains, are in distinct environments having different absorption maxima, one at 558 nm and the other at 595 nm. Despite an 80% impairment of ubiquinol oxidase activity in the cytochrome *d* complex following incubation with the myeloperoxidase system for 60 min, the dithionite-reduced spectrum of the cytochromes is intact (Rosen et al., 1987), suggesting that the damage to the cytochrome complex is subtle. The ubiquinol recognition site of the cytochrome *d* complex is associated with the cytochrome *b*-558 component and appears to be located at the extracellular surface of the cytoplasmic membrane (Kranz & Gennis, 1984; Gennis, 1986). Damage to the exposed ubiquinol recognition site seems an excellent candidate mechanism for explaining functional impairment of the cytochrome without notable alteration of the spectral properties of the complex. Cytochrome *d* terminal oxidase modified by brief (10 min) exposure to the myeloperoxidase-mediated antimicrobial system may prove useful for identifying, more precisely, the ubiquinol binding site of the *b*-558 complex.

The establishment of causal connections between loss of succinate oxidase activity and myeloperoxidase-mediated microbicidal activity remains a challenge. As previously shown (Rosen et al., 1987) and confirmed in this study, there is a strong relationship ($r = 0.97$) between the logarithms of microbial viability and succinate oxidase activity. At the same time, there is a strong linear relationship between loss of succinate–DCIP reductase activity and loss of microbial viability ($r = 0.98$). These correlations are consistent with a role for loss of electron transport as one mechanism of the myeloperoxidase-mediated microbicidal event(s). The possibility remains that the oxidations observed in the succinate oxidase system are parallel and not contributory to the microbicidal events. Nevertheless, they serve as prototypes for under-

standing the modification of native membrane structures by myeloperoxidase-derived oxidants and may be useful as markers for evaluating the activity of myeloperoxidase within the complex environment of the neutrophil phagocytic vacuole.

Registry No. Myeloperoxidase, 9003-99-0; succinate oxidase, 9014-35-1; succinate dehydrogenase, 9002-02-2; ubiquinol oxidase, 69671-26-7.

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