Differential Inactivation of *Escherichia coli* Membrane Dehydrogenases by a Myeloperoxidase-Mediated Antimicrobial System†

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ABSTRACT: Neutrophil myeloperoxidase, hydrogen peroxide, and chloride constitute a potent antimicrobial system with multiple effects on microbial cytoplasmic membranes. Among these is inhibition of succinate-dependent respiration mediated, principally, through inactivation of succinate dehydrogenase. Succinate-dependent respiration is inhibited at rates that correlate with loss of microbial viability, suggesting that loss of respiration might contribute to the microbicidal event. Because respiration in *Escherichia coli* can be mediated by dehydrogenases other than succinate dehydrogenase, the effects of the myeloperoxidase system on other membrane dehydrogenases were evaluated by histochemical activity stains of electrophoretically separated membrane proteins. Two bands of succinate dehydrogenase activity proved the most susceptible to inactivation with complete loss of staining activity within 20 min, under the conditions employed. A group with intermediate susceptibility, consisting of lactate, malate, glycerol-3-phosphate, and dihydroorotate dehydrogenases as well as three bands of glucose-6-phosphate dehydrogenase, was almost completely inactivated within 30 min. The relatively resistant group, including the dehydrogenases for glutamate, NADH, and NADPH and the remaining bands of glucose-6-phosphate dehydrogenase, retained substantial amounts of diaphorase activity for up to 60 min of incubation with the myeloperoxidase system. The differential effects of myeloperoxidase on dehydrogenase inactivation could not be correlated with published enzyme contents of flavin or iron–sulfur centers, potential targets of myeloperoxidase-derived oxidants. Despite the relative resistance of NADH dehydrogenase/diaphorase activity to myeloperoxidase-mediated inactivation, electron transport particles prepared from *E. coli* incubated for 20 min with the myeloperoxidase system lost 55% of their NADH oxidase activity. Generalized direct inactivation of microbial respiratory dehydrogenase is not a satisfactory explanation for myeloperoxidase-mediated microbicidal activity. Myeloperoxidase-derived oxidants may, however, uncouple dehydrogenases from downstream components of the respiratory chain, thereby contributing to an overall loss of respiratory activity.

Myeloperoxidase is a major component of the microbicidal armamentarium of neutrophils and monocytes. Cell-free systems composed of myeloperoxidase, H_2O_2, and an oxidizable halide cofactor, such as chloride, possess potent microbicidal activity against diverse microorganisms including bacteria, fungi, and viruses (Klebanoff & Clark, 1978). Although the mechanisms of microbicidal action are not fully defined, it has been determined that loss of microbial iron (Rosen & Klebanoff, 1982), destruction of membrane iron–sulfur clusters (Rosen & Klebanoff, 1985), oxidation of sulfhydryl groups (Thomas & Aune, 1978), impaired transmembrane nutrient transport (Albrich et al., 1986), and, possibly, altered cytoplasmic membrane permeability (Albrich et al., 1986; Sips & Hamers, 1981) are all associated with loss of microbial viability.

A recently active area of investigation has been the characterization of myeloperoxidase-mediated effects on microbial bioenergetics. Among the effects that have been observed are rapid hydrolysis of ATP to ADP and AMP (loss of energy charge) (Barrette et al., 1987), abolition of ATP production, inactivation of membrane F_0F_1 ATPase/ATP synthetase (Barrette et al., 1989), and loss of succinate- and glucose-dependent microbial respiration (Rosen et al., 1987). Many of the foregoing investigations have used an implicit kinetic argument: events that precede the loss of microbial viability are considered to be possible contributors to the microbicidal effect, while events that occur later are irrelevant.

The succinate-dependent respiratory system of *Escherichia coli* (succinate oxidase), which is inactivated by the myeloperoxidase-mediated antimicrobial system, contains two major protein clusters: succinate dehydrogenase (succinate:ubiquinone reductase) and a cytochrome *d* complex (ubiquinol oxidase). Succinate dehydrogenase is the only component of the succinate oxidase system to be inactivated at a rate sufficient to consider its inactivation to be a possible contributor to microbicidal activity (Rosen et al., 1987). The function of succinate dehydrogenase as a catalyst for ubiquinone reduction can also be assumed by many other dehydrogenases, including those for NADH, glycerol-3-phosphate, and lactate (Poole & Ingledew, 1987).

If loss of respiration through dehydrogenase inactivation is a factor in the loss of microbial viability, other dehydrogenases must also be inactivated at rates comparable to that of succinate dehydrogenase. Using nondenaturing polyacrylamide and agarose gel electrophoresis and histochemical activity stains, we investigated the kinetics of inactivation of these other dehydrogenases after exposure to the myeloperoxidase system.

Materials and Methods

Special Reagents. Chemicals were obtained from Sigma Chemical Co., St. Louis, MO, except for acrylamide and bis(acrylamide) National Diagnostics, Mannville, NJ) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED)[1] and aga-

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rose (standard low molecular weight) (Bio-Rad Laboratories, Richmond, CA), and were stored as previously described (Rakita et al., 1989).

Purification of Myeloperoxidase. The starting material, generously supplied by Dr. Dennis Hickstein (University of Washington, Seattle, WA), consisted of the insoluble residues from Nonidet NP-40 extracted leukocytes obtained from a patient with chronic myelogenous leukemia (Hickstein et al., 1987). The purification protocol was adapted from methods described by Merrill (1980) and Morita et al. (1986). Approximately 50 mL of dark green-brown residue, which had been stored for several months at −70 °C, was thawed in a water bath and blended for 90 s at room temperature with 150 mL of 1% cetyltrimethylammonium bromide (CTAB) in 100 mM sodium phosphate, pH 7.0. The homogenate was centrifuged, and the pellet was reextracted 5 times by vortexing with 50 mL of 0.05% CTAB in 50 mM sodium phosphate, pH 7.0. Extracts were combined and made 1 mM in Mg, Mn, and Ca, as the chlorides. The solution became slightly turbid and was clarified by rapid filtration through Whatman 1 filter paper. Concanavalin A bound to Sepharose gel (Sigma) was added (10 mL of gel per 100 mL of filtrate), and the mixture was stirred for several hours at 4 °C to allow binding of myeloperoxidase to the immobilized lectin. During this procedure, the A_{430} of the filtrate fell from 0.53 to 0.02. The intensely green gel was allowed to settle overnight at 4 °C, and the supernatant was decanted and discarded. The gel was resuspended in 100–150 mL of column wash buffer (0.1 M sodium acetate, pH 6.0, 0.1 M NaCl, and 0.05% CTAB) and poured into a 3.2-cm diameter column giving a column height of 3 cm. The column was washed at room temperature with 1 L of wash buffer at 10 mL/min, causing the A_{280} of the eluate to fall from 2.18 to <0.10. Myeloperoxidase was eluted by the addition of 0.5 M methyl α-D-mannoside to the wash buffer with an elution rate of 1 mL/min. Eluates were collected in 10-mL fractions until the A_{430} fell below 0.03. Combined eluates were brought to 85% saturation with solid ammonium sulfate, and the precipitated myeloperoxidase was dissolved in a minimal amount of 0.1 M NaCl/0.025 M sodium acetate, pH 4.7. The redissolved precipitate was applied to a 3.2 × 40 cm Sephacryl S-200 (Pharmacia) column and eluted at 4 °C with 0.1 M NaCl/0.025 M sodium acetate, pH 4.7, supplemented with 0.05% CTAB. Myeloperoxidase-containing fractions, detected by their green color, were combined, brought to 70% saturation with ammonium sulfate, and stirred for 2 h at 4 °C. The precipitate was dissolved in a minimal amount of distilled water and dialyzed extensively against water at 4 °C. The dialyzed enzyme was stored in small portions at −20 °C. Purity was estimated by the ratio of absorbances at 430 and 280 nm, which was 0.80, near the values of 0.82–0.83 for the crystalline enzyme (Morita et al., 1986; Agner, 1958). Enzymatic activity was estimated by the guaiacol method (Klebanoff et al., 1984).

Microorganisms. E. coli (ATCC 11775, American Type Culture Collection, Rockville, MD) were maintained on blood agar plates, grown in medium A, a minimal salts medium containing succinate as the sole carbon source, and harvested as previously described (Rosen et al., 1987). Viability of *E. coli* was determined by the pour-plate method using trypticase soy agar (Klebanoff et al., 1984).

Myeloperoxidase-Mediated Oxidation of E. coli. Nonenzymatic components indicated in the legends to figures were prewarmed in 250-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 110 mL. At indicated intervals, samples were removed, and the reaction was stopped by the addition of 0.01 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 0.1 M sodium phosphate buffer, pH 7.0.

Bacterial Sonicates. E. coli were suspended in 2.5 mL of 0.1 M sodium phosphate buffer, pH 7.0, containing 10 μg/mL deoxyribonuclease, 10 μg/mL ribonuclease, 10 mM $MgSO_4$, and 1 mM phenylmethanesulfonyl fluoride and sonicated by using the microtip of a Microson ultrasonic cell disruptor MS-25 (Heat Systems Ultrasonics, Inc., Farmingdale, NY), at 40% power, for 5 min with cooling in an ice bath. Sonicates were collected by centrifugation at 48000*g* for 30 min at 4 °C and stored at −70 °C overnight.

The pellets were thawed, suspended in 0.8 mL of 50 mM Tris, 4% Triton X-100, and 5 mM EDTA, pH 8.6, and sonicated at a 30% power setting for 1 min with cooling in an ice bath. Extracts were centrifuged at 8000*g* for 5 min, and the pellets were discarded. Extracts were used immediately for succinate dehydrogenase detection with agarose gels or were refrozen at −70 °C overnight.

Electrophoresis. Nondenaturing, nonreducing gel electrophoresis was performed using 7% polyacrylamide running gels (16 × 14 cm) and 3% polyacrylamide stacking gels, and the buffer systems of Laemmli (1970), modified by replacing sodium dodecyl sulfate throughout with 0.1% (w/v) Triton X-100. Samples of bacterial membrane extracts to be used for polyacrylamide gel electrophoresis were made dense with 5% glycerol (v/v), and 0.001% bromphenol blue was used as a tracking dye. Ten micrograms of protein was loaded per lane. Electrophoresis was carried out at 4–10 °C, 15 mA per gel, for 3–3.5 h.

Agarose gels were prepared by using 1% agarose in 0.025 M Tris/0.192 M glycine/1% Triton, with 0.025 M Tris/0.192 M glycine buffer in both electrode chambers. Samples were made dense with 5% Ficoll (w/v), and 10 μg of protein was loaded per lane. Electrophoresis was carried out at 4–10 °C, 180 V, for 2–3 h.

Histochemical Staining. Gels were stained for dehydrogenase activity by incubation in the dark at 37 °C, with gentle agitation, in solutions containing the appropriate substrate and the electron acceptor tetranitroblue tetrazolium. Where indicated, phenazine methosulfate was added as an electron carrier, and NAD or NADP were added as cofactors. Our procedures differed from those previously published (Owen, 1981) in that 100-mL total volumes were used, except for dihydro-L-orotate dehydrogenase (78 mL) and succinate dehydrogenase (200 mL). Lithium DL-lactate was used instead of the D-lactate enantiomorph, and NADH and NADPH were added directly in the solid form.

Staining times ranged from 20 min for malate dehydrogenase to 3–4 h for dihydroorotate and glucose-6-phosphate dehydrogenases. Reactions were stopped prior to maximal staining in order to detect differences in the staining rates among the different preparations. Prolonging staining time beyond 4 h merely increased nonspecific background staining. Stained gels were scanned with an LKB 2202 Ultrascan laser densitometer (LKB-Produkter AB, Bromma, Sweden), and integrated peak areas were determined. Because of the staining variability, a sample from bacteria removed

[1] Abbreviations: TEMED, *N,N,N′,N′*-tetramethylethylenediamine; CTAB, cetyltrimethylammonium bromide, ETP, electron transport particle.

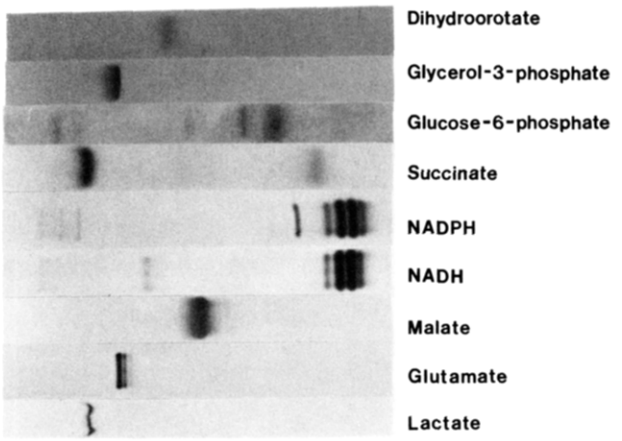

FIGURE 1: *E. coli* dehydrogenase activity in polyacrylamide and agarose gels. The complete myeloperoxidase system contained (per milliliter) 3×10^9 *E. coli*, 0.27 unit of myeloperoxidase, 0.37 unit of glucose oxidase, 0.1 M NaCl, 0.04 M sodium acetate, pH 5.0, 0.01 M glucose, 0.01 M Na_2SO_4, and 0.05 mg of gelatin. At $t = 0$, 20-mL samples were removed, pelleted, washed, sonicated, electrophoresed, and histochemically stained with the indicated substrate as described under Materials and Methods. All running gels were 7% polyacrylamide, except for succinate, which was 1% agarose. The top of the gel is to the left.

immediately from the reaction mixture was included in all gels, and enzyme activity was expressed as the percent of the densitometric peak area at $t = 0$.

Proteins. Protein concentrations were determined by using the bicinchoninic acid method (Hinson & Webber, 1988), using bovine serum albumin as standard.

Oxygen Consumption. Electron transport particles (ETPs) were prepared from *E. coli* exposed to the complete or control myeloperoxidase systems indicated in the legend to Table I. *E. coli* from the entire volume were pelleted, washed with 30 mM Tris, pH 8.0, and used immediately or stored at −70 °C. Pellets were resuspended in 2 mL of 30 mM Tris, pH 8.0, and sonicated with a Tekmar TM 300 sonicator at a power setting of 3, 20% pulse duty cycle, for 30 min with cooling in an ice bath. Unbroken bacteria were removed by centrifugation at 10000*g* for 10 min, and ETPs were collected by centrifugation at 48000*g* for 30 min.

ETPs were suspended in 30 mM Tris, pH 8.0, to a protein concentration of 0.1–0.4 mg/mL, and oxygen consumption was determined with a Clarke-type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) prior to and following stimulation with 31 mM sodium succinate, 62 mM DL-lactate, 62 mM glycerol 3-phosphate, or 0.94 mM NADH in 30 mM Tris, pH 8.0. 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).

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

RESULTS

Figure 1 is a composite of histochemically stained membrane proteins from *E. coli* that had been immediately removed ($t = 0$) from the myeloperoxidase reaction mixture described in the figure legend. Samples were pelleted, sonicated, and extracted with Triton/EDTA. Extracts were electrophoresed on polyacrylamide or agarose gels and stained for dehydrogenase activities. Single bands of dehydrogenase activity were observed when the substrates were dihydroorotate, glycerol-3-phosphate, malate, and lactate, while several closely spaced bands were visible with glutamate. Two bands of

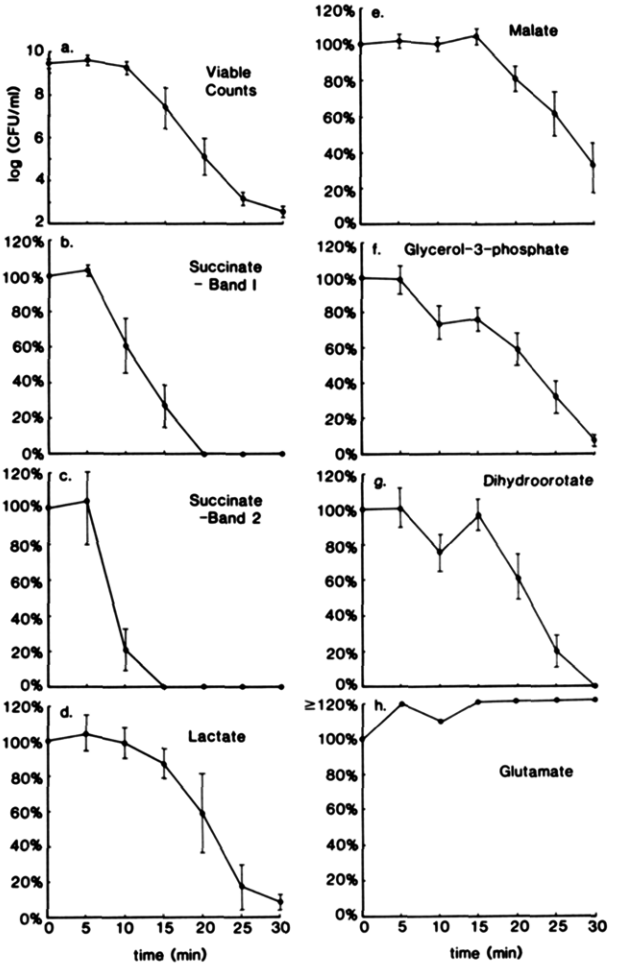

FIGURE 2: Microbial viability and dehydrogenase activities after incubation with the myeloperoxidase antimicrobial system. *E. coli* were incubated with the complete myeloperoxidase system described in Figure 1 or a control system (data not shown), in which NaCl was replaced by isosmolar Na_2SO_4. At the indicated times, samples were removed for determination of microbial viability (60-μL samples, panel a) and dehydrogenase activity (20-mL samples). The 20-mL samples were pelleted, washed, sonicated, electrophoresed, histochemically stained for dehydrogenase activity of succinate (panels b and c), lactate (panel d), malate (panel e), glycerol-3-phosphate (panel f), dihydroorotate (panel g), and glutamate (panel h), and scanned as described under Materials and Methods. Results, expressed as the percent of the densitometric peak area at $t = 0$, are the means ± SE of two to five experiments at each time point.

succinate dehydrogenase activity were present, the upper (band 1) much more prominent than the lower (band 2). Multiple bands of dehydrogenase activity for glucose-6-phosphate, NADH, and NADPH were present, and four bands were seen to have both NADH and NADPH diaphorase activity.

Incubation of *E. coli* with myeloperoxidase, glucose, glucose oxidase, and chloride resulted in loss of microbial viability (Figure 2, panel a), which was not observed when chloride was replaced by isosmolar sulfate (data not shown). The microbicidal effect was not detected by 10-min exposure to the myeloperoxidase system, but was significant at 15 min ($p < 0.05$) and was nearly complete at 30 min.

Comparison of enzyme activity to time of exposure to the myeloperoxidase system revealed three distinct patterns of enzyme susceptibility to inactivation. The two bands of succinate dehydrogenase had a rapid decline in activity (Figure 2, panels b and c). Significant loss of activity for both bands was present after 10-min exposure and had dropped to undetectable levels after 20 and 15 min for bands 1 and 2, respectively. The second group of dehydrogenases, consisting

Table I: Effect of Myeloperoxidase on *E. coli* Oxidase Activities[a]

substrate	oxidase act. control	oxidase act. complete system	oxidase act. % inactivation	diaphorase act. % inactivation
succinate, 31 mM	269 ± 30[b]	36 ± 17	88 ± 5	100 ± 0
lactate, 62 mM	54 ± 8	17 ± 6	66 ± 11	42 ± 21
α-glycerophosphate, 62 mM	23 ± 4	9 ± 3	58 ± 16	41 ± 6
NADH, 0.94 mM	118 ± 8	52 ± 10	55 ± 8	≤8 ± 10

[a] ETPs were prepared from *E. coli* incubated for 20 min with the control or complete myeloperoxidase systems described in Figure 2, except that 55-mL volumes were used, the myeloperoxidase concentration was 0.16–0.25 unit/mL, and the glucose oxidase concentration was 0.23 unit/mL. Diaphorase activities were calculated from the data in Figure 2. Geometric mean microbial viabilities were 1.1×10^9 for the control and 2.4×10^6 for the complete system. Oxygen concentration was determined with a Clarke-type O_2 electrode using substrates at the indicated concentrations. Results are the mean ± SE of five experiments for oxidase activity and three experiments for diaphorase activity. [b] Nanomoles of O_2 consumed per minute per milligram of protein.

of those for lactate (panel d), malate (panel e), glycerol-3-phosphate (panel f), dihydroorotate (panel g), and three bands of glucose-6-phosphate dehydrogenase (data not shown), had a slower rate of inactivation, with significant loss of activity first noted at 10 min for glycerol-3-phosphate and at 20–25 min for all others. However, all were nearly undetectable by 30 min. The third group, including dehydrogenases for glutamate (panel h), NADH, and NADPH and the other bands of glucose-6-phosphate dehydrogenase (data not shown), was relatively insensitive to the myeloperoxidase system.

Inactivation of the dye-reducing, or diaphorase, activities of the dehydrogenases would presumably be reflected by a similar loss of competence to participate in respiratory metabolism. However, the converse condition, uncoupling of the dehydrogenase from the respiratory chain with retention of diaphorase activity, remained a possibility. Further, for dehydrogenases with multiple bands of activity, it was not possible to determine which band was actually coupled to respiration. Thus, electron transport particles were prepared and characterized for their ability to utilize substrates selected from each of the above three groups for the reduction of molecular oxygen. Oxidase activities were determined as oxygen consumption after stimulation with succinate, lactate, glycerol-3-phosphate, or NADH (Table I). After 20-min exposure to the complete myeloperoxidase system, succinate oxidase activity fell by 88 ± 5%. The other three oxidases were not as sensitive: lactate oxidase activity declined by 66 ± 11%, glycerol-3-phosphate oxidase by 58 ± 16% ($p < 0.05$ vs succinate), and NADH oxidase by 55 ± 8% ($p < 0.01$ vs succinate). Viabilities were 1.14×10^9 and 2.40×10^6 colony forming units/mL for the control and complete systems, respectively.

Discussion

Detailed investigations of the inactivation of *E. coli* succinate oxidase by myeloperoxidase-dependent systems have indicated that succinate dehydrogenase is the most vulnerable link in this respiratory chain (Rosen et al., 1987; Rakita et al., 1989). Loss of succinate oxidase activity can be correlated with loss of microbial viability, and it is reasonable to consider whether the two phenomena are causally related. The terminal respiratory components, ubiquinone-8 and ubiquinol oxidase, are common elements to each *E. coli* respiratory chain. Substrate specificity is conferred by dehydrogenases unique to each pathway. At least nine primary dehydrogenases have been described (Poole & Ingledew, 1987). Inactivation of the dehydrogenase for just succinate should not inhibit respiration in nutrient-rich media if alternative dehydrogenases remain active. Thus, myeloperoxidase-mediated effects on these other dehydrogenase complexes were evaluated.

Respiratory dehydrogenases are notable for flavin and iron-centered cofactors that presumably contribute to the electron transport functions of the enzyme complexes. These sites represent potential targets of attack for myeloperoxidase-derived oxidants. Flavins have previously been considered resistant to myeloperoxidase-derived oxidants on the basis of the resistance to bleaching by HOCl of the fluorescent yellow isoalloxazine ring of riboflavin (Albrich et al., 1981). However, the physiologic flavin cofactors FMN and FAD contain in addition to the isoalloxazine ring ribose (FMN and FAD) and adenine (FAD) adducts. Adenine is highly susceptible to chemical modification by HOCl (Albrich et al., 1981), and we have observed that treatment of FAD with low concentrations of HOCl results in the disappearance of fluorescent FAD with the appearance of new fluorescent products. FMN is unchanged under the same conditions, suggesting that the site of modification is the nonfluorescent adenine ring (unpublished observation). On this basis, it would be expected that dehydrogenase enzymes might be susceptible to myeloperoxidase-mediated inactivation on the basis of their content of FAD but not FMN.

Similarly, myeloperoxidase-mediated killing of *E. coli* is associated with rapid, extensive destruction of iron–sulfur centers (Rosen & Klebanoff, 1985) and surface sulfhydryl groups (Thomas & Aune, 1977, 1978; Albrich et al., 1981). It would therefore be expected that membrane proteins with critical iron–sulfur components or essential sulfhydryls be among those highly susceptible to myeloperoxidase-mediated inactivation.

Covalently linked FAD is a component of succinate dehydrogenase and fumarate reductase (Condon & Owen, 1982), a closely related enzyme that is probably band 1 in our succinate-stained agarose gels (Figure 1 and unpublished data). Noncovalently bound FAD is a feature of lactate (Futai, 1973; Kaczorowski et al., 1978), glycerol-3-phosphate (Weiner & Heppel, 1972), and NADH dehydrogenases (Gutman et al., 1968; Jaworowski et al., 1981). The membrane-associated malate oxidase contains FAD (Narindrasorasak et al., 1979), but the nature of the FAD–peptide bond is uncertain.

The content of FAD cofactors did not correlate with loss of dehydrogenase/diaphorase activity under conditions employed in the present study. As previously observed, succinate dehydrogenases were rapidly inactivated at rates that corresponded to loss of microbial viability. At the other extreme, however, the NADH dehydrogenases exhibited marked resistance to inactivation of diaphorase activity. A distinguishing feature of the succinate dehydrogenases, compared to the more resistant dehydrogenases, is the covalent nature of the FAD–peptide bond. It has recently been determined that covalent linkage of the FAD cofactor is an absolute requirement for the succinate dehydrogenase activity of *E. coli* fumarate reductase (Blaut et al., 1989). Possibly, oxidation of covalently bound FAD results in inactivation of the susceptible enzyme whereas noncovalently bound FAD can be replaced, after oxidation, by intact cofactors from a nonoxidized cytoplasmic pool, thus preserving the functional integrity of the resistant dehydrogenases. At present, however, there is no direct evidence of FAD oxidation in intact *E. coli* by myeloperoxidase-derived oxidants.

Iron–sulfur centers are found in *E. coli*, in association with both isoenzymes of succinate dehydrogenase (Condon et al., 1985; Cole et al., 1985), with some but not all preparations

of dihydroorotate dehydrogenase (Larsen & Jensen, 1985; Kerr & Miller, 1968), and with at least one NADH dehydrogenase (Meinhardt et al., 1989; Gutman et al., 1968; Matsushita et al., 1987), although the issue of whether the iron–sulfur NADH dehydrogenase contributes to respiration is controversial (Owen et al., 1980a; Owen, 1981; Meinhardt et al., 1989). In addition to the better characterized iron–sulfur dehydrogenases, NADPH and glutamate dehydrogenases contain non-heme iron that has not been further characterized (Owen et al., 1980b). We feel that the data for iron–sulfur respiratory dehydrogenases is too scant to support conclusions about the role of iron–sulfur centers in respiratory inactivation by the myeloperoxidase antimicrobial system.

It appears that *global* oxidation of dehydrogenase FAD or iron–sulfur centers by myeloperoxidase-derived oxidants is not a suitable explanation for the loss of dehydrogenase/diaphorase activity. Indeed, if cofactor oxidation is important at all, it must occur in a discriminant fashion, attacking cofactors in some enzymes and sparing the same cofactors in others.

Alternatively, cofactors inactivated by the myeloperoxidase system might not be required for dehydrogenase/diaphorase activity but might still be important for linking the dehydrogenase to the rest of the respiratory chain. Accordingly, membrane oxidase activities for succinate, lactate, glycerol-3-phosphate, and NADH were determined for *E. coli* incubated for 20 min with the myeloperoxidase system (Table I). The dehydrogenase components of these respiratory chains spanned the range of susceptibility to myeloperoxidase inactivation from highly susceptible (succinate), to intermediate (lactate and glycerol-3-phosphate), to highly resistant (NADH). Each oxidase was inhibited, although to different degrees. Succinate oxidase was the most sensitive, with 88% inactivation, while the activities for lactate, glycerol-3-phosphate, and NADH oxidases dropped by 66%, 58%, and 55%, respectively. For succinate, lactate, and glycerol-3-phosphate, loss of oxidase activity corresponded well to the loss of histochemical staining. In contrast, none of the bands of NADH dehydrogenase seen in gel staining appeared to be inhibited by myeloperoxidase-derived oxidants, in contrast to the 55% inactivation of NADH oxidase activity.

The substantial loss of NADH oxidase activity with almost complete preservation of dehydrogenase activity is problematic. The decline in oxidase activity might reflect lesions downstream in the respiratory chain. A likely target would be the ubiquinol oxidase, which we have previously shown to be 60% inactivated after 20 min of exposure to the myeloperoxidase system (Rakita et al., 1989). This interpretation may be too facile, however, since the rate-limiting link in the respiratory chain is usually the dehydrogenase and not the ubiquinol oxidase (Lorence et al., 1987). Indeed, we have observed almost complete preservation of succinate oxidase activity, as long as the dehydrogenase is intact, despite a 50% decrease in ubiquinol oxidase activity (Rakita et al., 1989). Thus, loss of NADH oxidase activity may well reflect uncoupling of the respiratory NADH dehydrogenase from the remainder of the chain in a fashion not detectable by evaluation of diaphorase activity in polyacrylamide gels.

The data presented here confirm that the myeloperoxidase system has multiple effects at the microbial cell membrane. Effects on microbial respiratory dehydrogenases are diverse and do not afford a unifying explanation for a mechanism of myeloperoxidase-mediated bactericidal activity. The pattern of dehydrogenase inactivation does not support an obvious role for global FAD and iron–sulfur oxidation in these events. Inhibition of respiration remains a significant feature of myeloperoxidase-mediated bacterial oxidations, and a molecular basis remains to be elucidated.

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Isolation and Characterization of Cloned cDNAs Encoding Human Liver Chlordecone Reductase

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ABSTRACT: Chlordecone (Kepone), a toxic organochlorine pesticide, undergoes bioreduction to chlordecone alcohol in human liver. This reaction is controlled by a cytosolic enzyme, chlordecone reductase (CDR), which may be of the aldo–keto reductase family of xenobiotic metabolizing enzymes [Molowa et al. (1986) *J. Biol. Chem. 261*, 12624–12627]. To further investigate the primary structure and expression of CDR, we screened a library of human liver cDNAs cloned in the expression vector λgt11 and isolated an 800 bp cDNA that directed synthesis of a fusion protein recognized by polyclonal anti-CDR antibodies. Using this cDNA as a probe, we screened two human liver cDNA libraries and found several 1.2-kb cDNAs which would code for a polypeptide with 308 residues (35.8 kDa). However, a similar full-length cDNA, possibly the transcript of a pseudogene, contained an in-frame nonsense codon. The deduced protein sequence of CDR showed 65% similarity to the primary structure of human liver aldehyde reductase and 66% similarity to the inferred protein sequence of rat lens aldose reductase. A search of GenBank revealed significant nucleotide similarity to a cDNA coding for bovine lung prostaglandin f synthase and to a partial cDNA coding for frog lens ρ-crystallin. Southern blot analysis of human genomic DNA displayed between 45 and 65 kilobases of DNA hybridizable to CDR cDNA and demonstrated several restriction fragment length polymorphisms among 26 individuals. Northern blot analysis of RNA from human, gerbil, rabbit, hamster, mouse, and rat livers disclosed hybridization with CDR cDNA only for the first three species. These same three species' livers contain CDR activity and one or more proteins immunoreactive with anti-CDR antibodies. RNA from adult but not fetal human liver, and from the human hepatoma cell-line Hep G2, contained major (1.6 kb) and minor (2.8 kb) species hybridizable to a CDR cDNA. The relative amounts of these RNAs varied markedly among nine subjects. From this initial description of the nucleotide sequence for a human carbonyl reductase, we conclude that CDR and several related enzymes are part of a novel multigene family involved in the metabolism of such xenobiotics as chlordecone and possibly endogenous substrates.

The aldo–keto reductases are a family of cytoplasmic enzymes that convert many xenobiotic aldehydes and ketones to their corresponding alcohols. These enzymes have been purified from several tissues and species and are characterized by their broad, overlapping substrate specifities and lack of inducibility (Bachur, 1976). The multiplicity and diversity of these enzymes have made it difficult to define their specific molecular forms. In spite of their ubiquitous presence in mammalian tissues, the precise physiological functions of the aldo–keto reductases remain to be established.

Several years ago, we studied workers exposed heavily to the toxic, organochlorine pesticide 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one (chlordecone)[1] (Kepone). We proposed that the major pathway for metabolism of chlordecone was its bio-

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[1] Abbreviations: CDR, chlordecone reductase; bp, base pair(s); kb, kilobase(s); chlordecone, 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one; chlordecone alcohol, 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-ol.

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