

Loss of DNA–membrane interactions and cessation of DNA synthesis in myeloperoxidase-treated *Escherichia coli*

(DNA replication/microbicidal/hypochlorite/replication origin/bactericidal)

HENRY ROSEN*, JILL ORMAN†, ROBERT M. RAKITA*, BRYCE R. MICHEL†, AND DONALD R. VANDEVANTER†

*Department of Medicine, University of Washington, and †The Tumor Institute of Swedish Hospital Medical Center, Seattle WA 98195

Communicated by Seymour J. Klebanoff, September 19, 1990 (received for review August 8, 1990)

ABSTRACT Neutrophils and monocytes employ a diverse array of antimicrobial effector systems to support their host defense functions. The mechanisms of action of most of these systems are incompletely understood. The present report indicates that microbicidal activity by a neutrophil-derived antimicrobial system, consisting of myeloperoxidase, enzymatically generated hydrogen peroxide, and chloride ion, is accompanied by prompt cessation of DNA synthesis in *Escherichia coli*, as determined by markedly reduced incorporation of [³H]thymidine into trichloroacetic acid-precipitable material. Simultaneously, the myeloperoxidase system mediates a decline in the ability of *E. coli* membranes to bind hemimethylated DNA sequences containing the *E. coli* chromosomal origin of replication (*oriC*). Binding of *oriC* to the *E. coli* membrane is an essential element of orderly chromosomal DNA replication. Comparable early changes in DNA synthesis and DNA–membrane interactions were not observed with alternative oxidant or antibiotic-mediated microbicidal systems. It is proposed that oxidants generated by the myeloperoxidase system modify the *E. coli* membrane in such a fashion that *oriC* binding is markedly impaired. As a consequence chromosomal DNA replication is impaired and organisms can no longer replicate.

Human neutrophils and monocytes employ a diverse array of antimicrobial effector systems to support their host-defense functions. Among these is one comprised of myeloperoxidase, hydrogen peroxide, and an oxidizable halide cofactor, usually chloride. The best characterized microbicide of the system is the hypochlorous acid, HOCl, which gives rise to additional, derivative microbicidal oxidants, most notably chloramines (1, 2). Characterization of the pathways by which loss of viability is effected by these oxidants remains an area of active investigation.

Biochemical lesions induced by HOCl—in amounts just sufficient to cause loss of bacterial viability—are largely confined to the cell envelope. Membrane-associated functions that are inactivated in *Escherichia coli* include loss of F₀F₁ ATPase activity (3), succinate-dependent respiration (4, 5), nutrient transport (3, 6), and modification of proteins involved in remodeling the bacterial cell wall during growth and division (R.M.R. and H.R., unpublished observations). In contrast, cytosolic proteins with HOCl vulnerable sites, such as the sulfhydryl-containing enzyme aldolase, are oxidized in intact *E. coli* only by exposure to amounts of HOCl far in excess of those required to produce loss of viability (7, 8). Thus, it appears that injuries to cell envelope structures precede those to cytosolic structures and that the surface injuries are sufficient to effect loss of viability.

McKenna and Davies recently reported prompt cessation of DNA synthesis in *E. coli* incubated with low concentrations of HOCl (9). This inhibition was observed under con-

ditions where protein synthesis was relatively spared and where membrane integrity was fully preserved. Among several proposed mechanisms for loss of DNA synthesis was disruption of interactions between the cell membrane and chromosomal DNA that are required for DNA replication. The implication that the primary lesion responsible for cessation of DNA synthesis was at the level of the cell membrane would be consistent with the location of most other early HOCl-mediated oxidative events at the level of the cell envelope.

Initiation of chromosomal replication in *E. coli* begins at a specific base sequence designated the chromosomal origin of replication, *oriC*. Initiation is mediated by several cytosolic proteins and involves binding of the *oriC* complex to either proteins or phospholipids of the cell membrane (10). *oriC*–membrane interactions are markedly enhanced when adenines encountered in GATC sequences within *oriC* are methylated on just one strand of the newly formed duplex—so-called hemimethylated *oriC* (11).

The goals of the investigation described in this report were: (i) to establish whether the chloride-dependent myeloperoxidase antimicrobial system had effects on *E. coli* DNA synthesis similar to those of reagent HOCl; (ii) to determine whether there were parallel myeloperoxidase-mediated inhibitory effects on *oriC*–membrane interactions; and (iii) to relate changes in DNA synthesis and DNA–membrane interactions to the viability loss that is the cardinal effect of the myeloperoxidase system.

METHODS

Special Reagents. Human myeloperoxidase was prepared and assayed as described (5). Glucose oxidase (1130 units/ml, from *Aspergillus niger*; type V-S; Sigma) and xanthine oxidase (20 units/ml; Boehringer Mannheim) were used as received; activities specified by the suppliers were assumed to be accurate. The following buffer systems were used: HKE, 10 mM HEPES, pH 7.4/100 mM KCl/1 mM Na₂EDTA; HKE/Mg, HKE supplemented with 5 mM MgCl₂; TE, 10 mM Tris-HCl, pH 8.0/1 mM Na₂EDTA.

Bacterial Strains, Plasmids, and Growth Conditions. *E. coli* ATCC 11775 was maintained as a frozen stock in 40% (vol/vol) trypticase soy broth/30% (wt/vol) glycerol at –80°C. Plasmid pGO46, derived from pUC9, carries an insert of *oriC* and was constructed and characterized by Ogden *et al.* (11). *E. coli* strains RB258 (*dam*⁺) and RB264 (*dam*[–]) carrying plasmid pGO46 were the gift of Moselio Schachter (Tufts University, Medford, MA) and were grown as described (11). These strains were maintained as frozen stocks in 40% (vol/vol) Luria broth/12% glycerol at –80°C.

Hemimethylated pGO46 DNA. pGO46 plasmids were prepared from strains of *E. coli* that contained (*dam*⁺) or lacked (*dam*[–]) DNA adenyl methylase by using the alkaline lysis technique described by Maniatis *et al.* (ref. 12, pp 86–94) with the modification that, prior to equilibrium centrifugation in CsCl₂/ethidium bromide, the ethanol-precipitated DNA was

suspended in 4 ml of TE containing 4.2 g of CsCl₂ and 4 mg of ethidium bromide in 0.4 ml water and was cleared by centrifugation at 12,000 × *g* for 10 min at 20°C. Methylated and unmethylated plasmids were digested with *Pst* I (New England Biolabs), mixed in equal amounts, and subjected to alkaline denaturation/renaturation as described by Horiuchi and Zinder (13) with the modification that the renatured material was concentrated by precipitation with ethanol prior to the final dialysis step. The hemimethylated DNA was 3'-end-labeled by using 50 μCi (1 μCi = 37 kBq) of [α-³²P]dCTP as described by Maniatis *et al.* (12), and unreacted nucleotides were removed by gel filtration (Bio-Gel P-60; Bio-Rad) with TE buffer prior to storage of the labeled DNA at -20°C.

Filter Binding Assay. Nitrocellulose filters (7 mm, BA85; Schleicher & Schuell) were boiled for 15 min in distilled water and soaked for at least 30 min in HKE/Mg at room temperature prior to use. DNA-binding protein sources, indicated in the legends to figures and tables, were combined with 30–50 ng of ³²P-end-labeled pGO46 and 500 ng of competing calf thymus DNA in a final volume of 100 μl of HKE/Mg for 20–30 min at room temperature. The binding mixture was filtered by centrifugation filtration (microfilter holders; Schleicher & Schuell, SS009) at 2000 × *g* for 5 min, and the filters were washed once with 100 μl of HKE prior to Cherenkov counting for ³²P. Background retention of labeled DNA was determined by using bovine serum albumin at 5 μg/ml as the DNA-binding source, and these counts were subtracted from all other experimental values.

Large-Scale Preparation of Bacterial Membranes. *E. coli* (ATCC 11775) were grown with vigorous aeration to mid-logarithmic phase (*A*₅₄₀ = 0.8–1.0), in 2L trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) at 37°C. Washed organisms were suspended in 10 ml of 0.1 M Na₂SO₄ containing 10 mM MgSO₄, 10 μg of DNase (Sigma), and 10 μg of RNase (Millipore) and were sonicated for 90 min on ice at a low-power setting with the temperature below 10°C. Adequate sonication was associated with a marked decrease in turbidity of the suspension. Unbroken cells were removed by centrifugation for 10 min at 12,000 × *g*, and membrane material in the supernatant was pelleted by centrifugation for 30 min at 48,000 × *g*. Pelleted membranes were resuspended by brief sonication in distilled water and stored in 0.5-ml portions at concentrations of 5 mg of protein per ml at -80°C.

Microbicidal Systems. *E. coli* ATCC 11775 were grown overnight in 10 ml of trypticase soy broth, washed twice in 0.1 M Na₂SO₄, and resuspended in 0.1 M Na₂SO₄/0.05% gelatin to the required absorbance at 540 nm. Bacteria were incubated with components of the microbicidal systems, described in the legends to figures and tables, in an oscillating water bath at 37°C. Microbicidal reactions were initiated by the addition of: (i) myeloperoxidase and glucose oxidase, (ii) xanthine oxidase and acetaldehyde, or (iii) antibiotics, as appropriate. At the times indicated, 20-μl samples were diluted with 180 μl of 0.1 M Na₂SO₄ (supplemented with 1 mM NaN₃ when myeloperoxidase-dependent systems were used). Subsequent serial dilutions were plated in trypticase soy agar for determination of colony-forming activity (viability).

One-milliliter samples of myeloperoxidase-dependent reaction mixtures containing 10⁹ bacterial cells received 0.01 ml of 0.1 M azide and were kept on ice until cells could be processed for DNA binding as described below. In other microbicidal systems, microbicidal activity was stopped by immediate centrifugation (5- to 20-ml samples containing 10⁹ bacterial cells) with immediate further processing as described below.

DNA Binding Sources (Microbicidal Systems). *E. coli* cells (10⁹) were pelleted by centrifugation for 10 min at 12,000 × *g* and 4°C and were resuspended in 1 ml of cold 30 mM

Tris-HCl (pH 8.0). Lysozyme (0.1 ml; 50 mg/ml) and 1 M K₂EDTA (0.01 ml; pH 7.0) were added, and cells were maintained on ice for 10 min. Cells were incubated for an additional 20 min after the addition of 0.01 ml of 100× concentrated nucleases (DNase and RNase, 1 mg/ml each) and 0.01 ml of 1 M MgSO₄. Unbroken cells were removed by centrifugation for 10 min at 12,000 × *g* and 4°C. Membrane material was recovered by centrifugation for 60 min at 48,000 × *g* and 4°C and was resuspended in 0.25 ml of distilled water for use as a DNA-binding source. Filter binding assays used 10 μl of membrane suspension per 100 μl of binding mixture.

Thymidine Incorporation. One-milliliter samples containing 10⁹ bacterial cells were transferred to iced Microfuge tubes (containing 0.01 volume of 0.1 M sodium azide if cells had been incubated with a myeloperoxidase-dependent system). Cells were pelleted by centrifugation at 4°C and 11,000 × *g* for 3 min and resuspended for 60 min at 37°C in 1 ml of trypticase soy broth containing 0.5 μM [³H]thymidine (2 Ci/mmol; NEN). Thymidine incorporation was stopped by transferring 0.5 ml of the suspension to a Microfuge tube containing an equal volume of 20% (wt/vol) trichloroacetic acid. Trichloroacetic acid-insoluble material was pelleted by centrifugation, washed twice with 1 ml of 10% trichloroacetic acid, and assessed for ³H incorporation by liquid scintillation counting.

Statistics. Except as otherwise indicated, results are presented as means ± SEM.

RESULTS

Incubation of *E. coli* with the myeloperoxidase-mediated antimicrobial system described in Fig. 1 produced the anticipated decline in colony-forming activity (viability), which, for the first 8 min, was accompanied by a proportionate decline in

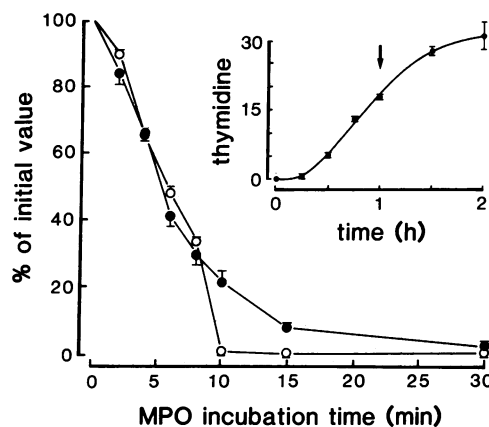


FIG. 1. Myeloperoxidase (MPO) effects on microbial viability and thymidine incorporation into DNA. The complete myeloperoxidase system contained (per ml) 10⁹ *E. coli* cells, 52 milliunits of myeloperoxidase, 226 milliunits of glucose oxidase, 10 mM glucose, 0.1 M sodium chloride, 10 mM sodium sulfate, and 40 mM sodium acetate (pH 5.0) in a total volume of 10 ml. At the indicated intervals samples were removed for determination of viability (○) and incorporation of [³H]thymidine into trichloroacetic acid-precipitable material (●). Results, which are means ± SE of three experiments, are expressed as the percent of initial values, which were 9.0 ± 0.1 × 10⁸ colony-forming units per ml for viability and 16.3 ± 0.5 pmol per 10⁹ cells per hour for thymidine incorporation. (Inset) Rate of [³H]thymidine incorporation by *E. coli* into trichloroacetic acid-precipitable material. Incorporation determinations were as described except that determinations were made at several time points. Results are expressed as nmol of thymidine incorporated per 10⁹ *E. coli* cells; data points are means of two experiments, and bars show the range of values. Incorporation at 1 hour (hr), indicated by the arrow, was used to evaluate myeloperoxidase effects on DNA synthesis, as described above.

bacterial incorporation of [^3H]thymidine into trichloroacetic acid-insoluble material. During this period, viability and thymidine incorporation fell in parallel to $33 \pm 1\%$ and $29 \pm 3\%$ of their initial values, respectively. In the next 2 min, viability declined to $1 \pm 0\%$, whereas thymidine incorporation was $21 \pm 4\%$ of the initial value but declined further to $2 \pm 0\%$ during the next 20 min. Omission of any of the components of the myeloperoxidase system—myeloperoxidase, chloride, glucose, or glucose oxidase—abolished both the antimicrobial and anti-DNA effects of the system (Table 1).

Incubation of *E. coli* with a separate oxidative microbicidal system, consisting of xanthine oxidase, acetaldehyde, and EDTA/Fe, also produced declines in microbial viability and DNA synthesis. However, in the xanthine oxidase system, cessation of thymidine incorporation lagged substantially behind loss of viability (kinetic data not shown). After a 30-min incubation with the xanthine oxidase system described in Table 1, viability was 1% of the initial value, while thymidine incorporation into DNA was 57%. Similarly, after a 30-min incubation with the gentamicin system described in Table 1, viability was 3% of the initial value, while thymidine incorporation was 46%. Accordingly, cessation of DNA synthesis is not a necessary, immediate consequence of loss of viability. However, microbicidal activity produced by the myeloperoxidase system, like that for reagent hypochlorite (9), correlates closely with cessation of DNA synthesis and also presumably with cessation of chromosomal DNA replication.

Initiation of chromosomal DNA replication involves a number of DNA-membrane interactions (10, 11, 14). The nature of many of these has been defined by using minichromosomes, episomes that incorporate the DNA base sequence of the chromosomal origin of replication, *oriC*. pGO46 is one such episome, constructed by insertion of an *oriC* sequence at the pUC9 cloning site (11). pGO46 contains both chromosomal and plasmid origins of replication and therefore is capable of relatively efficient replication in DNA methylase-deficient strains of *E. coli*. pGO46 was purified from *E. coli* strains containing and lacking DNA adenyl methylase. The fully methylated and unmethylated plasmids were linearized by cutting at their single *Pst* I restriction site, melted, and

reannealed to form a mixture of fully methylated, hemimethylated, and unmethylated DNA that in subsequent descriptions will be referred to as hemimethylated pGO46.

Filter binding assays have previously demonstrated a marked preference for membrane binding of hemimethylated *oriC* as compared with either the fully methylated or unmethylated forms (11). When filter binding assays were performed with membranes from *E. coli* 11775 and pGO46 DNA, the same preference was observed. Typically, hemimethylated pGO46 was end-labeled with ^{32}P and incubated with *E. coli* membranes in the presence of a 10- to 20-fold excess of unlabeled calf thymus DNA. In a typical series of experiments in which 35 ng of plasmid DNA was added to the binding mixture, 4.6 ± 0.2 ng ($n = 5$; mean \pm SE) was bound to the nitrocellulose filter in the presence of bacterial membranes. Replacement of *E. coli* membranes with bovine serum albumin reduced filter binding to 0.2 ± 0.1 ng. Specific binding of pGO46 DNA was calculated as [membrane-mediated binding minus albumin-mediated binding] and was 4.4 ± 0.1 ng. Binding of labeled hemimethylated pGO46 DNA was readily inhibited by excess cold hemimethylated pGO46 DNA but much less readily by fully methylated or unmethylated pGO46 (data not shown).

When membranes were prepared from *E. coli* that had been incubated with the myeloperoxidase-mediated antimicrobial system described in Fig. 2 *Upper*, there was a progressive decline in the ability of membranes to bind hemimethylated

Table 1. Effects of microbicidal systems on *E. coli* viability and DNA synthesis

Antimicrobial system	Viability*	Thymidine incorporation*
Complete MPO system	0 ± 0	0 ± 0
MPO omitted	108 ± 23	108 ± 4
Glucose omitted	121 ± 18	110 ± 2
Glucose oxidase omitted	114 ± 4	108 ± 6
Chloride omitted	108 ± 20	102 ± 4
Xanthine oxidase system	1 ± 0	57 ± 0
Gentamicin	3 ± 2	46 ± 5

E. coli, at 10^9 cells per ml, were incubated with the myeloperoxidase (MPO) system described in Fig. 1, and viability and thymidine incorporation were determined, also as described. Components of the system were omitted as indicated. Sodium chloride, when omitted, was replaced by isotonic sodium sulfate. The xanthine oxidase system contained (per ml) 5×10^7 *E. coli* cells, 40 milliunits of xanthine oxidase, 9 mM acetaldehyde, $10 \mu\text{M}$ FeSO_4 , $20 \mu\text{M}$ EDTA, 6.4 mM ammonium sulfate, 34 mM sodium sulfate, and 50 mM sodium phosphate (pH 7.0) in a total volume of 300 ml. The gentamicin system contained (per ml) 2×10^8 *E. coli* cells, 50 μg of gentamicin sulfate, 10 mM glucose, 0.1 M sodium chloride, and 40 mM sodium phosphate (pH 7.0) in a total volume of 55 ml. All incubations were for 30 min at 37°C, and results represent the means and range of values from two separate experiments. One-hundred percent thymidine incorporation was 15.3 ± 0.3 (mean \pm SE, $n = 6$) pmol per hour per 10^9 cells.

*Percent of values from cells not exposed to a microbicidal system.

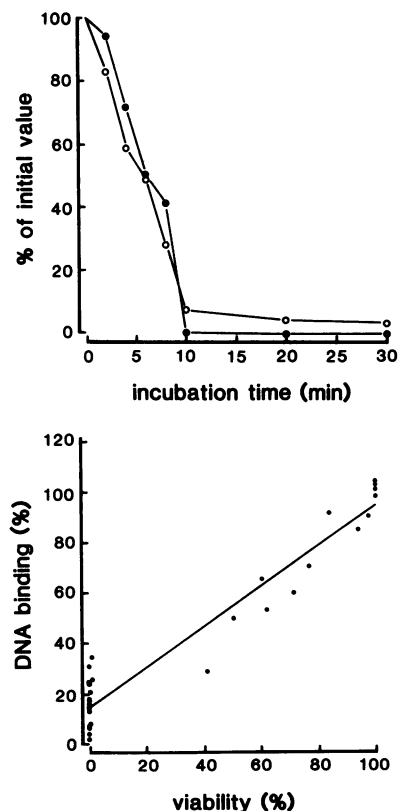


FIG. 2. Myeloperoxidase effects on microbial viability and bacterial membrane binding of *oriC* DNA. The complete myeloperoxidase system was as described in Fig. 1. At intervals, samples were removed to determine residual viability and bacterial membrane affinity for *oriC* DNA (filter binding assay) as described. (*Upper*) Results from one of four experiments with different sampling intervals, showing the rate of decline of both viability (\circ) and membrane DNA binding (\bullet). (*Lower*) Plot of DNA binding versus viability for all four experiments, $r = 0.97$. One-hundred percent viability was $9.0 \pm 0.4 \times 10^8$ colony-forming units per ml and 100% DNA binding was 4.5 ± 0.2 ng per filter (mean \pm SE, $n = 4$). Membrane protein per binding assay was $2.85 \pm 0.55 \mu\text{g}$ (mean \pm SD, $n = 41$).

Table 2. Effects of microbicidal systems on *E. coli* viability and membrane binding of *oriC* DNA

Antimicrobial system	Viability*	DNA binding*
Complete MPO system	0	2
MPO omitted	102	101
Glucose omitted	93	97
Glucose oxidase omitted	112	99
Chloride omitted	96	101
Xanthine oxidase system	1	99
Gentamicin	0	98
Ampicillin	0	98

E. coli were incubated for 30 min with the myeloperoxidase, xanthine oxidase, or gentamicin systems described in Table 1. The ampicillin system contained 2×10^8 *E. coli* per ml and 50 μ g of ampicillin per ml in 55 ml of trypticase soy broth at 37°C. Viability and DNA filter binding were determined as described. Results are from a single experiment (myeloperoxidase and ampicillin systems) or the mean of duplicate experiments (xanthine oxidase and gentamicin systems). One-hundred percent values for DNA binding were 4.7 ± 0.1 ng (mean \pm SE, $n = 6$) per filter.

*Percent of values from cells not exposed to a microbicidal system.

pGO46 DNA. The initial decline in DNA binding was rapid and corresponded well ($r = 0.99$) with the declines in microbial viability and, by inference from Fig. 1, DNA synthesis. Three additional experiments were performed with different sampling intervals, and correlation between loss of viability and membrane *oriC* binding was greater than $r = 0.96$ for each. The combined data, in which changes in DNA binding are plotted against viability, are shown in Fig. 2 Lower ($r = 0.97$). The decline in microbial viability and membrane binding of *oriC* DNA was not observed when any component of the myeloperoxidase system was omitted from the reaction mixture (Table 2). Further, microbicidal activity resulting from oxidants generated by the xanthine oxidase system, from gentamicin, or from ampicillin failed to impair membrane binding of *oriC* DNA (Table 2).

DISCUSSION

Neutrophils are microbicidal for a wide range of organisms including bacteria, fungi, and viruses (15). Insights regarding events that mediate these effects are not only of scientific interest but also have therapeutic potential. Awareness of essential but vulnerable microbial structures or metabolic pathways might guide development of antimicrobial therapies that pursue strategies characteristic of normal host cell systems. Such antimicrobial strategies might be especially effective in individuals with inadequate host cell numbers or function.

Lethal injuries mediated by myeloperoxidase-derived oxidants are probably multiple in nature and dependent upon the nature of the target organism and conditions of exposure. Multiple mechanisms of toxicity have been proposed, including oxidation of sulfhydryl groups (16), iron-sulfur centers (17), or conjugated diene lipids (8). Disrupted metabolic pathways include nutrient transport across the cytoplasmic membrane (6), membrane-associated ATP synthetase activity (3, 18), and aerobic electron transport (4, 5). Further, loss of membrane permeability (19), a disputed effect (6), also has been proposed as a lethal mechanism. Under these conditions of multiple injuries, causal relationships between a specific oxidative event and loss of viability have been difficult to establish. In general, loss of an indispensable structure or function simultaneously and proportionately with loss of viability is taken to imply causality. The criteria of indispensability, simultaneity, and proportionality have been met with various degrees of success in the above cited studies.

Cessation of DNA replication, as observed by McKenna and Davies in HOCl-treated *E. coli* (9), is an appealing

mechanism for loss of viability in any organism. For the myeloperoxidase system described in Fig. 1, cessation of DNA replication paralleled loss of viability almost exactly, to the point of 70% loss of each property. Thereafter, thymidine incorporation into DNA declined more slowly than did viability. The basis for the discordance is not known; however, it appeared at a time when other oxidant injuries (3–6, 8, 16–18), which would contribute additively to loss of viability, would be expected to become manifest.

Most or all early oxidant damage observed in *E. coli* treated with myeloperoxidase or reagent HOCl is confined to structures associated with the cell envelope (20). The functions of the cell envelope include regulation and initiation of chromosomal DNA replication (10, 14). It has been proposed from several lines of evidence that a DNA-membrane complex is essential for prokaryotic DNA replication (reviewed in ref. 14). A key replication-initiating enzyme, the product of *dnaA* (DnaA), appears to be absolutely dependent on phospholipids (cardiolipin) (21) and unsaturated fatty acids (22) found in the cell membrane. Further, a number of membrane proteins with specific affinity for *oriC* sequences have been identified (14, 23, 24), although their role in DNA replication remains to be established. Finally, efficient segregation of newly replicated chromosomal DNA into daughter cells appears to be dependent on DNA-membrane interactions (11, 14).

The weight of evidence supporting an important role for a DNA-membrane interaction in the initiation of chromosomal DNA replication together with the evidence that myeloperoxidase-derived oxidants affect principally membrane-associated targets prompted the evaluation of DNA-membrane interactions in myeloperoxidase-treated *E. coli*. The higher affinity of hemimethylated *oriC* for membrane structures (11) favored the use of hemimethylated preparations of pGO46 as a probe for the filter binding assays used to measure the interaction. The assays are based on the observation that, after appropriate treatment, nitrocellulose has low affinity for DNA but retains substantial affinity and capacity for protein (11). Probes were diluted 1:10 to 1:20 with randomly sheared mammalian DNA and incubated with *E. coli* membrane preparations. *oriC* sequences were retained on the filters by virtue of specific binding to membrane proteins, which in turn were bound to the nitrocellulose.

With increasing exposure to the myeloperoxidase system described in Fig. 2, the capacity of *E. coli* membrane preparations to bind *oriC* DNA fell markedly. The decline was proportionate to and simultaneous with the decline in microbial viability and, from Fig. 1, DNA synthesis. Accordingly, it is probable that the impaired DNA-membrane interaction contributed to the impairment of DNA synthesis and, consequently, to the decline in microbial replication. The declines in filter retention of *oriC* DNA must have been related to membrane oxidations because the DNA probes were not themselves exposed to the myeloperoxidase system. Further characterization of these membrane oxidations remains to be accomplished.

Impaired DNA replication in myeloperoxidase-treated *E. coli* might also have resulted from direct damage to chromosomal purine and pyrimidine bases, from depletion of appropriately phosphorylated nucleotide precursors or from damage to cytosolic enzymes responsible for chromosomal replication. These considerations were not evaluated in this study. While there is evidence for hydrolysis of ATP to ADP early in the course of microbicidal action of HOCl (18), there is little to suggest that cytosolic components, DNA, or enzymes are vulnerable to myeloperoxidase-derived oxidants during the early periods of viability loss considered here.

The xanthine oxidase system, used for purposes of comparison with myeloperoxidase, generates oxidants characteristic of myeloperoxidase-independent oxidative neutrophil microbicidal systems. Aerobic oxidation of acetaldehyde

mediated by xanthine oxidase results in the formation of superoxide anion and hydrogen peroxide. Interaction of these oxygen derivatives in the presence of catalytic amounts of Fe/EDTA results in the formation of highly reactive oxidants with properties much like hydroxyl radical. The fully constituted system is potently microbicidal (25, 26) but fails to impair either DNA synthesis to the degree that myeloperoxidase does (Table 1) or DNA-membrane interactions at all (Table 2). Gentamicin, which is microbicidal by virtue of its ability to bind ribosomes and inhibit protein synthesis, affects DNA synthesis and DNA-membrane interactions much like the xanthine oxidase system does. Ampicillin, which interferes with orderly cell wall synthesis and typically results in lysis of the target organism, substantially inhibits DNA synthesis (data not shown) presumably because of dispersion of DNA synthetic enzymes during cytolysis. Ampicillin had no effect on DNA-membrane interactions. Thus, among these antimicrobial systems, the myeloperoxidase system was unique in its concordant inhibition of DNA synthesis, DNA-membrane binding interaction, and microbial replication.

Although the data presented here have focused on the effects upon a single organism, *E. coli*, there is reason to believe that the microbicidal mechanisms described may be widely relevant among prokaryotes. DNA replication initiation at specific sites with AT-rich DNA repeats and the participation of initiator proteins like *E. coli* DnaA are a common theme among prokaryotes (10). Similarly, DNA-membrane complex formation appears to be a recurrent feature of chromosomal replication in these organisms (14). It should be noted that the concepts of microbicidal action considered here are not readily applicable to eukaryotic organisms, like fungi, and that there is no direct support for their relevance to prokaryotes other than *E. coli*.

In summary, DNA replication, which is necessary for bacterial cell replication, ceases rapidly upon exposure to myeloperoxidase-derived oxidants. Also, impairment of DNA-membrane interactions, loss of DNA synthesis, and loss of viability occur at the same time and to the same degree throughout the critical early period of myeloperoxidase action. Taken together, the evidence is compelling that inhibition of normal DNA-membrane interactions contributes substantially to the microbicidal effect of the cell-free myeloperoxidase system.

H.R. thanks Bernard M. Babior of Scripps Research Institute for discussions that prompted these investigations and Moselio Schaechter of Tufts University for providing the plasmid constructs and filter

binding methods. This work was supported, in part, by a grant, AI 25606, from the National Institute of Allergy and Infectious Diseases and by a donation from the M.J. Murdock Charitable Trust.

1. Thomas, E. L. (1979) *Infect. Immun.* **23**, 522-531.
2. Thomas, E. L. (1979) *Infect. Immun.* **25**, 110-116.
3. Barrette, W. C., Jr., Hannum, D. M., Wheeler, W. D. & Hurst, J. K. (1989) *Biochemistry* **28**, 9172-9178.
4. Rosen, H., Rakita, R. M., Waltersdorff, A. M. & Klebanoff, S. J. (1987) *J. Biol. Chem.* **262**, 15004-15010.
5. Rakita, R. M., Michel, B. R. & Rosen, H. (1989) *Biochemistry* **28**, 3031-3036.
6. Albrich, J. M., Gilbaugh, J. H., III, Callahan, K. B. & Hurst, J. K. (1986) *J. Clin. Invest.* **78**, 177-184.
7. Camper, A. K. & McFeters, G. A. (1979) *Appl. Environ. Microbiol.* **37**, 633-641.
8. Albrich, J. M., McCarthy, C. A. & Hurst, J. K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 210-214.
9. McKenna, S. M. & Davies, K. J. A. (1988) *Biochem. J.* **254**, 685-692.
10. Bramhill, D. & Kornberg, A. (1988) *Cell* **54**, 915-918.
11. Ogden, G. B., Pratt, M. J. & Schaechter, M. (1988) *Cell* **54**, 127-135.
12. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
13. Horiuchi, K. & Zinder, N. D. (1990) *Proc. Natl. Acad. Sci. USA* **69**, 3220-3224.
14. Firshin, W. (1989) *Annu. Rev. Microbiol.* **43**, 89-120.
15. Klebanoff, S. J. & Clark, R. A. (1978) *The Neutrophil: Function and Clinical Disorders* (North-Holland, Amsterdam).
16. Thomas, E. L. & Aune, T. M. (1978) *Antimicrob. Agents Chemother.* **13**, 1006-1010.
17. Rosen, H. & Klebanoff, S. J. (1985) *Infect. Immun.* **47**, 613-618.
18. Barrette, W. C., Jr., Albrich, J. M. & Hurst, J. K. (1987) *Infect. Immun.* **55**, 2518-2525.
19. Sips, H. J. & Hamers, M. N. (1981) *Infect. Immun.* **31**, 11-16.
20. Hurst, J. K. & Barrette, W. C., Jr. (1989) *CRC Crit. Rev. Biochem. Mol. Biol.* **24**, 271-328.
21. Sekimizu, K. & Kornberg, A. (1988) *J. Biol. Chem.* **263**, 7131-7135.
22. Yung, B. Y. & Kornberg, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7202-7205.
23. Jacq, A., Kern, R., Tsugita, A. & Kohiyama, M. (1989) *J. Bacteriol.* **171**, 1409-1416.
24. Kusano, T., Steinmetz, D., Hendrickson, W. G., Murchie, J., King, M., Benson, A. & Schaechter, M. (1984) *J. Bacteriol.* **158**, 313-316.
25. Rosen, H. & Klebanoff, S. J. (1979) *J. Exp. Med.* **149**, 27-39.
26. Rosen, H. & Klebanoff, S. J. (1981) *Arch. Biochem. Biophys.* **208**, 512-519.