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Biochemistry

Endonuclease IV of *Escherichia coli* is induced by paraquat

(*nfo* gene/superoxide/DNA repair/apurinic sites)

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ABSTRACT **The addition of paraquat (methyl viologen) to a growing culture of *Escherichia coli* K-12 led within 1 hr to a 10- to 20-fold increase in the level of endonuclease IV, a DNase for apurinic/apyrimidinic sites. The induction was blocked by chloramphenicol. Increases of 3-fold or more were also seen with plumbagin, menadione, and phenazine methosulfate. H_2O_2 produced no more than a 2-fold increase in endonuclease IV activity. The following agents had no significant effect: streptonigrin, nitrofurantoin, *tert*-butyl hydroperoxide, γ rays, 260-nm UV radiation, methyl methanesulfonate, mitomycin C, and ascorbate. Paraquat, plumbagin, menadione, and phenazine methosulfate are known to generate superoxide radical anions via redox cycling *in vivo*. A mutant lacking superoxide dismutase was unusually sensitive to induction by paraquat. In addition, endonuclease IV could be induced by merely growing the mutant in pure O_2. The levels of endonuclease IV in uninduced or paraquat-treated cells were unaffected by mutations of *oxyR*, a H_2O_2-inducible gene that governs an oxidative-stress regulon. The results indicate that endonuclease IV is an inducible DNA-repair enzyme and that its induction can be mediated via the production of superoxide radicals.**

Endonuclease IV (1) of *Escherichia coli* cleaves DNA at AP (apurinic or apyrimidinic) sites, and it is therefore referred to as an AP endonuclease (2, 3). AP sites are produced by spontaneous or enzymatic hydrolysis. They are commonly generated as intermediates in base-excision repair, a type of pathway in which glycosylases remove damaged bases from DNA (2, 3). Although endonuclease IV typifies the most common type of AP endonuclease found throughout nature, in *E. coli* it appears to be a relatively minor enzyme. In that organism, about 85% of the measurable AP endonuclease activity is due to a function of exonuclease III (4, 5). Thus, even though an *nfo* (endonuclease IV) mutation increases the sensitivity of *E. coli* to many DNA-damaging agents, in most cases this effect is readily apparent only in *xth* (exonuclease III) mutants (6). In addition, *E. coli* possesses endonuclease III (thymine glycol-DNA glycosylase), a minor AP endonuclease that has an associated glycosylase activity for ring-damaged pyrimidines (7, 8).

Plasmids bearing the cloned *nfo* gene were shown to specify overproduction of an AP endonuclease. It was identified as endonuclease IV on the basis of its EDTA resistance, sedimentation behavior, and persistence in mutants lacking endonuclease III and exonuclease III (6). Subsequently, we attempted to enhance enzyme overproduction by subcloning the gene in vectors that were thermoinducible for DNA replication, but the enzyme yields were low. This result led us to explore the possibility that the expression of the *nfo* gene might be regulated. In this report we show that endonuclease IV is induced by several agents that are known to generate O_2^- (superoxide) radical ions.

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MATERIALS AND METHODS

Genetic Nomenclature. *nfo* is a gene affecting endonuclease IV activity (6), *nth* is a gene affecting endonuclease III (thymine glycol-DNA glycosylase) (9, 10), and *xth* is the structural gene for exonuclease III (11). *nfo-1*::*kan* and *nth-1*::*kan* are insertion mutations created by cloning DNA segments that specify kanamycin resistance into the *nfo* and *nth* genes, respectively. *sodA* and *sodB* are genes affecting superoxide dismutases (12). *oxyR* is a gene controlling part of the adaptive response to oxidative stress (13).

Bacterial Strains and Plasmids. The strains used, which were derivatives of *E. coli* K-12, are listed in Table 1. Plasmid pWB21 is pBR322-*nfo*$^+$ (6). Plasmid pRPC53 (pBR322-*nth*$^+$), a gift of R. P. Cunningham, contained a 6.4-kilobase (kb) *Eco*RI–*Cla* I fragment of pLC9-9 (ColE1-*nfo*$^+$) (9). Plasmid transformation was performed with selection for ampicillin resistance (15).

Chemicals. Methyl methanesulfonate was obtained from Aldrich, streptonigrin from Flow Laboratories, and H_2O_2 from Fisher. Other inducing agents and antibiotics were obtained from Sigma.

Bacterial Growth. TY (tryptone-yeast) medium (16), VBC medium (13), and K medium (17) were as described. TYG medium is TY medium supplemented with 0.1% glucose. Unless otherwise stated, cultures were grown in 300-ml culture flasks (Bellco) possessing 14-mm-diameter sidearm cuvettes. The cultures (10 to 20 ml) were aerated by gyration at 300 rpm in a water bath at 37°C. Growth was monitored with a Klett colorimeter containing a no. 56 filter; 30 Klett units were equivalent to about 10^8 cells per ml.

Cell Treatments. Unless otherwise stated, the following general method was used. A saturated culture was diluted 100-fold in TY broth and grown with aeration at 37°C. At a cell density of 4×10^8 per ml, the culture was diluted 4- or 5-fold in fresh, prewarmed medium containing the agent to be tested (10 ml total volume), and the incubation was continued.

For heat shock at 50°C, the cells at 4×10^8 per ml were diluted 4-fold into prewarmed TY medium and aerated for 30 min at 50°C. The cultures were then diluted 2-fold with TY broth and grown at 37°C for 1 hr. For heat shock at 42°C, the cells were grown at 37°C, diluted into preheated medium, and aerated at 42°C for 1 hr.

Cultures that were to be subjected to γ-irradiation were grown with aeration in TYG medium. At a cell density of 2×10^8 per ml, they were collected by centrifugation, washed, and resuspended at the same density in 50 mM potassium phosphate buffer (pH 7.4) at 4°C. Two-milliliter samples were oxygenated and exposed to a ^{137}Cs irradiator at a dose rate of 1 kilorad/min (1 rad = 0.01 Gy) as previously described (9). The cells were then collected by centrifugation, resuspended in 10 ml of TYG broth, and grown at 37°C for 1.5 hr.

Abbreviation: AP, apurinic/apyrimidinic.

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Table 1. Strains used

Strain	Relevant genotype	Source or reference(s)
AB1157	*nfo*$^+$ *nth*$^+$ *xth*$^+$	10, 14
BW372	AB1157 *nth-1*::*kan*	9
BW434	AB1157 *nth-1*::*kan* Δ(*xth–pncA*)*90*	9
BW534	AB1157 *nfo-1*::*kan nth-1*::*kan*	6
GC4468	*sod*$^+$	12
K-12	*oxyR*$^+$	B. N. Ames*
KL16	*nfo*$^+$ *nth*$^+$ *xth*$^+$	14
QC774	GC4468 Φ(*sodA–lacZ*)*49* Φ(*sodB–kan*)*1*-Δ*2*	12
RK4936	*oxyR*$^+$	13
RPC500	AB1157 *nfo-1*::*kan*	6
RPC501	AB1157 *nfo-1*::*kan* Δ(*xth–pncA*)*90*	6
TA4110	K-12 *oxyR2* (constitutive)	13
TA4112	RK4936 Δ(*oxyR–btuB*)*3*	13

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For growth under N_2, 0.75 ml of a fresh saturated culture of BW372 was diluted into 150 ml of TY medium containing 1% glucose, 0.1 M potassium phosphate buffer (pH 7.4), and 0.01% Antifoam B (Dow Corning). The culture was placed in a 37°C water bath and bubbled with 95% N_2/5% CO_2 at 1 liter/min through a sintered-glass diffuser. As controls, cultures of BW372 and BW534 were grown in the same medium, exposed to air in a rotary shaker. Samples were collected during exponential growth at a cell density of about 2×10^8 per ml. Their final pH was 7.0–7.2.

Exposure to H_2O_2 at 37°C in K medium was as described (9). At 15 min, catalase was added to 0.2 μg/ml. After an additional 5 min of incubation, the cells were collected by centrifugation, resuspended at their original concentration in TYG medium, and grown for 1 hr at 37°C. H_2O_2 treatments in VBC medium were as described (13).

Cell Extracts. Culture samples were taken that were equivalent in mass to about 10^9 log-phase cells. The cells were washed by centrifugation at 4°C in 0.15 M NaCl/0.01 M Tris·HCl buffer (pH 8.0). The cell pellets were stored at −20°C and extracts were prepared by sonication (9).

Enzyme and Protein Assays. EDTA-resistant AP endonuclease activity (primarily endonuclease IV) was assayed in crude extracts as described (6). The assay measured the degradation of partially depyrimidinated DNA to acid-soluble fragments. The substrate was uracil-containing phage T4 [^{3}H]DNA that had been treated with uracil-DNA glycosylase. Catalase activity (18) was measured in the same sonicates. Enzymatic activities are expressed as units per milligram of protein, with the units as defined in the procedures cited. Protein was determined with the bicinchoninate reagent (19); bovine serum albumin was the standard.

RESULTS

Inducing Agents. We screened DNA-damaging agents for their ability to increase AP endonucleolytic activity in aerated, exponentially growing cultures of *E. coli*. The assays were performed in the presence of EDTA, which inhibits most DNases but not endonuclease III or IV (2). The agents included those known to induce DNA repair (20), to produce AP sites directly or indirectly (2), or to kill *nfo* mutants (6) preferentially. Each treatment was tested over a wide range of doses because a low dose might fail to induce an enzyme, whereas a high one might reduce general protein synthesis. We found marked increases in AP endonuclease activity after growth of the cells in the presence of paraquat (methyl viologen), plumbagin, menadione, or phenazine methosulfate (Table 2). These are all compounds that generate O_2^- ions by oxidative recycling *in vivo* (21). Two other producers of O_2^-—namely, streptonigrin (21) and nitrofurantoin (22)—were relatively ineffective, as were ascorbate [an H_2O_2 generator (23)] and *tert*-butyl hydroperoxide (Table 2). H_2O_2 produced up to a 2-fold increase in activity, but only under some conditions. The following additional agents (not listed in Table 2) failed to increase the measured DNase activity by more than 20%, even though they reduced cell growth: methyl methanesulfonate (0.2, 1, or 4 mg/ml), mitomycin C (0.5, 2.5, or 10 μg/ml), γ rays (4, 8, or 12 kilorads), bleomycin (0.08, 0.38, or 1.5 milliunits/ml), heat (42°C or 50°C), ethanol (2%, 4%, 6%, or 10% by volume), or 260-nm UV radiation (20, 40, or 60 J/m^2).

Table 2. Effect of redox-active compounds on EDTA-resistant AP endonuclease activity in *E. coli*

Treatment	Growth in 1 hr (fold increase)	Relative enzymatic activity
None	4.2	(1.0)
Paraquat		
0.25 mg/ml*	2.9	9.6
Plumbagin		
20 μg/ml*	1.8	3.1
Phenazine methosulfate		
60 μg/ml*	2.2	4.3
Menadione		
40 μg/ml*	2.2	4.2
H_2O_2 in TY medium		
0.4 mM	3.2	1.8
0.8 mM	2.9	1.7
1.5 mM	2.5	2.1
H_2O_2 in VBC medium		
60 μM	2.0†	0.9
H_2O_2 in K medium		
0.48 mM	3.3	1.2
2.4 mM	2.2	1.7
4.8 mM	1.5	1.5
19 mM	0.9	0.8
Ascorbic acid		
1.1 mM	4.1	0.9
5.7 mM	3.2	0.6
tert-Butyl hydroperoxide		
0.18 mM	3.6	0.8
0.70 mM	2.0	1.3
Nitrofurantoin		
2 μg/ml	3.7	0.9
5 μg/ml	3.1	1.0
10 μg/ml	1.9	1.0
Streptonigrin		
3 μg/ml	3.4	1.3
12 μg/ml	3.1	1.1
24 μg/ml	2.4	1.1

The strain used was KL16 (*nfo*$^+$ *nth*$^+$ *xth*$^+$). Treatments were as described in *Materials and Methods*. The relative enzymatic activity is the ratio of the specific activity of the treated culture (1 hr after exposure) to that of the untreated control in each experiment. The latter value ranged from 10 to 26 units/mg. Cell growth was measured by OD.

*Optimum concentration.

†In this medium, the untreated control grew 2.3-fold in 1 hr.

Induction Requires Protein Synthesis. The endonuclease activity of the growing cells increased steadily during the first 90 min of exposure to paraquat, and this induction was completely blocked by chloramphenicol (Fig. 1). Puromycin (1 mg/ml) had a similar effect when added 15 min before the paraquat (data not shown).

Identification of the Induced Enzyme. The endonuclease assay measured the degradation of partially depyrimidinated

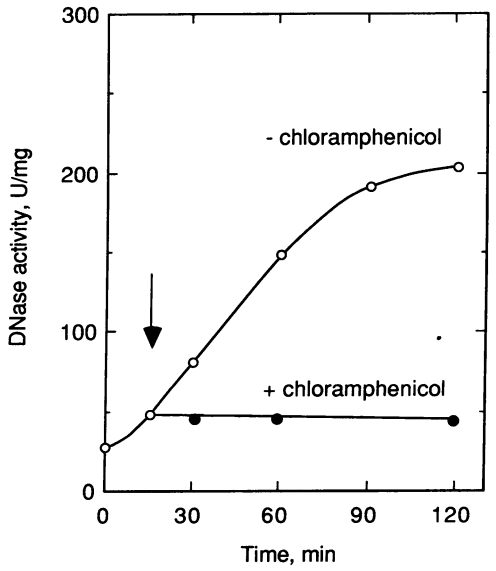

FIG. 1. Chloramphenicol prevents the induction of an endonuclease by paraquat. Paraquat (1 mg/ml) was added at zero time to a log-phase culture of strain KL16. Chloramphenicol (0.1 mg/ml) was added to one-half of the culture at 15 min (arrow). U, units.

DNA to acid-soluble fragments in the presence of EDTA, an inhibitor of exonuclease III (5). The assay detects both endonucleases III and IV in cell extracts, although it is more specific for the latter (6). To identify which enzyme was being induced by paraquat, we used a set of strains containing different doses of the *nfo* and *nth* (endonuclease III) genes (Fig. 2). Endonuclease activity was induced in an *nth* mutant but not in an *nfo* mutant (Fig. 2*A*). The highest levels of induced enzyme activity were in an *nth* mutant bearing a functional *nfo* gene on a multicopy plasmid (Fig. 2*B*). To exclude possible plasmid amplification as a contributing factor, the reciprocal experiment was performed—i.e., with an *nfo* mutant bearing an *nth*$^+$ plasmid. This endonuclease III-overproducer displayed an initially high level of AP endonuclease activity (76 units/mg), as expected, but the activity was not increased by paraquat (Fig. 2*B*). Therefore, the induced enzyme is endonuclease IV rather than endonuclease III.

The gene dose of *nfo* had a similar effect on the induction of AP endonuclease activity by other agents. Thus, plumbagin (20 μg/ml) caused an increase in endonuclease activity (from 150 to 699 units/mg) in strain BW554 (*nth xth*/pBR322-*nfo*$^+$); the final enzyme level was about 20–30 times that of plasmid-free cells. Although H_2O_2 (1.5 mM in TY broth) caused a 2-fold increase in endonuclease activity in a wild-type strain (Table 2), it failed to increase activity in strain RPC500 (*nfo*) (data not shown).

Induction in a *sodAB* Mutant. During aerobic metabolism, the redox cycling of paraquat and plumbagin generates O_2^- radicals that are rapidly converted to O_2 and H_2O_2 by superoxide dismutases (21). A *sodAB* mutant of *E. coli*, which lacks both the iron- and the manganese-containing superoxide dismutase, is therefore unusually sensitive to paraquat and to oxygen (12). Dose–response curves (Fig. 3) indicated that paraquat induced endonuclease IV activity more readily in the *sodAB* mutant than in its *sod*$^+$ parent. Moreover, endonuclease IV activity could be induced without drugs, merely by exposing a *sodAB* mutant to pure oxygen (Table 3). Thus, when grown in air in the absence of an inducing agent, the *sodAB* mutant had the same level of endonuclease IV as its parent. However, when the mutant was incubated in the presence of pure O_2, it exhibited an ≈4-fold increase in endonuclease activity, whereas that of its

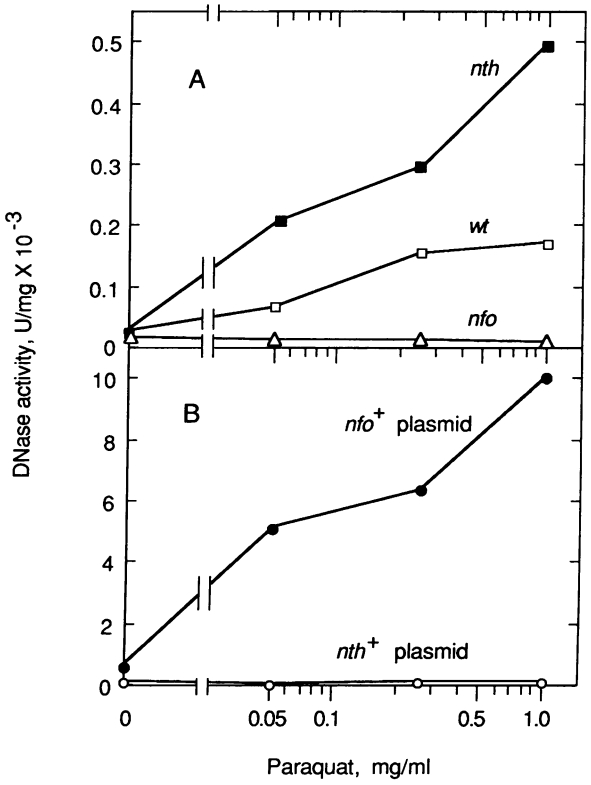

FIG. 2. Paraquat induction of AP endonuclease activity in endonuclease III (*nth*) and endonuclease IV (*nfo*) mutants (*A*) and overproducers (*B*). The cells were collected 1 hr after the addition of paraquat. The strains used were as follows: (*A*) AB1157 (*wt*, wild type), BW372 (*nth*), and RPC500 (*nfo*); (*B*) BW434/pWB21(*nfo*$^+$) and RPC501/pRPC53(*nth*$^+$). U, units.

congenic parent was not greatly affected. These results suggest that it was probably O_2^- that mediated the induction of endonuclease IV by the redox cycling agents. Nevertheless, streptonigrin, a known O_2^- -generator, not only failed to induce the enzyme in a wild-type strain (Table 2) but also failed to increase it more than 30% in the *sodAB* mutant at doses of 1.5, 6, or 24 μg/ml.

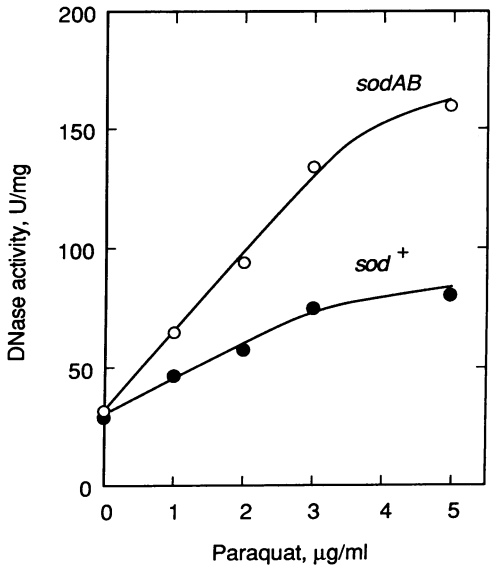

FIG. 3. Paraquat induction of endonuclease IV activity in a *sod* (superoxide dismutase) mutant. The strains used were GC4468 (*sod*$^+$) and QC774 (*sodAB*). U, units.

Table 3. Induction of endonuclease IV by O_2 in a *sodAB* mutant

Strain	Genotype	Time	AP endonuclease activity, units/mg	
			Air	O_2
GC4468	*sod*$^+$	1 hr	27	34
		2 hr	22	34
QC774	*sodAB*	1 hr	27	79
		2 hr	26	108

Cultures were grown in air at 37°C to a density of 4×10^8 per ml and diluted 4-fold at zero time with fresh TY medium containing 0.01% Antifoam B (Dow Corning). One sample of each culture was exposed to room air in a water bath shaker while pure O_2 was bubbled through the other. The cell densities were maintained at less than 4×10^8 per ml by repeated dilution.

Effect of Anaerobic Growth. Anaerobic growth is known to reduce levels of the manganese-containing superoxide dismutase, the *sodA* gene product (24). We therefore tested the effects of oxygen deprivation. To ensure rapid growth under nearly anaerobic conditions, we found it necessary to use a high level of CO_2 together with a buffered TY medium containing 1% glucose (see *Materials and Methods*). One culture of BW372 (*nth*) was grown under 95% N_2/5% CO_2, while another was grown in the same medium in room air. Under these conditions, the levels of endonuclease IV in the anaerobically and aerobically grown cells were 9.5 and 11.2 units/mg, respectively. A strain lacking endonuclease IV activity, BW534 (*nth nfo*) (6), had a background level of 4.9 units/mg when grown in air in the same medium. Therefore, oxygen deprivation had little effect on levels of endonuclease IV.

Induction Is Independent of *oxyR*. Exposure of *E. coli* or *Salmonella typhimurium* to 60 μM H_2O_2 induces an adaptive response to oxidative stress, which is accompanied by the increased synthesis of many proteins (13, 17, 25). A subset of these proteins is positively regulated by the *oxyR* gene and includes a catalase, an alkyl hydroperoxide reductase, glutathione reductase, and the manganese superoxide dismutase (13). We studied the effects of two mutations: *oxyR2*, which results in constitutive overexpression of the regulon, and the Δ(*oxyR–btuB*) deletion, which renders the regulon noninducible (13). We found that *oxyR* mutations did not significantly affect either the normal or the paraquat-induced expression of *nfo* (Table 4). For example, the untreated *oxyR*-constitutive mutant had a normal level of endonuclease IV activity in the face of a 15-fold overexpression of catalase. The independent regulation of these two enzymes was further supported by several other findings. For example, the induction of endonuclease IV in the *oxyR*$^+$ parental strains (Table 4) was not accompanied by an increase in catalase activity. Moreover, H_2O_2 and ascorbate had minor effects on endonuclease IV activity (Table 2) but were effective inducers of catalase; untreated cells had 9–10 units of catalase per mg of protein, whereas those treated with H_2O_2 (1.5 mM in TY broth) or ascorbate (1.0 mg/ml) had activities of 43 and 95 units/mg, respectively.

Table 4. Catalase and endonuclease (Endo) IV levels in *oxyR* mutants treated with paraquat (PQ, 1 mg/ml)

Strain*	Genotype	Enzymatic activity, units/mg			
		Catalase		Endo IV	
		− PQ	+ PQ	− PQ	+ PQ
K-12	*oxyR*$^+$	6	9	13	64
TA4110	*oxyR2*	90	102	18	66
RK4936	*oxyR*$^+$	6	7	18	70
TA4112	Δ*oxyR*	7	5	17	59

*Strain K-12 is the parent of TA4110, and strain RK4936 is the parent of TA4112. The *oxyR2* mutant constitutively overexpresses the *oxyR* regulon.

Exonuclease III Was Not Induced. By substituting 5 mM $MgCl_2$ for the EDTA in the reaction mixtures, we could measure the AP endonuclease activity of exonuclease III. In extracts of untreated, wild-type cells, exonuclease III constitutes about 85% of the enzymatic activity detectable by this assay, as determined by assays performed on *xth* mutants (B.W., unpublished data). The extracts produced in the experiments of Table 2 were tested for this activity. The untreated cells were found to have an average level of exonuclease III of 295 units per mg of protein. In terms of similar units, paraquat, by comparison, increased the levels of endonuclease IV up to as much as 493 units/mg in haploid cells (Fig. 2). Paraquat (tested in strain BW534), H_2O_2 *tert*-butyl hydroperoxide, streptonigrin, methyl methanesulfonate, mitomycin C, heat, and γ rays failed to increase exonuclease III activity by more than 70%. Plumbagin caused an increase no greater than that expected from its induction of endonuclease IV, which is also detected by this assay. Other agents were not tested.

DISCUSSION

The results indicate that endonuclease IV is an inducible enzyme. Although it was expressed constitutively in untreated cells (even under relatively anaerobic conditions), it was induced up to 20-fold with paraquat. Neither endonuclease III nor exonuclease III activity, however, was induced by any of the treatments tested. After induction, the level of endonuclease IV was about that of exonuclease III, which possesses the major AP endonucleolytic activity of *E. coli*. Therefore, endonuclease IV can no longer be considered a minor enzyme.

The compounds that produced the greatest increases in endonuclease IV activity have in common their ability to produce O_2^- radicals in the cell (21). Plumbagin, menadione, and streptonigrin are paraquinones, whereas paraquat and phenazine methosulfate are conjugated polycyclic compounds with quarternary ring nitrogens that serve as electron acceptors. In bacteria, these compounds are enzymatically reduced and then autoxidized, transferring electrons directly to O_2 to produce O_2^- (21). O_2^- is also generated during the aerobic oxidation of some normal cellular metabolites. To protect itself against these radicals, *E. coli* has two superoxide dismutases, enzymes that convert O_2^- to H_2O_2 and O_2 (26). One is a noninducible, iron-containing enzyme. The other is a manganese-containing enzyme that is metabolically regulated and that is induced by the same agents that induce endonuclease IV. A mutant lacking both enzymes (*sodAB*) is unusually susceptible to the lethal and mutagenic effects of these agents (12, 27).

It would be an oversimplification, however, to think of compounds like paraquat only as generators of O_2^-. *In vivo*, O_2^- and the other reduction products of O_2 are readily interconverted through the combined effects of enzymes and metalloprotein complexes (28). Conditions that produce O_2^- in the cell may lead to a chain of reactions that yield H_2O_2, hydroxyl radicals (OH·), singlet oxygen, and alkyl peroxides; any of these other reactive species might be a direct inducer of endonuclease IV. Particular attention has been given to OH·, which is highly reactive toward DNA *in vitro* (29, 30). It may arise from H_2O_2 (a product of O_2^- dismutation) via reactions with O_2^-, with reduced metalloproteins, or with reduced paraquat (28, 30–32). This possible interconversion may have been responsible for the small (at most 2-fold) increases in endonuclease IV activity produced under some conditons by H_2O_2 (Table 2). It remains to be seen, therefore,

whether O_2^- is the actual effector of the induction; instead, the inducer might be, for example, another active oxygen species generated from O_2^-, or it might be a DNA lesion that can be generated by various oxidants. Nevertheless, the following evidence strongly suggests that the induction of endonuclease IV may be mediated, at least in part, by O_2^-: (*i*) the enzyme was induced by compounds that generate O_2^-, (*ii*) a mutant for superoxide dismutase (*sod*) reacted to paraquat more readily than its *sod*$^+$ parent, and (*iii*) the enzyme was induced by growing a *sod* mutant in pure O_2.

While our data do not preclude an important role for H_2O_2 or OH· in the pathway of endonuclease IV induction, they provide no direct evidence for it. γ rays, which produce OH· and H_2O_2 as well as O_2^- (29, 33), failed to induce the enzyme under our conditions. The cells were also treated with H_2O_2 or ascorbate under a variety of conditions that have been reported either to induce catalase, to adapt the cells to otherwise lethal doses of H_2O_2, or to induce a DNA-repair system for H_2O_2-damaged phages (17, 23, 25). In each case, the treatments failed to induce endonuclease IV to the levels observed with O_2^- generators. It should be noted, however, that streptonigrin and nitrofurantoin, which are known to produce O_2^- (21, 22), also failed to induce the enzyme. It may be that the latter compounds block induction by exerting other toxic effects or that they are ineffective because of intracellular localization.

Our data indicate that the control of endonuclease IV is independent of the peroxide-inducible *oxyR* regulon, which controls a set of genes that are among those induced by oxidative stress (25). The products of both *sodA* (26) and *nfo* are induced by paraquat and plumbagin, suggesting that the genes might share a common regulatory pathway. Unlike *nfo*, however, *sodA* is more responsive to oxygen tension (26) and to *oxyR*-mediated stimulation by peroxides (13). However, different types of stress have been shown to produce overlapping patterns of induced proteins (25), and thus *sodA* may be under the control of more than one regulatory gene.

As an AP endonuclease, endonuclease IV would be expected to function in base-excision repair pathways, acting at the AP sites generated by glycosylases that remove damaged bases from DNA (2, 3). It can also excise the 3′-phosphoglycolate termini produced in DNA by oxygen-radical damage (34). In accordance with these expectations, studies with mutants (6) suggested that endonuclease IV assists exonuclease III in protecting cells from the lethal effects of ionizing radiation, bleomycin, H_2O_2, and *tert*-butyl hydroperoxide. Moreover, O_2^- generators are known to produce DNA lesions that are repaired in part by the action of the SOS regulon (35) and in part by an unknown inducible system that can reactivate DNA phages that have been damaged by O_2^- but not those damaged by H_2O_2 (36). Endonuclease IV may be part of this repair system.

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