Escherichia coli YqhD Exhibits Aldehyde Reductase Activity and Protects from the Harmful Effect of Lipid Peroxidation-derived Aldehydes*

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Evidence that Escherichia coli YqhD is involved in bacterial response to compounds that generate membrane lipid peroxidation is presented. Overexpression of yqhD results in increased resistance to the reactive oxygen species-generating compounds hydrogen peroxide, paraquat, chromate, and potassium tellurite. Increased tolerance was also observed for the lipid peroxidation-derived aldehydes butanaldehyde, propanaldehyde, acrolein, and malondialdehyde and the membrane-peroxidizing compound tert-butylhydroperoxide. Expression of yqhD was also associated with changes in the concentration of intracellular peroxides and cytoplasmic protein carbonyl content and with a reduction in intracellular acrolein levels. When compared with the wild type strain, an yqhD mutant exhibited a sensitive phenotype to all these compounds and also augmented levels of thiobarbituric acid-reactive substances, which may indicate an increased level of lipid peroxidation. Purified YqhD catalyzes the in vitro reduction of acetaldehyde, malondialdehyde, propanaldehyde, butanaldehyde, and acrolein in a NADPH-dependent reaction. Finally, yqhD transcription was induced in cells that had been exposed to conditions favoring lipid peroxidation. Taken together these results indicate that this enzyme may have a physiological function by protecting the cell against the toxic effect of aldehydes derived from lipid oxidation. We speculate that in Escherichia coli YqhD is part of a glutathione-independent, NADPH-dependent response mechanism to lipid peroxidation.

Oxidative stress is detrimental to a number of cellular macromolecules including proteins, nucleic acids, and membrane lipids. In eukaryotic organisms oxidative modification of cellular constituents has been implicated in the etiology of different pathological conditions such as diabetes, cataracts, pulmonary emphysema, arthritis, cancer, and aging (1, 2).

The electron-transport chain provides a constant supply of reactive oxygen species (ROS).4 Hydrogen peroxide (H2O2), superoxide (O2•−), and hydroxyl radical (OH•) are typical side products of the aerobic metabolism. ROS compounds are also formed during cell exposure to redox-cycling chemicals like paraquat or metals and metalloids like chromate and potassium tellurite (K2TeO3) (3–5). To alleviate ROS-generated oxidative damage, Escherichia coli cells induce the synthesis of a variety of antioxidant enzymes including catalases and superoxide dismutases (3, 6).

Except for mechanisms involved in membrane peroxidation, prokaryotic and eukaryotic cells use similar mechanisms to respond to oxidative stress. Lipid oxidation is a common consequence of the activity of free radicals on cell membrane components. Oxidation of polyunsaturated fatty acids by HO2, the protonated form of superoxide anion, or OH• leads to the formation of lipid peroxides (7). Enzymatic or chemical degradation of lipid peroxides results in the generation of toxic breakdown products like short-chain (C2-C9) aldehydes such as 2-alkenals (α,β-unsaturated aldehydes), 2-propanal (acrolein), 4-hydroxynonenal, and malondialdehyde (MDA) (7, 8). The toxicity of these “reactive aldehydes” lies in their ability to form Michael adducts with thiol and amino groups of proteins, thus affecting several cellular processes (9). For example, inactivation of glucose-6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and cytochrome c oxidase by reactive aldehydes that modify lysine and cysteine residues located in enzyme active sites has been reported (10–12). Reactive aldehydes can also generate DNA adducts causing an important increase of the rate of mutagenesis in E. coli and Salmonella (13, 14).

Mammalian cells use three distinct systems to cope with the harmful effect of reactive aldehydes. The glutathione S-transferase pathway fueled by the GSH or thioredoxin redox cycle conjugates aldehydes with glutathione (GSH), the aldo-keto reductase system reduces aldehydes to alcohols, and alcohol and aldehyde dehydrogenases oxidize aldehydes to carboxyl compounds. In animals, the main detoxification pathway is GSH-dependent, and it has been shown that the alcohol/aldehyde dehy-

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4 The abbreviations used are: ROS, reactive oxygen species; MDA, malondialdehyde; t-BOOH, tert-butylhydroperoxide; TBAR, thiobarbituric acid-reactive substance; qRT, quantitative real time; Cp, crossing point; wt, wild type.
Table 1

| Strain          | Relevant genotype                                                                 | Source or reference       |
|-----------------|------------------------------------------------------------------------------------|----------------------------|
| E. coli TOP10   | F⁻ tdrA Δ(mcrA-hudRM5-mcrBC) Δ880lacZΔM15 ΔlacY74 deoR napG recA1                 | Invitrogen®                |
| E. coli BW25113 | lacR mbrΔ1 ΔlaczW716 hisRS4 ArhABΔkuA3 ArhABΔkuA3                                  | Datsenko and Wanner (46)  |
| E. coli pBAD    | E. coli BW25113 harboring pBAD                                                     | This work                  |
| E. coli pBAD yqhD| E. coli BW25113 harboring pBAD yqhD                                               | This work                  |
| E. coli ΔyqhD   | E. coli BW25113 ΔyqhD (yqhD::Kan®)                                                | NARA Institute, Japan      |
| E. coli ΔyqhD pBAD yqhD | E. coli BW25113 ΔyqhD harboring pBAD yqhD                                          | This work                  |
| E. coli pDest17 yqhD | E. coli BL21 (DE3) harboring pDest17 yqhD plasmid                               | Sulzenbacher et al. (20)   |

**Plasmids**

| Plasmid          | Features                                                                 |
|------------------|--------------------------------------------------------------------------|
| pBAD TOPO        | Expression vector, Ap<sup>+</sup>                                         |
| pBAD yqhD        | pBAD harboring E. coli yqhD gene                                           |
| pGEM-T yqhDp     | Cloning vector, Ap<sup>+</sup>                                             |
| pGEM-T yqhD      | pGEMT harboring E. coli yqhD gene plus 300-bp upstream of yqhD translation start codon |
| pDest17 yqhD    | pDest17 harboring E. coli yqhD gene. Adds 6X His to YqhD and allows iso propyl 1-thio-β-d-galactopyranoside-induced over expression of YqhD |

**Primers**

| Primers | Forward (F) or Reverse (R), to amplify | 5'-3' Sequence |
|---------|----------------------------------------|----------------|
| yqhDF   | F, yqhD                                 | CCGGAAAGAGATATTAGATGGCCAGGTC |
| yqhDR   | R, yqhD                                 | AAAGTTTTACGCGCGGCCTCTTAGTATAC |
| sodDF   | R, sod                                 | ACCCTGCCGCCGCGCCTGTAGATTAT |
| sodDR   | R, sod                                 | CCTGGCATCTCCGCTGTAGCTTA |
| soxSFR  | F, soxS                                | TACCACGGCTGGGATATACGCTT |
| soxSR   | R, soxS                                | CAGGACCCCTTGGCAGCGCCCTT |
| 16F<sup>+</sup> | F, 16 S RNA                 | ATGGACGCGACACCTGGAGAC |
| 16R<sup>+</sup> | R, 16 S RNA                | TGACTTACAAACCGCCTG |
| gapAF    | F, gapA                                | GTAAAGTTGTTATACGGTTTGTG |
| gapAR<sup>+</sup> | R, gapA            | GGTTAGTGTGTTACGACAG |

<sup>*</sup> Primers used in qRT-PCR experiments. Primers were designed to generate a 300-bp product.
Cloning and Expression of yqhD and Protein Purification—Plasmid pBAD yqhD was constructed by cloning the PCR-amplified *E. coli* yqhD gene fragment into the expression vector pBAD TOPO (Invitrogen). *E. coli* BW25113 chromosomal DNA and primers described in Table 1 was used to amplify the yqhD gene. The PCR product was ligated to the cloning vector, and the resulting plasmid pBAD yqhD was introduced into *E. coli* Top10 by electroporation. Insert orientation was confirmed by Ncol digestion, and gene integrity was determined by nucleotide sequencing. Plasmid pBAD yqhD was then mobilized into *E. coli* BW25113 to carry out the resistance studies reported in this work.

*E. coli* BL21(DE3) cells harboring plasmid pDEST17 yqhD (a generous gift of Dr. C. Cambillau, CNRS, Marseille, France) were used to purify YqhD. After 5 h of induction with isopropyl 1-thio-β-D-galactopyranoside (1 mM), cells were harvested, resuspended in buffer 50 mM Tris-HCl, pH 7.0, and subjected to sonic disruption. Nucleic acids were precipitated with streptomycin sulfate (2%, w/v) on ice. After centrifuging at 13,000 × g for 5 min, the supernatant was loaded onto a Ni2+ column (nickel-nitrilotriacetic acid). Proteins were eluted with 0.5 M imidazole and fractionated by SDS-PAGE to estimate protein purity.

Assay for YqhD Dehydrogenase—The reaction mixture contained 50 mM potassium phosphate buffer, pH 7.0, 2 mM NADPH, a defined aldehyde (acetalddehyde, propanal, or butanal), or alcohol (butanol, propanol or isopropanol) as substrate and purified YqhD (10 μg/ml). The mixture was incubated for 2 min at 37 °C, and NADPH oxidation was determined at 340 nm using a UV-Visible Agilent 8453 spectrophotometer. One unit of enzyme activity was defined as the amount of protein that oxidizes 1 pmol of NADPH/min at 37 °C.

Minimal Inhibitory Concentrations—Cells from overnight cultures were diluted 100-fold with fresh LB medium. Ten μl of this dilution were used to inoculate 1 ml of LB medium containing the appropriate antibiotics (kan and/or amp) and the toxics to be tested. Cells were grown for 48 h at 37 °C with shaking.

Peroxide Intracellular Levels—The oxidant-sensitive probe 2′,7′-dichlorodihydrofluorescein diacetate was used to measure peroxide levels in cells exposed to potassium tellurite (0.5 μg/ml), hydrogen peroxide (1 mM), or glutathione (1 mM) (24). Cells were grown under aerobic conditions in LB medium amended with the compounds to be tested to an A600 nm ~ 0.3. After washing with 10 mM potassium phosphate buffer, pH 7.0, cells were incubated in the same buffer containing 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (dissolved in dimethyl sulfoxide) for 30 min. Cells were washed, suspended in the same buffer, and disrupted by sonication. Cell extracts (100 μl) were mixed with 1 ml of phosphate buffer, and fluorescence intensity was measured using an Applied Biosystems Cytofluor 4000 multi-well plate reader (excitation, 490 nm; emission, 519 nm). Fluorescence emission values were normalized to protein concentration.

Protein Carbonylation—Protein carbonyl content was determined according to Semchyshyn et al. (18). Nucleic acid-free extracts (100 μl) prepared from *E. coli* BW25113 cells treated and untreated for 30 min with K2TeO3 (0.5 μg/ml) or H2O2 (100 μM) were mixed with four volumes of 10 mM dinitrophenylhydrazine (dissolved in 2 M HCl) and incubated for 1 h at room temperature with vortexing every 10–15 min. Proteins were precipitated by adding 500 μl of 20% trichloroacetic acid and sedimented at 14,000 × g for 5 min. The pellet was washed at least 3 times with 1:1 ethanol:ethyl acetate to remove any unreacted dinitrophenylhydrazine. Finally, the pellet was dissolved at 37 °C in 450 μl of 50 mM dithiothreitol in 6 M guanidine HCl. Carbonyl content was determined spectrophotometrically at 370 nm using a molar absorption coefficient of 22,000 M−1 cm−1 (18).

TBARs—TBARs were determined in cell extracts as described by Semchyshyn et al. (18). Cells were grown at 37 °C in LB media to an A600 ~ 0.6 and exposed for 30 min to a particular toxic compound. Cell suspensions (1.0 ml) were then precipitated by the addition of 1.0 ml of 20% (v/v) trichloroacetic acid and centrifuged, and supernatants were mixed with 2.0 ml of a saturated solution of thiobarbituric acid dissolved in 0.1 M HCl and 10 mM butylated hydroxytoluene. Samples were heated at 100 °C for 60 min, and 1.5-ml aliquots were removed, chilled on ice, and mixed with 1.5 ml of n-butanol. After centrifugation at 4,000 × g for 10 min, the organic fraction was removed, and the A235 nm was measured. TBARs content was determined using a molar absorption coefficient of 156 mm−1 cm−1 (18, 25).

RNA Purification and Quantitative Real Time (qRT)-PCR—RNA purification was carried out using the Qiagen RNAeasy kit (Qiagen). *E. coli* BW25113 cultures were diluted 1:100, inoculated in 200 ml of LB medium, and incubated at 37 °C with shaking to an A600 ~ 0.6. Cultures were then amended with 0.5 μg/ml K2TeO3, 0.1 mM H2O2, 50 μg/ml paraquat, or 0.05% t-BOOH and incubated for 30 min. Cells were sedimented at 13,000 × g for 3 min and used for RNA purification.

Two μl of total RNA (~1 μg/μl) from control and treated cells were used in qRT-PCR experiments. Reactions (20 μl) used the LightCycler® RNA Amplification kit SYBR Green I (Roche Applied Science) and contained LightCycler® 0.4 μl of RT-PCR Enzyme Mix, 4 μl of SYBR Green I Reaction Mix, 3.2 μl of 25 mM MgCl2, 5.4 μl of H2O PCR grade, 4 μl of resolution solution, and 0.5 pmol of each specific primer. Amplification products were detected by increase of fluorescence. Crossing points (Cp) are inversely proportional to the RNA content and were determined using the software included in LightCycler® 2.0. Primers used to amplify yqhD are shown in Table 1. Induction of gene expression was expressed as the difference between the crossing points of each RT-PCR determination (Cp − Cptoxic). Positive numbers reflect higher amounts of the particular, specific mRNA in cells exposed to the toxic condition.

Acrolein Concentration—Acrolein concentration was determined as described by Slininger and Bothast (26). Acrolein reacts with tryptophan yielding a purple complex absorbing at 560 nm. Commercially available acrolein (Sigma) was used to standardize the assay and to make a calibration curve. “Tryptophan solution” consisted of tryptophan (2.05 g), 4.17 ml of 8.9 M HCl, and 2.5 ml of toluene in 1 liter of bi-distilled water. Approximately 300 μl of crude extracts (or acrolein standard) were mixed with 600 μl of 8.9 M HCl and 1.5 ml of tryptophan solution. Duplicated samples were incubated for 20 min at
TABLE 2
YqhD mediates resistance to potassium tellurite and other ROS elicitors in E. coli

| Strain                  | K2TeO3 | H2O2 | Paraquat | K2CrO7 | CdCl2 | Diamide |
|-------------------------|--------|------|----------|--------|-------|---------|
| E. coli pBAD            | 1.25   | 5    | 300      | 1.25   | 250   | 350     |
| E. coli pBAD yqhD       | 24     | 10   | 1000     | 5      | 250   | 350     |
| E. coli ΔyqhD           | 0.3    | 1.2  | 100      | 0.15   | 250   | 350     |

Table 3
YqhD is a NADPH-dependent aldehyde reductase

Enzyme activity (μmol of NADPH/min mg prot) was determined as described under “Experimental Procedures.” Each determination corresponds at least to three independent trials with each substrate concentration analyzed.

| Substrate     | Km     | kcat  | kcat/Km |
|---------------|--------|-------|---------|
|               | μM     | s-1   | s⁻¹M⁻¹  |
| Acetaldehyde  | 28.47  | 53.5  | 1880    |
| Propanaldehyde| 3.31   | 45.06 | 13613   |
| Butanaldehyde | 0.67   | 59.5  | 88018   |
| Acrolein      | 4.81   | 62.5  | 12994   |
| Malondialdehyde| 1.78  | 60.1  | 33764   |

RESULTS

YqhD Mediates Resistance to ROS Elicitors in E. coli—To determine whether YqhD plays a role in resistance to oxidative damage, minimal inhibitory concentrations for different ROS-generating compounds for E. coli strains lacking (ΔyqhD) and overexpressing the yqhD gene (pBAD yqhD) were determined (Table 2). Although cells overexpressing yqhD showed increased tolerance to compounds whose toxicity involves superoxide generation like paraquat and potassium tellurite (4, 27), the hydroxyl radical-generating chromate anion (5), and hydrogen peroxide, E. coli ΔyqhD showed increased sensitivity to all these compounds (Table 2). To assess whether YqhD-mediated resistance phenotype was also related to cytoplasm thiol depletion (especially GSH), the effect of YqhD on E. coli resistance to oxidant compounds that involve thiol oxidation like diamide and cadmium chloride (28) was determined. All E. coli strains tested exhibited similar resistance levels to these compounds, confirming the idea that YqhD protects E. coli from ROS-generated damage.

YqhD Is a NADPH-dependent Aldehyde Reductase—YqhD exhibits 40–50% sequence similarity with a number of bacterial alcohol dehydrogenases. However, it did not show any detectable enzymatic activity when tested with short-chain alcohols as substrates (20). In our hands, purified YqhD did not show NADP⁺-dependent dehydrogenase activity on methanol, ethanol, propanol, butanol, or isopropanol (not shown). YqhD showed enzymatic activity with short-chain aldehydes including a few with unsaturations or hydroxylations. Km values ranged from 0.67 mM for butanaldehyde to 28.5 mM for acetaldehyde. The kcat value was similar for all substrates (Table 3).

YqhD Elimination Increases TBARs Levels in E. coli—TBARs are routinely used to estimate malondialdehyde content. They are mainly generated during lipid peroxidation (17, 18, 25). We have previously shown that E. coli cells exposed to H2O2 or K2TeO3 have increased levels of TBARs (4). E. coli ΔyqhD exhibited increased content of TBARs even when H2O2 or K2TeO3 were not added to the culture, suggesting that YqhD may function in controlling the amount of membrane peroxidation products that are generated during the normal basal metabolism. E. coli ΔyqhD showed 2- (H2O2) and 4-fold (K2TeO3) TBARs increases when compared with the wild type strain, indicating that YqhD affects the content of reactive aldehydes within the cell (Fig. 1).

YqhD Protects E. coli from Protein Oxidation—Formation of carbonyl groups in proteins, a consequence of ROS and reactive aldehydes, is a commonly used marker to estimate oxidative stress. Anti-dinitrophenylhydrazine Western blotting was used to estimate the extent of cytoplasmic protein oxidation in E. coli pBAD yqhD and E. coli ΔyqhD. Probe activation was monitored in the presence or absence of the ROS-generating compounds K2TeO3, H2O2, paraquat, and chromate. E. coli cells lacking the yqhD gene showed an important increase in probe activation as compared with the wild type counterpart even in the absence of any compound. Overexpression of yqhD resulted in decreased peroxide levels in untreated cells. Peroxide levels were similar in wild type and pBAD yqhD cells (Fig. 2).
membrane peroxidation product in eukaryotic cells. Tellurite-, hydrogen peroxide-, or paraquat-mediated stress resulted in increased concentrations of acrolein in *E. coli* (Fig. 4). Acrolein content was proportional to the amount of K₃TeO₃, H₂O₂, or methyl viologen used to stress the cells (not shown). *E. coli ΔyqhD* showed increased levels of acrolein even in the absence of toxics, whereas *yqhD* overexpression prevented the ROS-mediated increase of acrolein concentration in *E. coli*. Genetic complementation of *ΔyqhD* cells restored the wild type acrolein phenotype (Fig. 4).

**Overexpression of yqhD Slightly Increases *E. coli* Resistance to Aldehydes Generated during Lipid Peroxidation**—To assess the physiological relevance of YqhD in vivo, *E. coli* resistance to aldehydes generated during membrane peroxidation was determined. *E. coli* resistance to acetaldehyde, propanal, butanal, and acrolein doubled as result of *yqhD* overexpression. A ~4-fold increase in resistance to MDA, the main peroxidation product, was also observed. Although *E. coli ΔyqhD* and the wild type strain showed identical minimal inhibitory concentrations for acetaldehyde, propanal, butanal, and MDA, *E. coli ΔyqhD* was slightly more sensitive to acrolein (Table 4).

The effect of *yqhD* expression on *E. coli* resistance to the classic membrane-peroxidizing compound t-BOOH was also determined. The hypersensitive phenotype to t-BOOH exhibited by *E. coli ΔyqhD* was reverted by genetic complementation. Overexpression of *yqhD* resulted in a significant increase in t-BOOH resistance (Table 4), again suggesting that YqhD is involved in *E. coli* defense against membrane lipid peroxidation.

**Transcription of yqhD Is Induced in Response to Conditions That Favor Lipid Peroxidation**—Induction of *yqhD* transcription in response to the oxidative stress elicitors K₃TeO₃ and paraquat was evaluated by qRT-PCR. Cp were determined for treated and untreated cells. A “Cp induction” value was defined as the difference between the Cp values obtained in each condition (see under “Experimental Procedures” for details). Increased amounts of *yqhD* mRNA were evidenced in cells exposed to individual toxics when compared with unexposed wild type cells (Table 5). Cp values for *yqhD* mRNA were ~1–2-fold higher in treated cells than those observed in untreated cells, reflecting an important difference in *yqhD* mRNA content between both conditions. The housekeeping gene gapA encoding glyceraldehyde-3-phosphate dehydrogenase showed a slight decrease in mRNA content in treated cells (not shown).

The *yqhD* Promoter Contains a Putative SoxS Binding Site—A putative SoxS binding site (CGTAAATTCTTAGGAATA) sharing 76% sequence identity with a SoxS binding sequence previously found in *E. coli* was identified 172 bp upstream of the *yqhD* ATG start codon (Promoter prediction program). Significant induction of *yqhD* mRNA synthesis was observed in *E. coli* lacking SoxS (Table 5), suggesting that induction of *yqhD* transcription is rather SoxS-independent.

**DISCUSSION**

Our interests in bacterial resistance to potassium tellurite lead us to investigate the synthesis of YqhD in *E. coli* grown in tellurite-amended media. We found that YqhD was highly induced when cells were grown in the presence of K₃TeO₃. This result and the fact that an important pathway of tellurite toxicity involves superoxide generation (4, 27) prompted us to evaluate the role that YqhD could have in *E. coli* resistance to tellurite and other oxidative stress elicitors.

To assess whether YqhD exhibits a general antioxidant function or a rather specific effect on ROS-caused oxidation, resistance to several oxidant agents was evaluated. *E. coli* resistance to ROS elicitors was directly related with *yqhD*
expression. Bacterial resistance to oxidants like diamide or CdCl₂ that do not involve ROS generation was not related to yqhD expression (Table 2). These results suggested that YqhD may play an antioxidant role by protecting E. coli from the oxidative damage caused specifically by ROS. This YqhD function would be of particular importance when cells encounter compounds that generate superoxide (tellurite) or hydroxyl radicals (chromate) (4, 5) since these toxins also affect cytoplasmic GSH levels (9, 29, 30). In this context, the hypersensitive phenotype to K₂TeO₃ and K₂Cr₂O₇ observed in E. coli ΔyqhD could be explained by a dual effect caused by ROS, namely the known direct damage on proteins, DNA, and membrane lipids and, indirectly, the inherent toxicity of peroxides and reactive aldehydes generated by membrane lipid peroxidation, which could not be scavenged in the absence of GSH.

Sulzenbacher et al. (20) have reported the crystal structure of E. coli alcohol dehydrogenase YqhD, and based on the special features exhibited by the bound NADPH cofactor (hydroxyl groups substituting the C5 and C6 carbons of the nicotinamide ring), they suggested a connection between YqhD and oxidative stress. Taking into account that YqhD shares similarity with a number of aldehyde dehydrogenases and the report by Zhang et al. (31) indicating that YqhD would be able of catalyzing the reduction of the toxic aldehyde 3-hydroxypropionaldehyde, we hypothesized that the antioxidant effect of YqhD could be related to reactive aldehydes generated by membrane lipid peroxidation. To test this assumption, YqhD was purified to homogeneity, and a number of potential substrates for the enzyme were tested in the presence of NADPH.

Kinetic parameters determined for YqhD showed that the enzyme is active with propanaldehyde, acetaldehyde, and butanaldehyde (Table 3). YqhD activity was also observed for the highly toxic aldehydes acrolein and MDA produced mainly during lipid peroxidation (2, 32). No activity was detected with short and medium chain alcohols. These results differ from those of Sulzenbacher et al. (20) who found that YqhD had no aldehyde dehydrogenase activity with acetaldehyde, butanaldehyde, and propanaldehyde. These authors also determined that YqhD exhibited the highest affinity with medium chain alcohols like propanol, butanol, and hexanol (Kₘ values 5–30 mM) suggesting that these compounds were not true substrates of the enzyme. We found that YqhD exhibited significant affinity for aldehydes (Kₘ values 0.6–4 mM for the best substrates) (Table 2). Based on the toxicity of the ROS elicitors K₂TeO₃ and K₂Cr₂O₇, which involves a strong effect in depleting the cellular GSH pool, we hypothesized that the observed YqhD activity with aldehydes could be of physiological relevance. In this context, detoxification of K₂TeO₃ and K₂Cr₂O₇ by E. coli (Table 3) corroborates our in vitro determinations and confirms that YqhD has aldehyde dehydrogenase activity on acrolein and MDA. Kinetic parameters determined for YqhD are consistent with those reported for a number of aldehyde dehydrogenases acting on reactive aldehydes produced by lipid peroxidation both in plant and mammalian cells (33, 34). However, although our results strongly support the idea that the enzyme functions as an aldehyde reductase in vivo as well as in vitro, the possibility of YqhD using other, yet unknown substrates in addition to acetaldehyde, propanaldehyde, butanaldehyde, MDA, and acrolein cannot be excluded.

Membrane peroxidation has been reported in bacteria exposed to oxidants like t-BOOH, TiO₂, H₂O₂, and K₂TeO₃ (4, 16–18). Determination of TBARs is routinely used to measure the major aldehyde produced during lipid peroxidation, MDA. To evaluate the role of YqhD in regulating the levels of reactive aldehydes, TBARs content was determined in E. coli ΔyqhD. This mutant showed higher levels of TBARs than the wild type strain. E. coli cultures entering the exponential growth phase or in the stationary phase as well have been shown to exhibit increased levels of aldehyde dehydrogenase activity (35). High aldehyde dehydrogenase activity has been also reported in cells exposed to stress conditions as low temperatures, salinity, UV radiation, and ROS elicitors (33). Thus, the increased TBAR content observed in E. coli ΔyqhD could reflect a role of YqhD in maintaining safe levels of reactive aldehydes within the cell.

To further investigate the relationship that may exist between YqhD aldehyde dehydrogenase activity observed in
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vitro and the level of reactive aldehydes generated by lipid peroxidation in vivo, we determined acrolein concentration in cells exposed to various ROS elicitors. Acrolein, or 2-propenal, is a highly toxic aldehyde generated as a byproduct during membrane peroxidation (32). Although acrolein generation has been scantily described in bacteria to date, its lethal effect for prokaryotes has been clearly demonstrated (13, 14). We found that E. coli exposed to H2O2, paraquat, K2Cr2O7, and K2TeO3 exhibited increased acrolein concentration (Fig. 4). As expected, cells that were exposed to low temperatures, a condition that increases membrane polyunsaturated fatty acid content (36), showed increased acrolein content (not shown) linking oxidative stress with acrolein production in E. coli. These results confirm that the aldehyde reductase activity of YqhD observed in vitro is probably related to the in vivo concentration of YqhD substrates.

Lipid hydroperoxides are intermediates of membrane lipid peroxidation that can generate reactive aldehydes (7, 8). Lipid hydroperoxides exhibit increased polarity and long lifetimes as compared with free radical intermediates, thus facilitating their interaction with intracellular targets (37). To cope with this highly reactive molecules, cells produce catalases, peroxidases, and/or glutathione transferases that transform peroxides in more stable molecules or unreactive adducts (19). We found that expression of yqhD directly affects intracellular peroxide levels in E. coli exposed to H2O2, K2TeO3, K2Cr2O7, or paraquat. Cells incapable of producing yqhD showed an increased concentration of intracellular peroxides suggesting that YqhD plays an important role in detoxifying reactive aldehydes that could increase intracellular peroxide generation or affect cellular defenses. This idea is consistent with the observation that cells overexpressing yqhD exhibited decreased levels of intracellular peroxides.

Another common marker used to assess oxidative stress is carbonyl group formation in proteins. This results from amino acid side chain modification preferentially caused by ROS and reactive aldehydes (18, 38). Because E. coli ΔyqhD contains increased levels of reactive aldehydes, determined as TBARs and acrolein, we hypothesized that the protective role of YqhD would be to prevent aldehyde-induced protein oxidation. We found that E. coli-defective in yqhD showed higher levels of oxidized proteins than wild type cells, suggesting that YqhD is somehow involved in protecting the cell from cytoplasmic oxidation and from oxidants generated under stress and also during the basal metabolism.

The respiratory electron transport chain is a major (and continuous) source of ROS in bacteria (3, 19). The “naturally generated” reactive oxygen species would be responsible for the increased levels of TBARs and oxidized protein found in E. coli ΔyqhD. Both phenotypes are reverted to wild type levels upon genetic complementation with yqhD. In addition, overexpression of yqhD resulted in decreased protein oxidation (Fig. 3B). This protective effect takes place in normally growing cells and is particularly high in cells subjected to conditions that favor lipid peroxidation. Activation of oxidative stress-inducible promoters by potassium tellurite supports this assumption. Recent results from our laboratory have shown that tellurite-mediated induction of the ibpA gene promoter, a gene related with oxidative response (39, 40), is blocked by yqhD overexpression. Similar findings were not observed with other stress-responsive promoters not related to oxidative stress as cspA, p3RpoH, and sulA.

Because in this work yqhD expression has been related to ROS resistance, TBARs, acrolein, peroxide, and especially oxidized protein levels, we hypothesized that the role of YqhD aldehyde reductase is to protect cytoplasmic macromolecules from aldehyde-mediated oxidation. E. coli resistance to lipid peroxidation-generated aldehydes revealed a correlation between yqhD expression and bacterial resistance, especially to acrolein (Table 4). These results are in agreement with the observed effect of yqhD on acrolein concentration in vivo as well as with enzyme activity exhibited by the purified protein. As proof that YqhD substrates come from membrane lipid peroxidation, we evaluated E. coli t-BOOH resistance. Although yqhD overexpression resulted in increased resistance, cells lacking yqhD showed a hypersensitive phenotype to this peroxide.

Because the lipid peroxidation pathway includes an elongation step in which oxygen reacts with a carbonyl radical to generate a lipoperoxide radical (41), we carried out K2TeO3 and H2O2 resistance analyses in E. coli under anaerobic conditions. No differences in resistance to K2TeO3 and H2O2 were observed between wt and yqhD-overexpressing cells in the absence of oxygen. TBAR determination suggested that lipid peroxidation does not occur in this condition, indicating that toxic YqhD substrates are not generated (unpublished results).

Transcription activation of the yqhD gene has been observed in cells treated with the oxidant Curvularia haloperoxidase system (23) when exposed to cold shock (21) or when grown in a limited-nutrient medium (22). Here we demonstrate that yqhD is also induced in cells exposed to conditions favoring lipid peroxidation, as are treatments with the ROS elicitors K2TeO3, H2O2, or paraquat or with the membrane lipid-peroxidating compound t-BOOH.

A highly conserved SoxS binding motif was identified in the promoter region of the yqhD gene (not shown). qRT-PCR studies carried out using RNA from wt and ΔsoxS E. coli strains suggested that yqhD induction is not dependent on SoxS. However, because gene activation may result from the action of various different transcriptional activators, this result does not rule out the functionality of the identified SoxS binding site.

In humans, a number of enzymes using cytotoxic reactive aldehydes as substrates have been described. As YqhD, these enzymes react with acrolein and MDA among others and are thought to form part of oxidative defense mechanisms able to neutralize the toxic effects of these lipid peroxidation-derived reactive aldehydes in heart (42), kidney (43), brain (44), and arterial tissues (45).

Some mechanistic insights have been communicated in Arabidopsis thaliana where a NADPH quinone oxidoreductase (P1–ZCR) has been involved in the detoxification of lipid peroxidation-derived reactive aldehydes (33). The authors proposed that this protein was part of a defense mechanism against

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oxidative stress by scavenging highly toxic lipid peroxidation-derived unsaturated aldehydes as acrolein and 4-hydroxynonenal. In addition, a NADPH-dependent AKR1C1 protein, which is induced by Michael acceptors and oxidative stress and that exhibits aldehyde reductase activity involved in cellular protection against reactive aldehydes, has been reported in humans (34).

To date no specific defense pathway dealing with lipid peroxidation-derived reactive aldehydes has been reported in bacteria. The results of this work allow us to speculate that YqhD is part of a response mechanism against lipid peroxidation-derived aldehydes. The role we propose for YqhD is especially relevant for *E. coli* cells that are stressed by ROS elicitors and by compounds that produce GSH oxidation. As mentioned before, the first cell defense mechanism coping with lipid peroxidation products is GSH-dependent and consists in a set of glutathione S-transferases, catalases, and peroxidases. In cells exposed to K$_2$TeO$_4$ or K$_2$Cr$_2$O$_7$, two compounds that deplete the intracellular GSH pool and generate ROS-mediated lipid peroxidation, this first defense mechanism would be absent because of glutathione oxidation. In this context, the GSH-independent, NADPH-dependent YqhD putative mechanism described here would be highly relevant in protecting the bacterial cell from being damaged by MDA, acrolein, and other aldehydes. In the absence of GSH, YqhD and probably other yet unknown aldehyde dehydrogenases or aldehyde reductases would act as cytosolic barriers against toxic products generated during lipid peroxidation in *E. coli*.

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YqhD Is an Aldehyde Reductase