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Enhanced sensitivity of ubiquinone-deficient mutants of *Saccharomyces cerevisiae* to products of autoxidized polyunsaturated fatty acids

(coenzyme Q/lipid hydroperoxide/antioxidant)

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ABSTRACT **Coenzyme Q (ubiquinone or Q) plays a well known electron transport function in the respiratory chain, and recent evidence suggests that the reduced form of ubiquinone (QH_2) may play a second role as a potent lipid-soluble antioxidant. To probe the function of QH_2 as an antioxidant *in vivo*, we have made use of a Q-deficient strain of *Saccharomyces cerevisiae* harboring a deletion in the *COQ3* gene [Clarke, C. F., Williams, W. & Teruya, J. H. (1991) *J. Biol. Chem.* 266, 16636–16644]. Q-deficient yeast and the wild-type parental strain were subjected to treatment with polyunsaturated fatty acids, which are prone to autoxidation and breakdown into toxic products. In this study we find that Q-deficient yeast are hypersensitive to the autoxidation products of linolenic acid and other polyunsaturated fatty acids. In contrast, the monounsaturated oleic acid, which is resistant to autoxidative breakdown, has no effect. The hypersensitivity of the *coq3*Δ strains can be prevented by the presence of the *COQ3* gene on a single copy plasmid, indicating that the sensitive phenotype results solely from the inability to produce Q. As a result of polyunsaturated fatty acid treatment, there is a marked elevation of lipid hydroperoxides in the *coq3* mutant as compared with either wild-type or respiratory-deficient control strains. The hypersensitivity of the Q-deficient mutant can be rescued by the addition of butylated hydroxytoluene, α-tocopherol, or trolox, an aqueous soluble vitamin E analog. The results indicate that autoxidation products of polyunsaturated fatty acids mediate the cell killing and that QH_2 plays an important role *in vivo* in protecting eukaryotic cells from these products.**

Coenzyme Q (ubiquinone or Q) is a lipid component of the electron transport chain that ferries electrons between complex I (or complex II) and the cytochrome b–c_1 complex (1, 2). In eukaryotic cells, Q performs these transport functions in the inner mitochondrial membrane, yet many intracelluar membranes contain Q (3, 4). The redox chemistry that allows the reversible cycling between the hydroquinone (QH_2) and Q in electron transport may also allow QH_2 to function as a lipid-soluble antioxidant. Many *in vitro* studies suggest that QH_2 scavenges free radicals and prevents lipid peroxidative damage in both mitochondrial and nonmitochondrial membrane fractions, liposome vesicles, and lipoproteins (5–7).

The mechanisms by which QH_2 functions as an antioxidant are incompletely understood. QH_2 may scavenge lipid peroxyl radicals and function as an antioxidant analogous to vitamin E (8, 9). QH_2 may also be involved in regenerating α-tocopherol (10–12). It is also possible that QH_2 may function to prevent initiation of lipid peroxidation as it has been reported to scavenge perferryl radicals (6). The content of QH_2 in low density lipoprotein particles is correlated with increased resistance to the initiation of lipid peroxidation (13–15). The level of QH_2 and other antioxidants in low density lipoprotein may thus have a profound influence on slowing the development of atherosclerosis, since oxidatively modified low density lipoprotein is thought to play an important role in the initiation of this disease (16, 17). Thus the "secondary" action of QH_2 as an antioxidant may play an important function in aging and in age-related degenerative diseases (18).

Despite the use of Q in a variety of clinical therapies and as a nutritional supplement, little is known regarding its mode of action in these settings (for example as antioxidant or electron transport component). To learn more about the functions of QH_2 *in vivo*, we have made use of yeast mutants that are completely deficient in Q. Tzagoloff and co-workers (19–21) have described eight complementation groups (*coq1–coq8*) of *Saccharomyces cerevisiae* mutants deficient in Q. These mutants lack Q and hence are respiratory defective and fail to grow on nonfermentable carbon sources. The *COQ3* gene of *S. cerevisiae* encodes the 3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase (22, 23). This methyltransferase is conserved among eukaryotes (24, 25), and yeast harboring a *COQ3* gene deletion (*coq3*Δ) do not synthesize Q (22).

In the current study we use *coq3*Δ yeast mutants to investigate the possible role of Q as an antioxidant. Since much *in vitro* evidence suggests that QH_2 functions as a lipid-soluble antioxidant, we were particularly interested in the susceptibility of the *coq3*Δ mutants to treatment with polyunsaturated fatty acids, which are known to generate lipid peroxides and peroxyl radicals by autoxidation reactions (26). The resulting lipid peroxides and lipid peroxyl radicals are chemically reactive, prone to further breakdown and rearrangements, and result in many products that are toxic to cells (27). Although *S. cerevisiae* does not normally produce polyunsaturated fatty acids, it does utilize polyunsaturated fatty acids when provided exogenously (28–30). In this study, we have compared the sensitivity of wild-type yeast and isogenic *coq3*Δ mutants to treatment with mono- or polyunsaturated fatty acids. The results show that Q-deficient yeast are hypersensitive to the products of polyunsaturated fatty acid autoxidation and suggest that QH_2 plays an important role *in vivo* as an antioxidant.

MATERIALS AND METHODS

Chemicals and Plasmid Constructions. Oleic acid, linoleic acid, linolenic acid, arachidonic acid, *t*-butyl hydroperoxide, cumene hydroperoxide, (+)-α-tocopherol, ammonium ferrous(II) sulfate, thiobarbituric acid, and glass beads (425–600 μm) were purchased from Sigma. Butylated hydroxytoluene

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Abbreviations: Q, ubiquinone; *atp2*Δ, yeast mutant harboring an *ATP2* gene deletion; BHT, butylated hydroxytoluene; *coq3*Δ, yeast mutant harboring a *COQ3* gene deletion; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; QH_2, ubiquinol; SOD, superoxide dismutase.

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(BHT), hydrogen peroxide, and paraquat (methyl viologen) were from Fischer. Xylenol orange was from Aldrich. Trolox (rac-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was from Fluka. The plasmid pCC-COQ3 was constructed by ligating the 2.2-kb *Sma*I fragment containing the *COQ3* gene from pRS12A (22) into the *Sma*I site of pRS313 (31).

Yeast Strains. Mutant strains of *S. cerevisiae* are described in Table 1. DO103 and CC304.1 were constructed by the one-step gene replacement procedure (32) with a 5.0-kb fragment from pMΔBL2, provided by D. Mueller (33). In pMΔBL2, 840 bp of the *ATP2* gene is replaced with a 3.0-kb *Bgl*II fragment of YEp13 containing the *LEU2* gene (33). The *COQ3* null mutation in CC303.1 was derived as described previously (22, 24). *ATP2* and *COQ3* null mutants failed to grow on media containing the nonfermentable carbon source glycerol. Disruptions of the *SOD2* and *SOD1* genes were performed as described previously (34, 35). All gene disruptions were verified by Southern blot analysis (37) of yeast genomic DNA.

Growth and Preparation of Yeast. Yeast were grown in YPD media (38) (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) at 30°C under atmospheric oxygen with shaking at 200 rpm. Yeast strains harboring the plasmids pRS313 or pCC-COQ3 were grown in synthetic complete media without histidine as described (38), to maintain selection for the plasmid. Cells were harvested in logarithmic phase (A_{600nm} = 0.1–1.0) and washed twice with sterile distilled water (4°C, 1000 × *g*, 5 min). Washed cell pellets were resuspended in sterile 0.1 M sodium phosphate, pH 6.2/0.2% dextrose, to a density of 10^6 or 10^7 cells/ml (A_{600nm} = 0.1 or 1.0, respectively). Cell dry weights were determined by drying cell samples in a 100°C oven overnight and weighing the dry cell samples.

Fatty Acid Sensitivity Assay. Yeast cell suspensions prepared above were aliquoted into 125-ml Ehrlenmeyer flasks, and either oleic (1:1 in ethanol) or linolenic acid (1:1 in ethanol) were added and then incubated at 30°C, 200 rpm under atmospheric oxygen (the final fatty acid concentration ranged from 8.2 to 820 μM). Samples were taken prior to the addition of fatty acids and after 1, 2, and 4 h of incubation and plated on solid YPD medium containing 2% agar. Viable colonies were counted after three days of growth at 30 °C to determine the percent survivors. 100% was defined as the number of cells capable of forming colonies before treatment with fatty acids.

Alternatively, cells were stressed with various concentrations of linoleic acid, arachidonic acid, hydrogen peroxide, *t*-butyl hydroperoxide, cumene hydroperoxide, or paraquat, and the percent survivors was determined as described. Assays were similarly carried out in which 200 μM trolox, vitamin E, or BHT were added to the cell suspensions prior to the addition of 82 μM linolenic acid. Experiments were also performed in which yeast cell suspensions treated with 820 μM oleic or linolenic acid were incubated in the presence of 100% nitrogen rather than atmospheric oxygen. For these experiments, N_2 gas was bubbled gently through water and then delivered to side-arm Ehrlenmeyer flasks via tygon tubing. Samples for plating assays were removed by syringe via a septum placed over the side-arm.

Table 1. Genotype and sources of *S. cerevisiae* strains

Strain	Genotype	Source
EG103	*α leu2-3,112 his3Δ1 trp1-289a ura3-52*	ref. 35
EG110	EG103-*sod2Δ::TRP1*	ref. 36
EG118	EG103-*sod1Δa::URA3*	ref. 36
DO103	EG103-*atp2Δ::LEU2*	This study
FW103	EG103-*coq3Δ::LEU2*	ref. 25
FW110	FW103-*sod2Δ::TRP1*	This study
CC2039	FW103-*sod1Δa::URA3*	This study
W303.1B	*α ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*	ref. 37
CC303.1	W303.1B-*coq3Δ::LEU2*	This study
CC304.1	W303.1B-*atp2Δ::LEU2*	This study

Preparation of Cell Lysates. Yeast cell suspensions (A_{600nm} = 1.0) were treated with 820 μM linolenic acid and incubated for various times as described. Samples (50 ml) were harvested either before or after incubation with 820 μM linolenic acid and washed twice with 40 ml of distilled water (4°C, 1000 × *g*, 5 min). Cell pellets were transferred to 13 × 100-mm glass culture tubes and resuspended in 0.3 ml of methanol/0.01% BHT. Glass beads (1 g) were added, and cells were lysed by vortexing (4 cycles, 30-s vortex, 30 s on ice), and the upper methanol layer was transferred to a microcentrifuge tube. The glass beads were washed once with 1 ml of methanol/0.01% BHT, the methanol layers were pooled, and following centrifugation (16,000 × *g*, 5 min, 4°C) the supernatants were assayed for autoxidation products.

Detection of Hydroperoxides. A modified ferrous oxidation/xylenol orange assay (39) was used to determine the levels of hydroperoxides in yeast cell lysates. Samples of yeast cell lysates (0.1 ml) were added to 0.7 ml of methanol/0.01% BHT. Then 0.1 ml of Reagent A (2.5 mM ammonium ferrous(II) sulfate/0.25 M sulfuric acid), and 0.1 ml of Reagent B (40 mM BHT/1.25 mM xylenol orange in methanol) were added. Samples were incubated (30 min, room temperature), and the absorbance at 560 nm was measured. The amount of hydroperoxides present in the yeast cell lysates was determined from a hydrogen peroxide standard curve (H_2O_2 $\varepsilon = 2.8 \times 10^4$ M^{-1} cm^{-1} in methanol at 233 nm) (40).

Detection of Thiobarbituric Acid Reactive Substances (TBARS). The levels of TBARS in yeast cell lysates were determined by the thiobarbituric acid assay (41) modified as follows. Samples of yeast cell lysates (0.1 ml) were mixed with 0.4 ml of methanol (0.01% BHT) and 0.5 ml of 1% thiobarbituric acid (prepared in 1% sulfuric acid) and incubated (100°C, 15 min). Samples were allowed to cool and after centrifugation (16,000 × *g*, 10 min); the absorbance at 532 nm was measured and corrected for by subtracting nonspecific turbidity at 600 nm (42). Levels of TBARS [malondialdehyde (MDA) equivalent] were determined with a MDA standard curve. MDA was prepared by incubating malonaldehyde bis-(dimethylacetal) in 1% sulfuric acid (MDA $\varepsilon = 1.37 \times 10^4$ M^{-1} cm^{-1} in 1% sulfuric acid at 245 nm) (43).

RESULTS

Sensitivity of Q-Deficient Yeast to Polyunsaturated Fatty Acids. Wild-type and Q-deficient (*coq3Δ*) yeast strains were treated with either the monounsaturated oleic acid or the polyunsaturated linolenic acid, and viable cells were determined after various periods of incubation (Fig. 1). Wild-type yeast were partially sensitive when exposed to 820 μM linolenic acid for 4 h in the presence of air. However, less than 1% of the *coq3Δ* yeast were viable after 4 h of incubation with 820 μM linolenic acid. Since *coq3Δ* yeast are unable to respire, the hypersensitivity to linolenic acid might result from the respiration-defective phenotype. To test whether another respiration-defective strain also exhibits hypersensitivity to linolenic acid, a null mutant in the *ATP2* gene (which encodes the β subunit of the ATPase) was constructed (*atp2Δ*). When treated with 820 μM linolenic acid, the *atp2Δ* strain showed sensitivity similar to that of the parental wild-type strain. None of the strains were sensitive to oleic acid or to the addition of 0.025% ethanol as a control (data not shown), suggesting that the sensitivity stems from the susceptibility to linolenic acid.

Treatment with a 10-fold lower concentration of linolenic acid (82 μM) generated results very similar to those shown in Fig. 1, while linolenic acid concentrations of 8.2 μM or lower resulted in no cell killing by 4 h (data not shown). Q-deficient

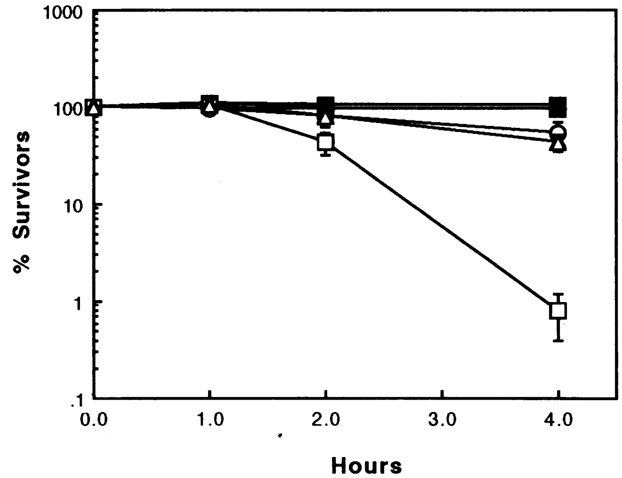

FIG. 1. Q-deficient (*coq3*Δ) yeast are hypersensitive to treatment with linolenic acid. Parental wild-type strain EG103 (○, ●), *coq3*Δ strain FW103 (□, ■), and *atp2*Δ strain DO103 (△, ▲) were incubated in 0.2% dextrose/0.1 M sodium phosphate (pH 6.2) supplemented with either 820 μM oleic (●, ■, ▲) or linolenic (○, □, △) acid at 30°C, 200 rpm in the presence of atmospheric oxygen. Samples were taken at various time points and plated on solid YPD medium to determine percent survivors. 100% is defined as the number of cells capable of forming colonies at the zero time point. Values are presented in log scale as mean ± SD of three independent experiments.

yeast were also hypersensitive to treatment with 820 μM arachidonic acid (5,8,11,14-eicostetraenoic acid) for 4 h, while under these conditions the wild-type and *atp2*Δ strains were only partially sensitive (data not shown). Results similar to Fig. 1 were also obtained when *COQ3* and *ATP2* deletion constructs were prepared in another wild-type background (W303–1B, Table 1; data not shown). These results suggest that hypersensitivity to polyunsaturated fatty acids results from Q deficiency and is independent of strain background or respiration competence.

To confirm that the sensitivity of Q-deficient yeast to linolenic acid was due to the *coq3* gene deletion, the *COQ3* gene on a single copy plasmid (pCC-COQ3.3), or the plasmid alone (pRS313) was introduced into FW103 (*coq3*Δ). As shown in Fig. 2, the *coq3*Δ strain harboring the *COQ3* gene on a single copy plasmid showed only partial sensitivity similar to that of the wild-type strain (Fig. 1), while the *coq3*Δ strain harboring the plasmid pRS313 was less than 1% viable by 4 h of incubation. Thus the *COQ3* gene when present at one copy per cell protects the *coq3*Δ strain from hypersensitivity to polyunsaturated fatty acids, suggesting that the hypersensitive phenotype results solely from the inability to produce Q.

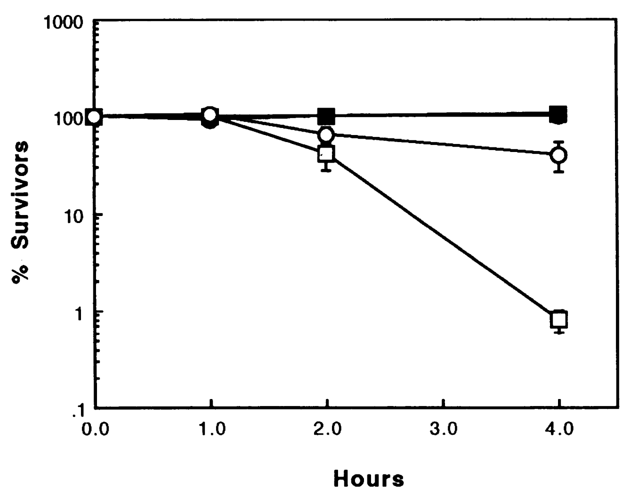

FIG. 2. Linolenic acid-sensitive phenotype of Q-deficient yeast can be rescued by the *COQ3* gene on a single copy plasmid. Yeast strains FW103:PRS313 (Coq⁻) (□, ■) and FW103:pCC-COQ3 (Coq⁺) (○, ●) were incubated in the presence of 820 μM oleic (●, ■) or linolenic (○, □) acid and atmospheric oxygen as described in Fig. 1. Values are expressed as mean ± SD of three independent experiments.

Autoxidation Products in Yeast Strains Treated with Polyunsaturated Fatty Acids. To confirm that the hypersensitivity of Q-deficient yeast was due to autoxidation of linolenic acid, the levels of lipid peroxidation products in yeast stressed with linolenic acid were determined. Wild-type, *coq3*Δ, and *atp2*Δ strains were treated with 820 μM linolenic acid, and the levels of hydroperoxides and TBARS in cells were determined over various periods of incubation (Fig. 3). Levels of hydroperoxides and TBARS were significantly higher in the hypersensitive *coq3*Δ strain than in wild-type or *atp2*Δ strain over the course of incubation. These results show a correlation between increased sensitivity to linolenic acid and levels of autoxidation products, suggesting that the hypersensitivity of the Q-deficient strain resulted from autoxidation of linolenic acid.

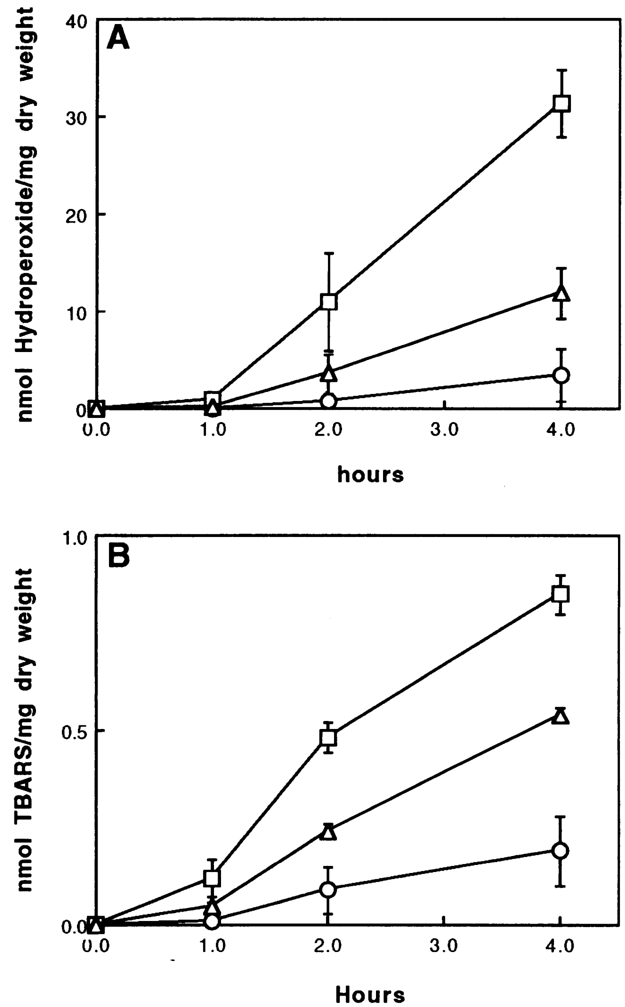

FIG. 3. Autoxidation products are elevated in Q-deficient yeast treated with linolenic acid. Wild-type EG103 (○), *coq3*Δ FW103 (□), and *atp2*Δ yeast DO103 (△) were incubated with 820 μM linolenic acid as described in Fig. 1. Cell lysates were prepared at various times, and the levels of hydroperoxides (*A*) and TBARS (*B*) were determined as described in *Materials and Methods*. (*A*) nmol of hydroperoxide detected per mg cell dry weight extracted. (*B*) nmol of TBARS detected per mg cell dry weight extracted. Values are expressed as mean ± SD of three independent experiments.

Sensitivity of Q-Deficient Yeast to Nonlipid Agents Inducing Oxidative Stress. The above results suggest that QH_2 plays an important role *in vivo* in protecting cells from the toxic effects of autoxidation products of polyunsaturated fatty acids. To investigate whether the Q-deficient yeast are sensitive to other types of oxidative stress, agents were examined that do not affect wild-type yeast but profoundly inhibit growth of yeast strains deficient in superoxide dismutase genes (44). Plate growth assays showed that both wild-type and *coq3*Δ yeast were resistant to treatment with 100% oxygen and to the redox cycling drug paraquat (data not shown). In other plate growth assays with *t*-butyl hydroperoxide or cumene hydroperoxide, the sensitivity of the *coq3*Δ and wild-type strains were identical. In fact in these assays, the variability between different wild-type laboratory strains of *S. cerevisiae* was greater than the difference between the isogenic wild-type and *coq3*Δ strains (data not shown). Survivor curves were also determined for wild-type and *coq3*Δ strains treated with hydrogen peroxide, *t*-butyl hydroperoxide, cumene hydroperoxide, or paraquat. In general, both wild-type and *coq3*Δ yeast were sensitive to these agents in a time- and concentration-dependent manner, with no significant differences in sensitivity between wild-type and *coq3*Δ strains (data not shown). These results indicate that the presence or absence of Q (and hence QH_2) makes no difference in the susceptibility to a variety of oxidative stress conditions.

Antioxidants Added Exogenously Partially Protect Q-Deficient Mutants to Linolenic Acid Treatment. To examine the effect of exogenously added antioxidants on the sensitivity of both wild-type and Q-deficient yeast to linolenic acid, BHT, α-tocopherol, and the more aqueous soluble α-tocopherol analog trolox were added to yeast prior to the addition of 82 μM linolenic acid. The effects after 4 h of incubation in the presence of air are shown in Fig. 4. Trolox, α-tocopherol, and BHT dramatically increased the percent survivors of both the wild-type and *coq3*Δ strains. The ability of free radical scavengers to protect cells from linolenic acid provides strong evidence that autoxidation products of linolenic acid are responsible for initiating cell killing. A low oxygen environment provided by flushing the culture flasks with nitrogen provided a degree of protection that was comparable with that afforded by antioxidants (data not shown).

Yeast Deficient in Superoxide Dismutase (SOD) Do Not Show Enhanced Sensitivity to Autoxidation Products of Linolenic Acid. Bilinski *et al.* (45) reported that wild-type yeast shifted from anaerobic to aerobic growth conditions were sensitive to treatment with linolenic acid; under these same conditions *sod1* mutants exhibited enhanced sensitivity. To determine whether combined deficiencies in SOD and Q result in enhanced sensitivity to linolenic acid, the *SOD1* or *SOD2* loci were disrupted in the *coq3*Δ strain. Fig. 5 shows that yeast strains with disruptions of either *SOD1*, *SOD2*, or both *SOD1* and *SOD2* (Table 1) (34, 35) are no more sensitive to linolenic acid treatment than the parental wild-type strain. In *coq3*Δ yeast strains, the additional deletion of *SOD1* or *SOD2* does not alter the hypersensitivity to linolenic acid. Thus in the total absence of Q, the presence or absence of SOD makes no difference with respect to the hypersensitivity to products of linolenic acid autoxidation.

DISCUSSION

These results show that Q-deficient (*coq3*Δ) yeast are hypersensitive to autoxidation products of polyunsaturated fatty acids. Less than 1% of *coq3*Δ cells are viable following a 4-h incubation with 820 or 82 μM linolenic acid. In contrast, about 50–70% of wild-type cells subjected to this treatment remain viable (Figs. 1 and 5). Q-deficient yeast are also sensitive to other polyunsaturated fatty acids that readily autoxidize (such as arachidonic acid) but are unaffected by the monounsaturated oleic acid that does not undergo this chemistry. The sensitivity to autoxidized polyunsaturated fatty acids is manifested in two strains of *coq3*Δ yeast from different backgrounds. Importantly, *coq3*Δ strains can regain resistance by the introduction of the yeast *COQ3* gene on a single copy plasmid, indicating that the sensitive phenotype results solely from the inability to produce Q (and hence QH_2). The hypersensitivity of *coq3*Δ strains is not a secondary effect of the inability to respire, since respiratory defective *atp2* null

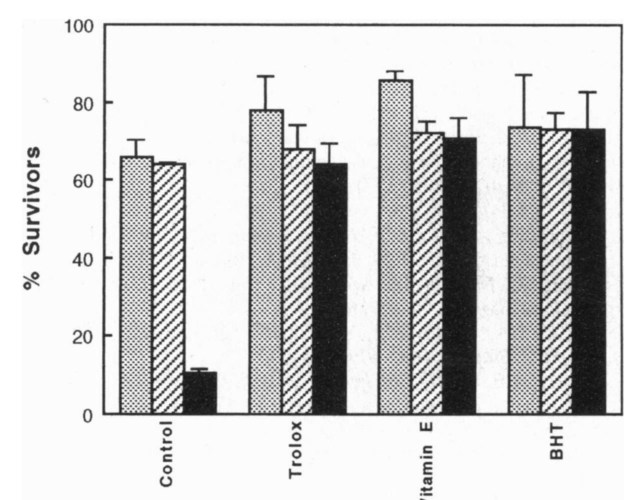

FIG. 4. Effect of exogenously added anti-oxidants on sensitivity to linolenic acid. Wild-type EG103 (▩), *coq3*Δ FW103 (■), and *atp2*Δ strain DO103 (▨) were treated with 82 μM linolenic acid (control) or prior to addition of linolenic acid, 200 μM of either trolox, vitamin E, or BHT were added to the cell suspensions. Incubations (5 ml) were performed in 17 × 150 mm test tubes, which afforded less aeration and resulted in slightly more survivors at the 4-h time point. After 4 h, aliquots were plated on solid YPD media to determine percent survivors. Values are expressed as mean ± SD of two independent experiments.

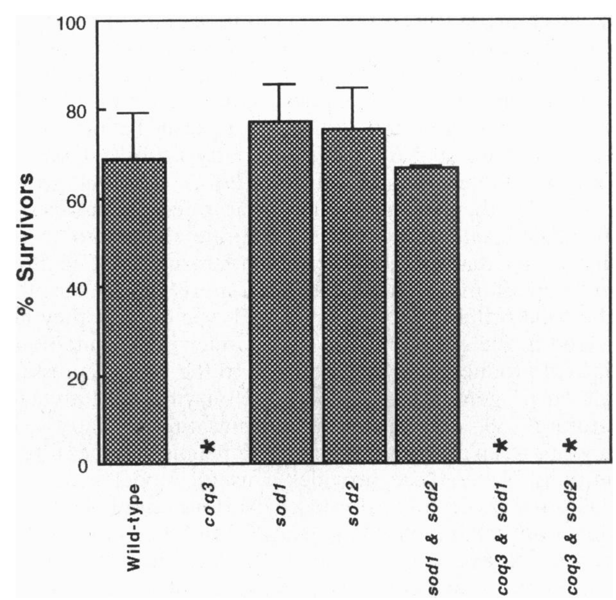

FIG. 5. Sensitivity of wild-type, Q-deficient and SOD-deficient yeast to linolenic acid. Yeast strains EG103 (wild-type), FW103 (*coq3*Δ), EG118 (*sod1*Δ), EG110 (*sod2*Δ), EG133 (*sod1*Δ, *sod2*Δ), CC2039 (*coq3*Δ, *sod1*Δ), and FW110 (*coq3*Δ, *sod2*Δ) were incubated with 82 μM linolenic acid as described in Fig. 1. Percent survivors determined after 4 h of incubation are as indicated or are less than 1% (asterisk). Values are expressed as mean ± SD of two measurements.

mutants (which fail to produce the β subunit of the ATPase) behave similarly to the parental wild-type strains.

Products of linolenic acid autoxidation are numerous and include hydroxy acids, oxo acids, epoxy acids, and aldehydes (46). The identity of the product(s) mediating the toxicity are not known. As a result of linolenic acid treatment, two different classes of autoxidation products, lipid peroxides and late stage aldehyde products (TBARS), were found to be markedly elevated in cell extracts of *coq3Δ* yeast as compared with wild-type yeast. Such products in the *atp2Δ* yeast accumulated to intermediate levels—both lipid peroxides and TBARS in the *atp2Δ* strain were significantly lower than in the *coq3Δ* strain, yet significantly elevated when compared with wild-type. Perhaps under conditions of respiratory deficiency (*atp2Δ*) it is more difficult for the cell to maintain adequate levels of QH_2. In this regard, it should be interesting to assess the effect of long term linolenic acid treatment on the *atp2Δ* and wild-type yeast strains. The hypersensitivity of the Q-deficient mutants can be rescued by the addition of a variety of antioxidants including BHT, α-tocopherol, or trolox, an α-tocopherol analog. Incubation of the yeast in the presence of nitrogen also rescues the sensitivity to linolenic acid. These data support the idea that the autoxidation products of polyunsaturated fatty acids mediate the cell killing and that QH_2 plays an important role *in vivo* in protecting eukaryotic cells from these products.

To investigate other aspects of protection that might be afforded by QH_2, the Q-deficient yeast strains were subjected to nonlipid oxidative stress agents. In contrast to the sensitivity to autoxidation breakdown products of polyunsaturated fatty acids, other types of oxidative stress do not specifically target the Q-deficient cells. For example, wild-type and *coq3Δ* yeast show very similar sensitivities to hydrogen peroxide, cumene hydroperoxide, and *t*-butyl hydroperoxide. Thus *in vivo* QH_2 does not appear to afford protection against hydroxyl or alkoxy radicals. Q-deficient and wild-type yeast are also equally resistant to exposure to 100% O_2 and to paraquat. Thus it seems unlikely that Q/QH_2 *in vivo* is involved in scavenging superoxide per se.

S. cerevisiae synthesize only monounsaturated fatty acids (28), which are very resistant to lipid peroxidation (26). The relative resistance of yeast like *Schizosaccharomyces pombe* and *S. cerevisiae* to oxidative stress in most laboratory cultures may stem from the absence of polyunsaturated fatty acids (47). However, polyunsaturated fatty acids can easily be introduced since yeast cells take up exogenous fatty acids and rapidly incorporate them into glycerolipids (29). *S. cerevisiae* grown aerobically in the presence of polyunsaturated fatty acids will preferentially internalize and incorporate them into membranes rather than expend energy synthesizing fatty acids *de novo*. Polyunsaturated fatty acids can comprise more than 50% of the total cellular fatty acids of wild-type yeast if they are provided in the growth media (30). Under these conditions, oleic acid production is inhibited due to the rapid repression of the *OLE1* gene, which encodes the enzyme Δ-9 fatty acid desaturase (30, 48). Since the polyunsaturated fatty acid composition can range from 0 to 50% depending on the culture conditions, *S. cerevisiae* provides a useful model system to evaluate the protection afforded by various antioxidants, including both small molecule scavengers and the enzymes that scavenge reactive oxygen species. In fact these organisms provide a means of ascertaining the targets of reactive oxygen species other than polyunsaturated fatty acids. For example, Janda *et al.* (47) found that hydrogen peroxide inhibits sugar transport in *S. pombe*, indicating a direct effect of the oxidant species on membrane proteins. In this regard it is interesting that QH_2 may also be involved in protein repair, as it has recently been found to be capable of reducing both ferrylmyoglobin and metmyoglobin to oxymyoglobin (49). There is evidence indicating that QH_2 may protect mitochondrial proteins and DNA against oxidative damage as well (50, 51).

The experiments reported here show that aerobically grown Q-deficient yeast are hypersensitive to linolenic acid. Our data provide an explanation for the previous observations of Bilinski *et al.* (45), that sensitivity of *S. cerevisiae* to treatment with linolenic acid required a transfer from anaerobic growth conditions to aerobic. Under such conditions, these investigators found that wild-type yeast were sensitive to linolenic autoxidation products, with *sod1* mutants exhibiting the most sensitivity. Q levels in anaerobically grown yeast are 30–300-fold lower than in aerobically grown cells (52, 53). Based on our findings with the *coq3Δ* yeast, it follows that the much lower QH_2 levels resulting from anaerobic growth conditions would be expected to sensitize both wild-type and SOD1-deficient yeast to the products of linolenic acid autoxidation. Under aerobic growth conditions (in the absence of anaerobic pretreatment) *sod1* and/or *sod2* mutants do not exhibit hypersensitivity to linolenic acid (ref. 45 and Fig. 5). This finding is consistent with the observation that the autoxidation of QH_2 (which impairs the effectiveness of QH_2 as an antioxidant, ref. 54) is independent of SOD (55). Our data also indicate that in the complete absence of Q, a deficiency in SOD1 or SOD2 has no additional affect on the hypersensitivity to products of linolenic autoxidation. This result indicates that SOD does not play a direct role in protecting cells from the products of autoxidized polyunsaturated fatty acids. Noack *et al.* (56) find a considerable protection of mitochondria against lipid peroxidation as long as respiratory substrates are present. Thus a fully oxidized state of Q may be analogous to either a profound decrease in Q (anaerobic growth) or the absence of Q (*coq3* deletion mutant) and may render membrane lipids susceptible to peroxidation. Many *in vitro* studies suggest that QH_2 functions as a potent lipid soluble antioxidant and inhibits the formation of lipid peroxidation products (5–7). The studies presented here confirm this idea and in addition provide evidence that QH_2 acts in this capacity *in vivo*.

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