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The Reduction of α-Tocopherolquinone by Human NAD(P)H: Quinone Oxidoreductase: The Role of α-Tocopherolhydroquinone as a Cellular Antioxidant

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SUMMARY

α-Tocopherolquinone (TQ), a product of α-tocopherol oxidation, can function as an antioxidant after reduction to α-tocopherolhydroquinone (TQH_2). We examined the ability of human NAD(P)H:quinone oxidoreductase (NQO1) to catalyze the reduction of TQ to TQH_2 in cell-free and cellular systems. In reactions with purified human NQO1, TQ was reduced to TQH_2. Kinetic parameters for the reduction of TQ by NQO1 (K_m = 370 μM; $k_{cat} = 5.6 \times 10^{3}\ min^{-1}$; $k_{cat}/K_m = 15\ min^{-1} \cdot \mu M^{-1}$) indicate that NQO1 can efficiently reduce TQ to TQH_2. A comparison of the rate of reduction of TQ and coenzyme Q_{10} by NQO1 showed that TQ is reduced more efficiently than coenzyme Q_{10}. Experiments with either Chinese hamster ovary (CHO) cells stably transfected with human NQO1 or CHO cell sonicates demonstrated a correlation between NQO1 activity and TQ reduction to TQH_2. CHO cells with elevated NQO1 generated and maintained higher levels of TQH_2 after treatment with TQ relative to NQO1-deficient CHO cells. TQH_2 generated from NQO1-mediated reduction of TQ prevented cumene hydroperoxide-induced lipid peroxidation in rat liver microsomes. In addition, cumene hydroperoxide-induced lipid peroxidation was inhibited more efficiently by TQ in CHO cell lines with elevated NQO1 activity. These data demonstrate that NQO1 can reduce TQ to TQH_2 and that TQH_2 can function as an efficient antioxidant. This work suggests that one of the physiological functions of NQO1 may be to regenerate antioxidant forms of α-tocopherol.

NQO1 (EC 1.6.99.2), or DT-diaphorase, is an obligate two-electron reductase that catalyzes reduction of a broad range of substrates (1, 2). It is a flavoprotein that exists as a homodimer and is biochemically characterized by its unique ability to utilize either NADH or NADPH as reducing cofactors and by its inhibition by the anticoagulant dicumarol (1, 3). The enzyme is generally considered as a detoxification enzyme because of its ability to detoxify reactive quinones and quinone-imines to less reactive and less toxic hydroquinones (1, 3). Such a two-electron reduction also bypasses semiquinone production and thus prevents the generation of reactive oxygen species derived from interaction of the semiquinone with molecular oxygen (4, 5). The ability of NQO1 to deactivate many reactive species, including quinones, quinone-imines, and azo compounds, demonstrates its importance as a chemoprotective enzyme (6–10). The other major protective effect of NQO1 is to function as a cancer preventive enzyme (6–9), which has been recognized for >30 years (11).

Whether NQO1 catalyzes the reduction of endogenous substrates remains unclear. A potential role for NQO1 in vitamin K metabolism has been suggested (12, 13), but studies with purified NQO1 do not support such a role (14). An important observation is that x-radiation and UV radiation, which are known to generate oxidative stress, induced NQO1 expression >30-fold in human cells (15). Our data show that hepatic loading of iron in rats generates oxidative stress and a concomitant induction of NQO1.[1] This suggests that in addition to limiting oxygen radical formation from exogenous quinones, NQO1 may play an endogenous antioxidant role. Recent work has suggested that NQO1 maintains ubiquinone (CoQ_{10}) in its quinol form, which can act as an antioxidant to protect membranes from oxidative stress (16).

We examined the role of NQO1 in the metabolism of α-to-

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[1] D. Siegel and L. Valerio, unpublished observations.

ABBREVIATIONS: TQ, α-tocopherolquinone; TQH_2, α-tocopherolhydroquinone; NQO1, NAD(P)H:quinone oxidoreductase; CoQ_{10}, coenzyme Q_{10}; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry, DCPIP, 2,6-dichlorophenol-indophenol; TBARS, thiobarbituric acid reactive substances; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

copherol derivatives. In this report, we demonstrate that TQ is an excellent substrate for human NQO1. TQ is produced via free radical attack of α-tocopherol (vitamin E) and has no intrinsic antioxidant activity (17–19). The product of NQO1-mediated reduction of TQ is TQH_2 which, unlike TQ, is a potent antioxidant (18, 19). Reduction of TQ to its TQH_2 derivative has been demonstrated in cellular systems, but the enzymes responsible have not been characterized (18–20). This work demonstrates a role for NQO1 in α-tocopherol metabolism and suggests that one of the physiological functions of NQO1 may be to regenerate antioxidant forms of α-tocopherol.

Materials and Methods

Chemicals. NADH, NADPH, cholic acid, dicumarol, DCPIP, arachidonic acid, butylated hydroxytoluene, α-tocopherol acetate, cumene hydroperoxide, 2-thiobarbituric acid, HEPES, and CoQ_{10} were purchased from Sigma Chemical (St. Louis, MO). TQ was obtained from United States Biochemical (Arlington Heights, IL). Stock solutions of TQ were prepared in 95% (v/v) ethanol unless otherwise stated. All other reagents were of analytical grade.

CHO cell lines and NQO1 purification. CHO cell lines stably transfected with human NQO1 cDNA were developed as previously described (21). CHO cell lines were grown in Ham's F-12 (GIBCO BRL, Gaithersburg, MD) supplemented with 10% (v/v) fetal bovine serum and 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (GIBCO BRL). Recombinant human NQO1 protein was purified from *Escherichia coli* using Cibacron Blue affinity chromatography as previously described (22). The specific activity of the purified NQO1 protein was 625 μmol of DCPIP/min/mg. NQO1 specific activity in the CHO cell lines and purified NQO1 protein was determined using DCPIP reduction as previously described (23). Protein concentrations were determined according to the method of Lowry (24).

Formation of TQH_2 in CHO sonicates and cultured cells. The reduction of TQ by CHO sonicates was performed by the addition of 650 μg of CHO sonicate to 50 mM potassium phosphate buffer, pH 7.4, containing 1% (w/v) cholic acid, 200 μM NADH, and 50 μM TQ (final volume, 250 μl) at 27°. After 30 min, the reactions were terminated, and TQ and TQH_2 analyses were performed as described below. The conversion of TQ to TQH_2 in the cultured CHO cell lines was determined using the following methods: CHO cell lines were grown to >90% confluency, the medium was removed, and fresh medium containing 50 μM TQ was added. After 6, 12, or 24 hr, the reaction was terminated by removal of the TQ-containing medium, washing of the cells with 10 ml of PBS twice, and scraping of the cells into 1 ml of PBS. The cells were pelleted by centrifugation, the supernatant was removed, and the cell pellet was snap-frozen in liquid nitrogen and stored at −70° until analysis by HPLC.

Analysis of TQ and TQH_2. HPLC was used to separate TQ and TQH_2 in reactions with either purified NQO1, CHO sonicates or CHO cell lines. Reactions (purified NQO1, CHO sonicates) were stopped with an equal volume of cold acetonitrile containing 0.01% (w/v) butylated hydroxytoluene and 200 μM α-tocopherol acetate (internal standard) and then centrifuged at 10,000 rpm for 2 min. The cell pellet (see above) was resuspended in 150 μl of acetonitrile containing 0.003% (w/v) butylated hydroxytoluene and 67 μM α-tocopherol acetate and then centrifuged at 10,000 rpm for 2 min. The supernatant was analyzed on a LiChrosorb RP-18 column (5 μm, 25 cm, Merck, Darmstadt, Germany) with the following gradient: buffer A, 1% (v/v) acetic acid; buffer B, 100% acetonitrile; gradient, 70–95% buffer B over 10 min and then hold at 95% buffer B for 25 min. Flow rate was maintained at 1.5 ml/min with UV detection at 280 nm. HPLC retention times were TQH_2, 14 min; TQ, 22.1 min; and internal standard, 33.3 min. TQH_2 was analyzed by capillary GC-MS on a Fisons MD800 instrument (Fisons Instruments, Beverly, MA) equipped with a Carlo Erba 8000 series gas chromatograph and a Fisons on-column injector and operated in the electron ionization mode at 70 eV. The HPLC peak corresponding to TQH_2 was collected, TQH_2 was extracted from the mobile phase with hexane, and the hexane was evaporated *in vacuo*. TQH_2 was converted to the tris-(*O*-trimethylsilyl) derivative as described (25). The derivative was analyzed by GC-MS on a 30 m × 0.25 mm DB-5ms column (J & W Scientific, Folsom, CA) with cold on-column injection (25). The tris-(*O*-trimethylsilyl) derivative of TQH_2 coeluted with an authentic standard and yielded a mass spectrum identical to that of the standard: m/z 665 [M+, 16%] 575 [21], 341 [8], 309 [100], 294 [20], 280 [10], 236 [9], 220 [6]. This spectrum is essentially identical to that we previously reported for TQH_2 (25). The molecular ion for the tris-(*O*-trimethylsilyl) derivative appeared at m/z 665 due to mass defect; the monoisotopic mass is 664.5 amu.

Kinetic analysis of TQ and CoQ_{10} reduction by NQO1. Kinetic analysis of TQ reduction by NQO1 was determined by spectrophotometrically monitoring NADH oxidation. Reactions (1 ml) were performed in 50 mM potassium phosphate buffer, pH 7.4, containing 5% (w/v) cholic acid, 0.4% (v/v) Tween-80, 200 μM NADH, 0.288 μg of NQO1, and 0.01–1.28 mM TQ. Reactions were monitored for 0–2 min at 27° at 340 nm. Kinetic parameters were determined using Enzfitter kinetic software (Biosoft, Cambridge, UK) from the mean of triplicate determinations. A comparison of the rate of reduction of TQ and CoQ_{10} (5, 50 μM) by NQO1 was determined by monitoring NADH oxidation as described above. Reactions (1 ml) were performed in 50 mM potassium phosphate buffer, pH 7.4, containing 5% cholic acid and 0.4% Tween-80, 200 μM NADH, and 7.2 μg of NQO1. For these experiments, initial stock solutions of TQ and CoQ_{10} (10 mM) were dissolved in *N*,*N*-dimethylformamide before dilution in buffer.

Lipid peroxidation in rat liver microsomes and CHO cells. Lipid peroxidation was measured by analysis of TBARS as previously described (26). Reactions with rat liver microsomes were performed as follows: 200 μM NADH, 3.6 μg of NQO1, 50 μM TQ, and 750 μg of microsomes were added to 50 mM potassium phosphate buffer, pH 7.4, containing 1% cholic acid (final volume, 0.5 ml) at 27°. After 30 min, cumene hydroperoxide (1 mM) was added to initiate lipid peroxidation. After 1 hr, the reaction was stopped by the addition of 100 μM butylated hydroxytoluene and 2 ml of 0.25 N hydrochloric acid containing 15% (w/v) trichloroacetic acid and 0.37% (w/v) 2-thiobarbituric acid. The sample was heated to 80° for 20 min and then centrifuged at 2500 rpm for 10 min, and the absorbance at 535 nm was determined. Results were expressed as TBARS equivalents using a molar extinction coefficient of 1.56×10^5 (26). Lipid peroxidation studies with CHO cell lines were performed as follows: CHO cell lines were grown to >90% confluency on 100-mm tissue culture plates. Before the initiation of lipid peroxidation, the medium was exchanged with fresh medium containing 250 μM arachidonic acid and 0, 1, or 5 μM TQ. After 14 hr, the TQ-containing medium was removed, the cells were washed with 10 ml of PBS, and 10 ml of Krebs-HEPES buffer, pH 7.2, was added. Lipid peroxidation was initiated by the addition of cumene hydroperoxide (1 mM), and after 2.5 hr, the cells were scraped into the medium and collected by centrifugation. The supernatant was removed, and the cell pellet was resuspended in 1 ml of PBS. An aliquot (10 μl) of cell suspension was removed for protein determination according to the method of Lowry (24), and lipid peroxidation was measured using the TBARS assay in the remaining cell suspension as described above.

Statistical analysis. Results are expressed as mean ± standard deviation. Statistical significance between sets of data was determined using the Student's *t* test.

Results

TQ as a substrate for purified NQO1. Initial experiments were performed to determine whether TQ could serve

as a substrate for purified NQO1. HPLC analysis of a reaction containing TQ, NADH, and NQO1 showed loss of the TQ peak (t_R = 22.1 min) and formation of a more polar product (t_R = 14 min; Fig. 1A). The product from the reduction of TQ by NQO1 was confirmed by GC-MS as TQH_2 (for details, see Materials and Methods). The reaction was NADH or NADPH dependent and dicumarol inhibitable (Fig. 1B). Kinetic values for the reduction of TQ by NQO1 were determined spectrophotometrically by monitoring the oxidation of NADH. Values for K_m, k_{cat}, and k_{cat}/K_m are presented in Table 1 and suggest that NQO1 efficiently reduces TQ to TQH_2. Kinetic parameters for the reduction of CoQ_{10} by NQO1 could not be determined due to the poor solubility of CoQ_{10}. Comparative studies on the reduction of TQ and CoQ_{10} by NQO1 were performed at substrate concentrations of 5 and 50 μM (Table 2). The rate of TQ reduction was ~36-fold greater at 5 μM and ~57-fold greater at 50 μM compared with the rate of CoQ_{10} reduction. These data demonstrate that NQO1 can reduce TQ considerably more efficiently than CoQ_{10}.

Reduction of TQ by NQO1-transfected CHO cell sonicates. The reduction of TQ by sonicates prepared from CHO cell lines stably transfected with NQO1 and expressing different amounts of NQO1 activity was examined by HPLC (Table 3). A relationship between TQ reduction and NQO1 activity was apparent. Sonicates prepared from the CHO cell line with the highest NQO1 activity (CHO_{812}) catalyzed the greatest amount of TQ reduction. Less TQ reduction was observed in sonicates from the CHO cell line CHO_{815}, which contains intermediate levels of NQO1, whereas sonicates from the NQO1-deficient CHO parent cell line (CHO_{glyA}) catalyzed only minimal TQ reduction. The reduction of TQ by the CHO_{812} and CHO_{815} sonicates was NADH or NADPH dependent and dicumarol inhibitable. The product of the reduction of TQ by CHO sonicates cochromatographed with TQH_2 prepared from the reduction of TQ by purified NQO1 (data not shown).

TABLE 1
Kinetic parameters for the reduction of TQ by NQO1
Reactions conditions are described in the text.

K_m	k_{cat}	k_{cat}/K_m
μM	min^{-1}	$min^{-1} \cdot \mu M^{-1}$
370	5.6×10^3	15

TABLE 2
Comparative rate of reduction of CoQ_{10} and TQ by NQO1
Reaction conditions are described in the text.

Substrate concentration	CoQ_{10}	TQ
μM	*nmol of NADH/min/mg of protein*	
5	7.9 ± 0.1	290 ± 7.4
50	52.0 ± 1.3	3000 ± 60

Values are mean ± standard deviation from three separate determinations.

TABLE 3
Reduction in TQ by NQO1-transfected CHO sonicates
Reaction conditions are described in the text.

Cell line	CHO-glyA	CHO-815	CHO-812
	nmol of DCPIP/min/mg of protein		
NQO1 activity	N.D.[a]	98	1300
TQ reduced[b]	0.3 ± 0.27	5.0 ± 0.54	11.0 ± 0.03

[a] Not detectable above control (minus sonicate) levels. Limit of detection <5 nmol of DCPIP/min/mg of protein.
[b] nmol of TQ reduced in 30 min.
Values are mean ± standard deviations from three separate determinations.

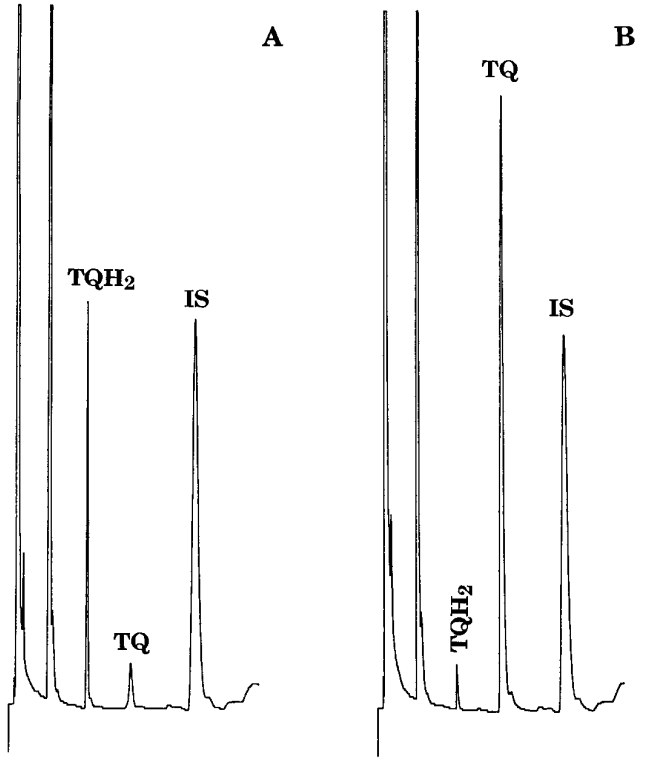

Fig. 1. HPLC analysis of the reduction of TQ by purified NQO1. Reaction conditions were 50 μM TQ, 200 μM NADH, and 7.2 μg of NQO1 in 50 mM potassium phosphate buffer, pH 7.4, containing 1% cholic acid. Reactions were performed in a total volume of 1 ml at 27° for 30 min in the absence (A) and presence (B) of 20 μM dicumarol. HPLC conditions are described in Materials and Methods.

Reduction of TQ by NQO1-transfected CHO cells in culture. Experiments were performed to examine TQ reduction and TQH_2 formation in NQO1-transfected CHO cells in culture (Fig. 2). Previous studies have shown that there were no significant differences between the CHO_{812} and CHO_{glyA} cell lines in the levels of one-electron reductases; NADPH:cytochrome P450 reductase, and NADH:cytochrome b_5 reductase (21). The NQO1-rich CHO_{812} and NQO1-deficient CHO_{glyA} cell lines were exposed to 50 μM TQ in culture, and the amounts of TQ and TQH_2 were determined by HPLC after 6 and 12 hr. The results are expressed as the ratio of TQH_2 to TQ and show that the high-NQO1 cell line, CHO_{812}, had significantly higher TQH_2-to-TQ ratios compared with the NQO1-deficient cell line, CHO_{glyA}. These data indicate that cells with high NQO1 can generate and maintain higher levels of TQH_2 than NQO1-deficient cells.

Antioxidant actions of TQH_2. Experiments were performed to determine the stability of TQH_2. HPLC analysis confirmed that TQH_2 formed in the reaction with NQO1 and stoichiometric amounts (50 μM) of TQ and NADH was stable under aerobic conditions. No autoxidation of TQH_2 back to TQ could be detected after 3 hr in 50 mM potassium phosphate buffer, pH 7.4, containing 1% cholic acid. The oxidation of TQH_2 to TQ, however, could readily be induced by unsaturated fatty acid peroxidation (Fig. 3A). In reactions in which

TQH_2 was formed after the reduction of TQ by NQO1, the addition of arachidonic acid, Fe^{2+}, and hydrogen peroxide catalyzed the oxidation of TQH_2 back to TQ. However, when arachidonic acid was absent or replaced by stearic acid, a saturated fatty acid, minimal oxidation of TQH_2 to TQ could be observed (Fig. 3, B and C). These data suggest that TQH_2 can be oxidized back to TQ via products of unsaturated fatty acid peroxidation.

Studies were performed to examine the antioxidant effects of TQH_2 on microsomal and cellular lipid peroxidation. Cumene hydroperoxide-induced lipid peroxidation was measured under aerobic conditions in microsomes supplemented with TQ, NADH, and NQO1 (Table 4). Minimal amounts of cumene hydroperoxide-induced lipid peroxidation were detected in reactions pretreated with NADH, NQO1, and TQ. In control reactions, however (minus NADH, NQO1, or TQ), substantial amounts of lipid peroxidation were detected. NADH alone did exhibit some antioxidant activity, which could be expected based on its known ability to scavenge organic radicals (27). These results indicate that TQH_2 formed from the reduction of TQ by NQO1 can function as an antioxidant and inhibit lipid peroxidation in microsomal incubations.

Experiments were then performed to examine the antioxidant properties of TQH_2 in a cultured cell system (Fig. 4). Because of the low unsaturated fatty acid content in cultured cells (28), the CHO cell lines were supplemented with arachidonic acid. The high-NQO1-expressing cell line, CHO_{812}, and the NQO1-deficient cell line, CHO_{glyA}, were loaded with arachidonic acid (250 μM) and 0, 1, or 5 μM TQ for 14 hr, after which the cells were exposed to cumene hydroperoxide (1 mM) in culture. The cells were harvested, and lipid peroxidation was measured. Essentially identical levels of lipid peroxidation were detected in the absence of TQ in the two cell lines (Fig. 4). Loading with TQ, however, inhibited lipid peroxidation 3-fold at 1 μM and 5-fold at 5 μM in the CHO_{812} cell line compared with the CHO_{glyA} cell line (Fig. 4). These studies indicate that cells with high NQO1 levels can efficiently reduce TQ to TQH_2 and that TQH_2 can then function as an antioxidant and inhibit cumene hydroperoxide-induced lipid peroxidation.

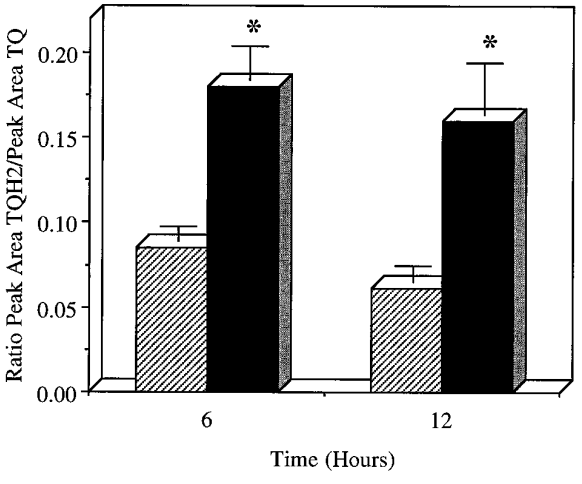

Fig. 2. The formation of TQH_2 in NQO1-transfected CHO cell lines. CHO_{GlyA} and CHO_{812} cell lines were treated with 50 μM TQ in culture; at 6 and 12 hr, the cells were harvested, and the amounts of TQH_2 and TQ were determined by HPLC. Data represent mean ± standard deviation values from three separate determinations (▨, CHO_{GlyA}; ■, CHO_{812}). *, CHO_{812} cells were significantly different from CHO_{GlyA} cells ($p < 0.05$).

TABLE 4

Inhibition of cumene hydroperoxide-induced microsomal lipid peroxidation by TQH_2

Reaction conditions are described in the text.

Treatment	TBARS
	nmol
Control reactions	
−NADH	4.2 ± 0.7
−TQ	2.2 ± 0.2
−NQO1	2.1 ± 0.4
Complete reaction[a] (NADH, TQ, NQO1)	0.4 ± 0.1

Complete system was significantly different from control (− NQO1) at $p < 0.05$.

Values are mean ± standard deviation from three separate determinations.

[a] Complete reaction to generate TQH_2.

Discussion

The data presented in this report indicate that TQ can be reduced to TQH_2 in cell-free and cellular systems by NQO1 and that TQH_2 can function as an effective antioxidant. Previous work has shown that TQ can be metabolized to TQH_2 by rat liver microsomal, mitochondrial, and cytosolic preparations (18–20). The reduction of TQ to TQH_2 in these preparations was either NADPH or NADH dependent, which suggests that many cellular reductases are capable of catalyzing this reaction. There are limited data available that

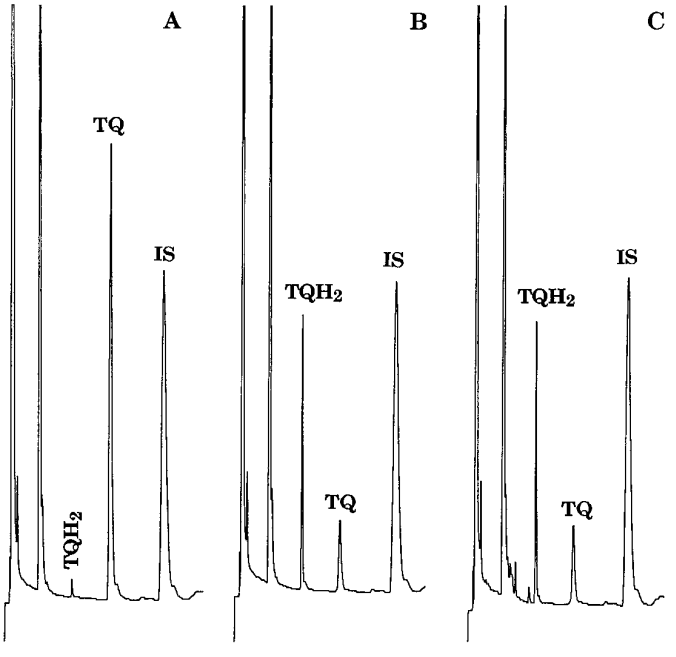

Fig. 3. HPLC analysis of the oxidation of TQH_2 by arachidonic acid peroxidation. Reaction conditions were 50 μM TQ, 50 μM NADH, and 7.2 μg of NQO1 in 50 mM potassium phosphate buffer, pH 7.4, containing 1% cholic acid. After 30 min, we added (A) 5 mM arachidonic acid, 50 μM ferrous sulfate, and 500 μM hydrogen peroxide; (B) 50 μM ferrous sulfate and 500 μM hydrogen peroxide; and (C) 5 mM stearic acid, 50 μM ferrous sulfate, and 500 μM hydrogen peroxide, and the reactions were allowed to proceed for 90 min. HPLC conditions are described in Materials and Methods.

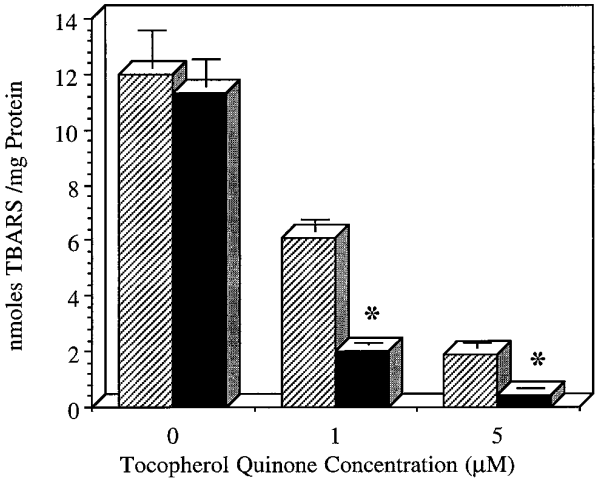

Fig. 4. Inhibition of cumene hydroperoxide-induced lipid peroxidation in NQO1-transfected CHO cell lines by pretreatment with TQ. CHO_{GlyA} and CHO_{812} cell lines were pretreated with 250 μM arachidonic acid and 0, 1, or 5 μM TQ and then exposed to 1 mM cumene hydroperoxide in culture. Reaction conditions are described in Materials and Methods. Data represents three separate determinations (± standard deviation: ▨, CHO_{GlyA}; ■, CHO_{812}). *, CHO_{812} cells were significantly different from CHO_{GlyA} cells ($p < 0.05$).

characterize the enzymes responsible for the reduction of TQ to TQH_2. One study has shown that purified NADPH-cytochrome P450 reductase can reduce TQ to TQH_2 (20), and a second study has suggested that TQ is a substrate for purified human brain carbonyl reductase (29).

Previous experiments using rat liver microsomes and purified NADPH cytochrome P450 reductase have suggested that TQH_2 is unstable under aerobic conditions, although paradoxically the authors also demonstrated that TQH_2 could be detected after aerobic incubations of isolated hepatocytes with TQ (20). In our experiments, reduction of TQ to TQH_2 by NQO1 proceeded readily under aerobic conditions, and the TQH_2 that was formed was stable with no significant autoxidation detected after 3 hr of incubation. The requirement for anaerobic conditions to detect significant quantities of TQH_2 after metabolism of TQ in previous studies using rat liver microsomes and purified cytochrome P450 reductase probably reflects the instability of the α-tocopherol semiquinone radical to oxygen. Indeed, we have found that incubation of rat liver microsomes with NADPH and TQ to generate the α-tocopherol semiquinone radical results in extensive oxygen uptake under aerobic conditions (data not shown). Because NQO1 is a two-electron reductase, it can directly form TQH_2 without the intermediacy of the α-tocopherol semiquinone radical.

The stability of TQH_2 is important because the hydroquinone form of TQ is responsible for the antioxidant function (18, 19). The current data show that TQH_2 is stable and does not undergo substantial autoxidation. However, the addition of an unsaturated fatty acid (arachidonic acid), Fe^{2+}, and hydrogen peroxide did catalyze oxidation of TQH_2 back to TQ. These data indicate that TQH_2, like α-tocopherol, is reactive primarily toward lipid-derived radicals. In a study comparing the inhibition of ascorbate/Fe^{2+}-induced lipid peroxidation in liposomes, TQH_2 was 5-fold more effective than α-tocopherol (18). The ability of a cell to maintain TQ in its reduced form will result in substantial antioxidant protection. TQ pretreatment has been shown to protect cultured cells from lipid peroxidation and cytotoxicity, presumably due to its reduction to TQH_2 (30–33). Our data demonstrate that cells with high NQO1 expression had higher levels of TQH_2 and were more resistant to lipid peroxidation than were cells lacking NQO1. The ability of cells to reduce TQ to TQH_2 via NQO1 therefore represents an effective protective mechanism against lipid peroxidative injury.

Beyer *et al.* (16) have shown recently that purified rat NQO1 is capable of reducing coenzyme Q derivatives to their quinol forms and that these reduced compounds have substantial antioxidant properties. In their study, the quinol forms of coenzyme Q (CoQ_9, CoQ_{10}) were shown to protect multilamellar vesicles against lipid peroxidation and isolated hepatocytes against Adriamycin-induced oxidative stress. We extended this work from rat to human NQO1 and compared the rate of reduction of CoQ_{10} and TQ. Under the conditions used in our study, the rate of TQ reduction by NQO1 was markedly greater (36–57-fold) than that observed with CoQ_{10}. The results obtained in both our work and that of Beyer *et al.* (16) clearly demonstrate that NQO1 can maintain endogenous quinones in a reduced antioxidative state.

It is conceivable that one of the functions of NQO1 may be to regenerate antioxidant forms of α-tocopherol (Fig. 5). A role for NQO1 in maintaining physiological levels of α-tocopherol from the reduction of α-tocopherones by NQO1 has previously been postulated (34). Oxidation of α-tocopherol by peroxyl radicals yields 8a-(alkyldioxy)tocopherones, which may either hydrolyze to TQ or be reduced to regenerate α-tocopherol (35). The regeneration of α-tocopherol from α-tocopherones occurs via a two-electron reduction, which could conceivably be catalyzed by a reductase such as NQO1 because this enzyme can catalyze the direct two-electron reduction of a broad range of substrates. Experiments are under way to examine the potential role of NQO1 in the reduction of α-tocopherones. In addition, the conversion of TQ into α-tocopherol in humans has recently been demonstrated (36); the authors suggest that α-tocopherol is regenerated from TQ after enzymatic reduction to TQH_2, although the enzyme(s) responsible have not yet been identified.

Recent work in our laboratory has shown that NQO1 expression is polymorphic due to an A-T substitution in exon 6 of the NQO1 gene (37, 38). This base pair substitution leads to a proline-to-serine change in the amino acid structure, which results in a total lack of NQO1 protein expression in individuals homozygous for this mutation (37, 38). The polymorphism demonstrates mendelian transmission (39) and

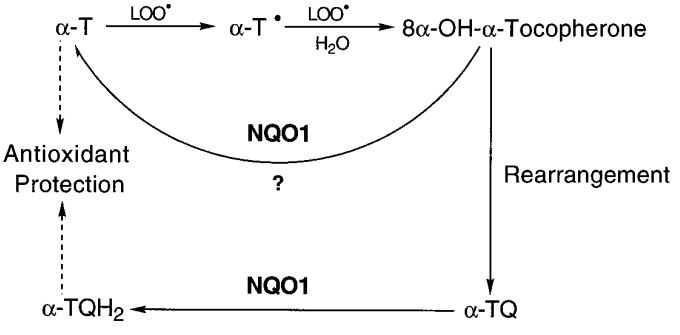

Fig. 5. Role of NQO1 in the regeneration of antioxidant forms of α-tocopherol (α-*T*). α-*T*•, α-tocopherol radical; α-*TQ*, α-tocopherolquinone; α-*TQH*$_2$, α-tocopherolhydroquinone.

occurs with a prevalence of ~6% in whites and ~18% in the Chinese (40). Our data suggest that individuals lacking expression of NQO1 may have a decreased capacity to protect against cellular oxidative damage, and this may have implications for chemoprotection and chemoprevention.

In summary, NQO1 has classically been considered a detoxification enzyme because of its role in the reduction of exogenous quinone substrates to their hydroquinone forms, thus bypassing reactive semiquinone radical formation. The ability of NQO1 to efficiently reduce TQ to TQH_2, however, suggests that one of the physiological functions of NQO1 may be to regenerate antioxidant forms of α-tocopherol.

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