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Redox Cycling of β-Lapachone and Related *o*-Naphthoquinones in the Presence of Dihydrolipoamide and Oxygen

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ABSTRACT. Lipophilic *o*-naphthoquinones (β-lapachone, CG 8-935, CG 9-442, CG 10-248, and mansonones A, C, E, and F), catalyze the oxidation of dihydrolipoamide (DHLA) by oxygen, whereas *p*-naphthoquinones (α-lapachone and menadione) are scarcely active. The greatest effects corresponded to β-lapachone and its analogues. Quinol production was demonstrated by (a) the absorption spectrum of the reduced quinone, and (b) the effect of pH variation on the rate of quinone-catalyzed DHLA oxidation. Superoxide dismutase (SOD) inhibited the rate of cytochrome *c* reduction and decreased the apparent rate of oxygen consumption by several DHLA/*o*-naphthoquinone systems. SOD also inhibited the rate of quinol oxidation by oxygen, after quinone reduction by a stoichiometric amount of DHLA. Catalase enhanced the effect of SOD, but in its absence catalase was inactive. It is concluded that quinone-catalyzed oxidation of DHLA implies a free-radical mechanism in which the quinol and superoxide radicals play an essential role. BIOCHEM PHARMACOL 51;3:275–283, 1996.

KEY WORDS. dihydrolipoamide; *o*-naphthoquinone; β-lapachone; mansonone; hydroquinone; auto-oxidation; redox-cycling; superoxide dismutase

Quinones effectively catalyze biological electron transfer, and naturally occurring quinones are widely used as chemotherapeutic agents [1–4]. A major reaction of quinones is their reduction to their semiquinone radical, which can transfer its free electron to an acceptor molecule or radical, under back-formation of the parent quinone [2, 4]. The electron acceptor may be another semiquinone radical, or molecular oxygen, which is reduced to superoxide anion.

Among the reductants capable of promoting quinone reduction is DHLA[§], the reduction product of lipoamide. DHLA can react with low potential quinones, such as *o*-phenanthrene quinone, thus promoting quinone redox cycling and oxy-radical production [5]. In the present study, we examined the same reactions using other *o*-naphthoquinones, some of which have biological activity and are of chemotherapeutic interest. Thus, β-lapachone and/or analogues produce: (a) inhibition of trypanosomatid growth [6, 7]; (b) inhibition of tumor cell growth [8]; (c) prevention of oncogenic transformation of CHEF/18A cells [9]; (d) inhibition of DNA, RNA and protein synthesis in *Trypanosoma cruzi* [10]; (e) production of strand breaks in the parasite DNA [10]; (f) induction of clastogenic chromosomal alterations [11] and (g) inhibition of topoisomerase I [12]. The quinones assayed may be grouped as follows, according to their structure (Fig. 1): (a) *o*-naphthoquinones with three oxygen atoms, two in C1 and C2 and one pyranic oxygen in position 1,2-*b* (β-lapachone and the CG quinones); (b) *o*-naphthoquinones with three oxygen atoms, two in C1 and C2 and one pyranic oxygen in position 1,8-*bc* (mansonones E and F); (c) an *o*-naphthoquinone with two oxygen atoms in C1 and C2 (mansonone C) and (d) an *o*-tetrahydronaphthoquinone with two oxygen atoms in C1 and C2 (mansonone A) [13]. For comparative purposes; α-lapachone, a *p*-naphthoquinone with two carbonyl groups in C1 and C4 and one pyranic oxygen in C2, was also examined.

MATERIALS AND METHODS

Reactants

DHLA was prepared by borohydride reduction of DL-lipoamide in aqueous methanol, as described [14]. CG *o*-naphthoquinones were obtained from the Pharmaceutical Division,

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§ *Abbreviations:* DHLA, dihydrolipoamide; DTT, dithiothreitol; GSH, reduced glutathione; SOD, superoxide dismutase; β-lapachone, 3,4-dihydro-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione; α-lapachone, 3,4-dihydro-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione; CG 8-935, 3,4-dihydro-2-methyl-2-ethyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione; CG 9-442, 3,4-dihydro-2-methylb-2-phenyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione; CG 10-248, 3,4-dihydro-2,2-dimethyl-9-chloro-2*H*-naphtho[1,2-*b*]pyran-5,6-dione; mansonone A, 5,6,7,8-tetrahydro-3,8-dimethyl-5-isopropyl-1,2-naphthoquinone; mansonone C, 3,8-dimethyl-5-isopropyl-1,2-naphthoquinone; mansonone E, 2,3-dihydro-3,6,9-trimethyl-naphtho[1,8-*bc*]pyran-7,8-dione; mansonone F, 3,6,9-trimethyl-naphtho[1,8-*bc*]pyran-7,8-dione; mansonone F, 3,6,9-trimethyl-naphtho[1,8-*bc*]pyran-7,8-dione; menadione, 2-methyl-1,4-naphthoquinone; and DETAPAC, diethylenetriaminopentaacetic acid.

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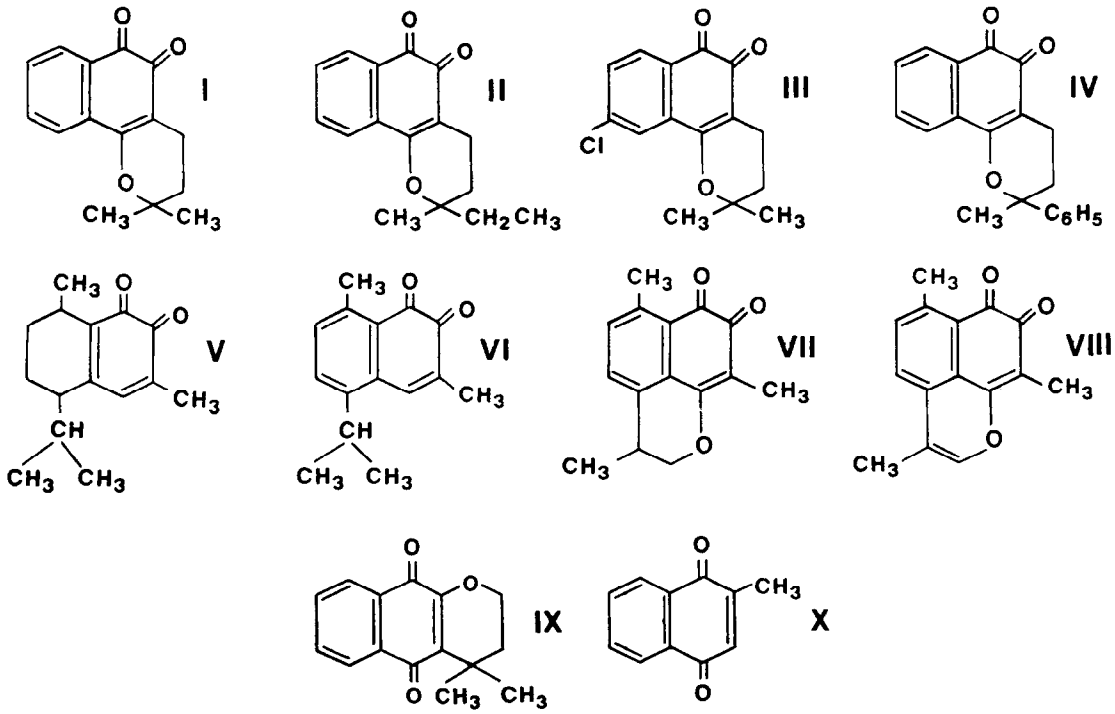

FIG. 1. Naphthoquinone structure. I, β-lapachone; II, CG 8-935; III, CG 10-248; IV, CG 9-442; V, mansonone A; VI, mansonone C; VII, mansonone E; VIII, mansonone F; IX, α-lapachone; and X, menadione.

CIBA-GEIGY Ltd., Basel, Switzerland; mansonones A, C, E, and F were obtained from the Instituto Superiore Di Sanità, Rome, Italy, through the courtesy of Dr. C. Galeffi; NADH, cytochrome *c*, catalase, SOD, pig heart lipoamide dehydrogenase, DTT, and GSH were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Other reagents were of analytical grade.

Assays

The rate of oxygen consumption during the reaction of DHLA or DTT with quinones was monitored using a Gilson Oxygraph, model 5/6 in 50 mM potassium-phosphate buffer solution, at the pH indicated under Results. Quinones and DHLA were dissolved in *N,N*-dimethylformamide, and aliquots (1–5 μL/mL) were added to the corresponding reaction mixtures, as described under Results. The reduction of cytochrome *c* was monitored according to Ref. 15, using the Aminco DW UV/VIS spectrophotometer. The reaction mixture contained: 0.1 M Tris–HCl pH 7.5, 1.0 mM DETAPAC, 50 μM cytochrome *c*, and 50 μM DHLA. SOD (20 U/mL) and CG 8-935 were added as indicated under Results. Lipoamide dehydrogenase activity was measured as described in Ref. 16. Quinone spectra were recorded using the Aminco DW UV/VIS spectrophotometer and reaction mixtures described under Results. All experiments were performed at 30°.

Expression of Results

Unless stated otherwise, the results presented are the average of duplicate measurements. Experimental values deviated from the mean value by less than 5%.

RESULTS

Quinone addition to a solution of DHLA resulted in a rapid consumption of oxygen. Figure 2 illustrates the kinetics of CG 8-935-catalyzed DHLA oxidation. The initial rate of oxygen consumption was relatively fast and depended on DHLA concentration (tracings A and B), whereas in the absence of quinone (tracing C) the rate of oxygen consumption was negligible.

Table 1 summarizes the pro-oxidant activity of several quinones, at fixed concentrations of both quinone and DHLA. For comparative purposes, results with DTT are also included, showing that the CG quinones and β-lapachone were the most effective catalysts of the oxidation of the thiol compounds, whereas the *p*-quinone α-lapachone was the least effective. Intermediate values were obtained with the mansonones, among which mansonone C was the most active. Generally speaking, reaction rates obtained with DTT were lower than those obtained with DHLA, allowance being made for mansonone A. Some differences between the quinones with regard to their effects were better observed with DTT than with DHLA, for example, the relatively lower activities of β-lapachone and CG 8-935 as compared with CG 9-442 and 10-248. Under similar experimental conditions, the quinones assayed did not enhance GSH (0.1 to 5.0 mM) oxidation to a significant degree (rate <1.0 μM/min). Production of lipoamide after incubation of DHLA with CG 8-935 was demonstrated by the decrease in the rate of the DHLA/NAD^+ reaction and the corresponding increase in the rate of the NADH/lipoamide reaction, when lipoamide dehydrogenase was added to the reaction mixture [17]. Enzyme activity was measured using

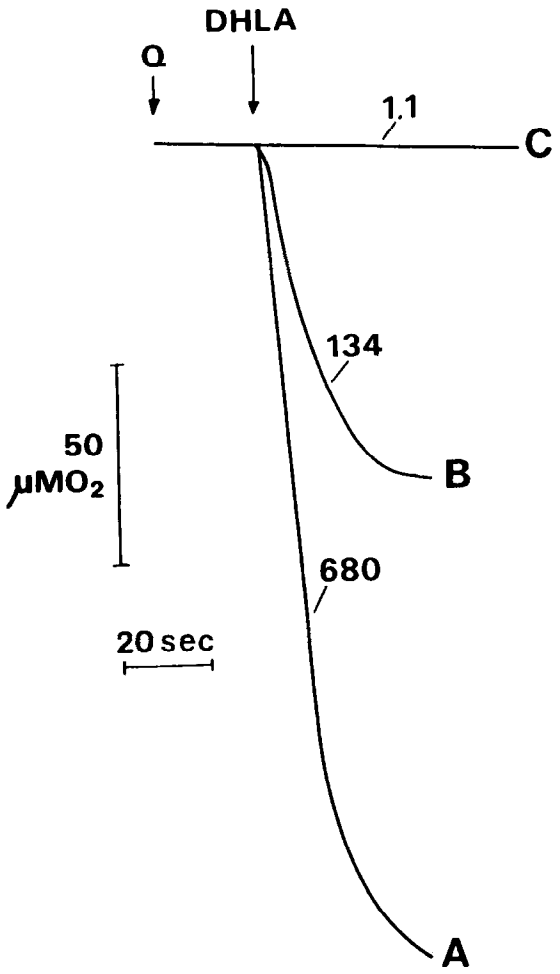

FIG. 2. Oxygen consumption after addition of DHLA to CG 8-935. Curve A: the reaction mixture contained 50 mM potassium-phosphate, pH 7.4, 50 μM quinone (Q) and 300 μM DHLA, added where indicated by the arrows. Curve B: same as A, except for DHLA concentration (100 μM). Curve C: same as A, without quinone (control tracing). Other conditions were as indicated under Materials and Methods. Figures near tracings indicate the rate of oxygen consumption (μM/min).

NAD^+ or NADH as substrates (Table 2). Under these experimental conditions, NAD^+ reduction indicated lipoamide production that was equivalent to NADH disappearance in the control sample, during the same time period [17].

Figure 3 shows the effect of DHLA on the quinone spectrum in the 250–410 nm range. The CG 8-935 spectrum closely resembled that of β-lapachone with a sharp peak at 260 nm and a broad one centered at 290 nm [18]. Addition of 300 μM DHLA produced the quinol spectrum with a trough centered at 275 nm and a broad absorption band with a maximum at roughly 330 nm. Further addition of 800 μM sodium borohydride failed to modify significantly the reduced quinone spectrum.

Table 3 shows the effect of medium pH on pro-oxidant activity of several *o*-naphthoquinones. Typical results with β-lapachone and mansonone E are illustrated in Fig. 4, where it is shown that pH variation in the 6.0 to 8.0 range markedly

TABLE 1. Effect of quinones on DHLA and DTT oxidation

Quinone (50 μM)	Oxygen consumption (μM/min)	
	DHLA	DTT
CG 9-442	990	315
CG 10-248	879	470
β-Lapachone	808	238
CG 8-935	733	208
Mansonone C	434	
Mansonone E	276	66
Mansonone F	58	14
Mansonone A	26	37
α-Lapachone	7	1
None	0	0

The reaction mixture contained 50 mM potassium-phosphate, pH 7.4, and 300 μM DHLA or DTT, as indicated above. Other experimental conditions were as described under Materials and Methods.

increased quinone pro-oxidant action. Furthermore, the quinone structure affected the response of DHLA-quinone systems to pH variation, since in the case of CG quinones and β-lapachone, the ratio of DHLA oxidation rates at pH 8.0 and 7.4, respectively, varied in the 1.3 to 1.7 range, whereas ratios for mansonones E and F were 2.5 and 3.2, respectively.

Figure 5 shows that at fixed quinone concentration the rate of quinone-catalyzed DHLA oxidation increased linearly as a function of DHLA concentration, the slope of the corresponding straight-lines depending on quinone concentration.

Figure 6 shows the effect of DHLA on the absorption spectrum of CG 8-935 in the 350–590 nm range, under air. It may be observed that addition of 300 μM DHLA to the quinone solution produced a marked decrease in absorbance, in close agreement with quinol production, as described in Fig. 3. Quinone reduction proved strongly dependent on DHLA concentration (Fig. 7). In fact, the percentage of reduced quinone immediately after DHLA addition was [DHLA concentration in parentheses (μM)]: 0 (50); 45 (200), and 88 (300). Somewhat different kinetics were recorded with mansonones E and F (Fig. 8). With these quinones, the redox cycle was relatively slower, particularly with mansonone F.

Figure 9 shows the effect of oxygen on quinone redox cycling. Thus, using 50 μM DHLA as reductant, under air, the

TABLE 2. Rates of lipoamide dehydrogenase reaction after oxidation of DHLA by CG 8-935

Time of DHLA–quinone incubation (min)	Rates of lipoamide dehydrogenase reaction (nmol/min)	
	Substrate	
	NAD^+	NADH
2.5	7.5	5.7
5	11.8	10.7
10	11.2	11.7

The reaction mixture contained 50 mM potassium-phosphate buffer, pH 7.4, 300 μM DHLA and 5 μM CG 8-935. At the times indicated above, 1.0 mM NAD^+ or 100 μM NADH was added. The lipoamide dehydrogenase reaction was initiated by adding 1 μg lipoamide dehydrogenase. Values represent the average of duplicate measurements.

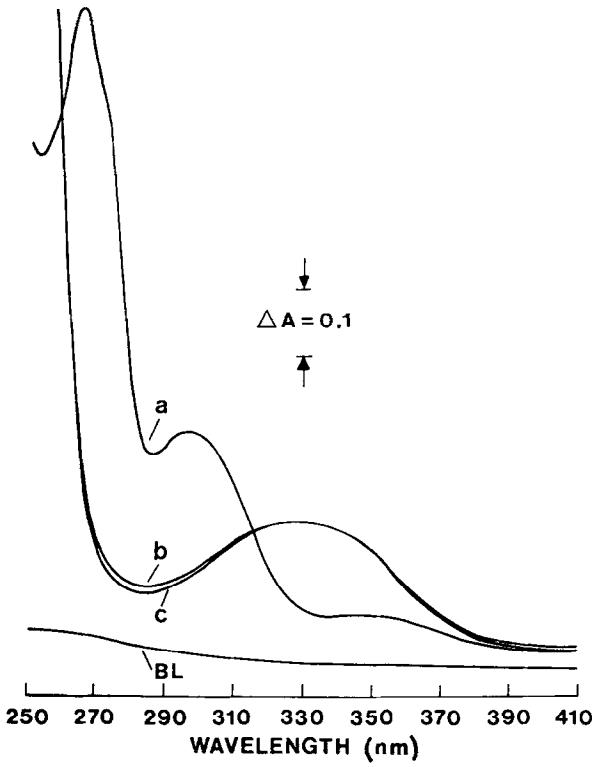

FIG. 3. Spectra of CG 8-935 and DHLA-reduced quinone. The reaction mixture contained 50 mM potassium-phosphate buffer, pH 7.4, and 40 μM CG 8-935. Key: (a) control spectrum; (b) same as (a) with addition of 300 μM DHLA; (c) same as (b) with addition of 800 μM borohydride. Spectra (b) and (c) were recorded immediately after reductant addition. BL, base line. Slit, 30 nm. Scanning velocity, 2 nm/sec.

quinol was oxidized immediately, whereas under argon the quinone was reduced permanently. Under the latter experimental conditions, 50 μM H_2O_2 did not oxidize the quinol (results not shown). Production of O_2^-, as a result of quinone-catalyzed DHLA oxidation, was confirmed by the effect of SOD on the reduction of cytochrome *c* by the DHLA/CG 8-935 system (Fig. 10). In this experiment, relatively low concentrations of reactants were used, in order to obtain measurable rates of cytochrome *c* reduction. The apparent effect of SOD was inversely related to quinone concentration, most

TABLE 3. Effect of pH on the rate of DHLA oxidation in the presence of quinones

Quinone (50 μM)	Oxygen consumption (μM/min)		
	pH 8.0	pH 7.4	pH 6.0
CG 8-935	821	572	32
CG 9-442	1204	707	63
CG 10-248	1160	902	82
β-Lapachone	814	592	31
Mansonone E	339	134	9
Mansonone F	87	27	3

The reaction mixture contained 50 mM potassium-phosphate buffer, pH and quinone as indicated above, and 50 μM DHLA. Other experimental conditions were as described in the legend of Fig. 3.

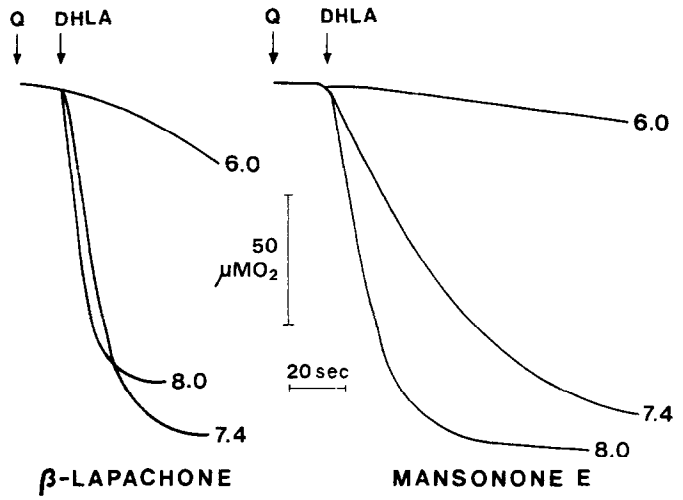

FIG. 4. Effect of pH on DHLA oxidation, catalyzed by β-lapachone or mansonone E. Experimental conditions were as described in the legend of Fig. 2, except for the DHLA concentration which was 50 μM and pH was as indicated near the tracings.

likely because of cytochrome *c* reduction by the semiquinone or the quinol [19, 20]. It should be noted that in the absence of quinone, the reduction of cytochrome *c* was insensitive to SOD. Table 4 summarizes the effect of SOD on cytochrome *c* reduction by several quinones. They all mediated electron transfer, though with different velocities, CG 8-935 proving by far the most active. Addition of SOD decreased the rate of cytochrome reduction to a similar degree, except with mansonone E. With CG 8-935, calculation of the superoxide-dependent cytochrome reduction yielded ca. 59% of total electron flux.

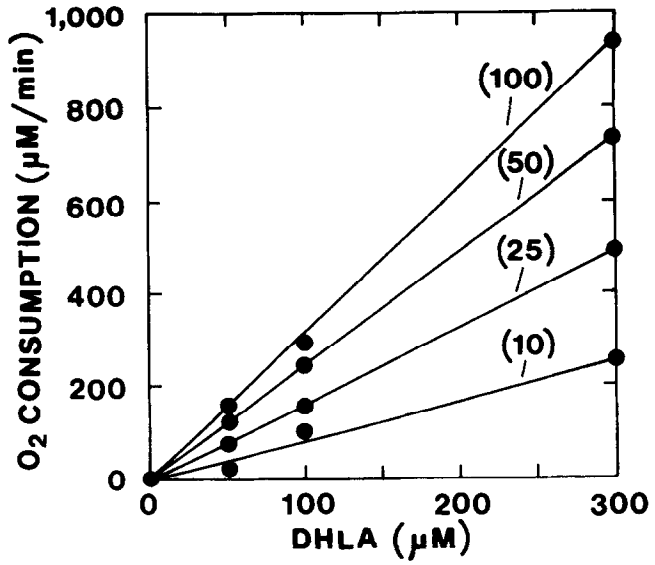

FIG. 5. Effect of DHLA and CG 8-935 concentrations on the rate of oxygen consumption. Experimental conditions were as described in the legend of Fig. 2, except for DHLA and quinone concentrations, which are indicated on the abscissa and by figures in parentheses (μM), respectively.

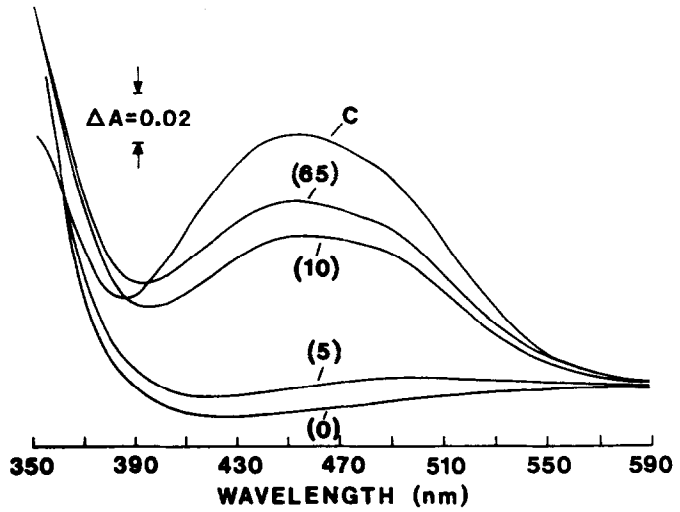

FIG. 6. Spectra of DHLA-reduced CG 8-935. The reaction mixture contained 50 mM potassium-phosphate, pH 7.4, and 50 μM CG 8-935. C, unreduced CG spectrum. Reduction was started by adding 300 μM DHLA. The figures in parentheses indicate the time (min) elapsed after adding DHLA. Scanning velocity, 2 nm/sec. Other conditions were as described under Materials and Methods.

Figure 11 shows the effect of SOD on the kinetics of CG 8-935 redox cycling, observed at 250–370 nm, using 40 μM DHLA as reductant. It may be seen that in the absence of SOD, quinone reduction proved undetectable, in contrast with spectra obtained when using SOD. Figure 12 shows the results of a similar experiment, where quinone reduction was measured at 450 nm, and increasing concentrations of SOD were used. In this case quinol oxidation was delayed significantly, as a function of SOD concentration, though heat-denatured SOD was ineffective. When both catalase and SOD were added, catalase markedly enhanced the effect of SOD.

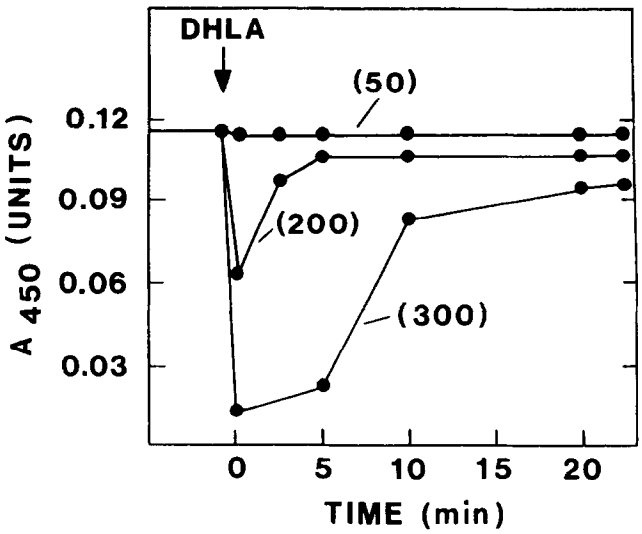

FIG. 7. Effect of DHLA concentration on the kinetics of CG 8-935 redox cycling measured at 450 nm. Experimental conditions were as described in the legend of Fig. 6, except for the DHLA concentration, which is indicated in parentheses (μM).

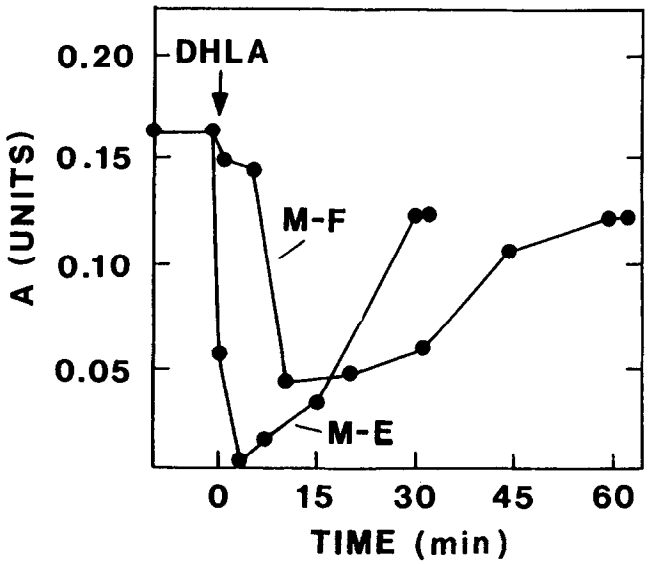

FIG. 8. Kinetics of mansonones E (M-E) and F (M-F) redox cycling. Experimental conditions were as described in Fig. 6. Absorbance was measured at 450 nm (mansonone E) and 570 nm (mansonone F).

Figure 13 shows redox-cycling kinetics of β-lapachone, as well as of mansonones A and E, in the presence of SOD. These results indicate that: (a) with β-lapachone, the kinetics were similar to those described in Fig. 12; (b) with mansonone E,

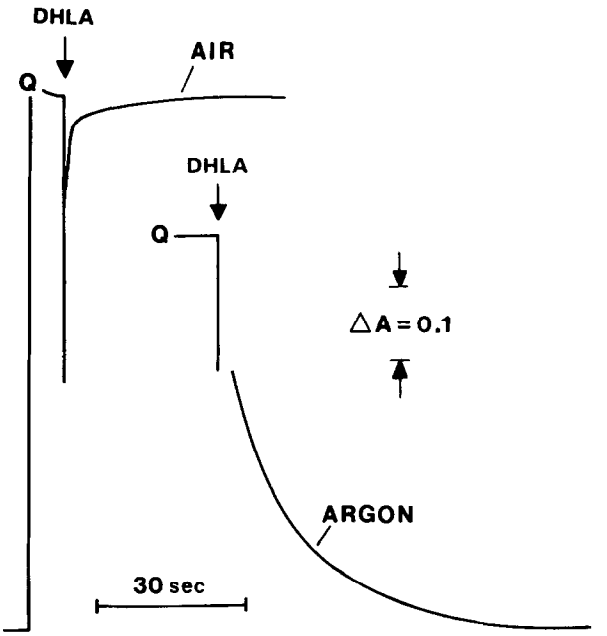

FIG. 9. Kinetics of CG 8-935 redox cycling in the presence of DHLA under air or argon. Air experiment: conditions were as described in Fig. 6, except for DHLA (50 μM); air in the gas space. Argon experiment: same experimental conditions except that the reaction mixture was gassed with argon 15 min before spectroscopic observation. DHLA (2 μL) was added through the cuvette cap. Argon was flushed in the gas space. Mixing of reagents was performed with a magnetic stirrer. Absorbance was measured at 450 nm.

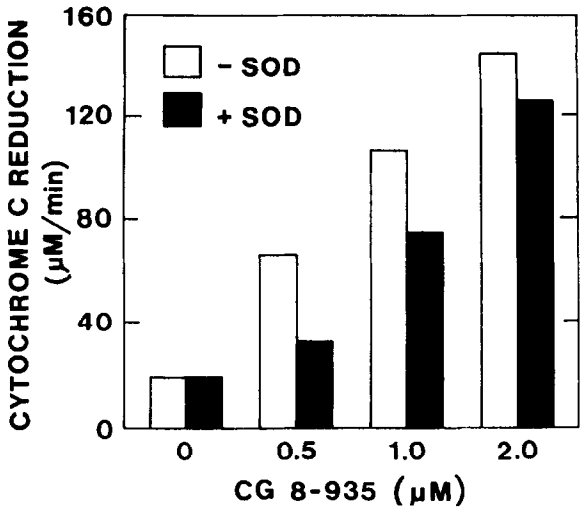

FIG. 10. Effect of SOD on cytochrome c reduction by the DHLA/CG 8-935 system. The reaction mixture contained SOD. Other conditions were as described under Materials and Methods.

the reduction rate was relatively slower; (c) with mansonone A, the rate of quinol oxidation was negligible; and (d) with α-lapachone, the rate of quinone reduction was also negligible.

Catalase decreased the rate of oxygen consumption by the DHLA/quinone system, as shown in Fig. 14. When catalase was added before DHLA (tracing B), its effect became manifest after a latent period, but thenceforth oxygen concentration in the reaction medium increased, instead of decreasing as observed with the control sample (tracing A). When both catalase and SOD were added together (tracing D), the increase in oxygen concentration found with catalase alone was not observed and the rate of oxygen consumption was lower than with SOD alone.

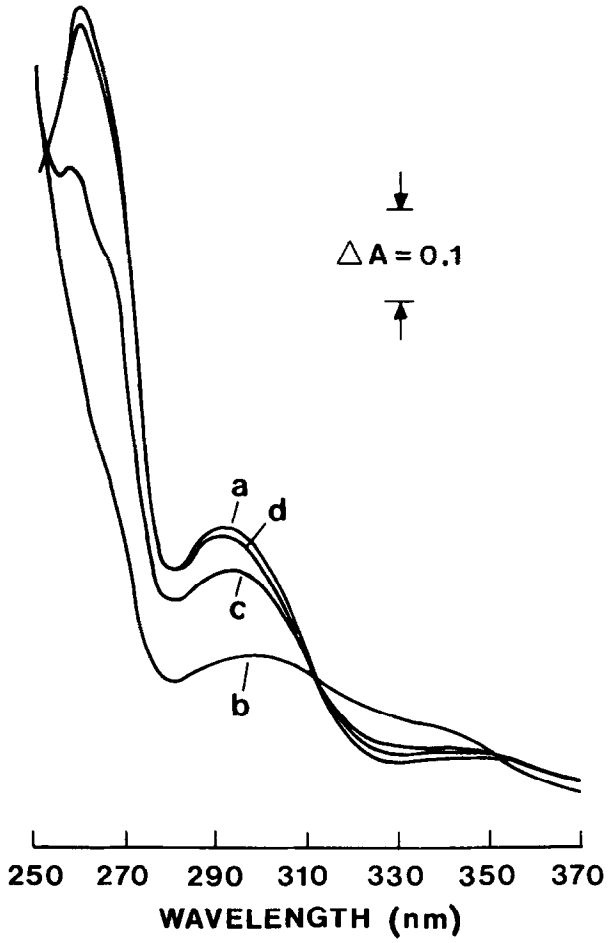

FIG. 11. Effect of SOD on CG 8-935 redox cycling. Experimental conditions were as described in Fig. 3, except for DHLA (40 μM). The reaction mixture contained 50 mM potassium-phosphate buffer, pH 7.4, 40 μM CG 8-935, and 30 U/mL SOD. Spectrum a: control spectrum. Spectra b–d were recorded at 0 (b), 2 (c), and 5 min (d) after 40 μM DHLA addition.

DISCUSSION

With the *o*-naphthoquinones used in the present study, an initial nucleophilic attack at the α,β-unsaturation linkage, to yield an adduct intermediate [21], is prevented by the *o*-naphthoquinone structure (Fig. 1). Accordingly, the *o*-naphthoquinone-catalyzed DHLA oxidation may be explained by the operation of a free radical chain mechanism [22, 23]. DHLA initially reduces the quinone (Q) to quinol (QH_2), the autooxidizable compound (Reaction 1). This reaction may proceed in two one-electron steps, with intermediate formation of a disulfide anion radical and the semiquinone anion radical. The disulfide anion radical should be a short-lived intermediate. Then, the radical mechanisms would continue according to Reactions 2 and 3. Reactions 4 and 5 represent chain propagation, and Reactions 6–8 represent chain termination.

TABLE 4. Cytochrome c reduction in the presence of DHLA/quinone systems

Quinone (2 μM)	Cytochrome c reduction (μM/min)	
	−SOD	+SOD
CG 8-935	199	118 (41)
Mansonone E	40	35 (15)
Mansonone F	31	17 (45)
Mansonone A	36	21 (42)
None	9	9 (0)

The reaction mixture contained 0.1 M Tris–HC, pH 7.5, 1.0 mM DETAPAC, 50 μM cytochrome c, 50 μM DHLA, 2 μM quinone, and 20 U/mL SOD as indicated. Figures in parentheses indicate percent inhibition of cytochrome c reduction. Other conditions were as described under Materials and Methods.

$$DHLA + Q \rightarrow LA + QH_2 \quad (1)$$
$$QH_2 + O_2 \rightarrow Q^{\cdot} + O_2^{-} + 2\,H^{+} \quad (2)$$
$$QH_2 + Q \rightarrow 2Q^{\cdot} + 2H^{+} \quad (3)$$
$$QH_2 + O_2^{-} \rightarrow Q^{\cdot} + H_2O_2 \quad (4)$$
$$Q^{\cdot} + O_2 \rightarrow Q + O_2^{-} \quad (5)$$
$$Q^{\cdot} + Q^{\cdot} + 4H^{+} \rightarrow 2QH_2 \quad (6)$$
$$Q^{\cdot} + O_2^{-} + 2H^{+} \rightarrow Q + H_2O_2 \quad (7)$$
$$O_2^{-} + O_2^{-} \rightarrow H_2O_2 + O_2 \quad (8)$$

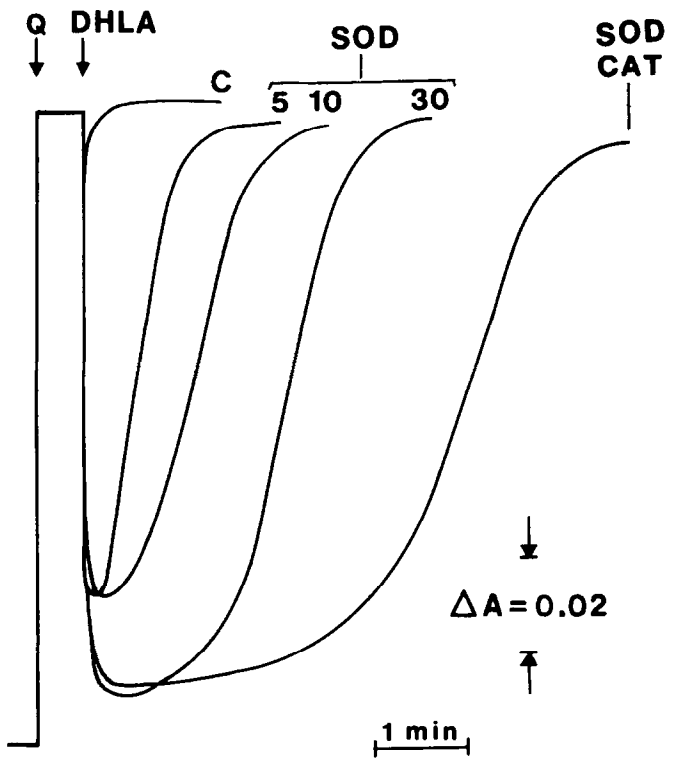

FIG. 12. Effect of SOD on the kinetics of CG 8-935 redox cycling, in the presence of DHLA. The reaction mixture contained 50 mM potassium-phosphate, pH 7.4, 50 μM CG 8-935 (Q) and 50 μM DHLA added where indicated by arrows. Line c, control sample or samples containing 30 U/mL heat-denatured SOD or 132 U/mL catalase; SOD concentration is indicated by the figures on the tracings; the SOD + CAT sample contained 30 U/mL SOD and 132 U/mL catalase. Absorbance was measured at 450 nm. Other experimental conditions were as described under Materials and Methods.

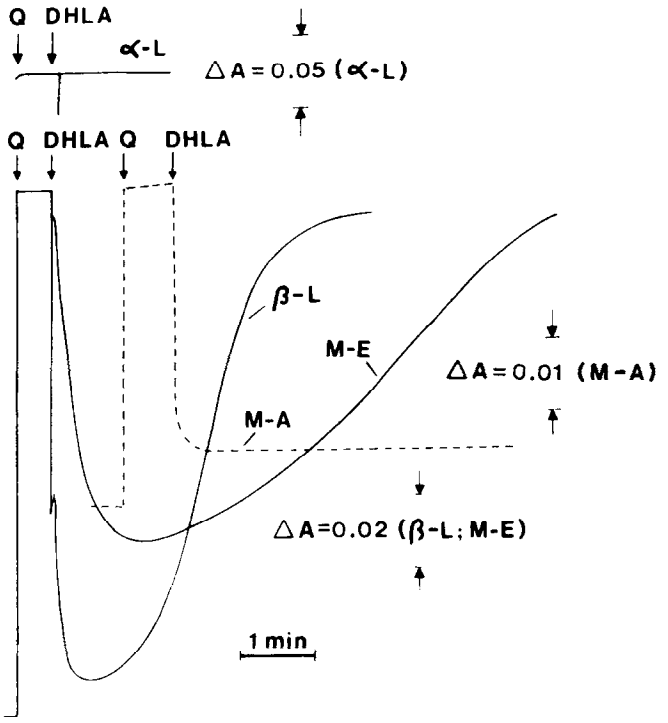

FIG. 13. Kinetics of β-lapachone (β-L), mansonone A (M-A), mansonone E (M-E) and α-lapachone (α-L) redox-cycling in the presence of DHLA and SOD. The reaction mixture contained 50 mM potassium-phosphate, pH 7.4, 20 U/mL of SOD, and 50 μM quinone (Q) as indicated near the tracings. The reaction was started by adding 50 μM DHLA as indicated by the arrows. Absorbance was measured at 450 nm. Other conditions were as described under Materials and Methods.

By this mechanism, the superoxide anion acts as a main chain propagator. If initiation reactions are slow, as compared with propagation, SOD will inhibit the chain operation, by accelerating Reaction 8, thus making O_2^- unavailable for chain propagation [22]. The postulated sequence of reactions is supported by: (a) the effect of the concentration of both DHLA and quinone on the rate of the oxidative process (Figs. 2 and 5); (b) the production of quinol, as a result of quinone reduction (Figs. 3 and 6–9); (c) the effect of pH variation on quinone activity (Fig. 4 and Table 3), in close agreement with the high reductant activity of deprotonated quinols [2, 5, 21]; (d) the essential role of oxygen for quinol oxidation and the lack of H_2O_2 action (Fig. 9); (e) the production of lipoamide as a result of DHLA oxidation (Table 2); (f) SOD inhibition of cytochrome c reduction (Fig. 10 and Table 4); (g) SOD inhibition of quinol oxidation (Figs. 11–13); (h) the decrease in oxygen consumption in the presence of SOD or catalase (Fig. 14); (i) the production of semiquinone by quinones in the presence of reductants [24]; and (j) the failure of H_2O_2 to oxidize DHLA [25].

The SOD effect may be explained by catalysis of superoxide dismutation (Reaction 8). At variance with SOD enhancement of 1,2-naphthohydroquinone oxidation [26], in the present study only an inhibitory effect of SOD was observed. Inhibition of quinol auto-oxidation confirms the role of superoxide in the free-radical chain, the rate of Reaction 4 prevailing over the rate of Reactions 2, 5, and 7 [23].

The effect of catalase implies H_2O_2 production. In this connection, it should be pointed out that the catalase effect required a certain time to manifest itself (Fig. 14), presumably because of the relatively slow rate of non-enzymatic O_2^- dismutation leading to H_2O_2 production. In the presence of SOD, catalase inhibition was immediate, as was to be expected from SOD-catalyzed production of H_2O_2. Catalase also increased the effect of SOD on quinol oxidation (Fig. 12), which may be interpreted by assuming that by decreasing H_2O_2, inhibition of SOD by its reaction product is prevented [27].

The diverse activities of quinones as catalysts of DHLA oxidation (Table 1) can be explained in terms of electronic symmetry and polarity, as illustrated by LUMO (lowest unoccupied molecular orbital) atomic charge coefficients. Corresponding values for quinones used in the present study have been calculated [28], showing that the carbonyl double bond is polarized, with the carbon atom tending to be electron deficient and hence more positive. In the case of *p*-quinones, symmetrically placed carbonyl groups oppose one another. In

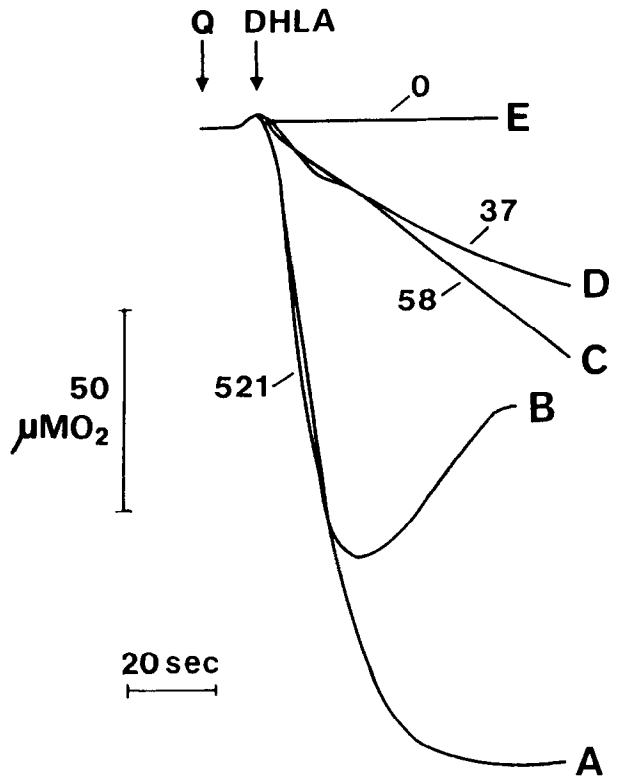

FIG. 14. Effect of catalase and SOD on oxygen consumption by the DHLA/CG 8-935 system. Experimental conditions were as described in the legend of Fig. 2. Line A, control sample containing 50 μM DHLA and 50 μM CG 8-935; B, same as A with 132 U/mL catalase added before DHLA; C, same as A with 20 U/mL SOD added before DHLA; D, same as A, with catalase and SOD added as in B and C, respectively; E, control sample containing only DHLA.

o-quinones, there is less symmetry, and at least partly for this reason *o*-quinones tend to be more reactive than *p*-quinones. The effect of oxygen atoms is due to their electronegativity, which is greater in the oxo group than in the enoyl-ether group, as illustrated in C2 by the very low activity of α-lapachone. The aromatic nature of the naphthalene ring is critical for the stability of the semiquinone radical, which rate-limits the velocity of the oxidation reaction, explaining the relatively low activity of mansonone A, which has a tetraline structure. The presence of a methyl group in C3 and the distinct linkage of the pyranic structure in mansonones seem to exert a negative influence on reactivity, as shown by mansonones E and F.

The dihydrolipoate/lipoate couple, which plays an essential role in mitochondrial dehydrogenase reactions, has recently gained attention as an antioxidant. α-Lipoic acid reacts with reactive oxygen species and protects membranes by interacting with vitamin C and glutathione, which may, in turn, recycle tocopherol. Moreover, the dihydrolipoate/lipoate couple also redox regulates NF-kB DNA binding activity [29]. The observations described here suggest that modification of the lipoate (or lipoamide) system redox level may contribute to *o*-naphthoquinone biological actions.

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