Published in final edited form as:

Toxicol Environ Chem. 2008 March 1; 90(2): 327–340. doi:10.1080/02772240701499778.

Ortho-quinone-enhanced ascorbate oxidation. Combined roles of lipid charge and the magnesium cation

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Abstract

Quinones are widely distributed compounds in nature. Of these, *ortho*-quinones are found to be involved in the pathogenic mechanism of Parkinson's disease, in oxidative deaminations to free-radical redox reactions, and as intermediates in the pathways implicated in the carcinogenicity of 2,3- and 3,4-catechol estrogens. Addition of $MgCl_2$ to solutions of the hydrophobic *ortho*-quinones, 1,10-phenanthroquinone (PHQ) and beta-lapachone (LQ) enhances ascorbate oxidation in the absence or presence of large unilamellar vesicles (LUVs) of the neutral lipid dimyristoylphosphatidylcholine (DMPC), although initial rates of ascorbate oxidation are smaller in the presence of lipid as compared to its absence. Addition of this salt to solutions of the *para*-quinone 1,4-naphthoquinone (NQ) did not affect the ascorbate rate of oxidation in the absence or presence of DMPC. Addition of $MgCl_2$ to semiquinone solutions of PHQ or LQ in the presence or absence of DMPC increases semiquinone stability, as detected from the semiquinone disproportionation equilibrium displacement to semiquinone formation. Furthermore, $MgCl_2$ increases the partition of the *ortho*-semiquinones into the aqueous phase, although no such effect is observed for the semiquinone of NQ. For all the quinones under study, smaller rates of ascorbate oxidation and of semiquinone equilibrium concentration occur in the presence of negatively charged LUVs composed of an equimolar mixture of DMPC and dimyristoylphosphatidic acid DMPA. Ascorbate oxidation rate enhancements correlate with an increase in semiquinone concentration with addition of $MgCl_2$, in the absence or presence of neutral lipid. This observation favors the proposition that ascorbate oxidation rate increases are caused by semiquinone thermodynamic stabilization. Thus, the ascorbate oxidation rate enhancement by $MgCl_2$ in solutions containing hydrophobic *ortho*-quinones is still possible in systems with hydrophobic environments analogous to that of DMPC.

Keywords

Ortho-quinone; semiquinone; magnesium; DMPC; membrane; ascorbate; DMPA; beta-lapachone; phenanthroquinone; naphthoquinone

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URL: <http://dx.doi.org/10.1080/02772240701499778>

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Introduction

Quinones are widely distributed compounds in nature. These are found in humans as endogenous compounds, such as coenzyme Q, the metabolites of estrogen hormones [1], or as exogenous compounds, such as those used clinically [2] or those produced as metabolites of polycyclic aromatic compounds [3]. Due to the importance of quinones in humans, and the high reactivity of quinones, substantial research on the chemistry and toxicology of these compounds has taken place. The two most common types of quinones are *ortho*- and *para*-quinones. Of these, *ortho*-quinones are found to be involved in the pathogenic mechanism of Parkinson's disease [4]. *o*-Quinone cofactors derived from tyrosine and tryptophan are involved in novel biological reactions that range from oxidative deaminations to free-radical redox reactions [5]. The *o*-quinone forms of 2,3- and 3,4-catechol estrogens have been implicated in the carcinogenicity of these hormones [6].

In a previous work, we reported the enhancement of ascorbate (AH^-) oxidation by *ortho*-quinones 1,10-phenanthroquinone (PHQ), beta-lapachone (LQ), but not by *para*-quinones naphthazarin (NZQ) and 1,4-naphthoquinone (NQ), in the presence of $MgCl_2$ and $CaCl_2$ at constant ionic strength [7]. Evidence was obtained which suggests that *ortho*-semiquinone-metal complex formation is responsible for the enhancement of the ascorbate oxidation by these metal salts in the presence of these *o*-quinones, i.e. (i) no interaction was detected between the corresponding quinones or the alkaline-earth metals with ascorbate, (ii) strong interactions were detected between the corresponding *ortho*-semiquinones and the alkaline-earth cations, (iii) no interactions were detected between the *ortho*-quinones and the alkaline-earth cations and, furthermore, (iv) neither the semiquinone nor the quinone of NQ was found to interact with the alkaline-earth cations. However, since both of these *o*-quinones are hydrophobic, it would be interesting to know if the observed enhancement is biologically relevant since these quinones will partition preferentially into lipid phases and presumably will not be accessible to ascorbate. Several other previous works have reported *o*-semiquinone stabilization upon complexing with bivalent cations [8–12]. Sato et al. [11] have described stabilization by calcium of the semiquinone form of pyrroloquinoline quinone (PQQ). Lebedev et al. [12] have shown that alteration of the redox state of the hydroxynaphthoquinone echinochrome A by Ca^{2+} accelerates free-radical oxidation of this quinone.

In this work, we have studied the possibility that these hydrophobic *o*-quinones enhance ascorbate oxidation in the presence of both neutral and negatively charged lipid membranes and in the presence and absence of an alkaline-earth cation. Of the studied quinones, LQ is the most biologically relevant. Beta-lapachone is a naturally occurring compound present in the bark of the South American Lapacho tree. It has antitumor activity against a variety of human cancers, including colon, prostate, promyelocytic leukemia, and breast [13–15]. Beta lapachone induces growth inhibition and apoptosis in bladder cancer cells [16]. It shows larger cytotoxicity in tumor cells with enhanced NAD(P)H: quinone oxido-reductase (NQO1, E.C. 1.6.99.2) activity [17].

Materials and methods

Materials

The compounds NQ, LQ and PHQ were purchased from Aldrich (Figure 1). Dimyristoylphosphatidic acid (DMPA), and dimyristoylphosphatidylcholine (DMPC) were obtained from Avanti Polar Lipids. High purity (99.999%) $MgCl_2$ and $CaCl_2$, and ascorbic acid (99%+) were purchased from Alfa Aesar, Wardhill, MA. Quinones were purified by double sublimation. Fresh stock solutions of these compounds were prepared in distilled, deionized, Chelex-treated water and were used the same day. Cacodylic buffer at pH 7.4 was used. This buffer, in addition to the distilled deionized water used, was treated with Chelex

resin to avoid transition metal trace contamination, following the method described by Buettner [18].

Liposome preparation

Multilamellar vesicles (MLVs) were first prepared as described previously [19,20]. In order to avoid lipid oxidation, DMPC and DMPA were used instead of egg yolk PC. A thin film of DMPC (100 to 150 mg), with or without DMPA in a 1: 1 ratio with DMPC and with or without quinone (quinone to lipid mole ratio of 40 μ mol: 20 mmol), was prepared in a septum-stoppered test tube by evaporating the solvent from its chloroform solution, using first a nitrogen gas flow followed by high-vacuum application for 30 min. Chelex-treated water was then added and the suspension strongly vortexed. This suspension was then submitted to five freeze-thaw cycles, as recommended by [21], followed by 10 filtration cycles through a polycarbonate filter of 100 nm pore diameter using a high pressure extruder to produce large unilamellar vesicles (LUVs).

Semiquinone generation

Semiquinones were produced as previously described [19,20]. Since most of the quinones under study here are only slightly soluble in buffer, semiquinones were first generated in absolute methanol by reacting from 10 to 20 mg of quinone with NaBH_4 in a convenient molar ratio such that semiquinone spectral intensity is maximized when transferred to aqueous buffer. The solvent was then evaporated to dryness using a dry nitrogen flow. The sample was submitted to high vacuum for at least 30 min. A nitrogen-purged aqueous sodium cacodylate buffer solution was then added to the dry semiquinone sample and the resulting solution equilibrated to pH 7.4 by addition of small aliquots of HCl or NaOH. This aqueous solution was diluted to 3.00 mL and centrifuged at 3500 rpm for 5 min. Two milliliters of the supernatant were then mixed with DMSO to avoid quinone or hydroquinone precipitation after salt addition. Aliquots of this solution were mixed with portions of MgCl_2 or NaCl and LUVs stock solutions to make 400 μ Ls of sample solutions containing 20 mM lipid, 150 mM NaCl or 50 mM MgCl_2 and 50 mM cacodylate buffer (pH 7.4), which were then submitted for EPR analysis. A final DMSO concentration of 1% (v/v) was used. The addition of larger concentrations of MgCl_2 produced precipitation of DMPC or DMPC/DMPA LUVs.

Attempts to detect the semiquinones by EPR spectroscopy using ascorbate as the reducing agent in samples with the quinone-containing liposomes described earlier were not successful, most probably due to the large semiquinone disproportionation constant in water at pH 7.4 (Equation 1, $K_1 > 10^{18}$) [22,23] and the low solubility of the quinones in water. Quinone reduction in methanol using NaBH_4 is a well-established procedure which yields EPR-detectable amounts of semiquinone at physiological pH and is suitable to detect semiquinone EPR spectral changes with changes in salts or lipids concentrations or identities. The nature of the semiquinone ion generated at physiological pH is, of course, not dependent on the quinone reduction method. The amounts of semiquinone and hydroquinone should depend on the semiquinone disproportionation constant at pH 7.4 and the initial NaBH_4 and quinone concentrations. Therefore, the effect of the presence of lipids or MgCl_2 on the semiquinone concentration or lipid/buffer partition is compared to the same batch of semiquinone solution but with different lipid or salt compositions.

Semiquinone EPR spectral simulation

First-derivative EPR spectra were acquired and analyzed using an EMX X-band Bruker EPR spectrometer coupled to a computer. Well-resolved semiquinone first-derivative EPR spectra in the absence of lipids, in the presence or absence of 50 mM MgCl_2 , were simulated and optimized (best-fitted to the experimental spectra) with WINSIM [24], using hyperfine coupling constants reported previously in the aqueous buffer, at pH 7.4 [7]. First-derivative

EPR spectra corresponding to lipid-containing samples, in which both the membrane-bound and the free or ion-paired semiquinone spectra were observed superimposed (hereby known as the composite spectra), were simulated and optimized in the same fashion starting with three superimposed different spectra: the well-resolved spectra corresponding to semiquinones in the aqueous phase (both free and ion-paired semiquinones) and a broad (membrane-bound) semiquinone spectrum. A single broad line with peak-to-peak width of 2 Gauss with identical g -value to that of the aqueous phase species was used as the starting broad spectral component in the optimization process. Linewidths, relative g -values, lineshapes and relative intensities were optimized in the composite spectra. The optimization process produced the EPR spectral intensities of all the semiquinone species involved, i.e. the free, ion-paired and membrane-bound semiquinones. Spectral simulation was performed on EPR spectra of at least three different samples of each composition. Semiquinone membrane–buffer partition coefficients K_1 were determined using Equation 1, where V_{aq} and V_{Lip} are the volumes of the aqueous and lipid phases and I_{aq} and I_{Lip} are the semiquinone EPR intensities of the aqueous and lipid phases, respectively.

$$K_1 = \frac{I_{Lip} V_{aq}}{I_{aq} V_{Lip}} \quad (1)$$

The lipid volume was determined from the lipid mass used and its density, assuming that the lipid phase density is equal to the aqueous phase density [25,26].

As observed previously, no spectral decrease in intensity was detected during the time period of the analysis of these samples [19].

Shifts in semiquinone disproportionation equilibrium

Shifts in the semiquinone disproportionation equilibrium (reaction (2)) were detected by monitoring the semiquinone concentration as a function of increases in divalent salt

concentration or liposome concentration, at constant ionic strength. Semiquinone concentrations were obtained by double integration of the first derivative EPR spectra and comparing this area with that of a 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxide (CTPO) spin standard.

Ascorbate oxidation

Ascorbate oxidation measurements were performed at $37.0 \pm 0.1^\circ\text{C}$ in 20 mM cacodylate buffer (pH 7.4). Stock and sample solutions were prepared with double distilled deionized water and decontaminated from traces of transition metals by exposure to Chelex 100 resin using the batch method [18]. Air-saturated sample solutions were used, containing LUVs composed of 20 mM DMPC or DMPC/DMPA (1: 1 mole ratio) plus 40 μM quinone, 1.0 mM ascorbate and 20 mM cacodylate buffer (pH 7.4) in the presence or absence of MgCl_2 (50 mM). Ionic strength was maintained constant and equal to that of a 150 mM NaCl solution by addition of this salt. Oxygen consumption rates were measured with a 5300 Oxygen Biological Monitor (Yellow Springs Instruments Co., USA) using a Clark electrode as a sensor in an YSI 5301 constant temperature bath. Rates were calculated from the initial constant slopes of $[\text{O}_2]$ traces, R_{ox} . Runs were started in the absence of ascorbate followed by ascorbate addition without interrupting oxygen consumption measurements.

Since oxygen solubility is decreased by the salting out effects of MgCl_2 and NaCl , runs were made by calibrating the initial oxygen concentration, $[\text{O}_2]_0$, to that corresponding to 150 mM NaCl or 50 mM MgCl_2 solutions. These were calculated using the equation proposed by Schumpe et al. [27] for mixed electrolyte solutions (Equation 3) where

$$\log\left(\frac{\alpha}{\alpha_0}\right) = \frac{1}{2} \sum c_i H_i z_i^2 \quad (3)$$

H_i is the relative salting-out parameter of oxygen for individual ions at 37.2°C , c_i is the ion molar concentration, z_i is the ion electrical charge, and α and α_0 are the oxygen solubilities in the presence and absence of salts, respectively. Relative salting-out parameters at this temperature were obtained from reference [28]. Thus, oxygen solubilities were calculated to be at 37°C , $195\ \mu\text{M}$ for 50 mM MgCl_2 and $191\ \mu\text{M}$ for 150 mM NaCl . Since such a small difference in oxygen solubilities exists between solutions containing these salts, this difference was not considered when comparing differences in R_{ox} between solutions containing MgCl_2 and those containing NaCl .

Results and discussion

Ascorbate oxidation

Representative traces of ascorbate oxidation are shown in Figure 2. As shown in this figure, the constant slopes observed in these traces through almost oxygen depletion are consistent with the “standard” behavior of PHQ and NQ reported by Roginsky et al. [29] in which the rate ascorbate oxidation is essentially independent of oxygen concentration and consistent with a two-step mechanism (Equations 4 and 5), being the first step rate-determining.

A constant slope is also observed for the O_2 traces belonging to LQ, again in agreement with that type of behavior for other studied naphthoquinones [29]. The presence of MgCl_2 and/or lipids did not change the standard behavior feature of the quinones used in this work. Initial rates of ascorbate oxidation, R_{ox} , are enhanced by the presence of quinone, as reported elsewhere [29]. As observed previously, R_{ox} is further enhanced if Mg^{+2} and an *ortho*-quinone (but not the *para*-quinone) are present (Table I) [7]. The average rate without quinone or lipid is $0.019 \pm 0.005\ \mu\text{M s}^{-1}$ and independent of the salt used. However, this enhancement is smaller if lipid membranes are present (Figure 2, and Table I). This is the expected behavior, since not all the quinone molecules are available at the liposome water–lipid interphase to be reduced by ascorbate. Extrapolation of R_{ox} to a quinone to DMPC molar ratio of zero, at constant lipid concentration (Figure 3), produces R_{ox} values at infinite dilution of the quinone in DMPC, $R_{\text{ox},0}$ (Table I). These values predict Mg^{+2} -induced enhancements even at very low quinone concentrations at the lipid phase for PHQ and LQ but not for NQ. If the lipid is negatively charged, R_{ox} is further decreased as compared to the presence of neutral lipid which, again, is understandable since the negative charge at the lipid interphase repels ascorbate. Upon addition of MgCl_2 to quinone-containing solutions in the presence of DMPC liposomes, R_{ox} is increased for samples containing PHQ or LQ but not NQ. Such a conclusion cannot be derived from

R_{ox} values of DMPC/DMPA-containing solutions due to the smaller R_{ox} values and the uncertainties obtained.

Ortho-semiquinone stabilization by Mg^{+2}

In a previous work, we have found that alkaline-earth cation chelation is observed for the *o*-semiquinones of LQ and PHQ but not for the *p*-semiquinones NQ and NZQ, while no interaction between the parent quinones or ascorbate and these salts was detected [7]. Such cation chelation by the *ortho*-semiquinones was evidenced by both the appearance of a distortion of the semiquinone EPR spectrum and from the shift in the semiquinone disproportionation equilibrium to the left (reaction (2)). It was therefore suggested that semiquinone–metal complexes are responsible for the enhancement in ascorbate oxidation by these metal salts in the presence of these *o*-quinones. Both of these observations indicate increased *o*-semiquinone stabilization upon chelation to divalent cations. The semiquinone disproportionation Gibbs free energy, ΔG_2^0 , is a function of the differences in the redox potentials, $E(Q/Q^{\cdot-})$ and $E(Q/QH_2)$ (Equation 6). Since the semiquinone is a negatively charged species and the hydroquinone is not,

$$\Delta G_2^0 = -2F \{E(Q/QH_2) - E(Q/Q^{\cdot-})\} \quad (6)$$

we could assume that $E(Q/QH_2)$ is not too dependent on alkaline-earth concentration as it should be for $E(Q/Q^{\cdot-})$. In fact, no interaction between the quinones used in the present work and Mg^{+2} was detected from the unchanged quinone absorption spectra with $MgCl_2$ addition [7]. Thus, a more positive ΔG_2^0 could be caused by a more positive $E(Q/Q^{\cdot-})$ value. Since quinones with more positive redox potentials produce larger R_{ox} values [29], this ascorbate oxidation enhancement by *o*-quinones in the presence of a divalent cation is primarily caused by an increase in the semiquinone stabilization, which results in a more positive quinone redox potential, i.e. $E(Q/Q^{\cdot-})$ is replaced by $E(Q, Mg^{+2}/Q^{\cdot-}, Mg^{+2})$. In this work, similar results are observed for the *ortho*-semiquinones $LQ^{\cdot-}$ and $PHQ^{\cdot-}$ but not for the *para*-semiquinone $NQ^{\cdot-}$, i.e. equilibrium (2) is shifted to the semiquinone for these *o*-semiquinones but not for $NQ^{\cdot-}$ upon addition of 50 mM $MgCl_2$, as compared to addition of 150 mM NaCl, in the presence of DMPC (Table I, nFigure 4 and 5). Similar explanations were given for the observed increase in the oxidation rate of ascorbate by hydrophobic quinones in the presence of positively charged phospholipids and for the oxidation rate increase of -alkyl-1,4-dihydronicotinamides by the lipophilic quinone, avarone, in the presence of CTAB micelles [30], i.e. semiquinone stabilization due to interaction of these species with positively charged lipids.

Semiquinone stability enhancements upon addition of $MgCl_2$ in the presence of DMPC/DMPA cannot be drawn from the collected data, within experimental error, due to the weaker EPR signals observed. Indeed, equilibrium (2) is shifted to the right for all of the semiquinones studied here upon changing the lipid electrical charge from neutral to negative, as previously observed [20], due to a repulsion of the semiquinone negative charge with the membrane charge while the more hydrophobic quinone and hydroquinone species partition into the membrane.

Semiquinone– Mg^{+2} formation versus oxygen reduction rates

The rate constant for the semiquinone reduction of oxygen, reaction (5), is in the order of $10^8 \text{ M}^{-1} \text{ s}^{-1}$ [31]. Thus, assuming a steady state semiquinone concentration of 10^{-6} M and an oxygen concentration of $200 \times 10^{-6} \text{ M}$, we can calculate an estimated rate for reaction (5) of 0.02 M/s. To our best knowledge, rate constants for ion-pair formation (or dissociation) between anions and metal cations in water have not been reported. The rate of ion-pair formation is, in general, assumed to be diffusion controlled, hence extremely fast [32]. Rate

constants of ion-pair formation between semiquinone anions and alkali metal cations in hexamethylphosphoramide have been measured and are in the order of $10^8 \text{ M}^{-1} \text{ s}^{-1}$ [33], and since water is near one-third as viscous as hexamethylphosphoramide [32], it is expected that diffusion in water is faster than in hexamethylphosphoramide. Thus, it is expected that at 50 mM MgCl_2 , the rate of ion-pair formation, reaction (1), should be near the range of 5 M/s or more. Thus, it is reasonable to expect ion-pair formation of the semiquinone before oxygen reduction.

Relative semiquinone concentrations shown in Table I are equilibrium concentrations while the semiquinone concentration formed during the R_{ox} measurements are not. During R_{ox} measurements, not all the quinones are exposed to ascorbate reduction and less if quinones are partitioned into lipid phases. Thus, a decrease in quinone–ascorbate encounters could mask environmental characteristics which could induce an increase in the rate of ascorbate oxidation due to semiquinone stabilization. Therefore, absolute values of semiquinone concentrations obtained by EPR measurements here do not have to correlate with absolute R_{ox} values when comparing systems with and without lipids (Table I). However, since magnesium cations can interact with semiquinones before electron transfer to oxygen during R_{ox} measurements, DMPC-containing samples with analogous composition show both increases in R_{ox} and in semiquinone equilibrium concentrations with the addition of MgCl_2 (Table I).

EPR study of semiquinone– Mg^{+2} interactions

Interactions of the semiquinones under study with Mg^{+2} and neutral and negatively charged lipids are shown in Figures 4 and 5. Compositions of solutions, for which EPR spectra are shown in these figures, are described in Table II. The resolved EPR spectrum of LQ^- , in the absence of lipids, shows the formation of an ion-paired species, $\text{LQ}^-, \text{Mg}^{+2}$ (as well as the free semiquinone), in the presence of 5 mM MgCl_2 (Figure 4(a)). Ion-paired semiquinones are also observed for PHQ^- (Figure 5(a)) but not for NQ^- (Figure 5(d)–(f)) upon addition of MgCl_2 , in agreement with our previous work. The asymmetry observed in PHQ^- spectra is evidence for the simultaneous observation of the free and ion-paired semiquinone species. In the presence of 20 mM DMPC and 5 mM MgCl_2 , both the free LQ^- and $\text{LQ}^-, \text{Mg}^{+2}$ are still observed, in addition to a broad component corresponding to semiquinones embedded in the membrane (Figure 4(c)). In the presence of 20 mM DMPC and 50 mM MgCl_2 only the ion-paired species, $\text{LQ}^-, \text{Mg}^{+2}$ (Figure 4(b)), and a mixture of free PHQ^- and $\text{PHQ}^-, \text{Mg}^{+2}$ (Figure 5(b)) are observed in the aqueous phase. Furthermore, in DMPC-containing samples, the semiquinone lipid–aqueous phase partition equilibrium is strongly displaced towards the aqueous phase (Figure 4(b)) as compared to 4(c) and Figure 5(b) as compared to 5(c) if the MgCl_2 concentration is increased, a feature not observed for NQ^- (Table II). Thus, not only the interaction of LQ^- or PHQ^- with Mg^{+2} is stabilizing these semiquinones but also improving their hydrophilic character. These observations of *ortho*-semiquinone– Mg^{+2} complex formation correlate with the observed enhancement of R_{ox} with Mg^{+2} addition in the presence of the corresponding *ortho*-quinones and DMPC, again implying that these complexes are improving the overall rate of ascorbate oxidation by stabilizing the semiquinone.

In the presence of 20 mM DMPC/DMPA and 150 mM NaCl, the resolved spectrum of the free aqueous LQ^- is mostly observed (Figure 4(d)). Thus, in this case, equilibrium (2) is displaced to the right and a relatively small semiquinone concentration is detected at the membrane. In a mixture containing 20 mM DMPC/DMPA and 5 mM MgCl_2 mostly the free LQ^- species is detected in the aqueous phase as determined from the hyperfine splitting constant set of the observed spectrum (Figure 4(e)) [7]. Thus, as opposed to the presence of DMPC with no DMPA, at least a fraction of the Mg^{+2} cations is interacting with the negatively charged membrane interface, thus decreasing the effective concentration of Mg^{+2} ions which are available to bind LQ^- at the aqueous phase. Since the lipid-localized semiquinone

concentration of both $LQ^{\cdot-}$ and $PHQ^{\cdot-}$ in negatively charged lipids is already low in the absence of Mg^{+2} , a decrease in the lipid-localized semiquinone concentration could not be detected, within experimental uncertainty, with $MgCl_2$ addition as detected for DMPC-containing samples.

In summary, the hydrophobic *ortho*-quinones PHQ and LQ, but not the *para*-quinone NQ, enhance ascorbate oxidation in the presence of $MgCl_2$ in the absence or presence of the neutral membrane DMPC, although rates of ascorbate oxidation are smaller in the presence of lipid as compared to its absence. In the presence of the negatively charged membrane, DMPC/DMPA rates of ascorbate oxidation are smaller than in the presence of DMPC. Addition of $MgCl_2$ to semiquinone solutions of PHQ or LQ in the presence or absence of DMPC increases semiquinone stability as detected from the semiquinone disproportionation equilibrium displacement to semiquinone formation. Furthermore, in the presence of neutral membranes, $MgCl_2$ addition increases the partition of the *ortho*-semiquinones into the aqueous phase although no such effect is observed for $NQ^{\cdot-}$. Ascorbate oxidation rate enhancements correlate with increases in semiquinone concentration upon addition of $MgCl_2$, in the absence or presence of neutral lipids. This favors the proposition that ascorbate oxidation rate increases are caused by semiquinone thermodynamic stabilization. The amplifying effects of $MgCl_2$ in the rate of ascorbate oxidation by hydrophobic *ortho*-quinones are still present in DMPC-containing systems.

Acknowledgments

The authors are grateful for grants No. SO6-GM008216, and P20 RR-016470 from NIH (USA) for financial support of this work.

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Phenanthroquinone (PHQ)

β -Lapachone (LQ)

1,4-Naphthoquinone (NQ)

Figure 1.
Quinones studied in this work.

Figure 2.

Oxygen consumption traces at 37°C of solutions containing 50 mM cacodylate (pH 7.4), 1.0 mM ascorbate and (1) quinone and $MgCl_2$, (2) quinone and NaCl, (3) quinone, $MgCl_2$ and DMPC, (4) quinone, NaCl and DMPC, (5) quinone, $MgCl_2$ and DMPC/DMPA, (6) NaCl or (7) $MgCl_2$. Salt concentrations are 50 mM $MgCl_2$ or 150 mM NaCl. Lipid concentration is 20 mM with a 40: 20,000 quinone to lipid mole ratio. Arrows indicate the instance when ascorbate was injected.

Figure 3.

R_{ox} dependence on quinone concentration. Samples contain 50 mM cacodylate (pH 7.4), 1.0 mM ascorbate and 20 mM DMPC LUVs with the specified quinone concentration. Dark symbols correspond to solutions containing 50 mM $MgCl_2$ and white symbols to solutions containing 150 mM NaCl.

Figure 4.

EPR spectra of deaerated solutions obtained after reduction of LQ in the presence or absence of MgCl_2 and in the presence or absence of 20 mM DMPC or DMPC/DMPA in 50 mM cacodylate, pH 7.4. Detailed composition of samples and relative amounts of semiquinone species are shown in Table II. Simulated and optimized spectra are shown with dotted lines. The optimization procedure is described in the “Experimental” section. EPR hyperfine splitting constants are those previously reported for the free and magnesium-complexed LQ^- [7].

Figure 5.

EPR spectra of deaerated solutions obtained after reduction of PHQ and NQ in the presence or absence of MgCl_2 and in the presence or absence of 20 mM DMPC or DMPC/DMPA in 50 mM cacodylate, pH 7.4. Detailed composition of samples and relative amounts of semiquinone species are shown in Table II. Simulated spectra are shown with dotted lines. The optimization procedure is described in “Materials and methods”. EPR hyperfine splitting constants are those previously reported for the free and magnesium-complexed $\text{PHQ}^{\cdot -}$ and for the free ion of $\text{NQ}^{\cdot -}$ [7].

Table I

Initial rates of oxygen consumption and semiquinone concentrations in the presence or absence of MgCl_2 and in the presence or absence of lipids.

Quinone ^a	Lipid	Salt added	R_{ox}^b ($\mu\text{M s}^{-1}$)	$[\text{Q}^-]$ (relative units ^d)	$R_{ox}^b \theta$ ($\mu\text{M s}^{-1}$)
NQ	DMPC	NaCl	0.16 ± 0.02	0.9 ± 0.1	-0.01 ± 0.01
		MgCl_2	0.19 ± 0.02	1.0 ± 0.1	0.00 ± 0.01
	DMPC/DMPA ^c	NaCl	0.03 ± 0.01	0.58 ± 0.07	
		MgCl_2	0.05 ± 0.01	0.63 ± 0.05	
LQ	Without lipid	NaCl	0.54 ± 0.06	0.8 ± 0.1	
		MgCl_2	0.57 ± 0.05	0.69 ± 0.07	
	DMPC	NaCl	0.09 ± 0.02	0.47 ± 0.08	0.01 ± 0.02
		MgCl_2	0.26 ± 0.03	1.0 ± 0.05	0.08 ± 0.03
PHQ	DMPC/DMPA ^c	NaCl	0.03 ± 0.01	0.19 ± 0.02	
		MgCl_2	0.10 ± 0.02	0.27 ± 0.03	
	Without lipid	NaCl	0.55 ± 0.02	0.19 ± 0.04	
		MgCl_2	1.29 ± 0.03	1.4 ± 0.5	
PHQ	DMPC	NaCl	0.09 ± 0.01	0.10 ± 0.01	-0.01 ± 0.01
		MgCl_2	0.30 ± 0.04	1.0 ± 0.1	0.018 ± 0.005
	DMPC/DMPA ^c	NaCl	0.07 ± 0.01	0.034 ± 0.008	
		MgCl_2	0.13 ± 0.03	0.04 ± 0.01	
Without lipid		NaCl	0.66 ± 0.01	0.04 ± 0.01	
		MgCl_2	1.5 ± 0.2	0.15 ± 0.02	

^aThe average rate without quinone or lipid is $0.019 \pm 0.005 \mu\text{M s}^{-1}$ and independent of the salt used.

^bSamples contained 1.0 mM ascorbate, 50 mM MgCl_2 or 150 mM NaCl in the presence or absence of quinone-containing LUVs with a quinone to lipid ratio of 40 μM Q: 20 mM lipid using 50 mM cacodylate buffer at pH 7.4.

^c1:1 DMPA to DMPA mol ratio used.

^dDetermined under anaerobic conditions. Semiquinones were generated as described in the "Experimental" section. Samples contained 20 mM lipid, 150 mM NaCl or 50 mM MgCl_2 and 50 mM cacodylate buffer (pH 7.4). Since different semiquinones may have different disproportionation constants, the semiquinone concentration is given relative to a value of 1 for the semiquinone concentration of each parent quinone in the presence of 20 mM DMPC and 50 mM MgCl_2 .

Table II

Semiquinone membrane–buffer partition constants and concentration ratio of aqueous ion-paired to free semiquinone in anaerobic samples studied.^a

Semiquinone	Sample composition	Figure	$K_1 \cdot 10^{-2}$	$[Q^{\cdot-}, Mg^{+2}]_{aq}/[Q^{\cdot-}]_{aq}$
LQ ^{•−}	5 mM MgCl ₂ , no lipid	4(a)	–	1.6 ± 0.5
LQ ^{•−}	50 mM MgCl ₂ , DMPC	4(b)	2.4 ± 0.4	7 ± 2
LQ ^{•−}	5 mM MgCl ₂ , DMPC	4(c)	7.9 ± 0.7	1.3 ± 0.2
LQ ^{•−}	No MgCl ₂ , DMPC/DMPA	4(d)	0.3 ± 0.2	–
LQ ^{•−}	5 mM MgCl ₂ , DMPC/DMPA	4(e)	0.3 ± 0.3	0.16 ± 0.08
LQ ^{•−}	50 mM MgCl ₂ , DMPC/DMPA	Not shown	0.8 ± 0.3	3.0 ± 0.8
PHQ ^{•−}	50 mM MgCl ₂ , no lipid	5(a)	–	3.2 ± 0.6
PHQ ^{•−}	50 mM MgCl ₂ , DMPC	5(b)	0.6 ± 0.1	2.1 ± 0.7
PHQ ^{•−}	No MgCl ₂ , DMPC	5(c)	5.9 ± 0.8	–
PHQ ^{•−}	No MgCl ₂ , DMPC/DMPA	Not shown	0.2 ± 0.1	–
PHQ ^{•−}	50 mM MgCl ₂ , DMPC/DMPA	Not shown	0.2 ± 0.1	4 ± 1
NQ ^{•−}	No MgCl ₂ , DMPC	5(d)	0.7 ± 0.1	–
NQ ^{•−}	50 mM MgCl ₂ , DMPC	5(e)	0.8 ± 0.1	<i>b</i>
NQ ^{•−}	50 mM MgCl ₂ , DMPC/DMPA	5(f)	0.44 ± 0.08	<i>b</i>

^aSemiquinones were generated as described in the “Experimental” section in N₂-saturated solutions. Samples contained NaCl or MgCl₂ and 50 mM cacodylate buffer (pH 7.4) with or without 20 mM of the indicated lipid mixture. Averages are shown of at least 3 repetitions.

^bIon-paired species not detected.