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Role of quinones on the ascorbate reduction rates of S-nitrosogluthathione

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Abstract

Quinones are one of the largest class of antitumor agents approved for clinical use and several antitumor quinones are in different stages of clinical and preclinical development. Many of these are metabolites of, or are, environmental toxins. Due to their chemical structure these are known to enhance electron transfer processes such as ascorbate oxidation and NO reduction. The paraquinones 2,6-dimethyl-1,4-benzoquinone (DMBQ), 1,4-benzoquinone (BQ), methyl-1,4-benzoquinone (MBQ), 2,6-dimethoxy-1,4-benzoquinone (DMOBQ), 2-hydroxymethyl-6-methoxy-1,4-benzoquinone (HMOBQ), trimethyl-1,4-benzoquinone (TMQ), tetramethyl-1,4-benzoquinone (DQ), 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UBQ-0), the paranaphthoquinones 1,4-naphthoquinone (NQ), menadione (MNQ), 1,4-naphthoquinone-2-sulfonate (NQ2S), juglone (JQ) and phenanthroquinone (PHQ) all enhance the anaerobic rate of ascorbate reduction of GSNO to produce NO and GSH. Rates of this reaction were much larger for p-benzoquinones and PHQ than for p-naphthoquinone derivatives with similar one-electron redox potentials. The quinone DMBQ also enhances the rate of NO production from S-nitrosylated bovine serum albumin (BSA-NO) upon ascorbate reduction. Density functional theory calculations suggest that stronger interactions between p-benzo- or phenanthrasemiquinones than those of p-naphthosemiquinones with GSNO are the major causes of these differences. Thus, quinones, and especially p-quinones and PHQ, could act as NO release enhancers from GSNO in biomedical systems in the presence of ascorbate. Since quinones are exogenous toxins which could enter the human body via a chemotherapeutic application or as an environmental contaminant, these could boost the release of NO from S-nitrosothiol storages in the body in the presence of ascorbate and thus enhance the responses elicited by a sudden increase in NO levels.

Keywords

quinone; nitrosothiol; nitrosogluthathione; nitric oxide; ascorbate; density functional theory

Introduction

Quinones form the second largest class of antitumor agents approved for clinical use in U.S.A. and several antitumor quinones are in different stages of clinical and preclinical development [1]. Many of these are metabolites of, or are, environmental toxins [2] [3-5]. A common feature in quinone-containing drugs is their ability to undergo reversible redox

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reactions to form semiquinone and oxygen radicals [3,6]. One electron reduction of a quinone (Q) gives the semiquinone radical ($Q^{\bullet-}$ or $QH^{\bullet}$) while two-electron reduction gives the hydroquinone (QH_2) [6]. The semiquinone can also be formed by a comproportionation reaction between a quinone and a hydroquinone, reaction (1) (the opposite of reaction 1 is the semiquinone disproportionation reaction).

Quinones can be enzymatically reduced by flavoenzymes. Some of these can catalyze a one electron reduction of quinones such as NADPH-cytochrome P450 reductase, NADH-cytochrome b_5 reductase or NADH/NADH dehydrogenase [7,8]. Xanthine oxidase catalyzes the reduction by one and two electrons of quinones [9,10]. The catalytic enhancement of ascorbate oxidation by quinones has been previously observed, including its dependence on the quinone one-electron redox potential (E^1_7) [11]. In addition, we have previously observed that quinones enhance the rates of ascorbate and xanthine/xanthine oxidase reduction of nitric oxide (NO) [12,13]

Nitric oxide is a physiologically relevant free radical that is involved in inflammation [14], neuronal transmission [15], and maintenance of vascular tone [16], and has also been implicated in the mechanisms of many pathologies including atherosclerosis [17] and ischemia/reperfusion injury [18]. In addition, NO is known for its antibacterial activity [19]. S-nitrosothiols (RSNO) are proposed as NO storage forms in biological media [20]. Protein S-nitrosylation has also been proposed as a fundamental mechanism by which nitric oxide regulates a wide range of cellular functions and phenotypes [21]. Nitric oxide release from thermal homolysis of RSNO is very slow at room temperature for all biologically relevant S-nitrosothiols [22]. Nitric oxide is released from S-nitrosothiols after reduction with xanthine/xanthine oxidase [23], ascorbate [24,25], photolysis [26], metal ions [27,28] and solvated electrons [29].

Ascorbate is a water-soluble compound which could act as antioxidant and/or reducing cofactor. It is actively accumulated in tissues [30] and higher levels of ascorbate are found in some tumors as compared to normal tissue [31]. The reduction of S-nitrosoglutathione, GSNO, by ascorbate has been studied before. At concentrations below 0.1 mM the products are GSSG and NO, while at larger ascorbate concentrations NO and GSH are produced [24]. Reactions under the latter conditions are postulated to occur via the nucleophilic attack by ascorbate at the nitroso-nitrogen atom, producing GSH and O-nitrosoascorbate. The latter decomposes, by a free-radical pathway, to produce dehydroascorbic acid and NO [24]. However, an outer-sphere mechanism for the ascorbate reduction of GSNO has also been proposed [32]. Furthermore, the ascorbate-GSNO reaction was found to be accelerated with increase in pH indicating that the anion and dianions of ascorbate are much more reactive than the acid form [32] and it is claimed that at pH 7.4 90% of the reaction occurs by the ascorbate dianion reduction of GSNO [24].

In view of the role of quinones in enhancing the rates of oxygen and NO reduction by ascorbate, we investigated the quinone-enhanced ascorbate reduction of GSNO. This work was done under anaerobic conditions as an approximation of hypoxic regions in tissues under abnormal conditions or of events of reduced oxygen supply, such as ischemia. Although a redox quinone-hydroquinone alkaline reactant that selectively releases NO from nitrosothiols has previously been studied [33], the identity of the quinone species provoking this release and the roles of the quinone redox potential and structure have not been determined.

Materials and methods

Chemicals

Quinones (Fig. 1) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO) and sublimed twice before use. GSNO was synthesized and purified as described by Hart [34]. The concentration of GSNO was determined by its absorbance at 334 nm, using the extinction coefficient $767 \text{ M}^{-1} \text{ cm}^{-1}$ [35]. Metal chelators DETAPAC and neocuproine were purchased from Alfa Aesar and used to avoid transition metal-catalyzed decomposition of GSNO [27]. The compounds 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH) and ascorbic acid were purchased from Aldrich Chemicals. Glutathione-S-transferase (GST) from human placenta (EC #: 2.5.1.18) was obtained from Sigma Chemical. Stock solutions of quinones and GSNO were prepared in water and used the same day of preparation. Deionized and Chelex-treated water was used in the preparation of all stock and sample solutions. Chelex treatment of water was monitored using the ascorbate test, as described by Buettner [36]. Care was always taken to minimize exposure of quinone-containing solutions to light.

S-nitrosylated bovine serum albumin (BSA-NO) was synthesized by S-nitrosylation of the Cys-34 in bovine serum albumin (BSA) by reaction of GSNO with BSA, as described by Huang et al. [37] Before nitrosylation, oxidized BSA was reduced using sodium dithionite with equimolar dithionite at 37°C for 1 h in 0.1 M Tris-HCl buffer (pH 8.0) containing 100 μM DETAPAC. This was followed by three repetitions of Sephadex G-50 spun column filtration of this solution as described elsewhere [38]. BSA concentration was determined using the BSA extinction coefficient (absorbance at 280 nm is 0.667 for 1 g BSA / ml [39]). This BSA solution was saturated with N_2 and then reacted for 1 hour with a N_2 -saturated GSNO solution using a GSNO:BSA molar ratio of 4:1 in HEN buffer (25 mM HEPES, pH 7.7, 0.1 mM EDTA, 0.01 mM neocuproine). GSNO was removed by three Sephadex G-50 spun column filtrations of this solution. This was followed by protein precipitation followed by washing with cold ethanol and redissolution in HEN buffer as described by Deutscher. [40] The precipitation step was repeated three times to remove excess GSNO. Absorption maxima were detected at 335 and 545 nm and the corresponding absorptivity at 335 nm, $3869 \text{ M}^{-1} \text{ cm}^{-1}$ [41], was used to quantitate BSA-NO concentration.

GSNO and BSA-NO reduction kinetics

These were monitored using a NO-specific electrochemical probe (ISO-NOP) inserted in a thermostated NO chamber (World Precision Instruments, Sarasota, FL, USA) at 37°C . The chamber was purged with high purity nitrogen followed by injection of 1.00 mL of a nitrogen-saturated solution containing GSNO or BSA-NO, quinone, 100 μM DETAPAC, 100 μM neocuproine, in 25 mM phosphate buffer (pH 7.4) with 25 % (v/v) DMSO added. This was followed by immediate exclusion of all gas bubbles out of the sample, through the chamber capillary. A small and concentrated aliquot of a N_2 -saturated ascorbate solution was then immediately added in the absence of a gas phase. Samples were continuously stirred using a spinning bar. Data acquisition was started before ascorbate addition. Basal voltage was calibrated to zero every day. The electrode was calibrated daily with known concentrations of NaNO_2 by reacting this salt with KI in sulfuric acid medium. Voltage output corresponding to a 20 μM NO solution was checked every day and the electrode membrane was replaced in case there was not agreement with previous outputs within 10 %. NO production data were collected in a computer and the initial rates of NO production (R_{NO}) were measured.

The same procedure, using GSNO, was repeated with hydroquinones without the presence of ascorbate, being the hydroquinone the last reagent added.

Determination of half-wave reduction potentials ($E_{1/2}$)

These were determined in nitrogen purged acetonitrile solutions containing 1 mM quinone, 0.1 M tetra-N-butylammonium perchlorate using differential pulse voltammetry (DPV). A BAS CV 50W voltammetric analyzer using a glassy carbon working electrode was used in these determinations. An Ag/AgCl(sat) electrode was used as the reference electrode ($E' = +0.22$ V vs. NHE) and a platinum wire as the counter electrode. Differential pulse voltammograms were obtained in the potential range of -2.00 to 0.00 volts, using a 50 mV pulse amplitude and 20 mV/s of scan rate. The reduction potential values were obtained from the DPV peak potential maxima. These are almost similar to the half-wave redox potentials, $E_{1/2}$, in normal polarographic measurements [42].

Glutathione (GSH) determination

The procedure described by Awasthi et al. [43] was used. In this procedure GSH is quantitatively conjugated to 1-chloro-2,4-dinitrobenzene (CDNB) by glutathione-S-transferase (GST) followed by HPLC analysis of the adduct produced. The advantage of this procedure over others is that GSNO is not exposed to an environment with pH greater than 7. GSNO is known to be more labile at alkaline than at neutral or acidic pH values [44,45]. For this purpose, N_2 -saturated samples containing 100 μ M DETAPAC, 100 μ M neocuproine, 6 U of GST /mL and 40 mM phosphate buffer (pH 7.4) were prepared. This was followed by addition of 500 μ M GSNO and immediate addition of the last reagent, 1.0 mM ascorbic acid. Samples were then stirred for 1 minute followed by immediate addition of 10 mM CDNB. After 4 more minutes of stirring, samples were immediately submitted to HPLC analysis. All reagents were added from N_2 -saturated stock solutions prepared the same day of analysis.

HPLC analyses were done using a Kromasil C_{18} (4.6×250 mm) column with a pre-column of the same material and eluted using a gradient from 1% trifluoroacetic acid in water to 1% trifluoroacetic acid in acetonitrile. The flow rate of elution was 1.5 mL/min. A Waters 1525 analytical HPLC system, equipped with a Waters 2487 absorption detector at 340 nm was used. The retention time of the corresponding GSH peak, 17.5 min, was determined using a GSH standard submitted to the same procedure as the sample. All determinations here were repeated at least three times and the average of these determinations $\pm$ standard deviation is reported.

Hydroquinone synthesis

This was done under nitrogen saturation conditions by reacting the quinone with an excess of $NaBH_4$ as described elsewhere [46]. Ten mgs of quinone were reacted with $NaBH_4$ in dry methanol until no further change in the quinone visible absorption band was detected. This solution was then purged with nitrogen to evaporate the solvent to dryness followed by addition of 2.0 mLs of DMSO. An aliquot of this solution was then transferred to another vial to prepare a final volume of 2.0 mLs of 10 mM hydroquinone in water. Before completing this volume, diluted HCl was added to a pH value of 3 in order to destroy any unreacted $NaBH_4$. This was followed by addition of diluted NaOH to increase the pH value to 7.4. The resulting solution was used to prepare a 1.0 mM hydroquinone stock solution.

Quinone aggregation

The absorbance at the appropriate wavelength maxima of solutions containing from 0 to 250 μ M quinone (depending on quinone solubility and absorbance at the corresponding wavelength) in 20 mM phosphate buffer (pH 7.4) and 25 % DMSO (v/v) were measured. The aggregation parameters were calculated from the nonlinear regression analysis of Eq.

(2) following the work of Yang et al. [47]. Where A is the total absorbance, ϵ_M and ϵ_D are the molar absorptivity coefficients of the

$$A = \epsilon_M \left[\frac{-1 + \sqrt{1 + 8K_D C_T}}{4K_D} \right] + \epsilon_D \left[\frac{C_T}{2} - \frac{(-1 + \sqrt{1 + 8K_D C_T})}{8K_D} \right] \quad (2)$$

monomer and dimer, respectively, C_T is the quinone analytical concentration and K_D is the dimerization constant. This method has been used for systems where the shape or relative intensity of the absorption bands of the different species in solution does not change with concentration of the molecule under study. To aid in the convergence of the non-linear regression, the value of ϵ_M was determined from linear regression of the low quinone concentration data ($< 20 \mu\text{M}$) and the initial value of ϵ_D used was estimated from linear regression of the absorbance data corresponding to the highest 5 concentrated solutions.

Quinone and semiquinone interactions with GSNO

Density functional theory (DFT) calculations were performed to obtain relative energies for the complexes of GSNO with the quinones PHQ, JQ, NQ, and UBQ and with their corresponding semiquinones. In these calculations GSNO was assumed to be ionized in analogy to GSH [48], at the pH conditions of this work. Thus the primary amine group of the glutamyl moiety is protonated and the two carboxylic groups of GSNO are deprotonated. The semiquinones of JQ [49], NQ [50], PHQ [51] and UBQ [52] are all anions at the pH conditions of this work, since their semiquinone pK_a values are close to 4-5. Geometry pre-optimizations were performed *in vacuo* with the PM3 semiempirical method using the Polak-Ribiere conjugated gradient protocol (1×10^{-5} convergence limit, $0.01 \text{ kcal}/\text{\AA}^* \text{mol}$ RMS-limit)[53]. The final optimizations in water were performed with DFT [B3LYP/6-31G(d) OPT SCRF=(PCM,Solvent=Water)] using Gaussian 03 at the High Performance Computing Facility (University of Puerto Rico, Rio Piedras). All conformational and thermodynamic parameters were obtained with a DFT single point calculation. The “stabilization energy” of the complexes was obtained from the difference of the Hartree energies, according to Eq. (3), where the Hartree to kcal/mol conversion factor is included.

$$\Delta E (\text{kcal/mol}) = (E_{\text{Complex}} - E_{\text{GSNO}} - E_{\text{Q}\cdot\text{Q}\cdot}) * 627.503 \quad (3)$$

Results and discussion

GSNO reduction kinetics

Changes in NO levels as a function of time in N_2 -saturated reaction mixtures containing ascorbate, GSNO, DETAPAC and neocuproine in phosphate buffer, in the absence and presence of various quinones, were monitored using a NO specific electrode. An example is shown in Fig. 2 corresponding to UBQ-0. Initial rates were measured from the initial slope of the [NO] traces. In the absence of quinone, a relatively small change in the NO levels as a function of time was noted in the reaction mixture. However, when quinones are included in this reaction a relatively fast increase in NO concentration was noted (Fig. 2). From the initial linear increase in NO concentration, the initial rates of NO production, R_{NO} , were determined (Fig. 2). Linear plots of R_{NO} , after subtracting initial rates in the absence of quinone, vs quinone concentration were obtained (Fig. 3), indicating that the quinone-enhanced reduction of GSNO is first order in quinone. From the slopes of plots in Fig. 3, the first order rate constants, K_{obs} , were obtained. Similar linear behaviors were obtained when R_{NO} values were plotted as a function of GSNO or ascorbate concentrations, while keeping

the other reagent concentration invariable (Fig. 4), thus indicating first order behavior for both ascorbate and GSNO.

When the Kobs values obtained from Fig. 3 were plotted against the quinone $E_{1/2}$ values, a bell-shaped curve was observed for samples containing p-benzoquinones (Fig. 5). That type of correlation between quinone reactivity and quinone redox potential was detected previously for the initial rates of the quinone-enhanced rate of ascorbate oxidation [11]. Interestingly, Kobs values for samples containing p-naphthoquinones, were smaller than those corresponding to p-benzoquinone- and PHQ-containing samples, when comparing quinones of similar redox potentials. Since such a difference in behavior was not detected in the quinone-enhanced ascorbate reduction of oxygen, this observation could only be explained by differences in possible interactions between the quinone and/or the semiquinone and GSNO. GSNO is known to degrade by itself in water to GSH, GSO_3H , and GSSG [54]. Amine and thiol groups from these compounds, as well as GSNO, could perform Michael addition to the quinone ring unless the quinone is protected by substituents [55-57]. To determine if there is an interaction between parent quinones and GSNO, we selected 2 quinones with no possibility for Michael addition. These are DQ and ETMNQ. Although the redox potential of ETMNQ is more positive than that of DQ, a larger Kobs is observed for DQ as compared to ETMNQ. Absorption spectra of N_2 -saturated samples containing 50 μM DQ or ETMNQ and 1.0 mM GSNO were not different from the spectra corresponding to the summation of both of the individual quinone and GSNO spectra, indicating that no interaction occurs between these quinones and GSNO (Fig. 6). Differences in reactivities between p-benzoquinones and p-naphthoquinones can not be ascribed to differences in the amounts and reactivities of possible thiol-substituted quinones and hydroquinones since the differences in reactivities between DQ and ETMNQ can not be ascribed to Michael addition products. In addition, glutathionyl-naphthohydroquinone derivatives autoxidizes 12 to 16 times faster than the parent naphthoquinone compounds and thus, should be oxidized by GSNO faster than the parent naphthoquinone, not slower.

Addition of 250 μM hydroquinone to an anaerobic solution containing 100 μM DETAPAC, 100 μM neocuproin, 500 μM GSNO and 3:1 (v/v) 20 mM phosphate buffer (pH 7.4): DMSO produced an initial rate of decomposition of GSNO which is, in all cases, smaller than 10 % of that observed for the corresponding 250 μM quinone + 1 mM ascorbate mixture (Table 1). This indicates that the contribution of the hydroquinone to this electron transfer reduction process is very minor. Interestingly, again, the rates of NO production when using QH_2 are smaller by one order of magnitude for p-naphthoquinones as compared to those of p-benzoquinones. Another possible explanation for the lower rate of ascorbate reduction of GSNO could be differences in aggregation extent of naphthoquinones and that of p-benzoquinones. Thus, we decided to measure the dimerization constant (K_D) of UBQ, NQ, PHQ and NQ2S. The reason for selecting these quinones is that UBQ has a similar redox potential as NQ and that of PHQ is similar to that of NQ2S. Non-linear regressions of plots of absorbance vs. quinone concentration produced the K_D values shown in Table 2. Those K_D values follow the expected behavior since the more hydrophilic quinones, i. e. UBQ and NQ2S (as noted from their water solubility), also have the smallest K_D values. Thus, K_D values do not correlate with the Kobs values in these cases.

GSH production

After five minutes of reaction, samples were submitted for GSH analysis. Again, a bell-shaped plot is detected for the amount of GSH formed as a function of quinone redox potential (Fig. 7). Also, smaller amounts were produced when p-naphthoquinones were used as compared to p-benzoquinones with redox potentials in the same range of values as those of p-benzoquinones, thus confirming the type of behavior shown in Fig. 5.

GSNO reduction stoichiometry

The stoichiometry of this reaction, in the absence of quinone, has been previously determined. Although a 1:2 ascorbate to GSNO mol ratio has been reported [24,59], a 1:1 mol ratio was also reported [32]. The anaerobic reaction of 500 μM GSNO with 250 μM ascorbate during 30 minutes and in the presence of 250 μM PHQ yielded a GSH produced to GSNO consumed mol ratio of 0.92 ± 0.05 (Fig. 8) and essentially no additional GSH is produced when using larger ascorbate concentrations. An identical behavior is observed if the same reaction is repeated but in the absence of quinone (Fig. 8). Thus, the 1:2 ascorbate to GSNO mol ratio is confirmed here. In addition, the fact that the GSH produced to GSNO consumed mol ratio is near 1 indicates that GSNO is essentially all converted to GSH, which is consistent with a previous report [32].

BSA-NO reduction kinetics

Initial NO production rates, R_{NO} , were measured after ascorbate addition to N_2 -saturated solutions containing DMBQ and 500 μM BSA-NO, in a similar fashion as measured for GSNO. Again, an increase in R_{NO} with DMBQ concentration increase is observed (Fig. 9). The linear dependence of R_{NO} on DMBQ concentration shows the first order behavior of this quinone in the BSA-NO reduction with ascorbate. Interestingly, the Kobs value for the mixture DMBQ-ascorbate-BSA-NO ($(0.00153 \pm 0.00002) \text{ s}^{-1}$) is about 25 % smaller than that found for the DMBQ-ascorbate-GSNO mixture ($(0.00214 \pm 0.00008) \text{ s}^{-1}$). This difference could be ascribed in part to the additional difficulty for the reductive cleavage of the S-NO bond which should be encountered in a macromolecule such as BSA-NO as compared to GSNO. This observation suggests that other quinones may enhance the reductive cleavage of NO from BSA-NO and, most probably, from other S-nitrosylated proteins.

Quinone and semiquinone interactions with GSNO

To verify if the differences in enhancement of the ascorbate reduction of GSNO by p-benzoquinones and PHQ as compared to p-naphthoquinones is mainly due to the interaction of the corresponding quinones and/or semiquinones with GSNO, the thermochemistry of this system was determined using quantum mechanics. The reason for selecting the quinones and semiquinones of PHQ, UBQ, JQ and NQ is that, as depicted in Fig. 5, PHQ and JQ have similar redox potentials, as well as, NQ and UBQ. However, the p-benzoquinone UBQ and PHQ in these 2 pairs of quinones show larger Kobs values, by an order of magnitude or more, than those of the corresponding p-naphthoquinones. Since, as stated above, no correlation between the K_D values of these quinones and Kobs was observed, interactions between the quinones or the semiquinones with GSNO could explain these differences in Kobs. According to these calculations the reduction of these quinones is thermally favored in solution with an average enthalpy change of at least -323 kcal/mol (Table 3).

Complex formation with GSNO, on the other hand, is not favored for Q (with the exception of UBQ, for which the calculation predicts some complex formation with $\Delta E = -4.8 \text{ kcal/mol}$). After reduction, $\text{Q}^{\bullet-}$ form more stable complexes with GSNO with $\Delta E < -13 \text{ kcal/mol}$. Moreover, the semiquinones of PHQ and UBQ in the GSNO-complexes are by far more stable than those formed by the other semiquinones. Therefore, $\text{PHQ}^{\bullet-}$ and $\text{UBQ}^{\bullet-}$ are expected to interact more strongly than $\text{JQ}^{\bullet-}$ and $\text{NQ}^{\bullet-}$ with GSNO, thus lowering the transition state energy for the electron transfer process to GSNO. This is in complete agreement with the observed data (Table 2 and Fig 5).

Since initial rates have been measured in this work to determine reaction orders, a possible mechanism can be postulated which does not consider the second electron oxidation step of ascorbate, Scheme 1. In addition, the formation of an association complex pre-equilibrium

between the semiquinone and GSNO is postulated (reaction 5), followed by a relatively slow intracomplex electron transfer and product formation (reaction 6). The latter is a reasonable expectation since appropriate orientations in this association complex are first needed to be formed before GSNO reduction and product formation occur. Thus, assuming step (6) as the rate-determining reaction and from pre-equilibria (4) and (5) it can be demonstrated that initial rates will follow a law of the type,

$$\frac{d[\text{NO}]}{dt} = k_3 [\text{Complex}] = k [\text{AH}^-] [\text{Q}] [\text{GSNO}] \quad (7)$$

where,

$$k = \frac{k_3 k_1 k_2}{k_{-1} k_{-2} [\text{AH}^-]} \quad (8)$$

A constant steady state concentration of the ascorbyl radical has been observed in previous works during the quinone-enhanced, [60] as well as in the iron- and methylene blue-catalyzed, [61] ascorbate oxidation. Such a behavior in the ascorbyl concentration will render a constant “k” value in Eq. 8.

In summary, the quinones under study here enhance the rates of GSNO and BSA-NO reduction with the consequent NO release. However, larger reactivity is observed from p-quinones and PHQ as compared to p-naphthoquinones. Thus, quinones, and especially p-quinones, could act as NO release enhancers from GSNO in biomedical systems in the presence of ascorbate. Since quinones are exogenous toxins which could enter the human body via a chemotherapeutic application or as an environmental contaminant, these could boost the release of NO from S-nitrosothiol stores in the body in the presence of ascorbate and thus enhance the responses elicited by a sudden increase in NO levels. Ascorbate concentrations can be rapidly increased in the body from micromolar to millimolar values when injected intravenously [62].

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List of Abbreviations

DMBQ	2,6-dimethyl-1,4-benzoquinone
BQ	1,4-benzoquinone
MBQ	methyl-1,4-benzoquinone
DMOBQ	2,6-dimethoxy-1,4-benzoquinone
HMOBQ	2-hydroxymethyl-6-methoxy-1,4-benzoquinone
TMQ	trimethyl-1,4-benzoquinone
DQ	tetramethyl-1,4-benzoquinone
UBQ-0	12,3-dimethoxy-5-methyl-1,4-benzoquinone
NQ	1,4-naphthoquinone
MNQ	menadione

NQ2S	1,4-naphthoquinone-2-sulfonate
JQ	juglone
PHQ	phenanthraquinone
GSNO	S-nitrosoglutathione
BSA-NO	S-nitrosylated serum albumin
GSH	glutathione
Q	quinone
Q^{•-}	semiquinone
QH₂	hydroquinone
RSNO	S-nitrosothiol
GSSG	oxidized glutathione
DETAPAC	diethylenetriaminepentaacetic acid
CDNB	1-chloro-2,4-dinitrobenzene
GST	glutathione-S-transferase
DFT	density functional theory

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MBQ; $R_1 = R_2 = R_3 = H$; $R_4 = CH_3$

DMBQ; $R_1 = R_2 = CH_3$, $R_4 = H$

HMBQ; $R_1 = CH_2OH$, $R_2 = OCH_3$, $R_3 = R_4 = H$

UBQ; $R_1 = CH_3$, $R_2 = R_3 = OCH_3$, $R_4 = H$

DMOBQ; $R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$

DQ; $R_1 = R_2 = R_3 = R_4 = CH_3$

NQ; $R_5 = R_6 = R_8 = H$

MNQ; $R_5 = CH_3$, $R_6 = R_8 = H$

JQ; $R_5 = R_6 = H$, $R_8 = OH$

NQ2S; $R_5 = SO_3^-$, $R_6 = R_8 = H$

ETMNQ; $R_8 = H$, $R_5 = CH_3$, $R_6 = SCH_2CH_3$

PHQ

Fig. 1.
Quinones used in this work.

Fig. 2.

Initial NO traces. Samples are saturated with nitrogen and contain 1.0 mM ascorbate, 500 μM GSNO, 100 μM DETAPAC, 100 μM neocuproine and the stated amount of UBQ in 25 mM phosphate buffer (pH 7.4) with 25 % (v/v) DMSO added. The arrow shows the instance in which ascorbate is added.

Fig. 3.

Determination of K_{obs} for different quinones. Samples are saturated with nitrogen and contain 1.0 mM ascorbate, 500 μ M GSNO, 100 μ M DETAPAC, 100 μ M neocuproine and quinone in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). Each point was determined 3 times and error bars are the corresponding errors in the mean values.

Fig. 4.

Determination of reaction orders corresponding to GSNO (a) and ascorbate (b). Samples are saturated with nitrogen and contain 100 μM DETAPAC, 100 μM neocuproine and 250 μM quinone in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). Each point was determined 3 times and error bars are the corresponding errors in the mean values.

Fig. 5.

Dependence of K_{obs} values with quinone one-electron redox potentials. Errors in K_{obs} were those obtained from linear regressions of plots in Fig. 3.

Fig. 6.

Absorption spectra of samples containing GSNO, in the presence or absence of DQ or ETMNQ, in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). Spectra in green are the summation of spectra corresponding to quinone in the absence of GSNO with that of GSNO in the absence of quinone.

Fig. 7.

Dependence of the amount of GSH concentration produced after 4 minutes of reaction after addition of ascorbate on quinone redox potential. Ascorbate is the last reagent added. Samples contained 1.0 mM ascorbate, 500 μ M GSNO, 100 μ M DETAPAC, 100 μ M neocuproine, 6 U of GST /mL, 250 μ M quinone, in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). After reaction, 10 mM CDNB was added before HPLC analysis. Each point corresponds to the average of at least 2 determinations and the errors are the differences between determinations.

Fig. 8.

Dependence of the GSH quantity produced on the ascorbate concentration. Samples contained 100 μM DETAPAC, 100 μM neocuproine, 500 μM GSNO, 6 U of GST /mL, and 250 μM quinone in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). Reactions were started by addition of ascorbate. This reaction was run under N_2 -saturation conditions. After 30 minutes of reaction, the GSH concentration was analyzed as described in **Materials and methods**. Each point corresponds to the average of at least 3 determinations and the errors are the corresponding errors in the mean values.

Fig. 9.

Dependence of the initial rate of NO production from BSA-NO on DMBQ concentration. Samples are saturated with nitrogen and contain 1.0 mM ascorbate, 500 μM BSA-NO, 100 μM DETAPAC, 100 μM neocuproine and DMBQ in 3: 1, 25 mM phosphate buffer (pH 7.4):DMSO (v/v). Each point corresponds to the average of at least 3 determinations and the errors are the corresponding errors in the mean values.

Scheme 1.

Postulated mechanism for the reaction under study in this work. AH^- represents ascorbate and $\text{AH}^\bullet$ the ascorbyl radical.

Table 1

Initial rates of GSNO decomposition in the presence of 250 μM quinone ($R_{\text{NO}, \text{Q}}$)^a or hydroquinone ($R_{\text{NO}, \text{QH}_2}$)^b.

Quinone	$R_{\text{NO}, \text{Q}} / 10^{-2} \mu\text{M s}^{-1}$	$R_{\text{NO}, \text{QH}_2} / 10^{-2} \mu\text{M s}^{-1}$
UBQ	5.1 ± 0.2	0.13 ± 0.01
DMOBQ	4.6 ± 0.2	0.094 ± 0.003
DMBQ	5.6 ± 0.3	0.11 ± 0.01
MBQ	4.0 ± 0.3	0.11 ± 0.02
HMBQ	4.6 ± 0.1	0.10 ± 0.03
NQ	0.45 ± 0.04	0.055 ± 0.004
JQ	0.71 ± 0.04	0.061 ± 0.002
MNQ	0.85 ± 0.05	0.030 ± 0.002
NQ2S	0.81 ± 0.03	0.058 ± 0.003
No Q or QH ₂	0.31 ± 0.07 ^c	0.014 ± 0.002 ^d

^a nitrogen saturated solutions 500 μM GSNO, 250 μM quinone, 100 μM DETAPAC, 100 μM neocuproine, in 25 mM phosphate buffer (pH 7.4) with 25 % (v/v) DMSO added. Initial rate measurement was done immediately after 1 mM ascorbate addition. Errors are of the mean of 3 determinations.

^b nitrogen saturated solutions 500 μM GSNO, 250 μM hydroquinone, 100 μM DETAPAC, 100 μM neocuproine, in 25 mM phosphate buffer (pH 7.4) with 25 % (v/v) DMSO added. Initial rate measurement was done immediately after hydroquinone addition. Errors are of the mean of 3 determinations.

^c Initial rates in the absence of quinone, but in the presence of 1.0 mM ascorbate.

^d Initial rates in the absence of both hydroquinone and ascorbate.

Table 2

Extinction coefficients and K_D values obtained from the non-linear regression fits of Eq. (2).^a

Quinone	$\varepsilon_M / 10^3 \text{ M}^{-1} \text{ cm}^{-1}$	$\varepsilon_D / 10^3 \text{ M}^{-1} \text{ cm}^{-1}$	K_D	R^{2c}
NQ (340) ^b	1.9 ± 0.5	6.1 ± 0.1	$(3.4 \pm 0.4) \times 10^3$	0.997
PHQ (327)	6.7 ± 0.2	6.5 ± 0.1	$(7.2 \pm .4) \times 10^3$	0.998
UBQ (410)	0.85 ± 0.03	0.76 ± 0.06	$(9.2 \pm 0.9) \times 10^2$	0.998
NQ2S (345)	2.81 ± 0.05	1.2 ± 0.9	1.1 ± 0.1	0.996

^a air saturated solutions containing from 0 to 2000 μM quinone (depending on quinone solubility and absorbance at the corresponding wavelength) in 20 mM phosphate buffer (pH 7.4) and 25 % DMSO (v/v). Errors are those obtained from the non-linear regression of plots.

^b number in parenthesis is the wavelength used in nm.

^c goodness of fit parameter

Table 3

Thermochemistry of quinones (Q), semiquinones (Q^{•-}) and their complexes with GSNO

Molecule ^d	E (Hartree)		μ (D)		ΔE (kcal/mol)	
	Q	Q ^{•-}	Q	Q ^{•-}	Q	Q ^{•-}
PHQ	-688.77797338	-688.90293214	8.9	12.8	--	-78.41
JQ	-610.35060463	-610.47293281	4.7	7.9	--	-76.76
NQ	-535.12944531	-535.25657472	1.9	4.8	--	-79.77
UBQ	-649.82125142	-649.95275856	1.6	1.9	--	-82.52
GSNO-PHQ	-1012.54397120	-1012.68059280	21.5	17.8	-6.19	-13.51
GSNO-JQ	-934.11759531	-934.24714382	8.9	7.8	-6.81	-11.34
GSNO-NQ	-858.89252027	-859.02748940	10.8	8.7	-4.36	-9.28
GSNO-UBQ	-973.585648724	-973.73596832	13.8	14.1	-5.19	-16.99

^aCorresponding values for GSNO are: E = -323.75613383 and μ = 12.4 D

^bThe stabilization energy for the formation of Q^{•-} is determined by $\Delta E = [E(Q^{\bullet-}) - E(Q)](627.503)$ kcal/mol. The goodness of these results was assessed with the formation energies for PHQ (-12.1 kcal/mol) and NQ (-23.1 kcal/mol). These values are in excellent agreement with the experimental values of -11.1 kcal/mol and -23.3 kcal/mol, respectively [63].