Some Aspects of Electron-Transfer Reaction of Ascorbate with Quinones

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A single-electron oxidation of ascorbate by a series of low-potential quinones was investigated. It was determined that bimolecular rate constants correlate with the single-electron reduction potential (E_7^1) of quinones. The results were interpreted in the framework of the "outer-sphere" electron-transfer theory given by Marcus. The consistency of Marcus model for these reactions was verified by comparison of the experimentally determined data with the predicted ones. Energetic considerations suggest that the single-electron transfer between ascorbate and quinone proceeds with a concomitant transfer of proton rather than *via* an electron transfer and a subsequent transfer of proton. This position to some extent was supported by the fact that over pH range, where no protonation/deprotonation of reagents occurs, the coefficient of proportionality ($\Delta \log\{k_{exp}\}\Delta pH$) for most active quinones in the ascorbate oxidation reaction was close to 0.5.

It is well known that depending on a situation ascorbate (vitamin C) in biological systems can exhibit either antioxidant or prooxidant activity. The former is associated with ability of ascorbate to readily react with variety of free radicals and active oxygen species (superoxide, hydroxyl radicals or hydrogen peroxide), while the latter results from the tendency of ascorbate to be oxidized by molecular oxygen in the presence of various catalysts [1—6]. It is generally accepted that in biological systems the limiting factor is most likely the ability of transition metal ions, which are absolutely required for the prooxidant activity. For example, the release of iron from protein complexes (transferrin, lactoferrin, hemoglobin, and ferritin) can provoke a free radical generation *via* the ascorbate—iron system [2]. Various iron chelates can substantially increase the efficiency of these processes [2, 4—7]. Ascorbate as well can markedly stimulate the oxygen uptake participating in reactions with low potential quinoidal compounds [1, 5, 6]. It is assumed that this effect could be ascribed to the ability of ascorbate to reduce quinones by a single-electron way to quinone radicals. The latter readily react with molecular oxygen and thereby catalyze the autooxidation. Even if the reaction is thermodynamically unfavourable, ascorbate can carry out a single-electron reduction of quinones since semiquinone radical products formed are continuously removed by reoxidation by oxygen or disproportionation [1]. Two-electron reduction of quinones leads to the formation of hydroquinones which are in general less readily autooxidized than quinone radicals and may be rapidly conjugated and excreted [5]. Thereby, for example, the two-electron reduction of quinones by DT-diaphorase (NAD(P)H: quinone oxidoreductase) is considered to be a mechanism for detoxication. However, the addition of ascorbate to these reaction mixtures increases the rate of redox cycling of quinones. It has been proposed that this effect can be influenced by a single-electron reduction of quinones by ascorbate which produces superoxide and thus can stimulate the DT-diaphorase-mediated redox cycling of quinones by initiating radical chain reaction [5]. The reaction of ascorbate with quinones has also been studied by electrochemical and ESR methods and the correlations between the cytotoxic activities of quinone/ascorbate mixtures and their ability to produce stable semiquinone radicals of low-potential quinones have been determined [6].

Numerous reports regarding the chemical redox behaviour of ascorbate, particularly the studies of electron-transfer (ET) mechanism between ascorbate and physiological/nonphysiological electron acceptors such as cytochrome c [8, 9], cytochrome b_{561} [10], cytochrome b_5 reductase or cytochrome reductase—cytochrome b_5 complex [11], and various inorganic complexes [12, 13] showed that reactions can proceed

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in accordance with the "outer-sphere" ET mechanism given by *Marcus* [14, 15] or "inner-sphere" (concerted electron/proton transfer) mechanism.

Thus, in attempt to elucidate the electron-transfer mechanism between quinones and ascorbic acid, we have tested a series of quinones with a wide variety of single-electron redox potentials in the ascorbate oxidation reaction and the data analyzed in the framework of the "outer-sphere" ET mechanism. As the private "outer-sphere" ET kinetic and thermodynamic parameters (electron self-exchange rate constants and single-electron redox potentials) for ascorbate and quinones are well known, the consistency of Marcus model for these reactions was verified by comparison of the experimentally determined data with the predicted ones.

EXPERIMENTAL

L-Ascorbic acid, 5-hydroxy-1,4-naphthoquinone (juglone), 2-hydroxy-1,4-naphthoquinone, 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin), 2,5-bis-(carboethoxyamino)-3,6-diaziridinyl-1,4-benzoquinone (diaziquinone), tetramethyl-1,4-benzoquinone (duroquinone) (Sigma, St. Louis, MO), 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin) (Fluka AG, Buchs, Switzerland), adriamycin, mitomycin C (Farmitalia, Milan, Italy) were used as received. 1,2-Naphthoquinone, 2,6-dimethyl-1,4-benzoquinone, 2,6-dimethoxy-1,4-benzoquinone, 1,4-benzoquinone, 9,10-phenanthrenequinone were obtained from Reakhim (Shostkino, Russia), purified by sublimation under vacuum or recrystallized from benzene or ethanol. 2,5,8-Trihydroxy-7-methoxy-2-methyl-1,2,3,4-tetrahydro-3-oxaanthraquinone (fuzarubin) was generous gift of Dr. A. G. Medentsev (Institute of Microorganism Physiology and Biochemistry, Puschino, Russia). 2-Dimethylamino-3-chloro-1,4-naphthoquinone was synthesized as described in [16].

All experiments were carried out in 0.1 M-(K_2HPO_4 and KH_2PO_4) buffer solution (pH 7.0) at (25 ± 0.1) °C. Aqueous solutions were prepared with double distilled water and buffer solution was purged from the traces of transition metals in accordance with the batch method using Chelex-100 resin [17]. The rate of the single-electron reduction of quinones by ascorbic acid (eqn (*1*)) was monitored according to the rate of oxygen consumption using Clark electrode as a sensor of membrane oxygen electrode due to immediate reoxidation of semiquinone radicals by oxygen to parent quinone compounds and superoxide. A volume of chamber of solution tested was 1.0 cm^3 The reaction was initiated by addition of quinones with a Hamilton syringe. Even if the reaction (*A*) is thermodynamically unfavourable, the equilibrium is shifted to the right side as the result of the rather high concentration of ascorbate with respect to the quinone [1].

The curve fittings were made by an iterative non-linear least-squares method.

RESULTS AND DISCUSSION

Oxygen consumption in the ascorbate oxidation reaction by quinones may be presented by the following schemes

$$Q + AsH^- \Leftrightarrow As^{\bullet -} + Q^{\bullet -} + H^+ \qquad (A)$$

$$Q^{\bullet -} + O_2 \Rightarrow Q + O_2^{\bullet -} \qquad (B)$$

$$Q^{\bullet -} + O_2^{\bullet -} + 2H^+ \Rightarrow Q + H_2O_2 \qquad (C)$$

$$O_2^{\bullet -} + HO_2^- + 2H^+ \Rightarrow HO_2^{\bullet} + H_2O_2 \qquad (D)$$

$$Q^{\bullet -} + H_2O_2 \Rightarrow Q + OH^- + OH^{\bullet} \qquad (E)$$

$$AsH^- + O_2 \Rightarrow As^{\bullet -} + O_2^{\bullet -} + H^+ \qquad (F)$$

$$AsH^- + O_2^{\bullet -} + H^+ \Rightarrow As^{\bullet -} + H_2O_2 \qquad (G)$$

where AsH^-, $As^{\bullet -}$ stand for ascorbic acid in reduced and semiquinone form, and $Q^{\bullet -}$, Q stand for quinones in semiquinone and oxidized form, respectively.

For low-potential quinones, the reactions (*B*—*D*) and (*G*), in comparison with the reaction (*A*), are fast ($k_2 \approx 10^7$—10^8 dm^3 mol^{-1} s^{-1} [18—20], $k_3 \approx 3.0 \times 10^5$ dm^3 mol^{-1} s^{-1} [21], $k_4 \approx 1.0 \times 10^8$ dm^3 mol^{-1} s^{-1} [21], $k_7 = (2.7$—$3.3) \times 10^5$ dm^3 mol^{-1} s^{-1} [22, 23]).

Thermodynamic calculations indicate that semiquinones having a single-electron reduction potentials between −0.33 V and −0.46 V can theoretically bring the single-electron reduction of H_2O_2 reaction (*E*) [24—26]. For example, k_5 estimated for adriamycin anion radical is $\approx 10^5$ dm^3 mol^{-1} s^{-1} [26]. The reduction of O_2 by ascorbate reaction (*F*) from the schemes above can be excluded as the rate is characterized by the very low rate constant ($(5.9$—$7.0) \times 10^{-4}$ dm^3 mol^{-1} s^{-1} at pH 7.0 [27, 28]). Thus, in the most general form one may suppose that the rate-determining stage in the oxygen consumption is the quinone reduction by ascorbate and, thereby, the kinetics of initial rate of oxygen consumption may lay account on the reactions (*A*) and (*B*) and be given by the simple relation

$$(d[O_2]/dt)_0 = k_{obs}[\text{quinone}] \qquad (1)$$

where the pseudo-first order rate constant (k_{obs}) can be expressed as follows

$$k_{obs} = (k_1 k_2 [AH^-][O_2])/(k_1[AH^-] + k_2[O_2]) \qquad (2)$$

Since, as can be seen from Fig. 1 (*a*, *c*), the direct proportionality between the initial rate of O_2 con-

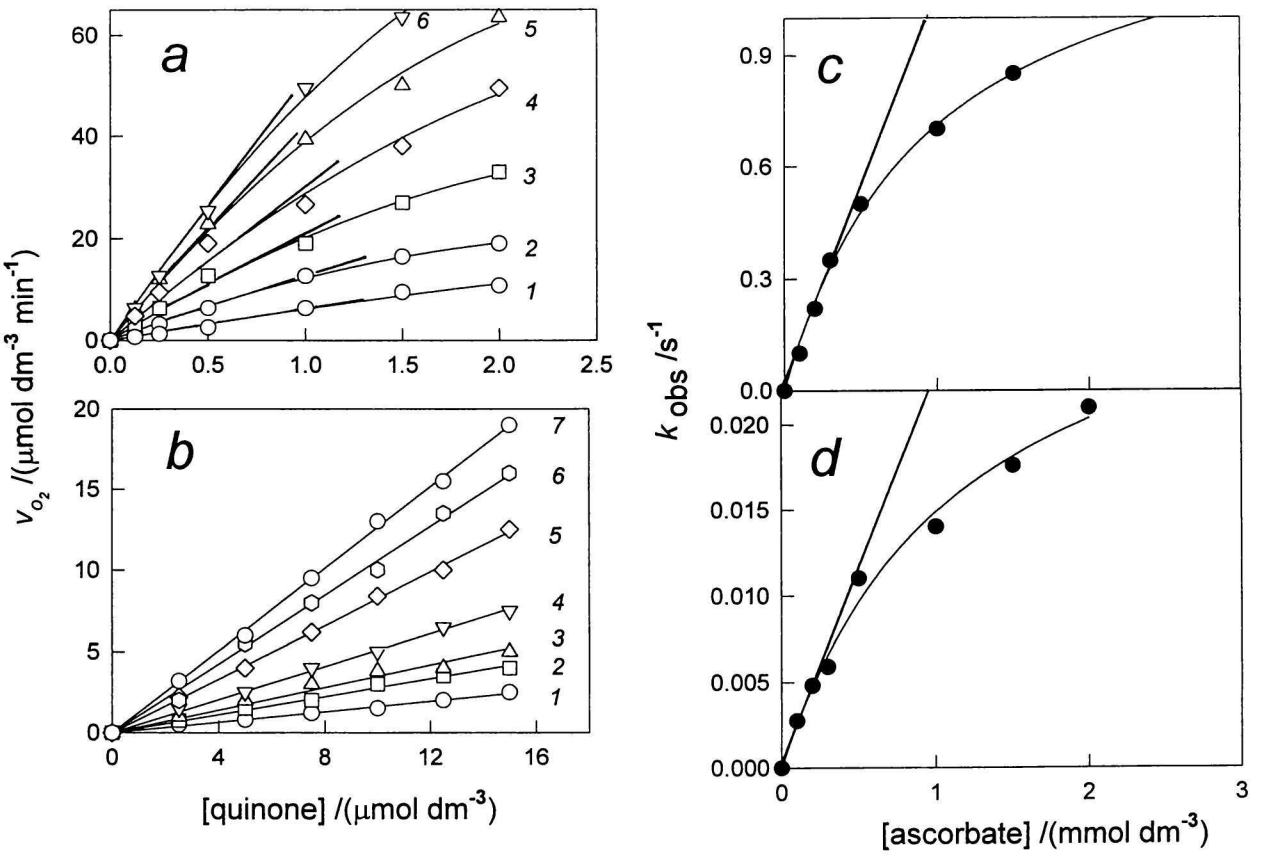

Fig. 1. The dependence of the initial rate of the oxygen consumption on the concentration of 2,3-dichloro-1,4-naphthoquinone (*a*) and 5-hydroxy-1,4-naphthoquinone (*b*) at the various concentrations of ascorbate, 0.1 mmol dm^{-3} (*1*), 0.2 mmol dm^{-3} (*2*), 0.3 mmol dm^{-3} (*3*), 0.5 mmol dm^{-3} (*4*), 1.0 mmol dm^{-3} (*5*), 1.5 mmol dm^{-3} (*6*), 2.0 mmol dm^{-3} (*7*). The apparent first-order rate constants (k_{obs}) for 2,3-dichloro-1,4-naphthoquinone evaluated from the slopes at the initial 2,3-dichloro-1,4-naphthoquinone concentrations. The dependence of k_{obs} on the ascorbate concentration for 2,3-dichloro-1,4-naphthoquinone (*c*) and 5-hydroxy-1,4-naphthoquinone (*d*). Bimolecular rate constants (k_{exp}) evaluated from the slopes at the initial ascorbate concentration. pH 7.0, 0.1 mol dm^{-3} K-phosphate buffer.

sumption and most active low-potential quinone (2,3-dichloro-1,4-naphthoquinone) was not maintained, k_{obs} was determined from the slopes at the initial region of quinone concentration. As k_2 is significantly higher than k_1, one may conclude that for ascorbate concentration used in the experiments k_2 [O_2] $\gg$ k_1 [AH^-] and eqn (*2*) must be simplified to $k_{obs} = k_1$ [AH^-] (k_1 will be further denoted as k_{exp}). However, the direct proportionality between k_{obs} and ascorbate was also not maintained (Fig. 1*c*, *d*). Obviously, this displays a more complicated character of the process under consideration. Thus, in order to determine bimolecular rate constant k_{exp}, we used the initial region of ascorbate concentration as shown in Fig. 1*c* and *d*. The bimolecular rate constants within the limits of their confidence determined by this method seem quite reasonable. For instance, the bimolecular rate constant evaluated from the initial rate of 2,6-dimethoxy-1,4-benzoquinone radical generation in the reaction with ascorbate by the method of EPR [6] is $\approx$ 0.7 dm^3 mol^{-1} s^{-1}, while that determined in our experiments by Clark electrode – (1.5 ± 0.9) dm^3 mol^{-1} s^{-1} The bimolecular rate constant determined for 2-dimethylamino-3-chloro-1,4-naphthoquinone ((0.05 ± 0.01) dm^3 mol^{-1} s^{-1}) is very close to the one determined by *Dikalev et al.* with the same assay system [4].

Quinones possessing single-electron potential ($E(Q/Q^{\bullet -})$) values between −0.415 V (2-hydroxy-1,4-naphthoquinone) and −0.036 V (2,3-dichloro-1,4-naphthoquinone) are reduced by semidehydroascorbate to semiquinones with spontaneous reoxidation by oxygen reactions (*A*) and (*B*). As can be seen from Table 1, the determined values of bimolecular rate constants of these reactions increase with increasing single-electron reduction potentials of quinones. As shown in Fig. 2*a*, these data were satisfactorily fitted by nonlinear least-square regression to the "outer-sphere" electron-transfer reaction equation given by *Marcus* [14, 15]

$$\ln\{k_{exp}\} = ln\{k^0_{exp}\} - (4\Delta G^{\#}(0)_{12} - nF\Delta E^1_7)^2 / /RT8\Delta G^{\#}(0)_{12} \qquad (3)$$

Table 1. Measured (k_{exp}) and Predicted from Marcus Eqns (*4*—*6*) (k_{calc}) Bimolecular Rate Constants of the Single-Electron Reduction of Low-Potential Quinones by Ascorbate and Single-Electron Reduction Potentials (E_7^1) of Quinones

	Quinones	k_{exp}	k_{calc}	E_7^1/V
		$dm^3\ mol^{-1}\ s^{-1}$	$dm^3\ mol^{-1}\ s^{-1}$	
1	2,3-Dichloro-1,4-naphthoquinone	980 ± 50	1.9×10^3	−0.036[a]
2	Diaziquinone	77.7 ± 4.5	7.57×10^2	−0.070[a]
3	1,2-Naphthoquinone	32.0 ± 2.1	5.72×10^2	−0.080[a]
4	2,6-Dimethyl-1,4-benzoquinone	28.0 ± 1.5	5.72×10^2	−0.080[a]
5	5-Hydroxy-1,4-naphthoquinone	30.5 ± 1.8	3.99×10^2	−0.093[a]
6	5,8-Dihydroxy-1,4-naphthoquinone	16.5 ± 1.1	2.5×10^2	−0.110[a]
7	9,10-Phenanthrenequinone	22.0 ± 1.8	1.9×10^2	−0.120[a]
8	1,4-Naphthoquinone	10.0 ± 1.0	79.8	−0.150[a]
9	2,6-Dimethoxy-1,4-naphthoquinone	1.5 ± 0.2	66.7	−0.150[a]
10	5-Hydroxy-2-methyl-1,4-naphthoquinone	3.2 ± 0.3	18.2	−0.156[a]
11	2,3,5,6-Tetramethyl-1,4-benzoquinone	0.2 ± 0.1	18.2	−0.240[a]
12	Fuzarubin	0.16 ± 0.1	2.9	−0.260[b]
13	Mitomycin C	0.08 ± 0.01	0.6	−0.310[a]
14	Adriamycin	0.08 ± 0.008	0.3	−0.330[a]
15	2-Dimethylamino-3-chloro-1,4-naphthoquinone	0.05 ± 0.01	0.1	−0.353[c]
16	2-Hydroxy-1,4-naphthoquinone	0.009 ± 0.005	0.02	−0.410[a]

a) E_7^1 values are from Ref. [1]. *b*) E_7^1 value is from Ref. [39]. *c*) Tentative E_7^1 value is calculated in accordance with the principle of additivity: the introduction of 2-dimethylamino group into 1,4-naphthoquinone lowers its E_7^1 by 0.26 V, whereby the introduction of 3-chloro group into this quinone increases its E_7^1 by 0.057 V.

where k_{exp}^0 is the limiting rate constant of electron transfer, $4\Delta G^{\#}(0)_{12}$ is the reorganization energy of electron transfer, n is the number of electrons transferred, and ΔE_7^1 is the difference in single-electron redox potentials of ascorbate and quinones. The single-electron redox potential of ascorbate was taken for the $AH^-/A^{\bullet -}$ couple (+ 0.33 V [29]) which is energetically more favourable than for the couple of $AH^-/AH^{\bullet}$ (+ 0.77 V [30]). Thus, for these reactions calculated values of $\Delta G^{\#}(0)_{12}$ were (0.22 ± 0.02) eV ((22.80 ± 4.77) kJ mol^{-1}) and $k_{exp}^0 = (7.95 \pm 0.78) \times 10^8$ $dm^3\ mol^{-1}\ s^{-1}$

As the kinetic and thermodynamic parameters for both ascorbic acid and quinones are well known, it was possible to predict bimolecular rate constants of the above reactions using the alternative cross-relation from the Marcus theory [14, 15], where the bimolecular rate constant for the electron transfer from ascorbate to quinones (k_{12}) can be expressed in terms of the electron self-exchange rate constant for ascorbate (k_{11}) and quinone compounds (k_{22})

$$k_{12} = (k_{11} k_{22} K_{12} f_{12})^{1/2} \qquad (4)$$

and

$$f_{12} = \exp[(\ln K_{12})^2 / 4 \ln(k_{11} k_{22} / Z^2)] \qquad (5)$$

where the equilibrium constant of reaction (K_{12}) is calculated from the difference of redox potentials (ΔE_7^1/V) of reagents

$$K_{12} = \exp[\Delta E_7^1 / (F/RT)] \qquad (6)$$

k_{11} value for semidehydroascorbate is taken as 8.0×10^5 $dm^3\ mol^{-1}\ s^{-1}$ [8] and k_{22} value for quinones – 1.0×10^8 $dm^3\ mol^{-1}\ s^{-1}$ [31]. Collision factor f_{12} is evaluated taking frequency factor Z as 10^{11} $dm^3\ mol^{-1}\ s^{-1}$.

As can be seen from data of Table 1, the values of calculated rate constants are markedly higher than experimentally determined rate constants. Nevertheless, orthogonal relationship between logarithms of experimental and calculated rate constants (Fig. 2*b*) enables us to suggest that these reactions may proceed in accordance with the "outer-sphere" ET mechanism.

Predicted value of $\Delta G^{\#}(0)_{12}$ as a function of k_{11} and k_{22} for these reactions calculated from eqn (*7*)* is 0.23 eV (22.93 kJ mol^{-1}), which is very close to the value determined from experimental data.

$$\Delta G^{\#}(0)_{12} = -0.5RT \ln\{(k_{11} k_{22})/Z^2\} \qquad (7)$$

The rate constants of ascorbate oxidation by high-potential 1,4-benzoquinone derivatives ($E_7^1 > 0$) (Table 2) cannot be determined by our assay system due to the slow reoxidation of semiquinones by O_2. The reduction of O_2 by high-potential 1,4-benzosemiquinone derivatives ($k_2 \approx 10^4$—10^7 $dm^3\ mol^{-1}\ s^{-1}$ [18]) is not appreciable because its back reaction is much faster ($k_{-2} = (0.98$—$1.0) \times 10^9$ $dm^3\ mol^{-1}\ s^{-1}$ [32]).

* This equation is simply derived from the Marcus assumption [14, 15] that the reorganization energy of ET reaction between reagents from different redox systems can be approximated as the average of reorganization energies of the individual self-exchange ET transfer reactions ($\Delta G^{\#}(0)_{12} = (\Delta G^{\#}(0)_{11} + \Delta G^{\#}(0)_{22})/2$).

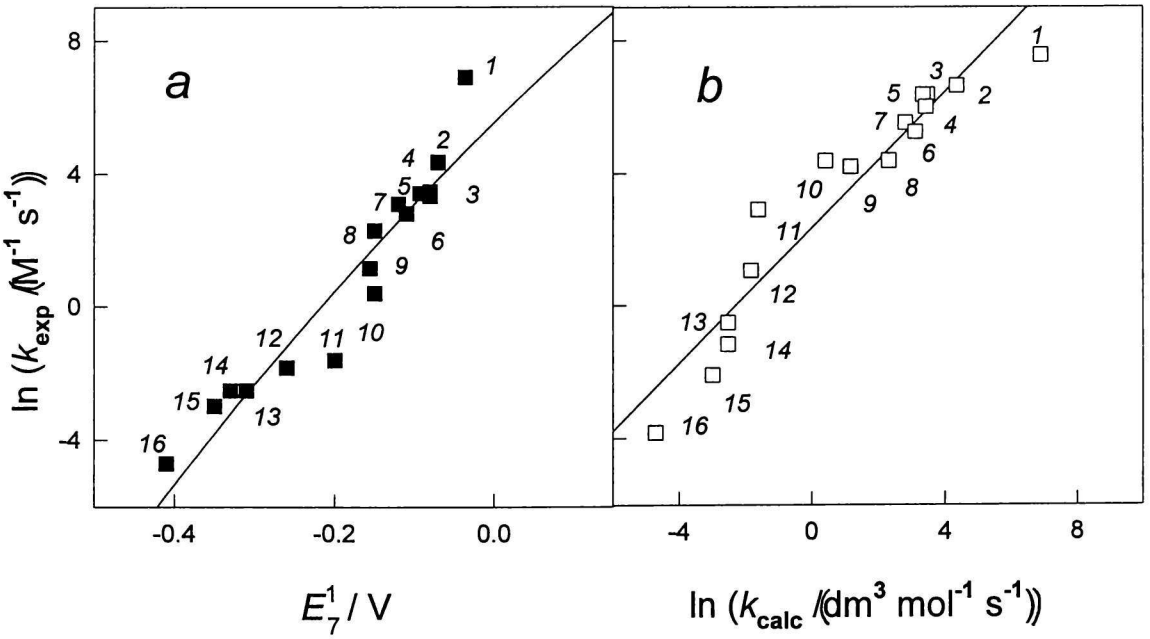

Fig. 2. The dependences of logarithms of determined bimolecular rate constants of the single-electron reduction of quinones by ascorbate on the single-electron reduction potentials (E_7^1) of quinones fitted to the Marcus eqn (*3*) (*a*). The orthogonality between logarithms of determined and predicted from the Marcus eqns (*4*—*6*) bimolecular rate constants using the values of electron self-exchange rate constants for quinones as 1.0×10^8 dm^{-3} mol^{-1} s^{-1} and ascorbate as 8.0×10^5 dm^{-3} mol^{-1} s^{-1} The single-electron redox potential for ascorbate was taken for $AsH^-/As^{\bullet -}$ couple (+ 0.33 V) (*b*). The numeration of quinones is as follows: 2,3-dichloro-1,4-naphthoquinone (*1*), diaziquinone (*2*), 1,2-naphthoquinone (*3*), 2,6-dimethyl-1,4-benzoquinone (*4*), 5-hydroxy-1,4-naphthoquinone (*5*), 5,8-dihydroxy-1,4-naphthoquinone (*6*), 9,10-phenanthrenequinone (*7*), 1,4-naphthoquinone (*8*), 2,6-dimethoxy-1,4-naphthoquinone (*9*), 5-hydroxy-2-methyl-1,4-naphthoquinone (*10*), 2,3,5,6-tetramethyl-1,4-benzoquinone (*11*), fuzarubin (*12*), mitomycin C (*13*), adriamycin (*14*), 2-dimethylamino-3-chloro-1,4-naphthoquinone (*15*), 2-hydroxy-1,4-naphthoquinone (*16*).

Table 2. Predicted Bimolecular Rate Constants (k_{exp}^{pred}) from the Experimental Curve (Fig. 2*a*) and Calculated Bimolecular Rate Constants (k_{calc}) from Marcus Eqns (*4*—*6*) of the Single-Electron Reduction of High-Potential Quinones by Ascorbate, and Single-Electron Reduction Potentials of Quinones (E_7^1)

Quinones	k_{exp}^{pred}	k_{calc}	$(E_7^1/V)^a$
	dm^3 mol^{-1} s^{-1}	dm^3 mol^{-1} s^{-1}	
1 Tetrachloro-1,4-benzoquinone	2.0×10^5	1.1×10^7	0.34
2 2,5-Dichloro-1,4-benzoquinone	1.45×10^4	8.9×10^6	0.22
3 1,2-Benzoquinone	1.3×10^4	8.7×10^6	0.21
4 1,4-Benzoquinone	1.1×10^3	6.9×10^4	0.09
5 2-Methyl-1,4-benzoquinone	1.5×10^2	1.4×10^4	0.02

a) E_7^1 values are from Ref. [1].

Radicals of the high-potential quinones can undergo disproportionation to quinones and hydroquinones at the rates (7.0—8.0) $\times$ 10^7 dm^3 mol^{-1} s^{-1} [33, 34] rather than reoxidation by O_2 or can be simultaneously reduced by another ascorbate molecule to hydroquinones, although one may suppose that the latter reaction is less probable. Nevertheless, one may be able to determine tentative bimolecular rate constant for the first electron transfer between ascorbate and high-potential benzoquinone derivatives using Marcus eqns (*4*—*6*) (Table 2).

Following the energetic considerations, the electron transfer between ascorbate and quinone followed by subsequent deprotonation would form a high-energy intermediate, the protonated ascorbate radical ($AsH^{\bullet}$). Thus, as the reduction potential of $AsH^{\bullet}/AsH^{\bullet -}$ couple (+ 0.77 V) is too high for the reduction to occur *via* this pathway, the stepwise electron/proton transfer mechanism (electron transfer with subsequent transfer of proton) for these reactions can be excluded, and one may argue that the electron and proton transfer occurs *via* the electron transfer with the concomitant transfer of proton. This supposition was verified over the pH ranges

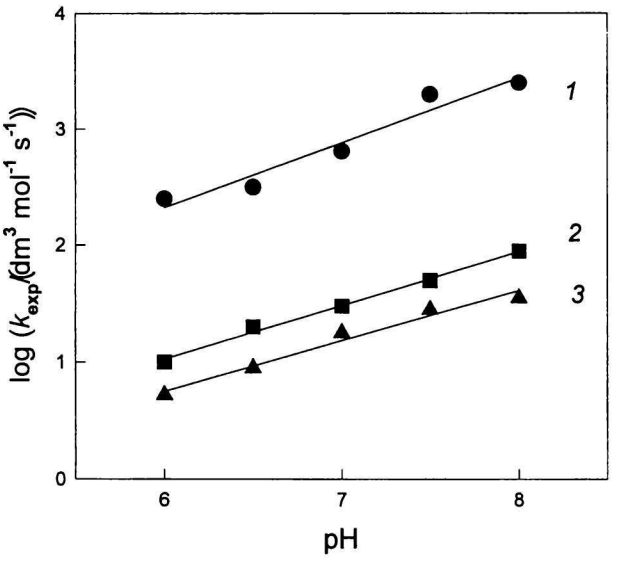

Fig. 3. The dependence of logarithms of bimolecular rate constant numerical values of the single-electron reduction of quinones by ascorbate on pH: 2,3-dichloro-1,4-naphthoquinone (*1*), 5-hydroxy-1,4-naphthoquinone (*2*), and 9,10-phenanthrenequinone (*3*).

where no protonation/deprotonation of the reagents occurs. Thus, in this case, if the proton is concomitantly transferred from the reducer with the electron, the rate constant must increase by one order on icreasing the pH value by a factor of 2, *i.e.* $\Delta \log\{k_{exp}\}/\Delta pH = 0.5$. In the region between pH 6.0 and pH 8.0, the initial rate of O_2 reduction by ascorbate is increased by 40—45 % (bimolecular rate constant between (3.4—4.5) $\times 10^{-4}$ dm^3 mol^{-1} s^{-1} and (8.1—9.8) $\times 10^{-4}$ dm^3 mol^{-1} s^{-1}) and, in comparison with the reaction between ascorbate and quinones, remains very negligible. As shown in Fig. 3, for the single-electron reduction of 2,3-dichloro-1,4-naphthoquinone by ascorbate the slope $\Delta \log\{k_{exp}\}/\Delta pH$ at pH 7.0 is equal to 0.54, for the reduction of 5-hydroxy-1,4-naphthoquinone: $\Delta \log\{k_{exp}\}/\Delta pH = 0.46$, and for 9,10-phenanthrenequinone: 0.43.

Numerous speculations exist attempting to explain ET reactions between ascorbate and cytochromes c [8, 9], b_{561} [10], or cytochrome b_5 reductase/cytochrome b_5 reductase—cytochrome b_5 complex [11], inorganic complexes, ET reactions between ascorbate and tocopherol or other free biological or nonbiological radicals [12, 13, 35]. The reaction between ascorbic acid and O_2 is also the subject of speculations in the framework of the "outer-sphere" ET model. For instance, the rate constant for the reduction of O_2 by ascorbate is (5.9—7.0) $\times 10^{-4}$ dm^3 mol^{-1} s^{-1} [27, 36], which exceeds the predicted rate constant of this reaction in the Marcus theory for subsequent electron/proton transfer (7.2 $\times$ 10^{-7} dm^3 mol^{-1} s^{-1}) taking self-exchange rate constant for $O_2/O_2^{\cdot-}$ couple as 100 dm^3 mol^{-1} s^{-1} [35]. Thus, in this case it is supposed that monoanion may reduce O_2 by the hydrogen atom transfer but the rate is so slow that it is not significant above pH 7.0 [35].

Thus, superoxide generation mediated by the reaction of the single-electron transfer between ascorbate and quinones may be easily speculated from the position of the "outer-sphere" electron-transfer model. So, as the electron self-exchange rate constant for quinones/semiquinone couple is much more higher (10^8 dm^3 mol^{-1} s^{-1}) than for O_2 (the electron self-exchange rate constant for O_2 is between 100 M^{-1} s^{-1} [37, 38] and 450 dm^3 mol^{-1} s^{-1} [39]), the electron will be readily transferred more to quinone compound than to O_2. It is supposed that the electron transfer between semiquinones and O_2 proceeds also in accordance with the "outer-sphere" electron-transfer mechanism [32]. Nevertheless, we calculated from eqn (*7*) that the experimentally determined value of reorganization energy of the single-electron transfer ($\Delta G^{\#}(0)_{12} = 0.21$ eV or 20.48 kJ mol^{-1}) of the above reactions drastically differs from the predicted value of reorganization energy which must be between 0.34 eV (32.81 kJ mol^{-1}) (taking self-exchange rate constant for O_2 100 dm^3 mol^{-1} s^{-1}) and 0.31 eV or 29.92 kJ mol^{-1} (taking self-exchange rate constant 450 dm^3 mol^{-1} s^{-1}).

Thus, in conclusion one must emphasize that ascorbic acid in the reaction with quinones can initiate "oxidative stress"-enhanced formation of active forms of oxygen due to the reoxidation of semiquinones by O_2 and the efficacy of the rate of superoxide generation for low-potential quinones ($E_7^1 \leq 0$) depends on their single-electron reduction potentials. High-potential quinones ($E_7^1 > 0$) are excluded from this regularity due to the slow reoxidation of their radicals by O_2.

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