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Reactions of Superoxide Ion with Redox-Active Substances of Physiological Importance: Generation of Semiquinone Radicals from Physiologically Important Quinones

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The reactions of superoxide ion, O_2^-, with physiologically important quinones were investigated in acetonitrile by electron spin resonance (ESR) spectroscopy. Superoxide ion could reduce quinones such as *p*-benzoquinone, duroquinone, vitamin E quinone, 1,4-naphthoquinone and vitamin K_3 to yield the corresponding semiquinone radicals. The fact that vitamin E quinone, an irreversible metabolite of vitamin E, was reduced by O_2^- to the semiquinone radical suggests that, like vitamin E, vitamin E quinone may also scavenge O_2^- and protect living cells from the effects of O_2^- in a hydrophobic environment. Further, in view of the apparent reversiblity of the reaction of O_2^- with vitamin K_3, it is unlikely that the *in vivo* toxicity of vitamin K_3 is solely due to O_2^- production, as has been suggested.

Keywords——superoxide ion; O_2^-; ESR; vitamin K_3; semiquinone radical; vitamin E quinone

Superoxide ion, O_2^-, is generated by several enzymatic systems such as xanthine oxidase.[1] It has been suggested that O_2^- functions as a mediator of electron flow from enzymes to electron acceptors such as cytochrome c (EC 1.9.3.1) or from electron acceptors to enzymes such as superoxide dismutase (SOD, EC 1.15.1.1) and is produced as an intermediate in the reactions catalyzed by oxygenase.[2] However, there are few reports on primary chemical reactions of O_2^- with biologically active substances.

It is expected that the reactions of O_2^-, which is a paramagnetic species, with physiological substances would yield a paramagnetic product at the first step. Therefore, we undertook to study the initial interactions of O_2^- with physiologically active substances using electron spin resonance (ESR) spectroscopy.

In a previous paper, we reported that vitamin E and its model compounds are oxidized by O_2^- to the corresponding chromanoxyl radicals.[3] In this paper, we report on the reactions of O_2^- with physiologically important quinones to yield semiquinone radicals.

Experimental

Preparation of O_2^-——Superoxide ion was prepared by electrolytic reduction of molecular oxygen in

acetonitrile at room temperature and its concentration was determined by spectrophotometry ($\lambda_{max}=255$ nm, $\varepsilon=1500\,M^{-1}\,cm^{-1}$).[4]

Materials——The quinones used were *p*-benzoquinone, duroquinone, vitamin E quinone (α-tocoquinone), 1,4-naphthoquinone and vitamin K_3 (menadione). The structures of these compounds are shown in Fig. 1. Vitamin E quinone was a gift from Eisai Co. Ltd. All the other quinones were commercial products and were used without further purification.

ESR Measurements——ESR measurements were performed at room temperature with a JEOL-PE-1X spectrometer (X-band) with 100 kHz field modulation. ESR parameters were calibrated by comparison with a standard sample of Mn^{2+} doped on MgO and diphenylpicrylhydrazyl (DPPH, $g=2.0036$).

The quinones examined were dissolved in acetonitrile. Immediately after mixing of the O_2^- solutions with quinones, the oxygen dissolved in the solutions was removed by bubbling pure nitrogen through the mixture, and then the reaction mixture was transferred to a flat quartz cell and ESR spectra were measured at room temperature.

Results

When the O_2^- solutions were added to acetonitrile solutions of *p*-benzoquinone and duroquinone, model compounds of vitamin E quinone, or acetonitrile solution of 1,4-naphthoquinone, a model compound of vitamin K, all the reaction products gave typical ESR spectra due to the corresponding semiquinone radicals, which were reported previously.[5-7] *p*-Benzosemiquinone was also produced by the reaction of O_2^- with hydroquinone.

When vitamin K_3 was mixed with the O_2^- solutions, a complicated ESR spectrum was

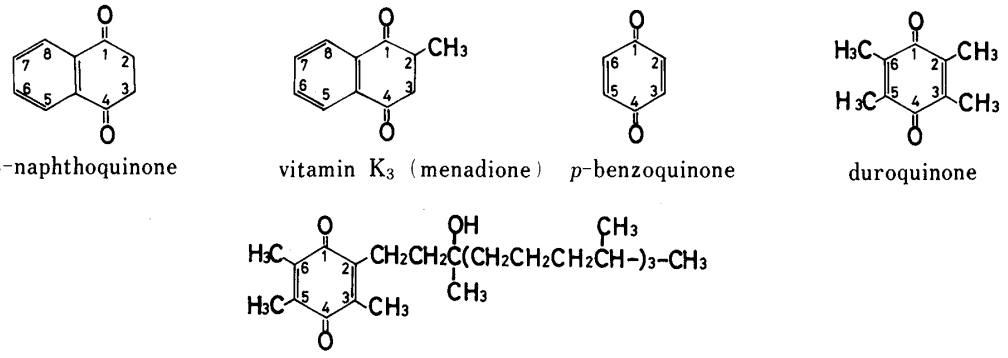


Fig. 1. Vitamin Quinones and Related Molecules Studied by ESR together with the Numbering of Their Carbon Atoms

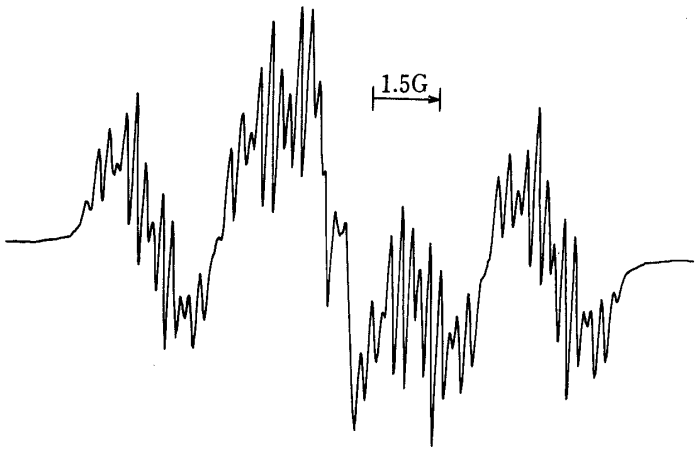


Fig. 2. ESR Spectrum Observed during the Reaction of O_2^- with Vitamin K_3

Reaction conditions: O_2^-, 7.6 mM; vitamin K_3, 50 mM. Measurements were made after bubbling nitrogen gas through the reaction mixture for 4 min. Instrument settings: microwave power, 10 mW; modulation amplitude, 0.25 G; time constant, 0.3 s; scan time, 16 min.

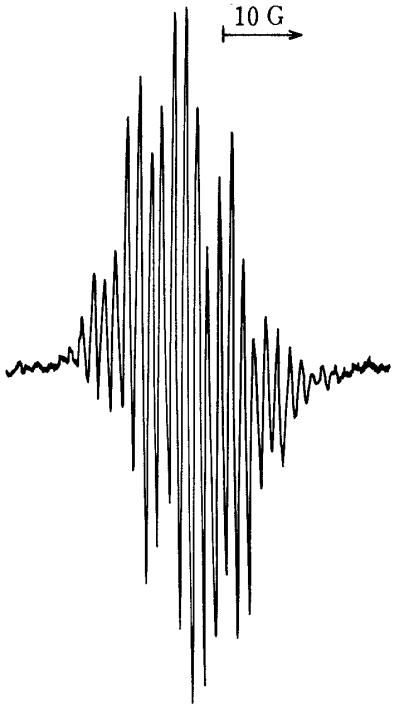


Fig. 3. Electron Spin Resonance Spectrum Observed during the Reaction of O_2^- with Vitamin E Quinone

Reaction conditions: O_2^-, 7.6 mM; vitamin E quinone, 100 mM; N_2 bubbling for 4 min. Instrument settings: microwave power, 10 mW; modulation amplitude, 0.25 G; time constant, 0.1 s; scan time, 8 min.

TABLE I. Proton Coupling Constants for Semiquinones

Semiquinone	Solvent	Proton coupling (gauss)	References
p-Benzoquinone	CH_3CN	$a_2^H=a_3^H=a_5^H=a_6^H=2.43$	This work
	DMSO	$a_2^H=a_3^H=a_5^H=a_6^H=2.419$	5
	DMSO	$a_2^H=a_3^H=a_5^H=a_6^H=2.35$	6
	Aprotic	$a_2^H=a_3^H=a_5^H=a_6^H=2.419$	7
1,4-Naphthoquinone	CH_3CN	$a_2^H=a_3^H=3.30$, $a_5^H=a_8^H=0.31$, $a_6^H=a_7^H=0.63$	This work
	DMSO	$a_2^H=a_3^H=3.31$, $a_5^H=a_8^H=0.300$, $a_6^H=a_7^H=0.633$	5
	DMSO	$a_2^H=a_3^H=3.25$, $a_5^H=a_8^H=0.31$, $a_6^H=a_7^H=0.62$	6
	Aprotic	$a_2^H=a_3^H=3.31$, $a_5^H=a_8^H=0.300$, $a_6^H=a_7^H=0.633$	7
Vitamin K_3	CH_3CN	$a_2^{H\,a)}=4.59$, $a_3^H=2.60$, $a_5^H=a_7^H=a_8^H=0.25$, $a_6^H=0.49$	This work
	DMSO	$a_2^{H\,a)}=a_3^H=2.69$, $a_5^H=0.22$, $a_6^H=0.76$, $a_7^H=0.62$, $a_8^H=0.37$	5
	EtOH	$a_2^{H\,a)}=2.911$, $a_3^H=2.467$, $a_5^H=0.480$, $a_6^H=0.780$, $a_7^H=0.560$, $a_8^H=0.700$	8
Duroquinone	CH_3CN	$a^{H\,a)}(12)=1.95$	This work
	DMF	$a^{H\,a)}(12)=1.91$	5
	EtOH	$a^{H\,a)}(12)=1.900$	8
Vitamin E quinone	CH_3CN	$a_3^H=a_5^H=a_6^H=1.88$, $a_2^{H\,b)}=0.84$	This work
	DMF	$a_3^H=1.60$, $a_5^H=a_6^H=2.21$, $a_2^{H\,b)}=0.81$	5
	EtOH	$a_3^H=a_5^H=a_6^H=1.905$, $a_2^{H\,b)}=0.910$	8

a) Methyl proton couplings. *b*) Methylene proton couplings.

observed as shown in Fig. 2. This spectrum is somewhat different from that obtained by the electrochemical reduction of vitamin K_3 in DMSO[5] or by the reaction of vitamin K_3 with potassium metal in ethanol.[8] However, on the basis of the calculated spin densities of vitamin K_3 semiquinone radical,[8] the redox potentials of vitamin K_3 (reduction potential, $E_{1/2} = -0.69$ V in DMF)[5] and O_2^- (O_2^-/O_2 couple, +0.07 V in aqueous solution),[9] and the results of a pulse radiolytic study of several semiquinones,[10] the ESR signals shown in Fig. 2 can be assigned to the semiquinone radicals ($a^H_{2\,(methyl)} = 4.59$ G, $a^H_3 = 2.60$ G, $a^H_5 = a^H_7 = a^H_8 = 0.25$ G, $a^H_6 = 0.49$ G).

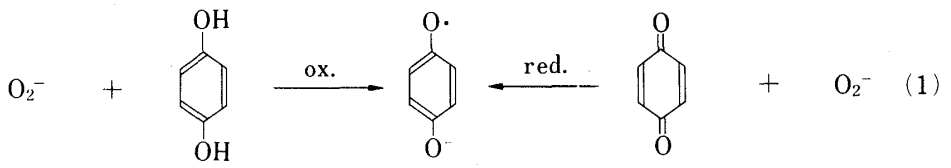
The radical species was also obtained from vitamin E quinone by reaction with O_2^-, and its ESR spectrum is shown in Fig. 3. This ESR spectrum resembles that of vitamin E semiquinone radical which was obtained by the reaction of vitamin E quinone with potassium metal.[8] Therefore, the ESR signal shown in Fig. 3 was assigned to vitamin E semiquinone radical.

The ESR parameters of these semiquinone radicals are summarized in Table I, along with the reported parameters.

These semiquinone radicals were stable and their ESR spectra could be observed even after the reaction mixtures had been kept for 2 h at room temperature.

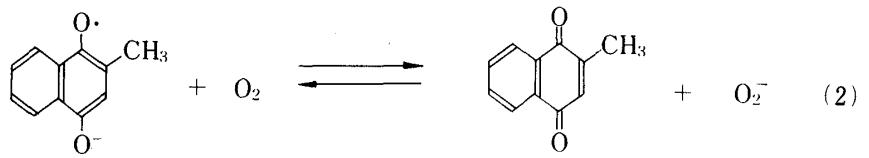
Discussion

The quinones examined were spontaneously reduced by O_2^- in acetonitrile solution to give fairly stable semiquinone radicals. The reactions of O_2^- with *p*-benzoquinone or hydroquinone gave the same ESR spectrum, suggesting that the same radical is produced by the reduction of benzoquinone with O_2^- and by the oxidation of hydroquinone with O_2^- (eq. (1)).



These results indicate that O_2^- can act as an oxidizing agent or a reducing agent and that its ability to do so is dependent on the redox potentials of substrates which react with O_2^-.

It is apparent from Table I that O_2^- has an ability to reduce several quinones to the corresponding semiquinone radicals. This is the first report that the semiquinone radical is produced by the reaction of O_2^- with vitamin K_3 or vitamin E. The semiquinone radical from vitamin K_3 (menadione) takes part in the production of O_2^- *in vivo*[11] and the toxicity of vitamin K_3 was suggested to be due to the production of O_2^-.[12] However, it now appears that the reaction may be reversible (eq. (2)).



Thus, it seems unlikely that the toxicity of vitamin K_3 can be solely a result of O_2^- production.

Vitamin E quinone, which is thought to be an irreversible metabolite of vitamin E, is also reduced by O_2^- to the semiquinone radical in acetonitrile. Vitamin E quinone, which occurs in tissues in small amounts, is suggested to function in a redox system.[13] Although the exact role of vitamin E quinone is not yet known, it is expected that, if O_2^- is formed, vitamin E

quinone would scavenge O_2^- and thus prevent cell damage.

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