EXPERIMENTAL DETERMINATION OF THE REDOX POTENTIAL
OF THE SUPEROXIDE RADICAL $\cdot O_2^-$

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SUMMARY. The redox potential of $\cdot O_2^-$ was determined based on the dependence of the electron transfer reaction from $\cdot O_2^-$ upon the known redox potential of various acceptors A (including a range of quinones, dyes and ferricyanide). The efficiencies and the rates of these electron transfer processes were determined, using the technique of pulse radiolysis, by monitoring the formation kinetics of the semiquinone radical anions at the appropriate wavelength. From the percentage efficiency versus $E^{\circ\prime}$ plot, an $E^{\circ\prime}$ value of $+ 0.15 \pm 0.01$ V at pH 7.0 and 22°C, or $E^{\circ} = + 0.57 \pm 0.01$ V, for the $\cdot O_2^-/O_2$ couple was obtained. The rate $k(\cdot O_2^- + A \rightarrow O_2 + \cdot A^-) = 9.8 \times 10^8$ M^{-1} sec^{-1} where A = p-benzoquinone. The E° value for the $\cdot HO_2$ radical is > 1.0 V. It was also found that hydroquinone can quantitatively reduce $\cdot O_2^-$ to H_2O_2, $k(\cdot O_2^- + QH_2 \rightarrow QH\cdot + HO_2^-) = 1.6 \pm 0.1 \times 10^7$ M^{-1} sec^{-1} at pH 7.0 and 22°C.

Molecular oxygen serves as an intermediate electron acceptor and electron transfer agent in many biological reactions (1-3). The properties of molecular oxygen important in oxidase mechanisms have been reviewed (3). Many oxidation reactions involving oxygen in the ground state proceed by one-electron steps via the superoxide radical $\cdot O_2^-$. The existence and importance of the role of $\cdot O_2^-$ in enzymic oxidation reactions has recently been reviewed by Fridovich (4). The redox potential $E^{\circ\prime}$ (pH 7.0) of the superoxide radical has been calculated for the $\cdot O_2^-/O_2$ couple and a value of $+ 0.59$ V has been derived (ref.3, p.34). No direct estimate for the potential of this couple has, however, been determined experimentally and various doubts and uncertainties have been raised (5) on the actual $E^{\circ\prime}$ value of this very important biological intermediate.
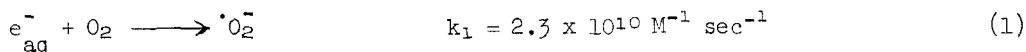
This communication presents experimental results aimed at determining the redox potential of the $\cdot O_2^-$ radical. The approach is based on determining the dependence of the electron transfer reactions from $\cdot O_2^-$ to a series of acceptors (A), as a function of the redox potential of the acceptors.

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EXPERIMENTAL. The technique of pulse radiolysis was used to produce $\cdot O_2^-$ radicals

in aqueous solutions (6,7). The radiolysis of water produces

$$H_2O \xrightarrow{\quad\quad} e_{aq}^- \ (2.8), \ OH(2.8), \ H(0.55), \ H_2O_2(0.71), \ H_2(0.45)$$
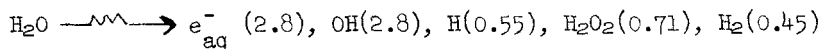
where the values in parenthesis are the G-values (yield produced per 100 eV of energy

absorbed by the water). In oxygen-saturated solution (1.3 mM), and in the presence of

1.0 M t-butyl alcohol to scavenge the OH radicals (6), the following reactions take

place:

$$e_{aq}^- + O_2 \longrightarrow \cdot O_2^- \qquad\qquad k_1 = 2.3 \times 10^{10} \ M^{-1} \ sec^{-1} \qquad\qquad (1)$$

$$H + O_2 \longrightarrow \cdot HO_2 \qquad\qquad k_2 = 2.0 \times 10^{10} \ M^{-1} \ sec^{-1} \qquad\qquad (2)$$

$$\cdot HO_2 \rightleftharpoons \cdot O_2^- + H^+ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (3)$$

with $pK_a = 4.8 \pm 0.1$ (8,9).

Determination of $E^{\circ\,\prime}$ Value of Superoxide Radical

The electron transfer reactions from $\cdot O_2^-$ to various acceptors A were monitored at

the wavelength of absorption of the corresponding semiquinone radical anions, $\cdot A^-$, see

Table I. For the quinones used as acceptors, the pK_a of the semiquinone radicals are

$\leqslant 5.0$ (10-12):

$$\cdot O_2^- + A \longrightarrow \cdot A^- + O_2 \qquad\qquad\qquad\qquad\qquad\qquad (4)$$

From the formation kinetics at the monitored wavelength, the rates of electron transfer

k_4 were determined (Table I). Since most of the acceptors used have a high affinity for

the hydrated electron, e_{aq}^-, the experimental conditions used were such that $k_1[O_2]/k_5[A]$

$\geqslant 20$ in all cases

$$e_{aq}^- + A \longrightarrow \cdot A^- \qquad\qquad\qquad\qquad\qquad\qquad\qquad (5)$$

The efficiency of electron transfer from $\cdot O_2^-$ to A, expressed as a percentage, was

determined based on the extent of formation of $\cdot A^-$ (for the same solution and the same

radiation dose) in the absence of oxygen, i.e., reaction (5) is taken as 100% efficiency.

These "blanks" were done for each acceptor just previous to studying the extent of electron

transfer according to reaction (4). The transient absorption spectra of the intermediates

produced via reactions (4) and (5) were found to be identical.

The percentage of electron transfer from $\cdot O_2^-$ as a function of the redox potential

of the acceptors, at pH 7.0, are shown in Figure 1(a). It can be seen that at $E^{\circ\,\prime}$ values

greater than + 0.23 V the efficiency of electron transfer is ~ 100%. At $E^{\circ\,\prime}$ below

+ 0.23 V, the efficiency decreases rapidly to almost zero. This change is interpreted

to the increased importance of the back reaction

$$\cdot A^- + O_2 \longrightarrow A + \cdot O_2^- \qquad\qquad\qquad\qquad\qquad\qquad (6)$$

TABLE I.   Rate Constants of the Electron Transfer Reaction from
$\cdot O_2^-$ to Various Acceptors in Aqueous Solution at pH 7.0
and at 22°C

No.	Acceptor, $A^{a)}$	$[O_2^-],\mu M^{b)}$	$E^{\circ\prime\ c)}$	Wavelength Monitored, nm	Rate of Electron Transfer$^{e)}$ $k(\cdot O_2^- + A)$, M^{-1} sec^{-1}
1	Ferricyanide (100)	4.6	- 0.055	420	-
2	Menaquinone (50)	2.3	+ 0.002	400	-
3	Methylene blue $(25)^{d)}$	1.0	+ 0.011	425,580	-
4	Toluidine blue O $(25)^{d)}$	1.0	+ 0.034	425,630	-
5	Duroquinone (50)	2.3	+ 0.068	430	-
6	Indophenol $(25)^{d,f)}$	1.0	+ 0.089	390	-
7	1,4-Naphthoquinone-2-sulfonate (50)	2.3	+ 0.118	400	6.6×10^8
8	1,2-Naphthoquinone (50)	2.3	+ 0.143	365	7.2×10^8
9	2,5-Dimethyl-p-bezoquinone (50)	2.3	+ 0.176	430	7.5×10^8
10	1,2-Naphthoquinone-4-sulfonate (50)	2.3	+ 0.217	365	8.4×10^8
11	2-Methyl-p-benzo-quinone (50)	2.3	+ 0.251	430	8.0×10^8
12	p-Benzoquinone (50)	2.3	+ 0.293	430	9.8×10^8
13	2,5-Dichloro-p-benzoquinone (50)	2.3	+ 0.310	430	1.1×10^9
14	Diphenoquinone (50)	2.3	+ 0.534	400	1.4×10^9

a) Values in parenthesis are the concentrations of A used in μM; b) concentration of $\cdot O_2^-$ generated under pulse radiolysis conditions; c) values at 30°C taken from ref. 17; d) in presence of 10 mM formate instead of $\underline{t}$-BuOH; e) values to better than $\pm 10\%$; f) determined at pH 9.0.

as the $E^{\circ\prime}$ of the acceptor decreases below that of the superoxide radical. From the
mid-point of the curve in Figure 1(a), the redox potential of the $\cdot O_2^-$ radical is found
to be $E^{\circ\prime} = + 0.15 \pm 0.01$ V or $E^{\circ} = + 0.57 \pm 0.01$ V. This value is in remarkably good
agreement with the thermodynamically calculated value $E^{\circ} = + 0.59$ V determined a number
of years ago (3).

Various acceptors have been used, including a range of quinones, dyes, and ferricyanid
in this determination. It is interesting to note that k_4 increases with increase in the
$E^{\circ\prime}$ value of the acceptor. The highest rate constant measured was 1.4×10^9 M^{-1} sec^{-1}
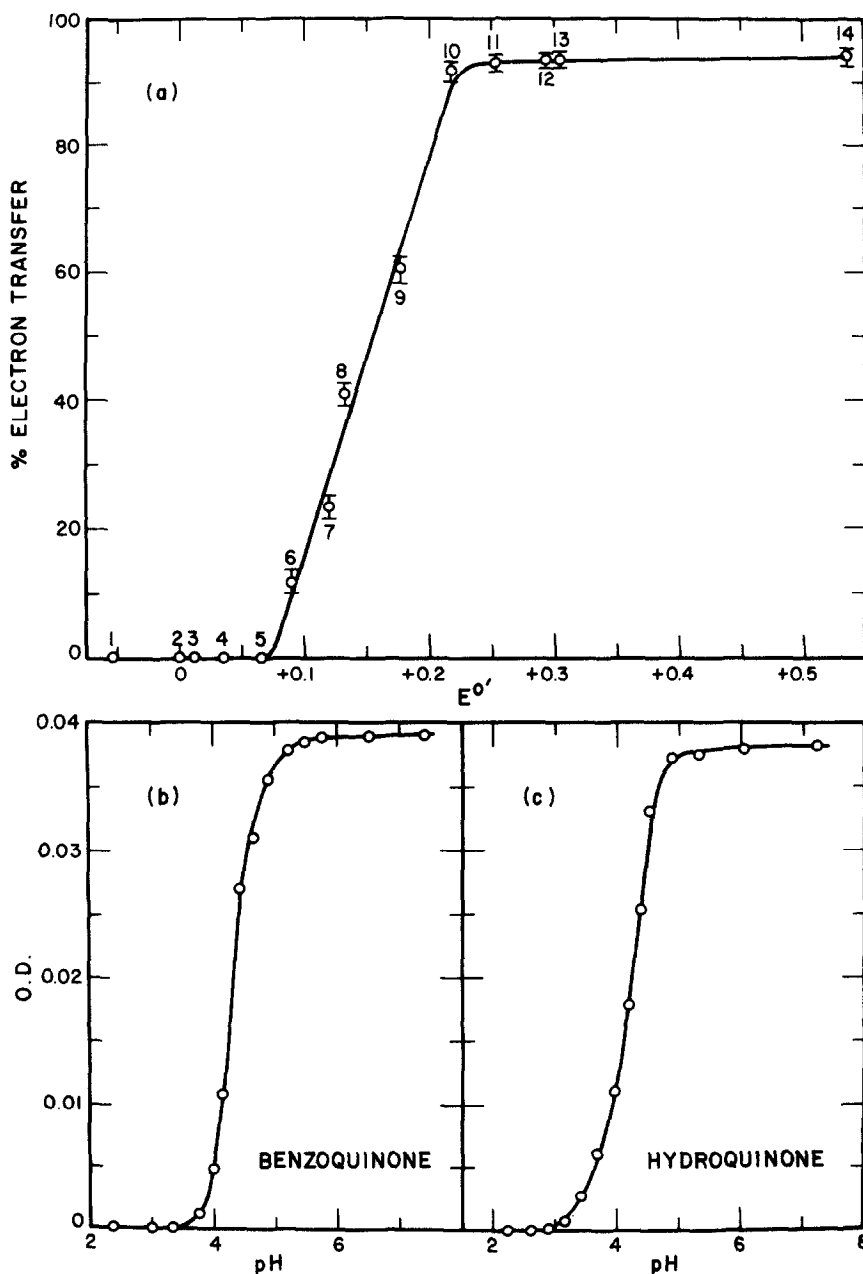for diphenoquinone $(E^{\circ\prime} = + 0.534$ V, Table I). This experimentally determined value of

FIGURE 1.   (A) Dependence of the efficiency (expressed as percentage) of the electron transfer reaction from $\cdot O_2^-$ upon the redox potential of the acceptor A. Experiments were carried out in oxygen-saturated aqueous solutions at 22°C, in presence of 1.0 M t-BuOH.   See Table I for the acceptors used corresponding to the indicated numbers. (B) Dependence upon pH of the absorbance at 410 nm of the semiquinone radical produced from the reaction of the superoxide radical with p-benzoquinone (BQ). Experimental conditions: 50μM p-BQ, 1.3mM O_2, 1.0 M t-BuOH, dose = 1.0 krad/pulse, equivalent to $[O_2^-]$ = 2.5μM.   (C) Dependence upon pH of the absorbance at 410 nm of the semiquinone radical produced from the reduction of the superoxide radical by p-hydroquinone. Experimental conditions: 1.0 mM hydroquinone, 1.3mM O_2, 1.0 M t-BuOH, dose = 1.0 krad/pulse, equivalent to $[O_2^-]$ = 2.5μM.

the $E^{\circ\prime}$ of $\cdot O_2^-$ brings the one-equivalent reduction close to the range in which it could

be driven by some biologically-occurring reductants (e.g., for cytochrome C, $E^{\circ\prime} = + 0.27$

V).

The determination of the redox potential of the conjugate acid $\cdot HO_2$ was attempted

at pH 2.0 using an O_2-saturated solution of $50\mu M$ diphenoquinone. No formation of the

semiquinone radical anion via

$$\cdot HO_2 + A \longrightarrow \cdot A^- - H^+ + O_2 \tag{7}$$
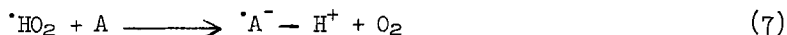
could be observed at 400 nm, indicating that the redox potential of the $\cdot HO_2$ radical

is > 1.0 V. In support of this conclusion, it was found that the apparent efficiency

of electron transfer from the superoxide radical $\cdot O_2^-$ to p-benzoquinone (BQ) was markedly

dependent upon the pH, Figure 1(b). As the pH decreases the $\cdot O_2^-$ radicals are converted

to $\cdot HO_2$, reaction (3), and these latter radicals do not transfer an electron to BQ (13)

to form $\cdot BQ^-$ or $\cdot BQ^- - H^+$. The mid-point in Figure 1(b) is ~ 4.35, lower than the pK_a

of $\cdot HO_2$, due to the shift of equilibrium (3) by reaction (4).

Electron Transfer from Hydroquinone to $\cdot O_2^-$

It should be possible to reduce the superoxide radical $\cdot O_2^-$ by hydroquinone ($E^{\circ\prime} =$

+0.28 V). The reduction of $\cdot HO_2$ by hydroquinone has already been suggested (14,15).

On pulse radiolysis of aqueous 1.0 mM solutions of hydroquinone, 1.0 M t-BuOH and

1.3 mM O_2 at pH 7.0, the characteristic transient spectrum of the semiquinone radical

anion with λ_{max} at 430 nm was observed.

$$QH_2 + \cdot O_2^- \longrightarrow QH^\cdot + HO_2^- \tag{8}$$
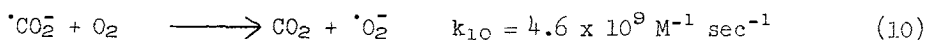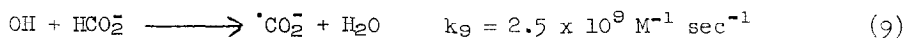$$QH^\cdot \longrightarrow Q^{\cdot-} + H^+$$
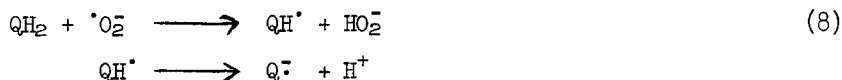
On irradiation of the same solution in the absence of oxygen, no formation of $Q^{\cdot-}$ was

observed at 430 nm. The formation of $Q^{\cdot-}$ is $\sim 90 \pm 2\%$ efficient giving, after correction,

an $\epsilon_{430} = 7.2 \times 10^3$ M^{-1} cm^{-1} in agreement with literature values (9,12). The rate of

this electron transfer reaction is relatively slow, $k_8 = 1.6 \pm 0.1 \times 10^7$ M^{-1} sec^{-1}.

Further support of the mechanism represented by reaction (8) was obtained from the

determination of the yield of H_2O_2. Using the iodide method (16), the $G(H_2O_2) = 4.35$

± 0.4 was obtained, based on $\epsilon_{350} = 2.635 \times 10^4$ M^{-1} cm^{-1} for I_3^-. This G-value corres-

ponds to the stoichiometry

$$G(H_2O_2) = G_{(e_{aq}^-)} + G_{(H)} + G_{(H_2O_2)}$$

and indicates that the peroxy t-BuOH radicals produced from the scavenging of OH radicals

do not contribute to the formation of either $Q^{\cdot-}$ or H_2O_2. Similarly, $Q^{\cdot-}$ radicals do not

produce H_2O_2 in presence of O_2. Furthermore, on pulse radiolysis of 1 mM hydroquinone, 1-3 mM O_2 in presence of 1.0 M HCO_2^-, the stoichiometry of $Q^{\cdot-}$ and H_2O_2 is in agreement with reactions

$$OH + HCO_2^- \longrightarrow {}^{\cdot}CO_2^- + H_2O \qquad k_9 = 2.5 \times 10^9 \ M^{-1} \ sec^{-1} \qquad (9)$$

$$^{\cdot}CO_2^- + O_2 \longrightarrow CO_2 + {}^{\cdot}O_2^- \qquad k_{10} = 4.6 \times 10^9 \ M^{-1} \ sec^{-1} \qquad (10)$$

followed by reaction (8).

The formation of $Q^{\cdot-}$ via reaction (8) was found to be pH - dependent, see Figure 1(c). Since the redox potential of $^{\cdot}HO_2$ is much higher than that of hydroquinone, reaction (11) was expected to occur

$$QH_2 + {}^{\cdot}HO_2 \longrightarrow QH^{\cdot} + HO_2^- \qquad (11)$$

The absence of the formation of the semiquinone radical under the pulse radiolysis conditions used is presumably due to $k_{11} \ll k_{12}$

$$^{\cdot}HO_2 + {}^{\cdot}HO_2 \longrightarrow H_2O_2 + O_2 \qquad (12)$$

$k_{12} = 8.5 \times 10^7 \ M^{-1} \ sec^{-1}$ (8). On γ-radiolysis of 2 mM QH_2, 1.0 M t-BuOH, 1.3 mM O_2 at pH 3.2 and low dose rates, the yield of hydrogen peroxide observed is $G(H_2O_2) \sim 4.3 \pm 0.4$, indicating that $^{\cdot}HO_2$ is reduced by QH_2 at very low $^{\cdot}HO_2$ concentrations, when reaction (11) competes favorably with reaction (12).

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