

COMPARISON BETWEEN THE ELECTRON TRANSFER REACTIONS FROM
FREE RADICALS AND THEIR CORRESPONDING PEROXY RADICALS TO QUINONES

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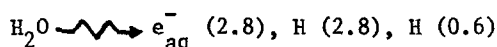
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SUMMARY: The fast reaction technique of pulse radiolysis was used to produce free radicals in aqueous solution from alcohols, deoxyribose, cytosine, uracil, thymine, dihydrothymine and histidine. The electron transfer reactions from these radicals to p-benzoquinone was observed from the formation kinetics of the semiquinone anion $\cdot\text{BQ}^-$ at 430 nm and the efficiency was found to be as high as 90% or more, with $k \sim 5 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$. In acid or neutral solutions in the presence of oxygen the peroxy radicals $\cdot\text{O}_2\text{RH}$ formed do not essentially transfer an electron to BQ, and the efficiency is <10%. The significance of these results in the fixation of radiation damage in photobiology and radiation biology are indicated. The reactions of the superoxide $\cdot\text{O}_2^-$ radical with BQ are also presented and discussed.

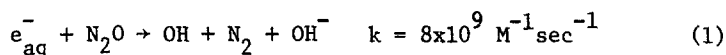
The role and importance of free radicals to oxygen in biological oxidation reactions has been clearly demonstrated¹⁻³. In spite of the considerable amount of work done, the mechanisms and kinetics of such reactions have not been defined due largely to experimental difficulties. Using fast kinetics techniques and model compounds these reactions can, however, be followed in vitro. Employing the technique of pulse radiolysis to produce free radicals in aqueous solution, it was recently⁴⁻⁷ found that a wide range of biologically important free radicals can efficiently transfer an electron to various acceptors in the absence of oxygen. The efficiency and rate constants of such electron transfer processes have been determined, and their dependence upon the nature of the acceptor has been indicated⁴⁻⁷. In this communication we present the effect of oxygen on the electron transfer reactions of some selected free radicals and evidence for the existence of certain peroxy radicals.

All the experiments were performed in aqueous solutions which upon irradiation give the following primary radicals with G-values (number/100 eV

absorbed) indicated in parenthesis.



The hydrated electron, e_{aq}^- , can be converted to OH radicals in presence of N_2O (26mM):



In solutions containing both N_2O and O_2 , the concentration ratio was chosen (13 mM N_2O and 0.7 mM O_2) in order for most of the e_{aq}^- (>90%) to react with N_2O and eliminate the formation of $\cdot\text{O}_2^-$ via $e_{\text{aq}}^- + \text{O}_2 \rightarrow \cdot\text{O}_2^-$ $k = 2.2 \times 10^{10} \text{ M}^{-1} \text{sec}^{-1}$ (ref. 8). Some representative free radicals were produced from the reaction of OH radicals with the substrates by H-atom abstraction or by addition to the substrate (Table I); [$\cdot\text{RH}$] $\sim 2 \mu\text{M}$ in all systems. The O_2 concentration was sufficient to produce the peroxy radicals $\cdot\text{O}_2\text{RH}$ quantitatively since $k(\cdot\text{RH} + \text{O}_2) \sim 5\text{--}50 \times 10^8 \text{ M}^{-1} \text{sec}^{-1}$.

The pulse radiolysis set-up used has been described^{9,10}. p-Benzoquinone, BQ, was chosen as the electron acceptor based on its strong oxidizing properties ($E^\circ = 0.699 \text{ V}$). The formation of its semiquinone anion, $\cdot\text{BQ}^-$, could be conveniently followed at 430 nm ($\lambda_{\text{max}} = 430 \text{ nm}$, $\epsilon_{430} = 7300 \text{ M}^{-1} \text{cm}^{-1}$)¹¹. The $\text{pK}_a = 4.0$ for^{11,12}



The $\cdot\text{BQ}^-$ was produced here either directly by reaction with e_{aq}^- , or by electron transfer from $\cdot\text{CO}_2^-$:



Alcohols and sugars react with OH radicals to form primarily α -hydroxy alkyl radicals and these react effectively with BQ to produce $\cdot\text{BQ}^-$:

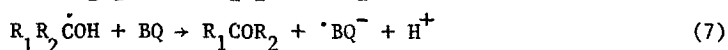
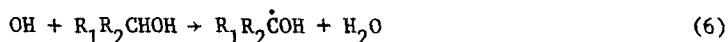


TABLE 1. EFFICIENCY AND RATE CONSTANT OF ELECTRON TRANSFER REACTIONS FROM FREE RADICALS AND THEIR CORRESPONDING PEROXY RADICALS TO P-BENZOQUINONE IN AQUEOUS SOLUTION.

Substrate	pH	Donor Radical	Electron Transfer ^{a)}			
			Percentage no-O ₂	Percentage O ₂	k, no-O ₂	M ⁻¹ sec ⁻¹ O ₂
HCO ₂ ⁻ (0.1 M)	6.9	•CO ₂ ⁻	100	--	7.0x10 ⁹	--
	6.9	•O ₂ ⁻	--	98	--	8.5x10 ⁸
t-BuOH (1.0 M)	6.9	•CH ₂ (CH ₃) ₂ COH	0	--	--	--
	5.10	•O ₂ CH ₂ (CH ₃) ₂ COH	--	0	--	--
Methanol (1.0 M)	6.2	•CH ₂ OH	88	--	4.8x10 ⁹	--
	6.2	•O ₂ CH ₂ OH	--	7	--	--
iso-Propanol (0.2 M)	6.9	(CH ₃) ₂ •COH	85	--	5.0x10 ⁹	--
	5.5	(CH ₃) ₂ C(OH)O ₂ •	--	4	--	--
Deoxyribose (10 mM)	5.6	dR•	76	--	2.7x10 ⁹	--
	5.6	•O ₂ dR	--	30	--	1.0x10 ⁹
Cytosine (1 mM)	6.9	•Cyt-OH	75	--	5.0x10 ⁹	--
	6.9	•O ₂ Cyt-OH	--	<15	--	--
Dihydrothymine (1 mM)	5.5	•Thy-H	53	--	4.5x10 ⁹	--
	5.5	•O ₂ Thy-H	--	~10	--	--
Thymine (0.5 mM)	5.5	•Thy-OH	35	--	--	--
	5.5	•O ₂ Thy-OH	--	~10	--	--
Histidine (2 mM)	7.7	•His-OH	93	--	1.8x10 ⁹	--
	5.8	•O ₂ His-OH	--	18	--	--

a) Percentage values to $\pm 5\%$, rates to $\pm 10\%$. Conditions of "no-O₂" refer to solutions containing 26 mM N₂O, and "O₂" to solutions containing 13 mM N₂O plus 0.7 mM O₂, see text. In all cases 20 μ M p-benzoquinone was used.

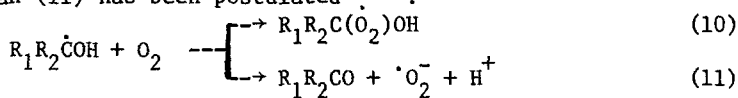
The percentages of electron transfer reactions were derived from the comparison of $\cdot\text{BQ}^-$ yields in the formate system (100%) and the systems under investigation. The electron transfer reaction (7) is not always 100% efficient since β and γ hydroxy radicals can be produced which do not transfer according to reaction (7), e.g. tert-butanol radicals. Some α -hydroxy radicals can also, in part, add to BQ without leading to the formation of $\cdot\text{BQ}^-$. The absolute rate constants of these electron transfer reactions were determined from the formation kinetics of $\cdot\text{BQ}^-$ at 430 nm. Similar electron transfer reactions from pyrimidine and amino acid radicals, with other quinones have been also observed⁴⁻⁷ and are not specific for BQ.

Because of its high redox potential, BQ reacts¹² specifically with $\cdot\text{O}_2^-$ in contrast with other quinones. We have rechecked this important reaction under well-controlled experimental conditions (0.1 M formate, 13 mM N_2O , 0.7 mM O_2 , 2×10^{-5} M BQ at pH 6.9). Reactions (1), (4), (8) and (9) take place:



Reaction (9) was found to be >98% efficient.

In the presence of oxygen, peroxy radicals $\cdot\text{O}_2\text{RH}$ are expected to be formed by addition of O_2 to $\cdot\text{RH}$. For α -hydroxy alkyl radicals reaction (10) rather than (11) has been postulated^{9, 13}:



The resulting free radical from the above reaction has been found not to transfer an electron to BQ in acid and near neutral solutions (see Table I), whereas $\text{R}_1\text{R}_2\dot{\text{C}}\text{OH}$ transfers quantitatively to BQ at any pH. This observation in conjunction with reaction (9) supports the conclusion that the addition of oxygen to α -hydroxy radicals takes place via reaction (10) in preference to electron transfer, via reaction (11).

The preferential addition of oxygen to free radical sites, due probably to a very small activation energy, is one of the interesting aspects of the oxidation mechanisms. Table I shows the results obtained from various sub-

strates (alcohols, deoxyribose, pyrimidines, and histidine). In all these cases, the radicals produced (e.g. by addition of OH to cytosine and histidine, or by H-atom abstraction from dihydrothymine) transfer effectively to BQ in the absence of O_2 . However, when O_2 is present, under conditions that no $\cdot O_2^-$ is produced, no formation of $\cdot BQ^-$ could be observed. These rather interesting observations may provide an explanation of the enhanced fixation of damage in photobiology and radiation biology in the presence of oxygen and have to be considered in all electron transfer systems.

It should be pointed out that the reported conclusions were deduced only because these peroxy radicals are poor electron donors, and do not transfer to BQ at a measurable rate ($<10^6 M^{-1}sec^{-1}$). The peroxy radicals, just like the corresponding $\cdot RH$ radicals, do, however, have acid-base properties ($\cdot O_2RH \rightleftharpoons \cdot O_2R^- + H^+$) and the pK_a values are significantly lower^{9,13,14} than those of the parent radicals. Furthermore, preliminary experiments indicate that $\cdot O_2R^-$ do transfer effectively to BQ. The relatively high (30%) transfer from deoxyribose $\cdot O_2dR$ radical to BQ (Table I) is presently explained on this basis.

The study of intermolecular electron transfer reactions appears to be a powerful tool in assignment of the structure of free radicals and the study of acid-base properties and reaction mechanisms. The use of quinones and other acceptors with high redox potentials to probe in these reactions are suggested.

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