

REACTION OF ALCOHOL RADICALS WITH CYCLIC DISULFIDES. AN OPTICAL AND CONDUCTIMETRIC PULSE RADIOLYSIS STUDY

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Abstract—The disulfides lipoamide (LIPA) and oxidized dithiothreitol (*ox*-DTT) react with methanol, ethanol, isopropanol and *t*-butanol radicals in aqueous solution at pH 10.8 to form disulfide radical anions. Electron transfer rates range from $ca\ 10^7\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}$ for *t*-butanol radicals with LIPA to $3.6 \times 10^8\ \text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}$ for methanol radicals with LIPA. The formations of the disulfide radical anions were followed by simultaneously monitoring absorption changes at 400 nm and changes in conductance with time. The electron transfer efficiencies are higher for LIPA than for *ox*-DTT increasing in the series *t*-butanol < isopropanol < ethanol ~ methanol and are less than the proportion of α -carbon radical formation on the alcohols.

INTRODUCTION

Electron transfer reactions of disulfide radicals may have some relevance to the functioning of enzymes such as lipoamide dehydrogenase and glutathione reductase which have disulfide structures near their active sites. Disulfide radicals in enzymes are stabilized by the tertiary structure of the enzyme⁽¹⁾ compared to amino acid disulfides⁽²⁾ and are well mimicked by cyclic disulfides such as lipoic acid⁽²⁻⁴⁾ and oxidized dithiothreitol, *ox*-DTT.⁽⁵⁻⁶⁾ In these optical pulse radiolysis studies, the disulfide radicals were produced either by direct scavenging of the e_{aq}^- in *t*-butanol solution or by electron transfer from the CO_2^- species generated from formate ions. Later studies have shown that the protonated disulfide radicals ($\text{p}K_a\ 5.6$) of both *ox*-DTT⁽⁷⁾ and lipoamide, LIPA⁽⁸⁾ undergo a chain reaction with formate ions making this method impracticable for quantitative work at $\text{pH} \leq 7$. The radicals of methanol, ethanol and isopropanol (formed by scavenging the oxidizing radicals) were found to give rise to different yields of disulfide radical while *t*-butanol radicals also react by a complex series of reactions with LIPA.⁽⁸⁾

In this study we have used simultaneous optical and conductimetric measurements to compare the yields of disulfide radical anion arising from reactions of various alcohol radicals with both *ox*-DTT and LIPA.

EXPERIMENTAL

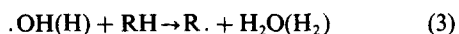
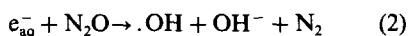
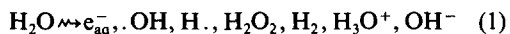
DL,6,8-Thioctic acid amid (lipoamide, LIPA) and trans-4,5-dihydroxy-1,2-dithiane (oxidised dithiothreitol, *ox*-DTT) were obtained from Sigma Chemical Co. and used as supplied.

The solutions were irradiated with 3.5 MeV electrons from a Van de Graaff accelerator using pulse widths of 10 and 30 ns. The charge per pulse was monitored with an inductive monitor⁽⁹⁾ and the dose was determined by the measurement of optical absorption at 472 nm in $10^{-2}\ \text{mol}\ \text{dm}^{-3}$ aerated potassium thiocyanate,⁽¹⁰⁾ assuming a molar absorptivity ϵ of $750\ \text{m}^2\ \text{mol}^{-1}$ and a radiation chemical yield of $0.30\ \mu\text{mol}\ \text{J}^{-1}$; doses of 1.2 and 3 Gy per pulse were used. The samples were introduced into the cell using a flow system which empties the cell between radiation pulses. The cell (path length $2 \pm 0.05\ \text{cm}$) and associated optical detection system (250 W Xe arc lamp, Spex Minimate single-pass monochromator, 3 nm bandwidth, 1P28 photomultiplier) are described elsewhere.^(11,12) The cell constant ($0.359\ \text{cm}^{-1}$) was measured by the simultaneous observation of optical (350 nm) and conductance changes in $10^{-3}\ \text{mol}\ \text{dm}^{-3}$ tetranitromethane and isopropanol ($0.2\ \text{mol}\ \text{dm}^{-3}$) adjusted to pH 4.6 with perchloric acid.⁽¹³⁾ The solution temperature was not regulated but was monitored ($21 \pm 0.5^\circ\text{C}$). The conductance changes were measured using a balanced d.c. conductivity detection method^(11,12) using a polarisation voltage of 100 V, applied $ca\ 2\ \text{ms}$ before the electron pulse for $ca\ 10\ \text{ms}$. The detection system, which employs a hybrid transmission-line transformer, has a time resolution of $ca\ 5\ \text{ns}$ and is capable of rejecting the interfering transient signal at the cell electrodes due to the electron pulse charge deposited in the cell. The low-frequency response of the transformer and associated amplifier permits observations to be made up to $ca\ 100\ \mu\text{s}$ after the radiation pulse. Typically 1-4 transients were averaged at either polarising voltage polarity, using a Tektronix 7612D digitiser (5 ns per slot, 3.5 ns rise-time) interfaced to a Digi-

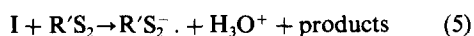
tal PDP11/34 computer. Optical transients were acquired simultaneously on the second digitiser input channel.

RESULTS AND DISCUSSION

The alcohol radicals ($R\cdot$) were produced by the pulse radiolysis (1.2–3 Gy in 30 ns) of the alcohols (RH, 0.2 mol dm^{-3}) in N_2O -saturated aqueous solution adjusted to pH 10.8 (sodium hydroxide). A high pH was chosen to ensure the fast neutralisation of radiolytic H_3O^+ [from reaction (1)] to enable concurrent changes in conductance and absorption to be directly compared (see later).



In the presence of both *ox*-DTT and LIPA ($\text{R}'\text{S}_2\cdot$), characteristic disulfide radical anion ($\text{R}'\text{S}_2\cdot^-$) absorption spectra were produced and the rate of exponential growth monitored at 400 nm (Fig. 1), was dependent on disulfide concentration (Fig. 2). A previous study using ethanol and isopropanol⁽⁸⁾ has proposed that $R\cdot$ reacts quickly with LIPA to form an intermediate (I) which reacts with further disulfide to yield $\text{R}'\text{S}_2\cdot^-$ [reaction (5)] in competition with unimolecular decay [reaction (6)].

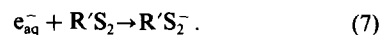


Rate constant $k(5)$ for ethanol was determined at $1.0 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ over the pH range 5–9 while $k(6)$ (determined from the intercept of plots of dose/absorbance against the reciprocal of disulfide concentration) decreased from *ca* $2.5 \times 10^4 \text{ s}^{-1}$ to $1.3 \times 10^4 \text{ s}^{-1}$ at pH 9.⁽⁸⁾ Our data at high pH (Fig. 2) do not indicate within experimental error that an intercept [arising from reaction (6)] exists and we cannot distinguish between the above mechanism and reaction (5a).



(Our study was carried out at low dose (1.2–3 Gy) to ensure minimal loss of the alcohol radicals through radical–radical reactions). The rate constants for reaction (5a), determined from Fig. 2, are presented in Table 1. Data for ethanol and isopropanol radicals reacting with LIPA are similar to that previously found.⁽⁸⁾

The rate constant for direct scavenging of the e_{aq}^- by LIPA is *ca* a factor of 3 greater than that for scavenging the e_{aq}^- with N_2O .⁽³⁾ As disulfide concentrations in Table 2 are *ca* 4% (for LIPA) and 8% (for *ox*-DTT) of the N_2O concentration we would expect *ca* 10% of the total radical yield to give disulfide radicals directly, reaction (7).



This small initial absorption could only be clearly seen for *t*-butanol as its is disguised by reaction (5a) with the other alcohols and probably accounts for the previously reported observation⁽⁸⁾ rather than

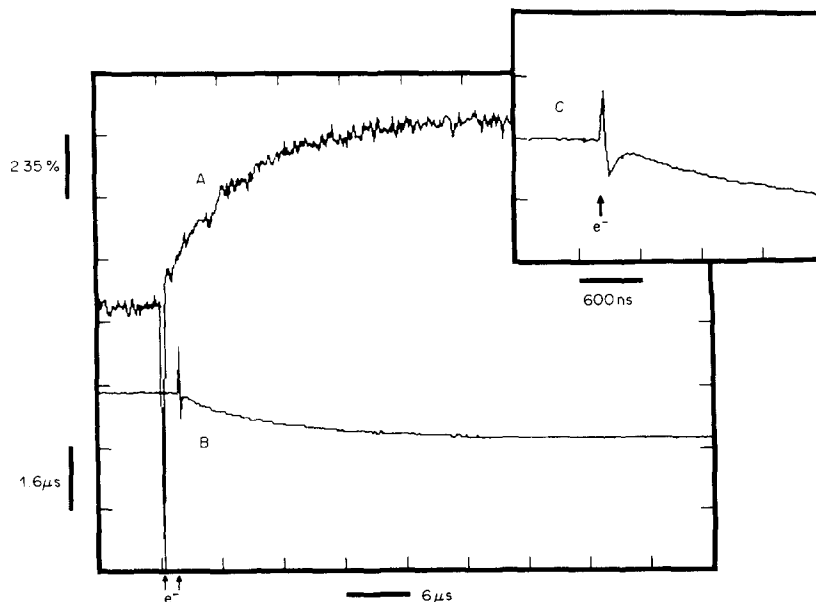


Fig. 1. Typical (A) optical and (B) conductance transients obtained with *ox*-DTT (2 mmol dm^{-3}) and ethanol (0.2 mol dm^{-3}) solution saturated with N_2O at pH 10.8 (3 Gy in 30 ns pulse). The insert (C) shows the conductance signal on an expanded timescale. The transients represent the average of four experiments (two acquired with +100 V polarisation and two with –100 V polarisation). Arrows indicate the time of the radiation pulse; traces A and B, acquired simultaneously, have been offset in time for ease of identification.

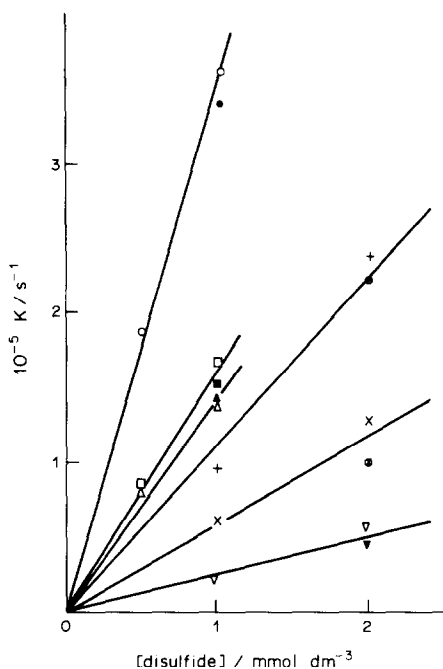


Fig. 2. First order rate constants for the appearance of absorption at 400 nm following pulse radiolysis (open symbols, 3 Gy; closed (and circled) symbols, 1.2 Gy) of N_2O saturated solutions at pH 10.8 containing alcohols (0.2 mol dm^{-3}) and disulfides. LIPA \circ (methanol), \square (ethanol), \triangle (isopropanol); *ox*-DTT; + (methanol, \times (ethanol), ∇ (isopropanol).

the formation of a precursor which absorbs weakly at 400 nm. We observe the same proportion of the initial absorption to the built-up absorption over the spectral range 250–500 nm. While a small proportion

of *t*-butanol radicals (ca 5%) underwent electron transfer with LIPA [reaction (5a), $k = \text{ca } 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$] this was not the case for *ox*-DTT where only second-order decay kinetics ($2k = \text{ca } 2.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) was observed for the initially formed disulfide radical.

The absorption at 400 nm arising from the reaction of alcohol radicals with the disulfides can be compared with that following direct electron transfer from the CO_2^- species. Such a comparison gives an estimate of the percentage yield of disulfide radical anion arising from the alcohol radicals (Table 2).

The change in conductivity (σ_t) at time t following the pulse is given by

$$\sigma_t = k_c G_c(t) = 10^{-3} \sum_i c_i |z_i| \Lambda_i \quad (8)$$

where k_c is the cell constant, G_c the measured conductance change and c_i is the concentration of all species of charge z_i and equivalent conductivity Λ_i produced by the pulse.

At pH 9.8 H_3O^+ produced by the pulse, reaction (1), is quickly neutralized while nitrous oxide converts e_{aq}^- to $\cdot\text{OH}$ and OH^- . These fast reactions ($t_{1/2} < 70 \text{ ns}$) occur in parallel and consecutively with the scavenging of $\cdot\text{OH}$ and H^\cdot by the alcohols which in turn react with the disulfides. The rate of loss in conductance parallels the build-up of optical absorption at 400 nm (Fig. 1), and is consistent with the formation of the radical disulfide anion [reaction (5a)] in parallel with the neutralization of its counterion.

The maximum change in conductance for a radical yield of $0.68 \mu\text{mol J}^{-1}$ produced under the above conditions⁽¹⁴⁾ can be calculated using equation (8). At 21°C Λ for $\text{OH}^- = 188 \text{ S cm}^2 \text{ mol}^{-1}$ and for the disulfide radical anions can be estimated as ca $28 \text{ S cm}^2 \text{ mol}^{-1(15)}$ giving $G_c(\text{max}) = -0.30 \mu\text{S Gy}^{-1}$. The measured loss of conductance can be compared with this calculated value to give an estimate of the % change in conductance (Table 2).

It is seen that the % transfer from the alcohol radicals to LIPA as determined from optical measurements (corrected for the direct scavenging of e_{aq}^-

Table 1. Rate constants for the reaction of alcohol radicals with disulfides [$k(5a)$] at pH 10.8

Alcohol	$k(5a)/\text{dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	
	LIPA	<i>ox</i> -DTT
Methanol	3.6×10^8	1.1×10^8
Ethanol	1.6×10^8	0.6×10^8
Isopropanol	1.4×10^8	0.2×10^8
<i>t</i> -butanol	ca 10^7	$< 10^6$

Table 2. Yields of disulfide radical anion at pH 10.8 from optical and conductimetric measurements

		O.D. Gy^{-1}	% transfer ¹	$\mu\text{S Gy}^{-1}$	% change in ² conductance
<i>ox</i> -DTT (2 mmol dm^{-3})	Formate	0.0090 ± 0.0001	100	—	—
	Methanol	0.0065 ± 0.0004	72 (69)	-0.22 ± 0.04	73
	Ethanol	0.0062 ± 0.0007	69 (66)	-0.22 ± 0.02	73
	Isopropanol	0.0033 ± 0.0006	37 (31)	-0.15 ± 0.01	49
	<i>t</i> -Butanol	0.0008 ± 0.0001 (0.0008)	9 (0)	-0.052 ± 0.003	17
LIPA (1 mmol dm^{-3})	Formate	0.0106 ± 0.0001	100	—	—
	Methanol	0.00083 ± 0.0002	78 (76)	-0.23 ± 0.01	76
	Ethanol	0.0074 ± 0.0002	70 (67)	-0.24 ± 0.01	77
	Isopropanol	0.0072 ± 0.0002	68 (65)	-0.21 ± 0.01	68
	<i>t</i> -Butanol	0.0014 ± 0.0001 (0.0010)	12 (4)	-0.019 ± 0.001	6

¹Maximum observed yields relative to transfer from CO_2^- species. Figures in brackets are yields corrected for the initial formation of disulfide radical anion through direct scavenging of the e_{aq}^- (9% for *ox*-DTT, 8% for LIPA based on *t*-butanol data).

²Calculated using equation (7) assuming a maximum radiation chemical yield of $0.68 \mu\text{mol J}^{-1}$ for the radicals.

by the disulfide) agrees well with the observed % change in conductance. The yields for *ox*-DTT are in good agreement in the case of methanol and ethanol only. The higher observed % change in conductance measured for isopropanol and *t*-butanol compared to % absorption indicates that charged species other than the disulfide radical anion are also formed.

Some information on the early events in the systems under study can be obtained from fast conductivity observations (Fig. 1, insert). A fast loss of conductance beyond neutralization of the radiolytic H_3O^+ [formed in reaction (1)] is observed. Some of this loss would be due to the neutralization of the counterion to the disulfide radical anion initially formed through competitive scavenging of the e_{aq}^- . Also the hydroxyl group $\text{p}K_{\text{a}}$ of alcohol radicals are ≥ 10.6 ^(16,17) and may also account for some loss of conductance. However control experiments with water alone at pH 10.8 also show a rapid decrease in conductance followed by a fast restoration on an equivalent timescale as seen in Fig. 1. The reason for these fast conductance changes are currently under further study.

The measured yields of disulfide radical (Table 2) are far less than the proportion of the alcohol radicals that are formed by hydrogen abstraction from the α -carbon, e.g. α -carbon radical formation for methanol is 93% and isopropanol is 85%.⁽¹⁸⁾ These results imply that the alcohol α -carbon radicals undergo at least two reactions with the disulfides, one of which yields the disulfide radical. The lower yields of disulfide radical anions for *ox*-DTT compared to LIPA might be related to *ox*-DTT possessing two α -carbon hydrogens adjacent to the disulfide group to only one on LIPA. Abstraction of these hydrogens by the alcohol radicals in competition to electron transfer might not lead to disulfide radical formation. Hydrogen abstraction from either of the other carbons of *ox*-DTT would result in the formation of carbon radicals in the α -position to the hydroxyl

groups. The $\text{p}K_{\text{a}}$ of these hydroxyl groups would then be expected to be lowered and might account for the observed greater % change in conductance compared to % electron transfer for *ox*-DTT.

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