THE NATURE OF THE SUPEROXIDE ION IN DIPOLAR APROTIC SOLVENTS:

The electron paramagnetic resonance spectra of the superoxide ion in *N,N*-dimethylformamide – evidence for hydrated forms

Martin R. GREEN, H. Allen O. HILL and David R. TURNER
Inorganic Chemistry Laboratory, South Parks Road, Oxford, England

Received 9 May 1979

1. Introduction

Since the discovery by McCord and Fridovich [1] of the role of the cupreins as superoxide dismutases, the chemical and physical properties of its substrate have received considerable attention [2–6]. It has become apparent that the properties of the superoxide ion are dependent on both the solvent and the counter-ion [4,5,7,8] particularly the former. Of special importance are the differences between its properties in aqueous and non-aqueous solutions since the ion may be formed in environments related to either in biological systems. The effects of hydration have already been considered and a difference in solvation enthalpy [5] between aprotic and aqueous solution has been estimated as 25 $kJ.mol^{-1}$. The first, second and third gas-phase hydration enthalpies have been determined [9] as 52.3, 40.6 and 29.3 $kJ.mol^{-1}$, respectively. Thus it is likely that the degree of hydration will have a significant effect on the properties [4,5] of the ion, e.g., its nucleophilicity and redox potential.

The electron paramagnetic resonance (EPR) spectrum of the ion [4,10–12] is particularly responsive to its environment. Thus whilst the *g*-values of the free ion [21] are theoretically $g_{\parallel} = 4$ and $g_{\perp} = 0$, the effect of any chemical environment will dramatically alter these. The *g*-values for the superoxide ion have been [4,10,13] satisfactorily accounted for by inclusion of the effect of the 'ligand' field, the term 'ligand' in this respect being taken to represent the effect of solvation, ion-pairing, complex formation. In most environments all three *g*-values are close to the free electron value, any departure being accounted for by a change in the chemical environment. Thus EPR spectroscopy is a technique well-suited to an investigation of the solvation of the superoxide ion.

2. Experimental

N,N-Dimethylformamide [DMF] was purified with regard to the recommendations [14] of the Commission of Pure and Applied Chemistry. The solvent was dried over activated AW500 molecular sieve and left to stand at 4°C in the dark over anhydrous $CuSO_4$ with occasional stirring for $\geqslant 3$ days. The DMF was distilled in vacuo using a packed column, the middle 60% cut being collected. The purified solvent was stored at –20°C over activated AW 500 molecular sieve under argon in a container designed so that the DMF could be expelled by a top pressure of argon. The DMF was normally used within 36 h though deterioration of the solvent over a period of weeks was not normally a problem.

Solutions containing the superoxide ion were generated [15,16] by the electrochemical reduction of oxygen. Cyclic voltammetry and controlled potential electrolyses (CPE) were carried out using a PAR 173 potentiostat, the charge passed being measured by a PAR 179 digital coulombmeter. The three-electrode electro-chemical cell was of conventional design holding 6 ml. of solvent in the working compartment. The platinum foil secondary electrode was separated from the working compartment by a fine glass frit. The reference electrode was Ag–10^{-3} M

 Elsevier/North-Holland Biomedical Press

$AgNO_3$, 0.1 M tetraethylammonium perchlorate (TEAP) in purified acetonitrile. CPE were carried out using a clean platinum foil electrode of ~ 8 cm^2 surface area at a potential 0.15 V more negative than the peak reduction potential for O_2 [−1.45 V] as determined by cyclic voltammetry at a platinum disc electrode. Solutions were constantly stirred during CPE by a stream of dried oxygen gas and for quantitative work the charge passed during an experiment was corrected for the background current in the absence of any oxygen. The supporting electrolyte was 0.1 M TEAP which had been dried for ⩾ 36 h at 80°C under vacuum. Oxygen was dried by passage through columns of activated 4A and 5A sieves.

EPR spectra were recorded on a Varian E109 spectrometer with 100 kHz field modulation operating in the first derivative mode. Spectra at 77 K were measured using a Varian liquid nitrogen dewar insert and other variable temperature spectra were obtained by passing cold nitrogen gas through a Varian low temperature insert. The concentration of the superoxide ion in the solutions was measured by its reaction with tetranitromethane (TNM) [1,17] by monitoring the A_{350} increase due to formation of the nitroform anion [ϵ_{350} = 14.8 × 10^3 $M^{-1}.cm^{-1}$). As solutions of TNM tend to deteriorate on standing best results were obtained when an aliquot of a solution containing the superoxide ion in DMF was added directly to 3 ml of TNM solution in a cuvette. The cuvette was rapidly shaken and the absorbance increase immediately noted. Freshly prepared TNM solution (~ 2 × 10^{-2} M) was in distilled ethanol. Superoxide solutions for EPR were typically ~ 5 mM and almost colourless. Optical measurements were made using a Cary 17 spectrometer.

Reagent grade DMF, acetonitrile, Analar $CuSO_4$ TNM and Linde 4 A molecular sieve were from BDH Chemicals Ltd., Poole, Dorset. Coloured impurities were removed from the TNM shortly before use by washing with water; TEAP was obtained from Eastman Kodak Co. and Union Carbide AW500 molecular sieve was from Fluka AG. Water was purified by a reverse osmosis technique (Millipore Corp., Bedford, MA) and had a specific conductivity of 18 $M\Omega.cm^{-1}$.

3. Results

Figure 1 shows the result of titrating a solution of

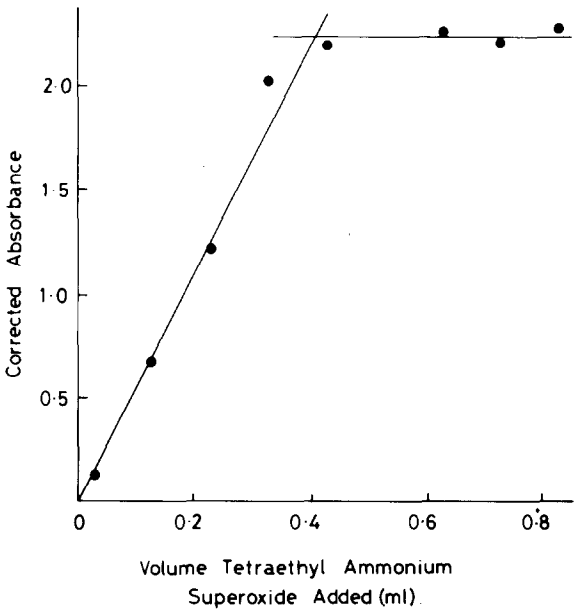


Fig.1. Addition of a solution of electrochemically generated tetraethylammonium superoxide in DMF to 2.4 ml of tetranitromethane (1.5 × 10^{-4} M) in ethanol in a cuvette of 1 cm pathlength. The ΔA_{350}, due to the formation of the nitroform anion, has been corrected for volume changes.

TNM (1.5 × 10^{-4} M) with a solution containing the superoxide ion at a concentration determined colometrically as 9.3 × 10^{-4} M. The end-point of the titration using this spectrophotometric method gives a value of 8.9 × 10^{-4} M. Thus contrary to [18], it is possible to prepare solutions containing the superoxide ion in DMF as long as the solvent is carefully purified. The EPR spectrum of such a solution at 77 K is shown in fig.2a. It is similar to that in [16] and consists of two overlapping sets of resonances. One (I) is characterised by three g-values, g_z = 2.038, g_y = 2.008 and g_x = 2.002. The other (II) is broader and appears to have two $g_{\parallel}$ ($\equiv g_z$) components at g = 2.23 and 2.20. Addition of water converts the I into II. Only resonance II is obtained if undried supporting electrolyte, solvent or oxygen is used or if the sample is melted and exposed to air. Failure to take stringent precautions to exclude water will result in only resonance II being observed.

As shown in fig.2 the g-values of resonance II depend on the amount of water added. Other differences between resonances I and II are apparent.

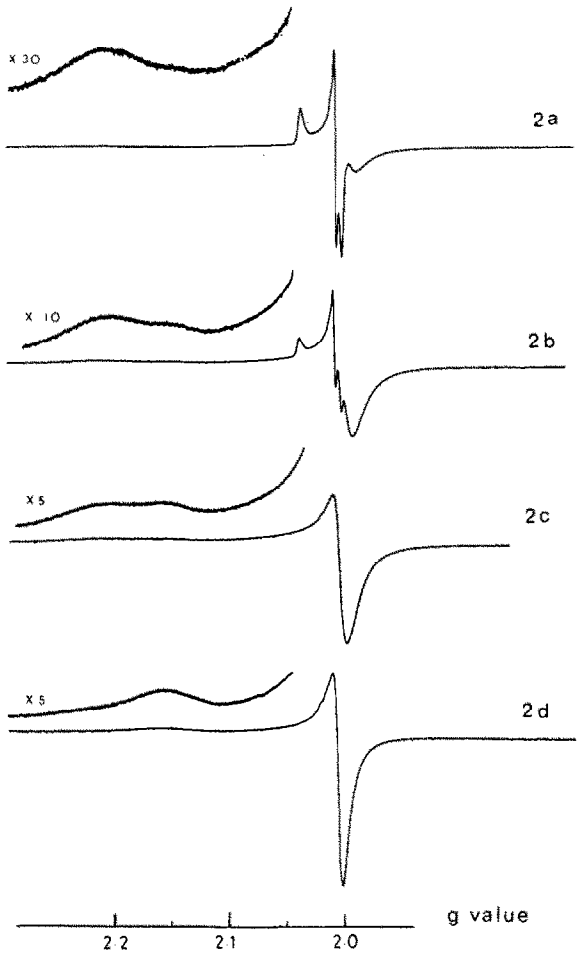


Fig.2. EPR spectra of solutions of the superoxide ion in DMF at 77 K frozen as glasses. (a) 5 mM tetraethylammonium superoxide; (b) + 18 mM H_2O; (c) + 55 mM H_2O; (d) + 220 mM H_2O. Field 3290 G; power 12.5 mW; modulation 1 G; frequency 9.268 GHz; scan rate $1G.s^{-1}$; time constant 0.250 s. Gain; (a) 3.2×10^2; (b) 5×10^2; (c,d) 6.3×10^2.

They have different dependencies on temperature (fig.3). The signal at 77 K is identical to that in fig.2b but with increasing temperature the intensity of resonance II decreases more rapidly than that of resonance I, the effect being fully reversible. The spectra shown in fig.2 and fig.3a,b are those of glassy solutions. The spectrum of an amorphous sample is quite different (fig.3c); melting and re-freezing this sample to re-form the glass yields the original spectrum. Figure 4 shows a plot of signal height

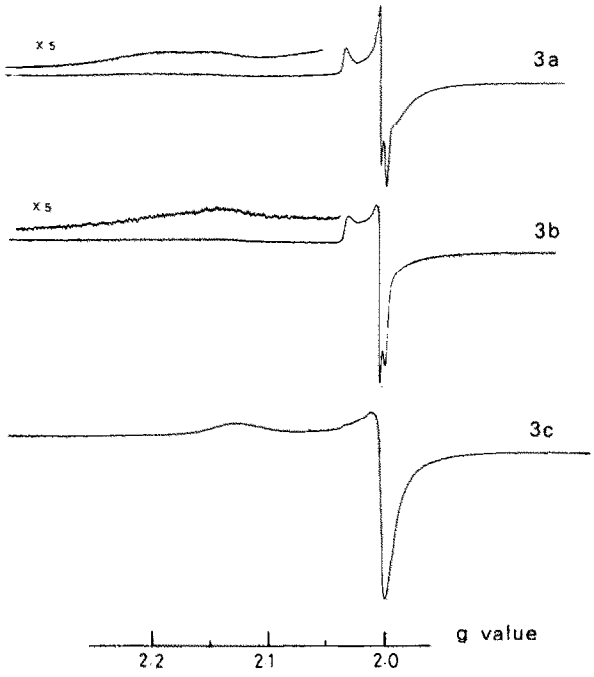


Fig.3. Variable temperature EPR spectra of tetraethylammonium superoxide (5 mM O_2^- + 18 mM H_2O) in frozen matrices of dimethylformamide. (a) 106 K (glass matrix); (b) 143 K (glass matrix); (c) 106 K (amorphous matrix). Field 3300 G; power 12.5 mW; modulation 1 G; frequency 9.370 GHz; scan rate $2G.s^{-1}$; time constant 0.128 s. Gain: (a) 6.3×10^2; (b,c) 2×10^3.

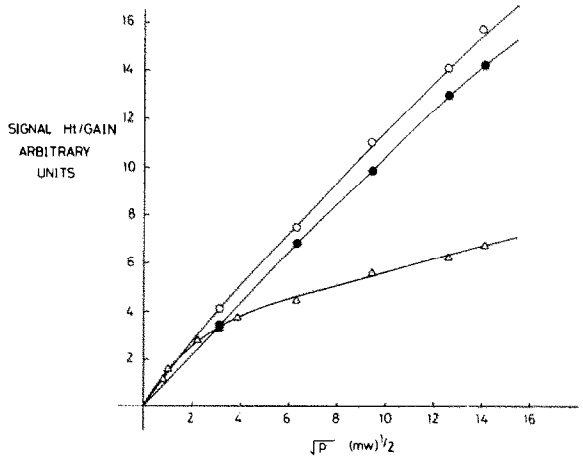


Fig.4. A plot of signal height/gain (arbitrary units) against (Power)$^{1/2}$ (mW)$^{1/2}$ for solutions of tetraethylammonium superoxide in DMF at 77 K in varying chemical environments. (Δ) resonance I (unhydrated superoxide); (•) resonance II (hydrated superoxide) in a glass matrix; (○) superoxide in an amorphous matrix.

Table 1
The g-values and peak to peak widths $\Delta G_{\perp}^{p}$ of tetraethyl ammonium superoxide frozen in DMF matrices at variable temperatures and in a number of chemical environments

		II $g_{\parallel}$	I g_z [$\equiv g_{\parallel}$]	$g_{\perp}$	II $\Delta G_{\perp}^{p}$ (gauss)	I $\Delta G_{\perp}^{p}$ (gauss)
5 mM O_2^-	77 K	2.22 (4) 2.20 (0)	2.038	g_x = 2.002 g_y = 2.008	~ 34	~ 10
+ 18 mM H_2O	77 K	2.20 (9) 2.16 (2)	2.038	2.007	~ 33	~ 13
+ 55 mM H_2O	77 K	2.20 (3) 2.15 (6)	–	2.001	~ 20	–
+ 220 mM H_2O	77 K	2.14 (8)	–	2.004	~ 15	–
5 mM O_2^- + 18 mM H_2O	106 K	2.20 (7) 2.15 (3)	2.039	2.009		~ 12
+ 18 mM H_2O	143 K	2.14 (8)	2.039	2.010		~ 15
+ 18 mM H_2O	143 K	2.12 (9)[a]		2.009[a]	$\Delta G_{\perp}^{p}$ = ~ 23[a]	

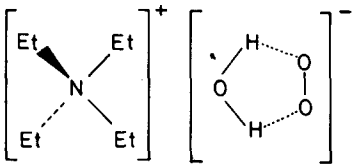
[a] Amorphous matrix (see text)

Resonances in columns headed by I are attributed to the 'unhydrated' superoxide ion and those in columns headed by II are attributed to hydrated superoxide species. The superoxide solutions are frozen as glasses unless otherwise indicated

against the square-root of the microwave power. Resonance II and those of the amorphous samples show a linear dependence but that of resonance I is much more easily saturated.

4. Discussion

The experiments described above show that it is possible to prepare solutions of DMF containing the superoxide ion. They also show that the EPR spectrum of the superoxide ion is very sensitive to its environment. The most straightforward interpretation of the data is that resonance I arises from the unhydrated O_2^-, presumably as the ion pair $Et_4N^+O_2^-$ solvated by DMF. The broad signals associated with resonance II presumably arise from the hydrated forms $Et_4N^+O_2^-(H_2O)_n$ and it is possible that the component $g_{\parallel}$ = 2.23 is due to $Et_4N^+O_2^-(H_2O)$. The low value of g_z (table 1) derived from resonance I suggests [8,10,11] that there is a considerable assymmetry in the environment of O_2^- in the unhydrated form associated perhaps with the ion-pair. This interaction would be reduced in a water-separated ion-pair and it is possible that the structure (A) suggested [19] for the monohydrate $O_2^-(H_2O)$ in the gas phase may be relevant here



(A)

Two aspects of this work are relevant to the study of the superoxide ion in biological systems:

(1) It can exist in a number of different solvated forms and that the EPR spectra of these may differ significantly and could easily be misassigned. They are even sensitive to the method of sample preparation.

(2) The superoxide ion will exist in water as the hydrate $O_2^-(H_2O)_n$ and it is very likely that this, and not O_2^-, is the substrate for superoxide dismutase.

The attendant solvent molecules could therefore provide a convenient source of protons [2,4,20] for the disproportionation, that is:

$$Cu(II)SOD + O_2^- (H_2O)_n \rightarrow Cu(I)SOD + O_2 + n\ H_2O$$

$$Cu(I)SOD + O_2^- (H_2O)_n \rightarrow Cu(II)SOD + H_2O_2 + 2\ OH^- + (n-2)\ H_2O$$

Acknowledgements

We are grateful to the Science Research Council for studentships to M.R.G. and D.R.T. and to the Medical Research Council for support. This is a contribution from the Oxford Enzyme Group of which H.A.O.H. is a member.

References

[1] McCord, J. M. and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055.
[2] Bors, W., Saran, M., Lengfelder, E., Spöttl, R. and Michel, C. (1974) Curr. Top. Rad. Res. 9, 247–309.
[3] Lee-Ruff, E. (1977) Chem. Soc. Rev. 6, 195–214.
[4] Hill, H. A. O. (1978) in: New Trends in Bioinorganic Chemistry (Williams, R. J. P. and Da Silva, J. J. R. F. eds) pp. 173–208, Academic Press, London.
[5] Fee, J. A. and Valentine, J. S. (1977) in: Superoxide and superoxide dismutases (Michelson, A. M. et al. eds) pp. 19–60, Academic Press, London.
[6] Fridovich, I. (1974) Horizons Biochem. Biophys. 1, 1–37.
[7] Bray, R. C., Mautner, G. N., Fielden, E. M. and Carle, C. I. (1977) in: Superoxide and superoxide dismutases (Michelson, A. M. et al. eds) pp. 61–75, Academic Press, London.
[8] Eastland, G. E. and Symons, M. C. A. (1977) J. Phys. Chem. 81, 1502–1504.
[9] Arshadi, M. and Kebarle, P. (1970) J. Phys. Chem. 74, 1483–1485.
[10] Lunsford, J. H. (1973) Catal. Rev. 8, 135–157.
[11] Bennett, J. E., Mile, B. and Thomas, A. (1968) Trans. Farady Soc. 64, 3200–3209.
[12] Clarkson, R. B. and McClellan, S. (1978) J. Phys. Chem. 82, 294–297.
[13] Kanzig, W. and Cohen, M. H. (1959) Phys. Rev. Lett. 509–510.
[14] Juillard, J. (1977) Pure Appl. Chem. 49, 885–892.
[15] Peover, M. E. and White, B. S. (1965) Chem. Commun. 183–184.
[16] Maricle, D. L. and Hodgson, W. G. (1965) Anal. Chem. 37, 1562–1565.
[17] Czapski, G. and Bielski, B. H. J. (1963) J. Phys. Chem. 67, 2180–2184.
[18] Fee, J. A. and Hildenbrand, P. G. (1974) FEBS Lett. 39, 79–82.
[19] Spangler, G. E. and Collins, C. I. (1975) Anal. Chem. 47, 393–402.
[20] Fridovich, I. (1976) Adv. Exp. Med. Biol. 74, 530–539.
[21] Bennett, J. E., Ingram, D. J. E., Symons, M. C. R., George, P. and Griffiths, J. S. (1955) Phil. Mag. 46, 443–444.