EPR Study of the Cr(V) and Radical Species Produced in the Reduction of Cr(VI) by Ascorbate

DAVID M. L. GOODGAME and A. MARTIN JOY

Chemistry Department, Imperial College of Science and Technology, London SW7 2AY, U.K.

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Abstract

X-Band EPR studies of the interaction between potassium dichromate and ascorbate anion in aqueous solution at pH 7–8.5 have shown that relatively long-lived Cr(V) species are produced, but that generation of the ascorbate radical is favoured as the pH is raised within that range. New, additional Cr(V) signals are produced when TRIS–HCl is used as buffer reagent, and it is suggested that TRIS forms a Cr(V) complex and is, therefore, an unreliable buffer in such systems.

Introduction

There is growing interest in the chemistry of Cr(V) not only because of its involvement and use in synthetic chemistry [1] but also because of its formation when Cr(VI) reacts with molecules of biological relevance [2–5]. In the latter context, particular interest centres on the species involved when Cr(VI), which is known to be carcinogenic and mutagenic [6], is reduced by naturally occurring reductants to its final, bound form as Cr(III).

We [5], and others [4], have previously observed by EPR spectroscopy the formation of Cr(V) by the interaction of aqueous solutions of Cr(VI) with reduced glutathione, a major cellular reductant. Those observations have prompted us to extend our studies to other naturally occurring reductants and we report here the results of an EPR study of the Cr(VI)/ascorbate system at pH values close to those of biological relevance.

Experimental

Potassium dichromate (AnalaR grade, Hopkin and Williams Ltd.) and sodium ascorbate (Aldrich Chemical Co.) were used as supplied. Aqueous buffer solutions were prepared with TRIS–HCl [tris(hydroxymethyl)aminomethane·HCl] (1 M) or HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid] (0.1 M).

Aqueous solutions containing various Cr(VI):ascorbate ratios were prepared by the addition of potassium dichromate solution (2 cm^3, 0.034 M) to a solution of the required amount of sodium ascorbate in 2 cm^3 of water or buffer solution. (When using TRIS-buffer the $K_2Cr_2O_7$ was dissolved in 0.5 M $KOH_{(aq)}$ (2 cm^3)). The pH of the resultant solution was adjusted to the required value by the addition of 0.5 M KOH or 0.5 M HCl solutions.

The X-band EPR spectra of the solutions were then immediately recorded at room temperature (24 °C) using a Varian E12 X-band (9.5 GHz) spectrometer and a Heraeus quartz flat cell. Frequency calibration was made with a Sanders WM 16 wavemeter and field calibration with a Varian E 500 NMR gaussmeter.

Results and Discussion

Studies were made varying the Cr(VI):ascorbate mole ratios within the range 2:1 to 1:5, at various pH values 7.0–8.5, and employing both unbuffered and buffered (HEPES and TRIS–HCl) solutions. Depending on the solution pH, relative mole ratio of reactants, and the buffer system used (if any), the Cr(VI)/ascorbate reaction gave four types of X-band spectra, which we shall designate A (Fig. 1), B (Fig. 2), C (Fig. 3), and D (Fig. 4) respectively (see summary in Table I).

Unbuffered solutions containing equimolar amounts of Cr(VI) and ascorbate at pH 7.0 gave spectra of type A, comprising the typically sharp band (1.5 gauss peak-to-peak derivative width) at g = 1.979, due to Cr(V), with four weak ^{53}Cr (9.55% abundance, $I = 3/2$) hyperfine bands at 17.7 gauss spacing. This ^{53}Cr nuclear hyperfine spacing is very similar to the values (~18 gauss) found by Hayman [7] for the Cr(V) species formed by the reaction of Cr(VI) with ribonucleotides, in which O-donors are also thought to form the Cr coordination environment. Much smaller A values (8.5 gauss) were observed [5] for the Cr(V) species produced by the reduction of Cr(VI) by glutathione, where coordinated sulphur atoms are probably involved.

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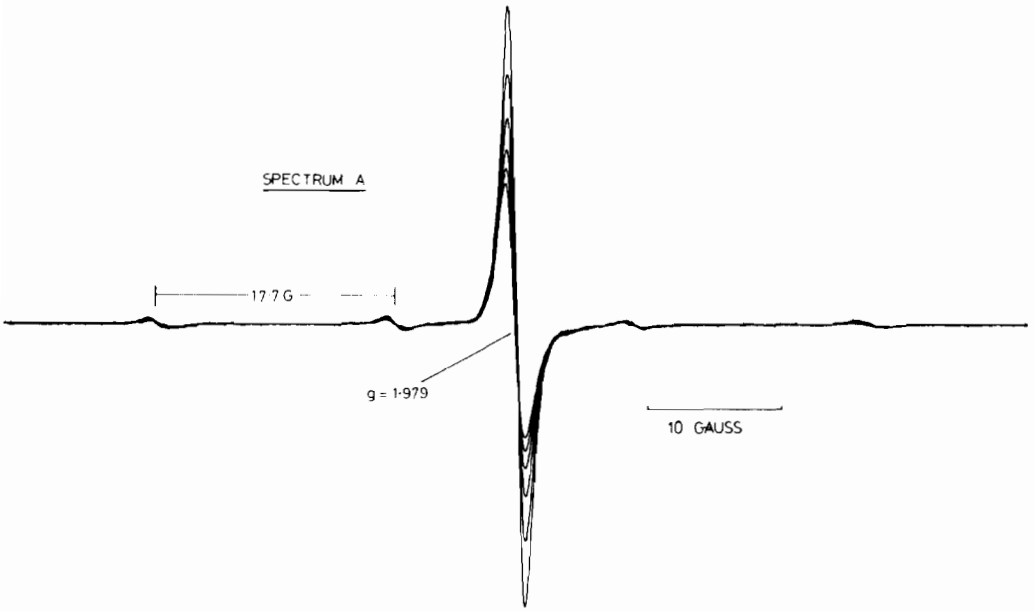

Fig. 1. X-Band EPR spectrum of an aqueous solution (pH 7.0) of Cr(VI) and ascorbate (2:1 mole ratio). Spectra taken at 253 s scan intervals.

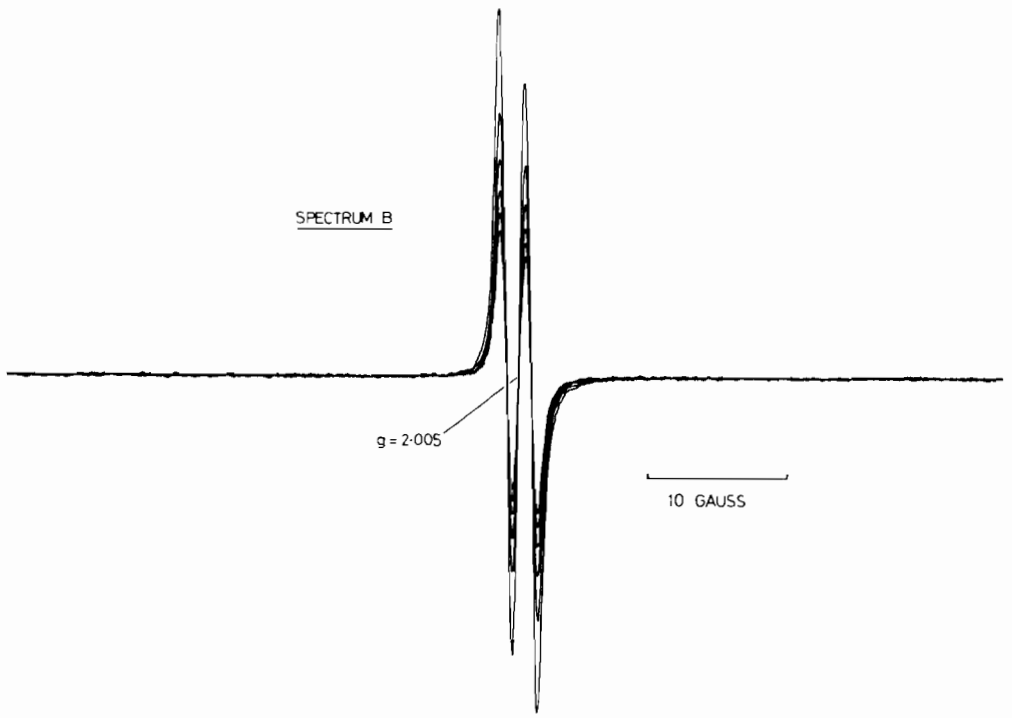

Fig. 2. X-Band EPR spectrum of an aqueous solution (pH 8.0, unbuffered) of Cr(VI) and ascorbate (1:1 mole ratio). Spectra taken at 253 s scan intervals.

The intensity of these Cr(V) signals decreased with time, with an approximate half-life of *ca.* 15 min.

On raising the pH to 7.5 the Cr(V) signal was replaced by a sharp doublet centred at $g = 2.005$. (The transition between the two types of spectra was quite sharp and only in a narrow pH range just below 7.5 could both signals be seen). The doublet signal is due to the ascorbate radical [8, 9] and the value of the hyperfine splitting (1.7 gauss, due to the proton on C_4) is in agreement with the major radical species, termed radical I by Laroff *et al.* [9], in which the

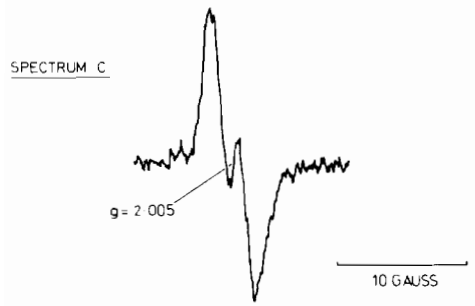

Fig. 3. X-Band EPR spectrum of an aqueous solution (pH 7.0) of Cr(VI) and ascorbate (1:5 mole ratio).

electron is spread over the carbonyl groups of the radical anion.

Variation of the Cr(VI):ascorbate mole ratio at pH = 7.0 gave a Cr(V) signal (Fig. 1) when Cr(VI) was in excess, but not for [Cr(VI)]:[ascorbate] ratios of 1:2 or 1:5. In these last two cases spectra of type C were observed comprising ascorbate radical signals, but with broader lines than in the type B spectra referred to above.

The spectral observations for solutions with pH control depended upon the buffer reagent used. The use of HEPES as buffer gave generally similar results to those obtained for unbuffered solutions, except that the Cr(V) and ascorbate radical signals co-existed over a wider range of pH values. Thus when HEPES is used with Cr(VI):ascorbate in 1:1 mole ratio Cr(V) and radical signals were both seen in the pH range 8.0 to 8.5 (Fig. 5), but only Cr(V) bands were observed in the pH range 7–8.

Connett and Wetterhahn have recently reported the results of a kinetic study [10] of the Cr(VI)/ascorbate interaction by UV–Vis spectroscopy in which TRIS–HCl was used as a buffer reagent. Under the conditions employed it was stated [10] that little or no Cr(V) was observable by EPR spectroscopy. We therefore carried out EPR studies in which TRIS–HCl replaced HEPES as buffer reagent. Quite different spectra were obtained (Type D, Fig. 4). Under conditions where, with unbuffered solutions and with HEPES buffer, type A spectra were obtained, the use of TRIS–HCl gave an extra Cr(V) signal at $g = 1.976$ in addition to the $g = 1.979$ band. The species responsible for the $g = 1.976$ band is probably a Cr(V) complex involving TRIS, as the hydroxy groups in the TRIS molecule are good potential donors to Cr(V). However we observed no Cr(V) species when Cr(VI) was mixed with TRIS–HCl alone in aqueous solution. It appears that the $g = 1.976$ signal is due either to a ternary Cr(V)/ascorbate/TRIS complex or to a Cr(V)(TRIS) complex formed by reaction of TRIS with the Cr(V)(ascorbate) species responsible for the $g = 1.979$ band. This evidence for the direct involvement of TRIS in a Cr(VI)/Cr(V) system is important as TRIS is a commonly used buffer reagent. In our view, it should now be regarded as a 'non-innocent' buffer reagent in systems of this type.

The mechanism advanced by Connett and Wetterhahn [10] for the reaction of Cr(VI) with ascorbate

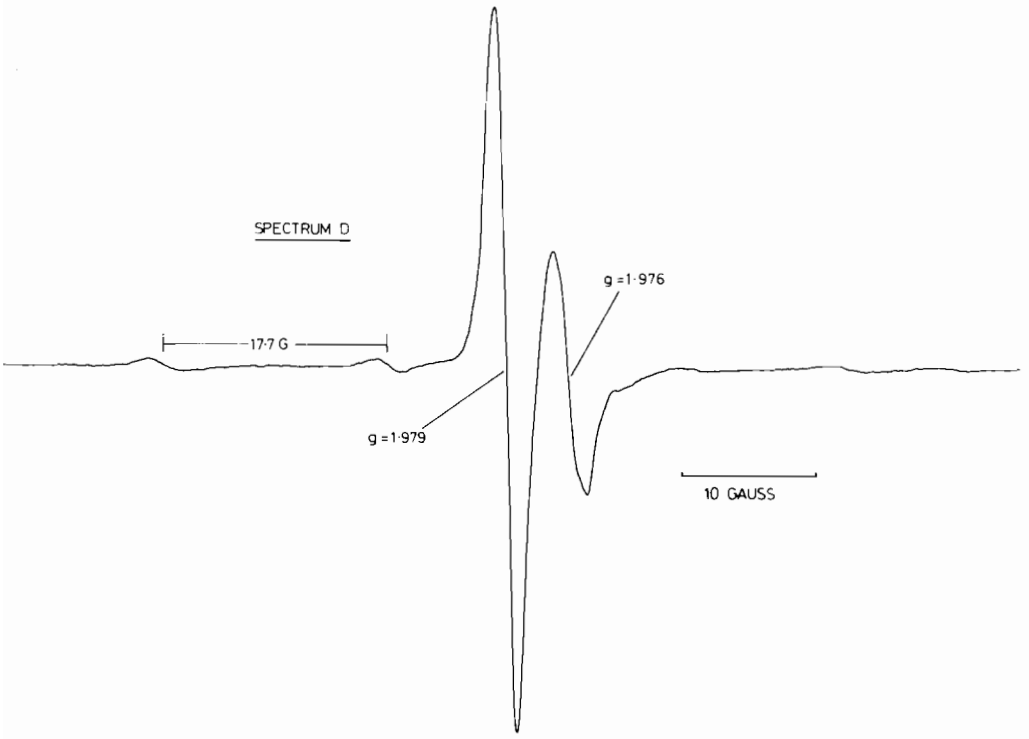

Fig. 4. X-Band EPR spectrum of an aqueous solution (pH 7.0, TRIS–HCl buffered) of Cr(VI) and ascorbate (2:1 mole ratio).

TABLE I. Summary of Types of Spectra

[Cr(VI)]: [Ascorbate]	Buffer[a]	pH	Spectral type[b]
1:1	H	7.0	A
1:1	H	7.5	A
1:1	H	8.0	A + B
1:1	H	8.5	A + B
2:1	H	7.0	A
1:2	H	7.0	C
1:5	H	7.0	C
1:1	N	7.0	A
1:1	N	7.5	B
1:1	N	8.0	B
2:1	N	7.0	A
1:2	N	7.0	C
1:5	N	7.0	C
1:1	T	7.0	D
1:1	T	7.5	D
1:1	T	8.0	C
2:1	T	7.0	D
1:2	T	7.0	C

[a]H = HEPES, N = no buffer, T = TRIS–HCl. [b]See Figs. 1–4 for spectral types.

(HA^-) at pH 7.4 involves the initial formation of a Cr(VI) ester, HACr(VI), as the rate determining step, followed by a rapid unimolecular redox reaction with the formation of Cr(IV) and dehydroascorbate. However, a recent theoretical study of the general oxidation mechanism of ascorbic acid invokes the formation of the ascorbate radical as the initial product in neutral or basic solutions [11].

Our results suggest that, under the conditions we have employed, which are close to those of physiological relevance, the mechanism of the Cr(VI)–ascorbate redox system may be more complex than either of the two postulated mechanisms [10, 11]. The ascorbate radical is certainly present at the higher end of the pH range we have examined but we do not observe it near pH 7. This contrasts sharply with the formation of the radical over a wide pH range (5–10) when generated by radiolysis [9]. Moreover there is also clear evidence for the presence of Cr(V) as well, so such species should be taken into account in assessing the possible mechanism(s) for the Cr(VI) oxidation of ascorbate and in the likely generation of Cr(V) from Cr(VI) by ascorbate in biological systems.

Acknowledgements

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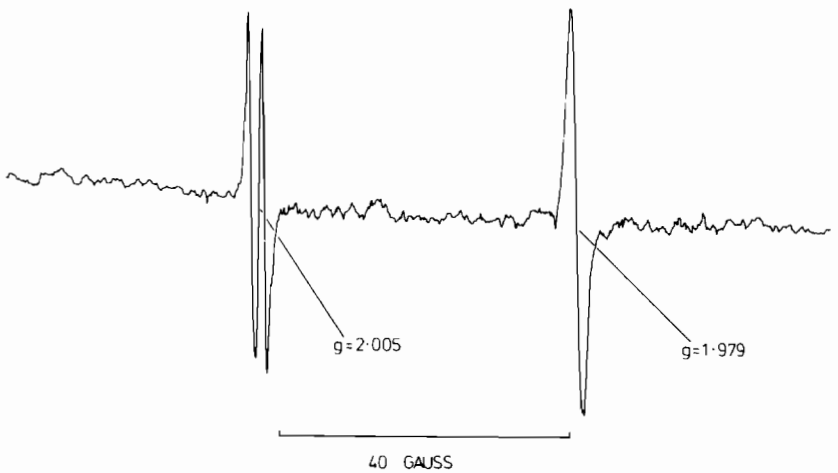

Fig. 5. X-Band EPR spectrum of an aqueous solution (pH 8.5, HEPES buffered) of Cr(VI) and ascorbate (1:1 mole ratio).