Formation of Water-Soluble Chromium(V) by the Interaction of Humic Acid and the Carcinogen Chromium(VI)

D. M. L. GOODGAME, P. B. HAYMAN

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

and D. E. HATHWAY

Imperial Chemical Industries, P.L.C., Central Toxicology Laboratory, Alderley Park, Cheshire, SK10 4TJ, U.K.

Received June 28, 1983

The interaction of chromium(VI) with humic acid in water in the pH range 4 to 9 has been shown by e.p.r. to produce water-soluble chromium(V) species, but at lower pH values solids containing chromium(III) are formed.

Introduction

Compounds of chromium(VI), particularly as chromates, have extensive industrial use, but the use and subsequent disposal of some of them present a potential environmental hazard because of their mammalian carcinogenicity and toxicity [1–4].

The cellular uptake of readily transportable chromium(VI) [5] can be followed by reduction to chromium(III) by biological reductants [6, 7] and chromium(III) is probably the ultimate form of the metal that is bound in cells [*e.g.*, 7, 8]. Recently, however, detectable chromium(V) species have been observed from the incubation of chromate with rat liver microsomes and NADPH [9] and from the reactions of chromium(VI) with a range of nucleic acid components [10]. Jennette has suggested [9] that chromium(V) should be considered as a species involved in chromium carcinogenicity, but, at present, this appears to be based on the existence of this species in microsomal systems *in vitro*. In view of the fact that chromium(VI) may inadvertantly find its way into soil, there arises the question as to whether naturally occurring reductants can generate chromium(V) species from chromium(VI).

We report here the results of an e.p.r. study of the reaction between chromium(VI) and humic acid, which is known [11, 12] to have reducing properties.

Results

Most of the previously reported e.p.r. studies of the interactions between transition metal ions and humic acids have been on solid materials [12–15] frequently obtained under quite acid conditions. As we were particularly interested in identifying the water-soluble species derived from chromium(VI)/humic acid interactions, we have studied the e.p.r. spectra of solutions as well as solids, and over a range of pH values.

The X-band e.p.r. spectrum of humic acid alone, in water in the pH range 5–9.2, measured over an applied magnetic field range of 0 to 1T, showed only the well-known, sharp (~0.4 mT linewidth) free radical signal at $g = 2.004 \pm 0.001$. A typical spectrum in the relevant region is shown in Fig. 1A. [All spectra in Fig. 1 are with constant concentrations of humic acid (0.0125 g per cm^3) and of chromium(VI) (0.125 *M*) and constant spectrometer settings, to aid direct comparison]. The e.p.r. spectrum of potassium chromate and humic acid at pH 9.2 was identical to that of humic acid alone (Fig. 1B; as before, the 0–1T range was studied but only the $g = 2$ region is shown). As the pH was successively lowered to 4 (Figs. 1C–G) the humic acid/chromium(VI) solutions afforded a new signal on the high-field side of the free-radical absorption. In no case were any additional features observed elsewhere in the 0–1T range.

The new absorption consists of a set of three features whose sharpness and g-values (g_1 1.984, g_2 1.979, g_3 1.972) are characteristic of chromium in the +5 oxidation state. The presence of three components in the chromium(V) signal suggests that there is more than one Cr(V) species present. This might be expected in view of the complexity of humic acid. It is interesting to note in this connection that Goodman and Cheshire have recently found [14] that two molybdenum(V) species are produced in solid samples obtained by humic acid reduction of sodium molybdate.

Most chromium(V) compounds produced in solution by reduction of chromium(VI) with organic substrates are relatively short-lived. In contrast to this, the signals from the chromium(V)/humic acid species are relatively persistent. As shown in Fig. 2A–C for a pH of 5.6, detectable amounts of chromium(V) were present in solution up to at least 5

0020-1693/84/$3.00

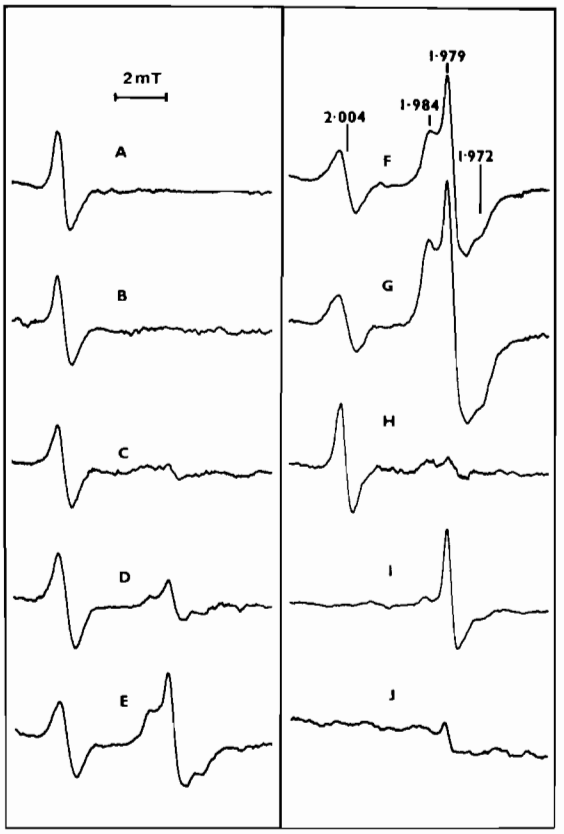

Fig. 1. X-Band e.p.r. spectra in the g ≈ 2 region of aqueous solutions of: A, Humic acid (0.0125 g per cm^3) (= HA) at pH 9.2; B, HA + K_2CrO_4 (0.125 *M*) at pH 9.2; C, as B but at pH 8.0; D, as B but at pH 7.0; E, as B but at pH 6.0; F, as B but at pH 5.0; G, as B but at pH 4.0; H, HA and K_2CrO_4 held at pH 4.0 for 2 mins. then brought to pH 9.2; I, supernatant liquid after removal of solid formed from HA and K_2CrO_4 at pH 3.0; J, as I but formation at pH 2.0.

days after mixing humic acid with chromium(VI). Decay of the chromium(V) absorption is also accompanied by decay of the free radical signal.

The pH dependence of the intensity of the chromium(V) signal is at least partly reversible. Figure 1G shows the spectrum of the chromium(V)/humic acid species at pH 4. If such a solution is prepared at pH 4, held at that pH for two minutes and then brought to a pH of 9.2 the signal due to chromium(V) is very weak [Fig. 1H]. We have not, however, attempted a rigorous quantitative study of the combined pH and time dependence of the chromium(V) species.

Reduction of the pH below ~3.8 caused the formation of a precipitate. The e.p.r. spectra of the supernatant liquids from such mixtures showed no free radical signal, and greatly diminished chromium(V) absorptions as the pH was lowered towards 2 (Figs. 1I and J). The solids obtained at these low pH values (after triple washing with 0.2 *M* HCl and drying in vacuo) had e.p.r. spectra showing very

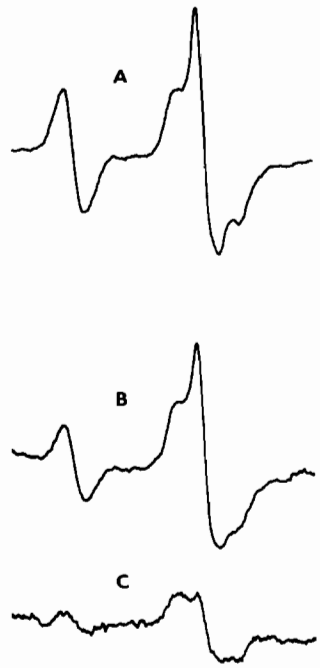

Fig. 2. X-Band e.p.r. spectra in the g ≈ 2 region of aqueous solutions of humic acid (0.0125 g per cm^3) and chromium(VI) (introduced as $K_2Cr_2O_7$, 0.125 *M*) at pH 5.6; A, 10 minutes after mixing; B, 90 minutes after mixing; C, 5 days after mixing.

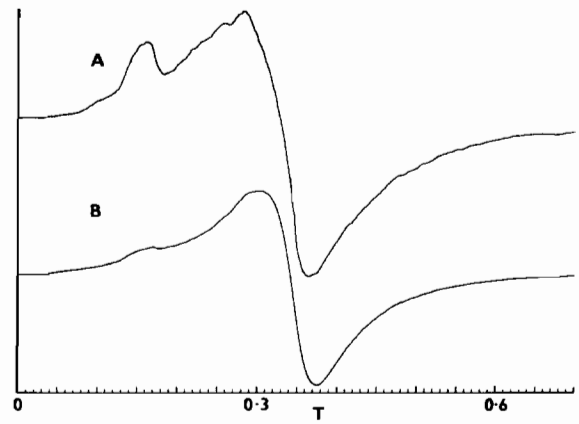

Fig. 3. X-Band e.p.r. spectra (0–0.7T) of: A, the solid 'chromium(III) humate' obtained from humic acid + K_2CrO_4 at pH 2.0; B, solid 'chromium(III) humate' obtained from humic acid + chromium(III) nitrate.

broad absorptions of the type generally observed for chromium(III) (Fig. 3A). A spectrum of this type has previously been reported for solid 'chromium(III) humate' by Lakatos and co-workers [12], and we have been able to produce such spectra from the solid products from direct reaction between humic acid and *e.g.*, chromium(III) nitrate in the pH range 2–3 (*e.g.*, Fig. 3B).

Discussion

In our view, the most interesting result of the present work is that a relatively persistent chromium(V) signal is detectable as a result of the interaction of chromium(VI) with humic acid. We recognise that small quantities of chromium(III) species may have also been formed in these solutions. However, the detection of low concentrations of chromium(III) in solution would be very difficult because the breadth of their absorptions would cause any low intensity Cr(III) bands to merge into the spectral baseline. Conversely, the solid humate compounds formed at low pH values contain appreciable amounts of chromium(III) and, although there was no evidence for the presence of any chromium(V) in such solids, the strength of the Cr(III) absorption may have obscured any small amount of Cr(V).

However, with these provisos in mind, it appears that chromium(V) species are a feature of the aqueous solution behaviour of chromium(VI)/humic acid interactions. Moreover, the amount of such species increases as the pH is lowered (prior to the point of precipitation). The oxyanions of chromium(VI) are known to exhibit complex equilibria in aqueous solution involving the species CrO_4^{2-}, $HCrO_4^-$, H_2CrO_4, and $Cr_2O_7^{2-}$, with an equilibrium between $HCrO_4^-$ and $Cr_2O_7^{2-}$ predominating between pH 2 and 6, and the CrO_4^{2-} ion becoming favoured at higher pH values. Accordingly, the pH dependence of the chromium(V) formation we observed may reflect a greater reactivity of $HCrO_4^-$ and/or $Cr_2O_7^{2-}$, as compared with CrO_4^{2-}, with humic acid. However, in view of the extremely ill-defined nature of the complex sets of potential metal ion binding sites in the humic acids, there is the added complication that changes in pH could alter the extent of protonation of one or more of these sites. Indeed, it was necessary to check that the production of chromium(V) was not limited to one particular humic acid. We therefore examined the behaviour with Podsol B humic acid, and this also gave soluble chromium(V) species.

The detection of these persistent chromium(V) signals is of interest, but clearly additional studies, involving other soil components and employing alternative techniques, are needed to determine if these species have a significant role in the biological reduction of chromium(VI).

Experimental

The chromium(VI) compounds used were potassium chromate, and potassium dichromate of AnalaR grade. The humic acid used for most of the work was obtained as the sodium salt, from Aldrich, but some control experiments were made with Podsol B humic acid (see Discussion). The precipitates which formed at low pH were separated by centrifuging, triple washed with 0.2 *M* HCl, with centrifuge separation between washings, and dried in vacuo for 24 hrs.

The e.p.r. measurements were made on a Varian E12 X-band spectrometer at ambient temperatures with the solutions in a Heraeus flat cell. Frequency calibration was made with a Sanders WM16 wavemeter, and field calibration with a Varian E500 n.m.r. gaussmeter.

Acknowledgement

We thank the SERC and Imperial Chemical Industries, P.L.C., for a CASE studentship to P.B.H., and the SERC for e.p.r. equipment.

References

1 L. Tomatis, C. Agthe, H. Bartsch, J. Huff, R. Montesano, R. Saracci, E. Walker and J. Wilbourn, *Cancer Res., 38,* 877 (1978).
2 J. M. Davies, *Lancet,* 384 (1978).
3 A. Furst, M. Schlauder and D. P. Sasmore, *Cancer Res., 36,* 1779 (1976).
4 A. G. Levis and F. Majone, *Br. J. Cancer, 44,* 219 (1981) and refs. therein.
5 K. W. Jennette, *Biol. Trace Element Res., 1,* 55 (1979).
6 F. L. Petrilli and S. DeFlora, *Mutat. Res., 54,* 139 (1978).
7 J. E. Gruber and K. W. Jennette, *Biochem. Biophys. Res. Commun., 82,* 700 (1978).
8 M. J. Tsapakos, T. H. Hampton and K. W. Jennette, *J. Biol. Chem., 256,* 3623 (1981).
9 K. W. Jennette, *J. Am. Chem. Soc., 104,* 874 (1982).
10 D. M. L. Goodgame, P. B. Hayman and D. E. Hathway, *Polyhedron, 1,* 497 (1982).
11 M. Szilagyi, *Soil Sci., 111,* 233 (1971).
12 B. Lakatos, T. Tibai and J. Meisel, *Geoderma, 19,* 319 (1977).
13 B. A. Goodman and M. V. Cheshire, *Nature new Biol., 244,* 158 (1973).
14 B. A. Goodman and M. V. Cheshire, *Nature, 299,* 618 (1982).
15 B. A. Goodman and M. V. Cheshire, *J. Soil Sci., 27,* 337 (1976).