Formation of Chromium(V) during the Slow Reduction of Carcinogenic Chromium(VI) by Milk and some of its Constituents

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Compounds of chromium(VI) present a potential environmental hazard because of their mammalian carcinogenicity and toxicity [1–4], though they are important industrial materials. We [5–7], and others [8, 9], have recently observed that the reduction of Cr(VI) by cellular reductants or soil components [10, 11] frequently generates water soluble Cr(V) species of unexpected stability.

In this context, we were interested in a recent report [12] of unusually high mortality in cattle grazing in a field next to a dump containing chromium compounds as waste material from a chemical incinerator. Chemical waste spillage from the dump was reported [12] to contain very high concentrations of chromium, inferred [12] to be Cr(VI), and 'relatively high' Cr concentrations (e.g. 127–138 ppm) were found in the pasture soil near the dump. As stated by Smith and Lloyd [12], one cannot necessarily conclude that such pollution contributed to the cattle death, and alternative assessments of the cause, and of the amounts of chromium released, particularly in the Cr(VI) oxidation state, have been advanced by Eduljee [13]. However, high values of chromium (1300 ppb; stated normal <20 ppb) were also reported (independent analysis quoted in ref. 12) in the milk of cows grazing on the field in question. Soil ingestion during grazing has been advanced as a possible explanation [13].

Although no information is available as to the oxidation state(s) of the chromium in the milk samples cited above, our interest in the biological and ecological aspects of the reduction of Cr(VI) to Cr(III) has led us to examine, by EPR spectroscopy, the reaction between Cr(VI) and milk and some of its components. We report here the results of that work.

Experimental

The source of Cr(VI) used was potassium dichromate (AnalaR grade, Hopkin and Williams Ltd.). The milk samples were standard, pasteurized supplies from retail food stores.

In a typical measurement, potassium dichromate (10 mg in 0.5 cm^3 water) was added to milk (2 cm^3) and the X-band (*ca.* 9.5 GHz) EPR spectrum recorded, as described previously [7], straight after mixing and at various intervals thereafter. Studies of the reaction between Cr(VI) and aqueous solutions of lactose (concentration 40 mg cm^{-3}) and glucose (concentration 20 mg cm^{-3}) were made in the same way.

Results and Discussion

The interaction of Cr(VI) with milk at room temperature gave, within a few minutes, a small, sharp EPR band at $g = 1.980$, characteristic of Cr(V). The signal grew over a period of several hours and reached its maximum intensity after *ca.* 1 day. Subsequent signal decay was very gradual, occurring over a period of several weeks for samples stored at room temperature in unsealed EPR tubes.

The main $g = 1.980$ band was a multiplet, comprising four principal components with *ca.* 0.9 gauss spacing (Fig. 1A). Its set of four ^{53}Cr hyperfine bands

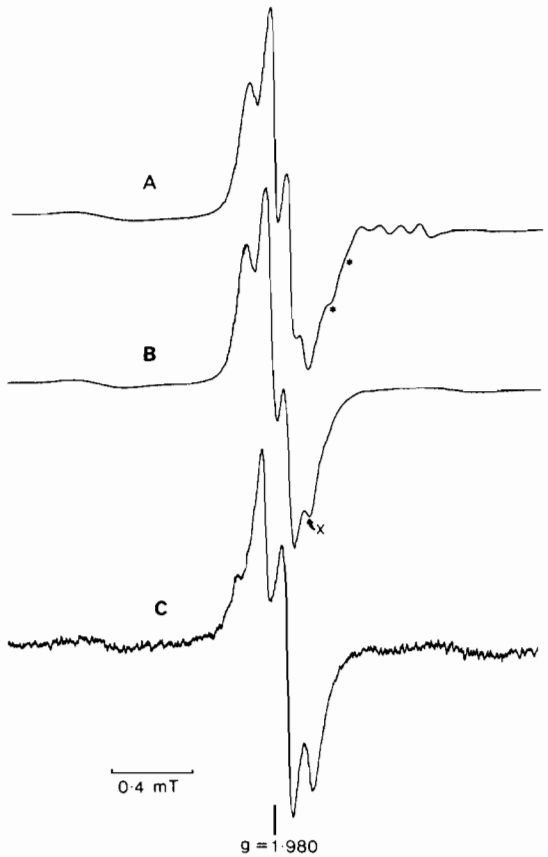

Fig. 1. Room-temperature X-band (*ca.* 9.5 GHz) EPR spectra of aqueous solutions of potassium dichromate and: A, Milk, 2 h after mixing the reactants; B, Lactose, 1 h after mixing; C, Glucose, 10 min after mixing.

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was also observed and these had 17.9 gauss spacing, a value very similar to those observed for Cr(V) in oxygen donor atom environments [7, 14]. There was also a further set of at least six bands (two occurring as weak shoulders on the main band, marked * in Fig. 1A) with *ca.* 1.0 gauss spacing, centred at g = 1.977.

Various species in milk are potentially able to reduce Cr(VI) to Cr(V) and to bind to the Cr(V) so formed, but lactose is the most likely component, in view of its relatively high concentration (*ca.* 4.9%) in cow's milk [15]. Vitamin C, which also reduces Cr(VI) to Cr(III) via Cr(V) species [7], is also present, but in much lower concentration (typically 16 mg l^{-1} [15]. Moreover, although the Cr(V) EPR band generated by vitamin C occurs at g = 1.979 it does not possess the multiplet structure observed for the Cr(V) complex in milk [7]. We therefore examined the EPR spectra of aqueous solutions of lactose with Cr(VI), using a lactose concentration equivalent to that obtained in the experiments using milk.

The EPR spectrum of the lactose/Cr(VI) reaction mixture immediately after mixing corresponded closely to the main Cr(V) band observed in milk, comprising four components of *ca.* 0.9 gauss spacing with ^{53}Cr hyperfine bands at 17.9 gauss spacing. One difference from the spectrum observed with milk is that lactose did not show the weak shoulders marked * in Fig. 1A, although there was some band broadening in that region. Moreover the weak, but clearly resolved set of bands at g = 1.977 in the milk/Cr(VI) mixtures was seen only very faintly at very much higher instrument settings in the lactose/Cr(VI) spectra.

The Cr(V)/lactose band increased somewhat in intensity within *ca.* 1 h after the reactants were mixed, but there was also a slight decrease in the apparent, relative intensity of the component at highest field (marked X in Fig. 1B) over a period of up to *ca.* 24 h. The spectrum then remained unchanged for several days.

Similar experiments in which Cr(VI) was reacted with D-glucose, the reducing monosaccharide unit in lactose, gave the Cr(V) spectrum shown in Fig. 1C. This, again, consisted of a four-component band at g = 1.980, generally similar to those observed with milk and with lactose. With glucose, however, over a period of *ca.* 2 h the band structure became less well resolved.

From these results it appears that the formation of the chromium(V) species produced in the reaction of Cr(VI) with milk results from Cr(VI)/lactose interaction, with the metal binding to hydroxyl oxygen atoms of the glucose unit. The splitting of the principal band may be attributed to hyperfine splitting from the ring protons. The small changes in the relative intensities of their hyperfine components when lactose is replaced by glucose in the reaction mixture

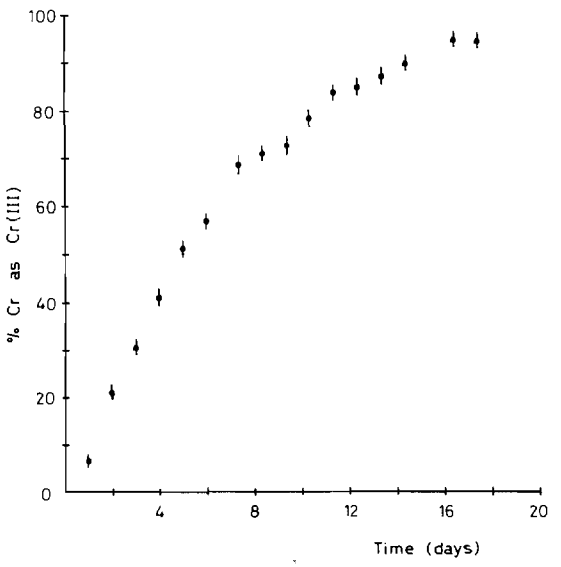

Fig. 2. Time dependence of the conversion of Cr(VI) to Cr(III) by milk as measured by integration of the Cr(III) EPR absorption.

presumably reflect differences in the effects of metal binding on the sugar conformations.

We have been unable to identify the origin of the set of very weak EPR bands on the high field edge of the principal Cr(V)/lactose signal produced in milk. Chromium(VI) is necessary for their formation as they were absent from the EPR spectra of milk alone, measured with the same spectrometer settings. They presumably arise from a Cr(V) complex of one, or more, of the many minor components of milk.

The reduction of Cr(VI) by milk results in its eventual conversion to Cr(III). By integrating the EPR signal of the Cr(III) produced, we were able to measure the time dependence of the production of Cr(III) (Fig. 2.). It appears that, under the experimental conditions we have used, the total conversion of Cr(VI) to Cr(III) is quite slow, with only *ca.* 50% conversion after 5 days. The further environmental consequences of Cr(VI) ingestion by dairy cattle will obviously depend on the rate at which the chromium is transported to the milk produced and on the extent to which biological reduction to Cr(III) occurs en route. Our results suggest that any abnormal Cr(VI) amounts reaching milk are not reduced rapidly by it, and that Cr(V) species are produced, for an appreciable time, as part of that reduction process.

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