EVIDENCE FOR FREE RADICAL INVOLVEMENT IN THE TOXICITY AND CARCINOGENICITY OF CHROMATE DUSTS

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INTRODUCTION

Epidemiologic studies of workers in chromate-ore related industries,[1-3] and stainless steel welding and related occupations[4] have shown that they have about 20–40 times higher risk of throat or respiratory track cancer than controls. Although the actual carcinogenic substances were not identified in these statistical studies, Cr(VI) compounds (for example, calcium chromate, zinc chromate, and lead chromate) were implicated as the causative agents, whereas Cr(III) compounds were not suspected as carcinogens.[5] These suggestions were supported by laboratory studies wherein many Cr(VI) compounds produced sarcomas at the implant or injection sites.[1,5] Squamous cell carcinomas and adenocarcinomas closely resembling human lung cancer were induced by intrabronchial implants of calcium chromate in rats,[6,7] whereas Cr(III) oxide and Cr(III) sulfate did not induce any tumor formation.[6,7]

While the mechanism of the chromate-induced carcinogenicity is not fully understood, it is generally thought[8] that it involves some damage to DNA. Specifically, it has been reported that: a) the chromate ion, henceforth referred to as chromate, can pass through the cell membrane and enter the cell while CR(III) does not,[9] b) chromate does not interact with either native or denatured DNA[10] while Cr(III) does,[11] amd c) the final Cr-DNA complex isolated from cellular reactions of chromate is Cr(III)-DNA, with Cr(III) binding to the phosphate groups.[12,13] Thus the important question is: since Cr(III) cannot pass through the membranes, how does Cr(III)-DNA complex form? For this to happen, Cr(VI) must be reduced to its lower oxidation states,[14] ultimately to Cr(III), by some reductants in the cellular environment. Unless this reduction occurs, the DNA would not he damaged and therefore no chromate carcinogenicity would ensue. Thus the reduction of chromate to its lower oxidation states seems to be a key step in the chromate carcinogenicity.[15] One of the major reductants in cellular environments is thought to be glutathione (GSH), both outside and inside the cells.[16-18] Some evidence for the role of GSH in the chromate toxicity was provided by recent studies showing that exposure of hamster cells to non-toxic levels of added selemite increases the levels of GSH as well as the Cr(VI)-induced DNA strand breaks,[19] and that such DNA strand breaks in hepatocytes also change in direct proportion to the GSH content.[20,21] These observations were interpreted as implying that the reduction of chromate by GSH to some reactive intermediate is an important step in the chromate carcinogenicity.[20,21] In the present undertaking[22] we have used electron spin resonance (ESR) and spin-trap methodology to investigate the reduction of chromate by GSH and find evidence for the involvement of the glutathionyl radical (GS•) as well as Cr(V)-intermediates.

MATERIALS AND METHODS

ESR spectra were obtained at X-band (~9.7 GHz) using a Bruker ER200D ESR spectrometer. The magnetic field was calibrated with a self-tracking NMR Gaussmeter (Bruker, Model ER035M) and the microwave frequency was measured with a Hewlett-Packard (Model 5340A) frequency counter. The spin probes, α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (4-POBN) and 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), were purchased from Aldrich, and used without further purification since very weak or no spin-adduct signals were obtained from the purchased sample when used alone. $K_2Cr_2O_7$, purchased from Fisher, was used as a source for the chromate ion. All measurements were made at room temperature.

RESULTS AND DISCUSSION

Figure 1 shows some typical ESR spectra obtained. While an aqueous solution of 0.1 M spin trap, 4-POBN, containing either chromate or GSH alone, did not give any ESR signal, mixtures of chromate, GSH and 4-POBN together gave a spectrum which was composite of the spin adduct signal (sharp doublets of triplets) and those of Cr(V) (the broad peaks at g = 1.995 and g = 1.985)[23-26] (Figure 1a). About ten minutes later, when the signal from Cr(V) had decayed, a clear spectrum at g = 2.0061, consisting of only doublets of triplets, was obtained, whicb is assigned to the 4-POBN-GS adduct because of its strong similarity to the spectrum reported earlier[25] for the same adduct, the GS• radical being produced via reaction of GSH with α-chromanoxyl radical. The analysis of the spectrum (doublets of triplets) in Figure 1a gave the nitrogen hyperfine coupling a_N = 15.0 G and proton hyperfine coupling a_H = 2.3 G, which compare well with those (a_N = 15.13 G and a_H = 2.32 G) reported earlier.[25]

Additional support for this identification was obtained from spin-trap studies with DMPO. The ESR spectrum obtained using DMPO was composite of that of the spin adduct, a 1:2:2:1 quartet, and that of Cr(V), the broad peaks at g = 1.995 and g = 1.985, Figure 1b. The analysis of the spin-adduct spectrum gave a_N = 15.2 G and a_H = 15.9 G. These values are fairly close to those (a_N = 15.4 G and a_H = 16.2 G) reported earlier[26,27] for the DMPO-GS spin adduct.

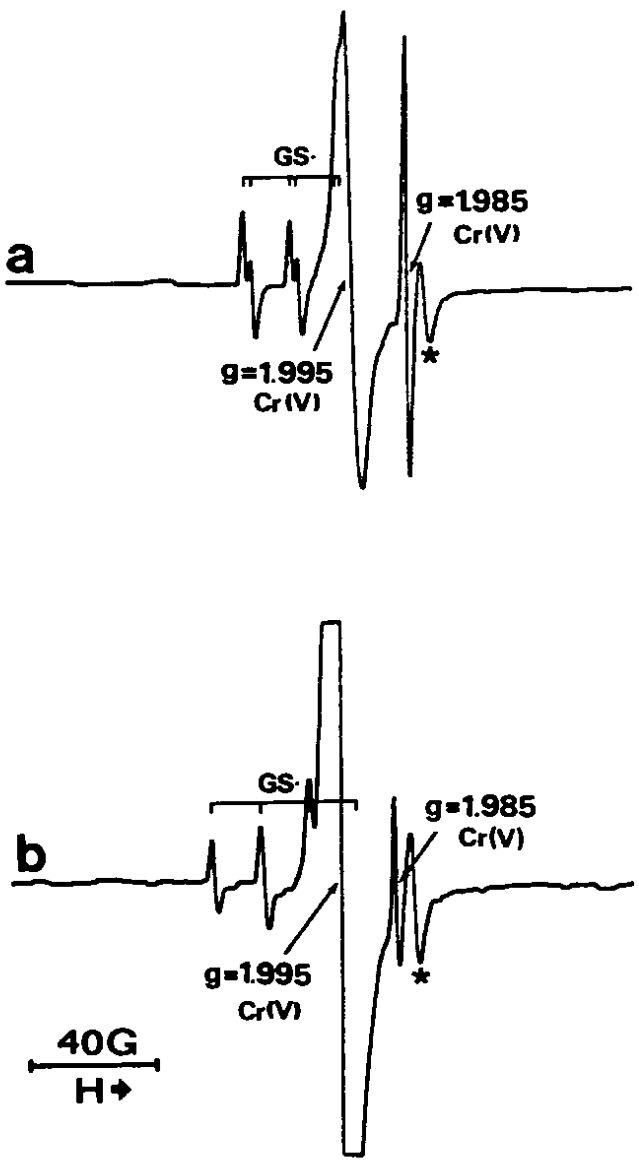

Figure 1. ESR spectra recorded 2 minutes after mixing a solution of $[K_2Cr_2O_7]$ = 0.015 M, [glutathione] = 0.15 M; (a) [4-POBN] = 0.1 M; pH = 4.0; (b) [DMPO] = 0.1 M; pH = 7.2. The asterisks indicate minor Cr(V) species.

Moreover the spin-adduct spectrum showed a rapid decrease with time essentially as described previously.[26,27]

The spin-trap studies showed that an increase in the amount of GSH causes to an increase in the spin adduct ESR signals until the intensity leveled off at a molar ratio of about fifteen to one of GSH to $K_2Cr_2O_7$. No spin-adduct ESR signal was detected for the molar ratio of less than one. We also find direct evidence for the formation of a fairly long-lived Cr(V) intermediate, but at molar ratios of higher than one of GSH to $K_2Cr_2O_7$, in contradictiom to an earlier report.[16] In agreement with other studies,[23,24] however, several different Cr(V) complexes were observed depending on the reaction conditions, as indicated by asterisks in Figure 1. We were able to isolate the dominant, g = 1.995, species with a yield of about 50 percent. The measured g-values for the powder spectrum are $g_{\parallel}$ = 2.007 and $g_{\perp}$ = 1.989, with little variation with temperature from 115 to 310 K. These values are typical of Cr(V) solids.[28] ESR measurements on samples redissolved in water gave spectra identical with those from the reaction mixtures (before isolation), showing the stability of this isolated product, and reaffirming its Cr(V) identification.

The above results also help understand two recent reports[20,21] showing that increased levels of GSH in the cells result in increased DNA damage by Cr(VI). Our detection of the formation of GS · and Cr(V) at high GSH levels, as true for the *in vivo* conditions,[10] suggests that the synergistic reactions of the Cr(V) intermediate and GS · are perhaps responsible for the increased Cr(VI)-induced DNA strand breaks at high GSH levels. It is thus felt that these results open up new avenues for understanding the mechanism of chromate-related carcinogenesis.

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Abbreviations used here: GSH, glutathione; GS·, glutathionyl radical; 4-POBN, α-(4-pyridyl-1-oxide)-N-tert-butylnitrone; DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; ESR, electron spin resonance.

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