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In vitro interaction of mutagenic chromium(VI) with red blood cells

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The interaction of mutagenic Cr(VI) with red blood cells has been studied by ESR spectroscopy. Signals of two Cr(V) species are observed almost immediately after contacting red cells with chromate(VI) aqueous solution at pH 7.4. The signal at $g_o = 1.985$, which decays within one hour, is attributed to a Cr(V) complex formed by glutathione due its reducing and chelating ability. The other signal at $g_o = 1.979$, which is distinctly more persistent, may indicate that some immobilization of the formed Cr(V) ions takes place on the macromolecular cell components, e.g. glycoproteins.

Glutathione; Chromium; ESR; (Red blood cell)

1. INTRODUCTION

Although chromium compounds are well recognized as potent inorganic mutagens or even carcinogens [1–5], the precise description of the active species as well as the mode of its action is still not available. It seems to be generally accepted that Cr(VI) is the initial oxidation state in the mutagenic pathway of this metal ion. It seems also to be chemically intelligible that the ultimate form bound to small cellular moieties like DNA or proteins is the Cr(III) ion. It was shown, among others, that Cr(III) ions may cross-link nucleic acids or condensate them very effectively [6–9]. A number of works also indicated very clearly that the intermediate oxidation state Cr(V), may play a critical role in the biochemical and chemical behaviour of chromium in the living systems [10–13]. Cr(V) is a highly reactive species and most likely is easily transported into the cell organella. Many molecules present in the living system, both in the membranes and inside the cells, may act as reducing agents for Cr(VI) ions. The extensive work of Connett and Wetterhahn [14] indicated several possibilities for chromium(VI) reduction by biomolecules. One of the most important representatives of reducing biomolecules is the tripeptide glutathione (γ-Glu-Cys-Gly, GSH) which is a very abundant molecule in the living cell. Although the detailed mechanism of the reduction of Cr(VI) to Cr(V) in the presence of GSH is not yet available [13,14,16,17], it is evident that some relatively long-lived species of Cr(V) are produced. Cr(V) ions can be stabilized very effectively by several natural ligands like e.g. glucose or other sugars [15,18]. In blood cells the interaction between GSH and chromium ions seems to be very likely (see e.g. [16]) and the direct interaction between the GSH and Cr(VI) may be an important source of Cr(V) ions in these cells. In order to clarify the problem of chromium reduction in the cell, we have performed the studies on the behaviour of Cr(VI) in the blood cells using the ESR technique and the results are presented in this communication.

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2. EXPERIMENTAL

Blood, with EDTA as anticoagulant, of normal adult was used. Plasma and buffy coat were carefully removed by aspiration after centrifugation. Red blood cells were washed three times in the buffer prior to use. Equal volumes of packed red blood cells and buffer containing 10 mM Cr(VI) as chromate were mixed and immediately transferred into a flat ESR cell. In order to block GSH, *N*-ethylmaleimide (NEM) was added to the red blood cell suspension [19] before addition of Cr(VI) ions (final concentration ranging from 0.5 to 1.5 mM). Lysates were made by freezing-thawing under nitrogen in order to avoid oxidation by air. The ESR spectra were recorded at room temperature on a Bruker 220 D instrument. The microwave frequency was 9.4 GHz, the radiation power 10 mW and the modulation amplitude 0.4–4.0 G.

3. RESULTS AND DISCUSSION

The interaction of Cr(VI) with red blood cells results in two ESR signals (fig.1) already several minutes after mixing chromate with the red cells. The narrow ESR signal at $g_o = 1.985$ decays within one hour. The other one at $g_o = 1.979$ is distinctly broader and vanishes within one day. In the samples treated with *N*-ethylmaleimide only one spectrum was observed at $g_o =$

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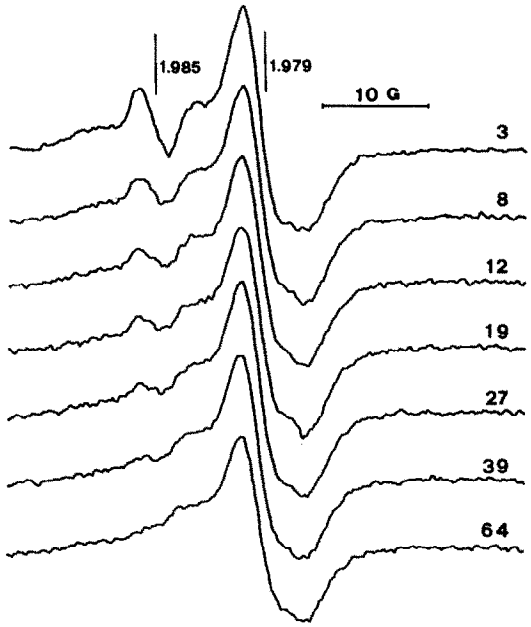

Fig.1. ESR spectra of Cr(V) species formed by interaction of chromate (10 mM) with red blood cells as a function of time (min).

1.979. In the latter samples the GSH reduction ability is inhibited totally by added NEM (due to covalent blocking of the GSH molecule). Thus, the signal at g_o = 1.985 can be attributed to the Cr(V)-GSH system. A further support to this assignment derives from the fact that equimolar (2.5 mM) aqueous solutions of GSH and chromate at pH 7.4 yield only the narrow spectrum at g_o = 1.985 (fig.2b), which has also been described previously [13]. This species also decays rapidly yielding Cr(III) as the final reduction product. Furthermore, the addition of GSH to the sample containing red blood cells and Cr(VI) leads to a distinct increase of the signal at g_o = 1.985. This unequivocally indicates that the GSH molecule is a reducing agent of chromium(VI) in the cell but not the only one. GSH (or its oxidized form) is also a chelating agent for Cr(V) ions produced [12,13] although the formed species easily decay towards Cr(III) complexes. It is very likely that Cr(V) ions obtained by the reduction of chromate with GSH are transferred to the chelating agents more effectively than GSH itself or that GSH reduces Cr(VI) ions bound to other molecules. The experiment shown in fig.3a clearly suggests such a possibility. The addition of a sugar ligand to the chromate-GSH solution results almost immediately in the formation of the Cr(V) complexes with the sugar molecules and these latter species are distinctly more stable than those with glutathione. The reduction ability of sugar molecules is much less effective since within the same time no Cr(V) ESR signal is observed in the solutions containing only chromate and sugar. The more detailed description of the latter experiments will be published elsewhere [20].

The ESR spectrum at g_o = 1.979 indicates very clearly that there are other reducing agents too, in the red blood cells. The broader shape of the signal may indicate that some immobilization of the formed Cr(V) ions takes place on macromolecular components, e.g. glycoproteins, as shown by the comparison with the spectrum of a Cr(V) species bound to a polymeric matrix (fig.3b). These components can more effectively stabilize this oxidation state. Cr(VI) reduction in the blood plasma which does not contain reduced glutathione results in the spectrum centered at g_o = 1.979. This experiment again provides evidence of the direct involvement of GSH in the reduction of chromate inside the cell, and also supports the presence of other efficient Cr(VI) reductants (possibly enzymes) in the living systems. The ESR spectrum of Cr(VI) added to the lysed cells is identical to those obtained for the intact red blood cells. This result substantiates the fact that chromate ions may easily enter the cells by crossing the membrane and then undergo the reduction processes as discussed above.

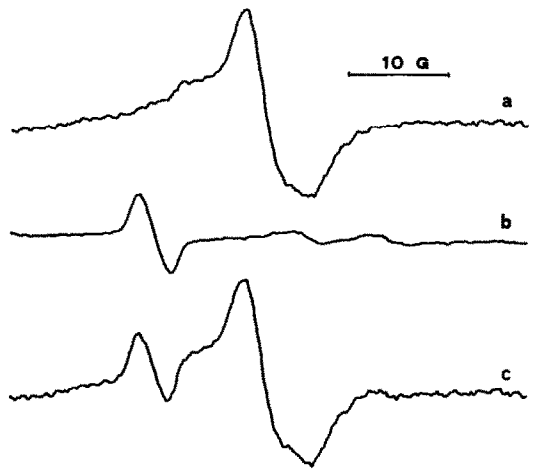

Fig.2. ESR spectra of Cr(V) species formed: (a) by interaction of chromate (10 mM) with red blood cells after 64 min (cf. fig.1); (b) by interaction of chromate (2.5 mM) with GSH in 1:1 molar ratio. Spectrum c is obtained by addition of the spectra a and b.

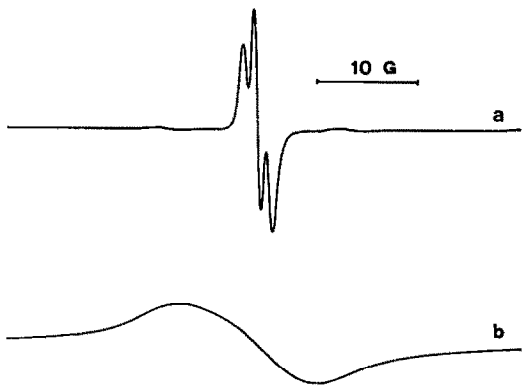

Fig.3. ESR spectra of Cr(V) species obtained a few minutes after interaction of chromate with (a) D-mannose or (b) polygalacturonic acid at pH 7.4 in the presence of GSH. Spectrum a was recorded in solution, spectrum b was recorded in the solid state.

4. CONCLUSIONS

Chromium(VI) ions penetrate red blood cells undergoing reduction to Cr(V) intermediates and further to Cr(III) ions. Glutathione is an important intracellular reducing agent for Cr(VI) ions. It does not, however, stabilize effectively the Cr(V) species formed. GSH, however, is not the only reductant inside the cell. Sugar containing molecules could be competitive in both reduction of Cr(VI) and stabilization of the Cr(V) intermediate. It is thus likely that part of the chromium ions which undergo the reduction process with GSH is already bound to the other ligands which are more effective in the stabilization of the (+5) oxidation state than glutathione itself. The further reduction of the Cr(V) ion anchored to a biomolecule to Cr(III) can also modify the chemical structure of the ligand, altering the primary function of the natural system.

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