

Reaction of Chromium(VI) with Ascorbate Produces Chromium(V), Chromium(IV), and Carbon-Based Radicals

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Reaction of potassium dichromate with sodium ascorbate was studied by EPR spectroscopy at room temperature, in 0.10 M *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES), phosphate, cacodylate, and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffers at pH 7.0, in the presence of 0.10 M spin trap [5,5-dimethyl-1-pyrroline 1-oxide or 2-methyl-*N*-(4-pyridinylmethylene)-2-propanamine *N,N'*-dioxide]. Chromium(V), ascorbate radical, CO₂⁻, and other carbon-based spin trap-radical adducts were observed. Chromium(V), CO₂⁻, and the carbon-based radicals were observed at low ratios of ascorbate to chromium, and ascorbate radical was observed at high ratios of ascorbate to chromium. The presence of Cr(IV) was detected indirectly by reaction with Mn(II) and a subsequent decrease in the Mn(II) EPR signal. More Cr(IV) was found for the higher reaction ratios of ascorbate to Cr(VI). The only buffer effect observed was a relative decrease of the Cr(V) signal in Tris-HCl vs HEPES, phosphate, and cacodylate buffers, no change in the radical adducts was observed. There was no evidence for reactive oxygen species as intermediates in this reaction. Addition of the singlet oxygen trap 2,2,6,6-tetramethyl-4-piperidone hydrochloride showed no 2,2,6,6-tetramethyl-1-piperidinyloxy radical formation. The Cr(V) species did not react with dioxygen, and dioxygen did not affect the formation of carbon-based radicals. A mechanism consistent with these observations is discussed.

Introduction

Chromium(VI) is a known carcinogen in humans and animals (1, 2); however, Cr(VI) does not react with DNA *in vitro* unless reducing agents are added. The uptake-reduction model (3) proposes that Cr(VI) is reduced intracellularly, producing reactive intermediates that target DNA. These potentially genotoxic species include Cr(V), Cr(IV), Cr(III), free radicals, and reactive oxygen species. Although glutathione has been the focus of major study (4), there has been renewed interest in the reaction between Cr(VI) and ascorbate (vitamin C) since a number of reports have suggested that ascorbate is also involved in the reductive pathway of Cr(VI) *in vivo*. Ascorbate was found to be more reactive than glutathione for reduction of Cr(VI) in rat lung (5). Ascorbate has been shown to be the major reductant of Cr(VI) in rat lung, kidney, and liver ultrafiltrates (6, 7). Cr-DNA binding resulting from the reaction of Cr(VI) with DNA in the presence of rat lung ultrafiltrates was correlated to ascorbate-dependent metabolism of Cr(VI) (7). Increases in the ascorbate levels of V-79 cells affected relative levels of Cr(VI)-induced DNA damage, specifically decreasing alkali-labile sites and increasing DNA-protein cross-links and cytotoxicity (8). Ascorbate was found to block extracellular dissolution of lead chromate particles and to decrease genotoxicity of Cr(VI) in Chinese hamster ovary cells (9).

One basic question that remains to be fully answered is the role of cellular reductants such as ascorbate and glutathione toward activation of Cr(VI) to reactive Cr(V), Cr(IV), or radicals vs detoxification of Cr(VI) by reduction to the stable end product Cr(III) and/or scavenging of

radicals. It is a major hypothesis in this laboratory that Cr(VI)-induced DNA damage will be dependent on the relative concentrations of these small molecule reductants, and that the different types of DNA damage observed in different animal tissues and cell lines will be related to different intracellular stoichiometries of Cr(VI) and reducing agents. One manifestation of the different reactivity of Cr(VI) with cellular reductants may be two pathways of Cr(VI)-induced DNA damage, namely a chromium-mediated pathway and a free radical pathway. Although the understanding of Cr(VI) metabolism *in vivo* will likely involve a combined effect of ascorbate and glutathione, one of the first steps is to understand the reaction of Cr(VI) with ascorbate alone.

In light of these goals the previous literature on Cr(VI)/ascorbate chemistry is incomplete. A previous EPR study (10) in 1 M Tris-HCl and 0.10 M *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES)¹ buffers at pH 7-8.5, at room temperature (RT) had shown that the *in vitro* reduction of Cr(VI) by ascorbate produces Cr(V) and ascorbate radical as unstable intermediates; however, spin traps were not used to look for more reactive free radicals. The interaction of Tris-HCl buffer with Cr(V) was reported (10) but the effect of buffer on the stability of Cr(V) was not determined, which is crucial for evaluating the potential reactivity of Cr(V) toward DNA *in vitro* (11), nor was an attempt made to quantitate the Cr(V) or ascorbate radical formed. While

¹ Abbreviations: asch⁻, ascorbate; asc⁻, ascorbate radical; DHA, dehydroascorbate; DKG, 2,3-diketogularic acid; DMPO, 5,5-dimethyl-1-pyrroline 1-oxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; EHBA, 2-ethyl-2-hydroxybutyric acid; HEPES, *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]; POBN, α -(4-pyridyl 1-oxide)-*N*-*tert*-butyl nitron, 2-methyl-*N*-(4-pyridinylmethylene)-2-propanamine *N,N'*-dioxide; R, carbon-based radical; RT, room temperature; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy.

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