

Reduction of Chromium(VI) by Ascorbate Leads to Chromium–DNA Binding and DNA Strand Breaks in Vitro[†]

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Received July 12, 1994; Revised Manuscript Received November 7, 1994[⊗]

ABSTRACT: Chromium(VI) is a known human carcinogen which requires intracellular reduction for activation. Ascorbate (vitamin C) has been reported to function as a major reductant of Cr(VI) in animals and cell culture systems. The reaction of Cr(VI) with varying concentrations of ascorbate was studied under physiological conditions in vitro in order to determine the types of reactive intermediates produced and to evaluate the reactivity of these intermediates with DNA. Reactions of 1.8 mM Cr(VI) with 0–18 mM ascorbate at pH 7.0 in *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES; 0.10 M) and tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl; 0.050 M) buffers were studied by electron paramagnetic resonance and UV/visible spectroscopy. Cr(V) and carbon-based free radical adducts of 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) were observed at 0.5 to 1 and 1 to 1 reactions of ascorbate to Cr(VI). Levels of Cr(V) were higher for reactions in HEPES buffer, and levels of carbon-based radicals were higher in Tris·HCl buffer. Levels of Cr(IV) and Cr(III) increased with increasing concentration of ascorbate in both buffers. Reaction of Cr(VI) with varying ascorbate in the presence of calf thymus DNA or pBR322 DNA resulted in Cr–DNA adducts and plasmid relaxation, respectively. Maximum binding of Cr to DNA was observed for the 1:1 reaction ratio of Cr(VI) with ascorbate in both HEPES and Tris·HCl buffers, but total Cr bound to DNA was 8-fold lower in Tris·HCl than HEPES buffer. Preincubation of Cr(VI) with ascorbate before reaction with DNA decreased Cr–DNA binding to background levels. Preincubation of Cr(III) with ascorbate resulted in only low Cr–DNA binding. Levels of Cr–DNA binding were higher with single-stranded vs double-stranded DNA. Reactions with ¹⁴C-labeled ascorbate produced no cross-linking of ascorbate to DNA. Maximum plasmid relaxation was observed for the 1:1 ascorbate to Cr(VI) ratio in both buffers; however, single-strand breaks were 2-fold higher in Tris·HCl than HEPES buffer. Reactions with plasmid in the presence of DMPO quenched formation of single-strand breaks. Interpretation of these results in light of the spectroscopic studies suggested that Cr(V) and carbon-based radicals were responsible for Cr–DNA adducts and DNA single-strand breaks, respectively.

Chromium(VI) is a known carcinogen in humans and animals (IARC, 1987), and chromate compounds are mutagenic and genotoxic (De Flora & Wetterhahn, 1989). Although a link between Cr(VI) and lung cancer was established in the 1930s (reviewed in Léonard & Lauwerys, 1980), the mechanism of Cr(VI)-induced cancer as well as the ultimate genotoxic species are unknown. Chromium(VI) requires intracellular reduction for activation (Connett & Wetterhahn, 1983). The primary reducing agents based on kinetics and cellular concentrations are ascorbate (vitamin

C) and glutathione (GSH;¹ γ -glutamylcysteinylglycine) (Connett & Wetterhahn, 1985), but other small molecules and enzyme systems are capable of reducing Cr(VI) under physiological conditions. The in vivo reduction of Cr(VI) can theoretically produce many reactive intermediates that could target DNA, namely, Cr(V), Cr(IV), free radicals, and reactive oxygen species, as well as the final end product Cr(III) (Connett & Wetterhahn, 1983). The DNA damage resulting from Cr(VI) exposure has recently been reviewed (De Flora & Wetterhahn, 1989; De Flora et al., 1990). The major lesions observed in vivo include DNA–protein cross-links, DNA interstrand cross-links, Cr–DNA adducts, and single-strand breaks. Oxidative nucleotide base modification as 8-oxo-2'-deoxyguanosine has recently been observed in Cr(VI)-treated chick embryos (Misra et al., submitted for publication). These Cr(VI)-induced DNA lesions presum-

[†] Supported by PHS Grant CA34869 awarded by the National Cancer Institute, DHHS (K.E.W.). The EPR spectrometer was purchased with funding from NSF Grant CHE-8701406. D.M.S. was supported by a postdoctoral fellowship from the Norris Cotton Cancer Center, Dartmouth/Hitchcock Medical Center, and an NRSA fellowship (CA59292) from the National Cancer Institute, DHHS. L.J.K. and K.D.C. were supported by the Research Experiences for Undergraduates Program funded by the National Science Foundation (NSF CHE-9100493). K.D.C. was also supported by a Dartmouth College Presidential Scholar Research Assistantship. P.H.G. was supported by a Howard Hughes Undergraduate Biological Sciences Research Internship. L.S.P. was supported by a Dartmouth College Presidential Scholar Research Assistantship and a Waterhouse Research Grant.

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[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1994.

¹ Abbreviations: asc^{•-}, ascorbate radical anion; CT, calf thymus; DABA, diamminobenzoic acid; DETAPAC, diethylenetriaminepentaacetic acid; DHA, dehydroascorbate; DKG, 2,3-diketogularic acid; DMPO, 5,5-dimethyl-1-pyrroline 1-oxide; EHBA, 2-ethyl-2-hydroxybutyric acid; EPR, electron paramagnetic resonance; GSH, glutathione, γ -glutamylcysteinylglycine; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; RNase A, ribonuclease A; RT, room temperature (24–26 °C); TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy radical; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride.