Potentially Deadly Carcinogenic Chromium Redox Cycle Involving Peroxochromium(IV) and Glutathione

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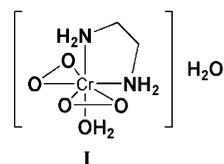
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Abstract: Peroxochromium(IV) complexes are putative DNA-damaging and mutagenic agents in chromium(VI)-mediated carcinogenesis. The reaction between aquaethylenediaminebis(peroxo)chromium(IV) and glutathione at neutral pH exhibits a cyclic redox process displaying a persistent recycling of Cr(IV) and Cr(VI) with the intervention of chromium(V) intermediates. The coordination by a glutathione molecule triggers an autooxidation of the Cr(IV)−peroxo complex to Cr(VI) via an internal electron-transfer process followed by reduction to Cr(IV) via metastable chromium(V) intermediates. The cycle is repeated by the second peroxo species. The Cr(V) and -(IV) intermediates have been characterized as mono- and bisglutathionato complexes with or without a coordinated peroxo moiety. These intermediates are capable of damaging DNA, as evidenced by single strand breaks and DNA oxidation. The implication here is that the potential for a persistent, if not perpetual, deadly chromium carcinogenic cycle exists in the cellular milieu through the assistance of molecular oxygen and glutathione.

It is universally accepted that chromium(VI) compounds are highly carcinogenic and mutagenic agents.[1] However, the effect of chromium on human health is perhaps one of the most controversial issues because of the dual role of chromium as a carcinogen and a nutritional supplement.[2] Since neither Cr(VI) nor Cr(III) species alone contribute to DNA damage, hypervalent chromium species, usually Cr(V) and Cr(IV), are considered to be the putative DNA-damaging agents.[1] The correlation between oxygen consumption and single strand breakage (SSB) in DNA by Cr(VI) suggests the participation of an active oxygen-coordinated chromium species in this process.[3] Among many potentially DNA-damaging species, peroxochromium(IV) complexes have been implicated in damaging and mutating DNA.[4] We have extensively studied DNA damage by oxochromate(V) species,[5] but because of the paucity of stable and water-soluble Cr(IV)−peroxo complexes, their redox properties and ability to damage DNA have not been studied. More importantly, it is unknown how these peroxo complexes might interact with cellular reducing agents and generate additional potential carcinogens. To the best of our knowledge, the only water-soluble and stable peroxochromium(IV) complexes that exist to date are those reported by Hoffman[6] and House and Garner.[7] Although the redox properties of these complexes have been studied by Gould and co-workers,[8] mostly in acidic conditions, no reports to date have dealt with their reactions with glutathione (GSH) at neutral pH. Since GSH is a key player in chromium carcinogenesis because of its abundance and its ability to reduce chromate to metastable chromium carcinogens, we have examined the reaction of aquaethylenediaminebis(peroxo)chromium(IV) hydrate (**I**) with GSH at neutral pH. Here we report an unusual reaction between **I** and GSH that exhibits a redox process displaying a persistent recycling of Cr(IV) and Cr(VI) with the intervention of chromium(V).

I

In compound **I,** chromium is coordinated to two peroxo anions, an ethylenediamine molecule and a water molecule, giving the formulation $[Cr(H_2O)(en)(O_2)_2]$. X-ray diffraction data[9] support a pentagonal bipyramidal geometry for the compound. Furthermore, Dalal and co-workers[10] used magnetic susceptibility measurements to establish that the ground state of compound **I** is a spin triplet, as expected for the +4 oxidation state of chromium. Compound **I** is stable at or near neutral pH, as evidenced by the lack of changes in the UV−vis spectra over 24 h. The existence of an acid−base equilibrium in the pH range 6−8 was not detected in the UV−vis spectra, since no spectral changes were observed in this pH region.

The reaction[11] between **I** and a 10-fold molar excess of GSH at pH 7.0 resulted in a Cr(III) product coordinated to GSH. Molecular mass determination by mass spectrometry revealed that the Cr(III) product contains up to four glutathione molecules, reminiscent of a Cr(III) complex reported by O'Brien[12] from a reaction between Cr(VI) and GSH. Spectroscopic titration at 372 nm revealed that each mole of diperoxo complex consumed 9 mol of GSH, consistent with the reaction shown in eq 1.

$$2\mathbf{I} + 18GSH \rightarrow 2[Cr(III)(en)(GS)_4]^- + 5GSSG + 8H_2O + 2H^+ \tag{1}$$

The reaction was monitored by UV−vis spectroscopy at 372 nm and exhibited a triphasic kinetic profile with two distinct phases of increase followed by a decrease in absorbance, indicating the presence of at least two intermediates. The two-phase increase in absorbance lasted ∼3 h, while the decrease in absorbance was observed up to 12 h. Three Cr(V) species were detected as intermediates by EPR spectroscopy as well. These species exhibited *g* values of 1.996, 1.986, and 1.983 (Figure 1), and their intensity−time profiles and hence the relative distributions were dependent on the concentration of GSH relative to Cr(IV).

In addition to chromium(V) intermediates, a thiyl radical rather than a hydroxyl radical was detected using the spin trap DEPMPO.[14] One of the signals (*g* = 1.983) was formed and disappeared more quickly than those the other two Cr(V) intermediates. The other two signals at *g* = 1.986 and *g* = 1.996 persisted for a longer time (∼2 h) and showed similar intensity−time profiles, implying that

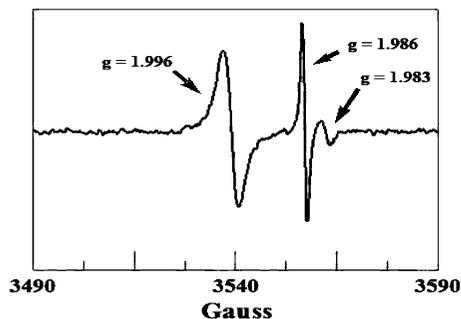[†] Present address: Department of Ophthalmology, Mayo Clinic, Rochester, MN 55905.

Figure 1. X-band EPR[13] spectrum of the reaction mixture containing **I** (1.0 mM) and GSH (40.0 mM) in phosphate buffer (100 mM, pH 7.0). The g values and line widths of the signals are g = 1.996, 4.84 G; g = 1.986, 1.24 G; and g = 1.983, 2.20 G.

these intermediates were formed in a parallel process. In addition, a very broad signal (peak-to-peak separation = 260 G) at g = 1.975 was observed when nearly all of the Cr(V) species were depleted.

The observation of a triphasic kinetic profile and detection of three Cr(V) species can be adequately described by the following sequence of events. First, chromium(IV) is oxidized to Cr(VI) by a coordinated peroxo moiety, and that process must be aided by GSH since the Cr(IV) center alone does not undergo self-oxidation. Although such a process must go through a sequential one-electron transfer, we did not detect any hydroxyl radicals, so the intervening Cr(V) complex must be quickly oxidized to Cr(VI). The chromium(VI) species thus generated undergoes reduction to Cr(IV) via an intervening Cr(V) species by successive one-electron reduction processes involving two GSH units. These Cr(V) and Cr(IV) species most likely contain peroxo and GS$^-$ anions, and the Cr(V) species most likely is one of the observed intermediates captured by EPR spectroscopy before it is reduced to Cr(IV). Subsequent oxidation of the Cr(IV) species containing a coordinated GSH by the second peroxo unit takes it back to Cr(VI) again, with and without the aid of another GSH unit, thus generating two additional Cr(VI) transients. In fact, the existence of a Cr(IV)−monoperoxo species coordinated to two GSH units was evident in the mass spectrum, as discussed below. Reductions of the two Cr(VI) species by GSH is expected to generate two additional Cr(V) species, which are then reduced to Cr(III) through Cr(IV). Therefore, the two relatively longer-lived Cr(V) intermediates can be identified as mono- and bisglutathionatoCr(V) complexes not containing a peroxo moiety.

Various chromium(V) species have been reported by several groups in reactions between Cr(VI) and GSH.[12,15−20] Two of these species observed in our reaction at g = 1.986 and 1.996 are in agreement with those reported by Shi and Dalal,[16,17] who also observed the dependence of signal intensity on GSH concentration. We agree with the earlier assignments that these species are likely to be mono- (g = 1.986) and bisglutathionato (g = 1.996)[16,17] chromium(V) complexes. The signal at g = 1.983 can be attributed to a peroxochromate(V) species coordinated to a GSH molecule on the basis of previous reports in which an intermediate with a similar g value was observed when mixtures of chromate and GSH were exposed to saturated oxygen or hydrogen peroxide.[15]

The broad EPR peak that persisted near the end of the reaction after the depletion of the Cr(V) was not visible without selecting an unusually large magnetic field width. Although Cr(IV) species are not usually observed at room temperature, because of either a large zero-field splitting or the large spin−orbit coupling constants exhibited by the triplet ground states, Dalal and co-workers[21] have reported EPR spectra of some oxochromium(IV) complexes that do show very broad signals. The broad EPR signal at g = 1.975

perhaps represents a Cr(IV) species. This assignment is consistent with the MS/MS characterization (Figure 2) of an intermediate that most likely represents a Cr(IV) species. Both the intensity−time profile and fragmentation patterns of the ion with m/z 757 support the formulation of this species as a Cr(IV) intermediate, which then fragments into a variety of species, as shown in Figure 2.
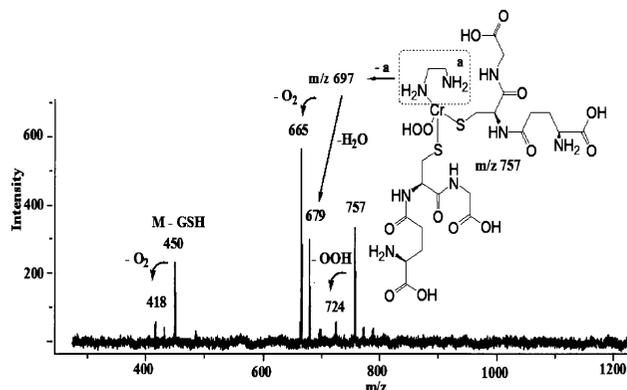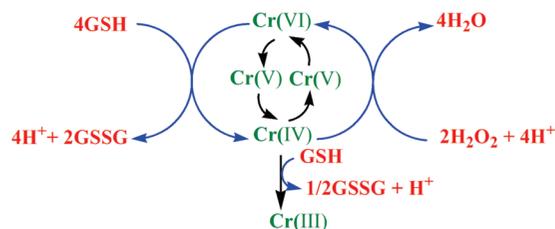

Figure 2. Proposed fragmentation patterns of the transient m/z 757 peak detected by positive-ion MS.[13] The existence of the positive ion might be due to either protonation of peroxo species as shown or protonation of an uncoordinated end of ethylenediamine or GSH. The fragmentation pattern includes a species (m/z 697) not seen in the spectrum, perhaps because of a lack of stability.

The fragmentation can be rationalized with the loss of the peroxo ligand via three different pathways. The observation of this additional intermediate by mass spectrometry may not be inconsistent with the UV−vis kinetic profile. The Cr(V)− and Cr(IV)−monoperoxo glutathione complexes may display similar molar absorptivities, and therefore, a third intermediate may not be detected in the absorbance−time profile. Scheme 1 depicts the cyclic nature of the reaction.

Scheme 1. Schematic Representation of a Cyclic Reaction between Aquaethylenediaminebis(peroxo)chromium(IV) and Glutathione That Generates Metastable Cr(V) and Cr(IV) Intermediates[a]

[a] Glutathione triggers the oxidation of the metal by the coordinated peroxo groups, producing Cr(VI) via transient Cr(V). Next, this Cr(VI) is reduced to Cr(V) and then to monoperoxoCr(IV). The cycle is repeated in excess glutathione until the complex is reduced to tetraglutathionatochromium(III) complexes.

The present study clearly pinpoints the potential for the formation of both Cr(V)− and Cr(IV)−peroxo species in the presence of glutathione in a cyclic manner. During this cyclic process, if additional peroxo compounds were to form by reactions with activated oxygen, a persistent, if not perpetual, and deadly chromium carcinogenic cycle could potentially occur in the cellular milieu. Finally, the ability of GSH-assisted DNA breakage by the peroxo-chromium(IV) complex was ascertained from a reaction between **I** and the 28-mer 5′-GATCTAGTAGGAGGACAAATAGTGTTTG-3′. Compound **I** alone did not show SSB, while such breakage was indeed observed by ^{31}P NMR spectroscopy in the presence of GSH (Figure 3).
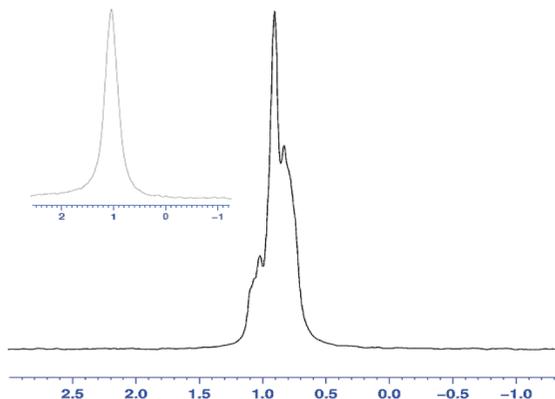
Figure 3. ^{31}P NMR spectrum[13] of a 28-mer in the presence of **I** (0.15 mM) and GSH (0.45 mM), exhibiting three distinct signals and two shoulders due the cleavage of the oligomer. The inset in the figure is the spectrum of the oligomer and **I** in the absence of GSH.

In the absence of GSH, a broad ^{31}P resonance at 0.95 ppm due to heterogeneity of the chemical shifts of magnetically nonequivalent phosphorus nuclei in the sequence was observed. In the presence of GSH, three signals at 1.05, 0.91, and 0.84 ppm were observed. We do not know, however, which intermediate is responsible for such breakage. Moreover, extensive DNA damage was observed when the reaction was carried out in the presence of hydrogen peroxide and GSH, signifying the importance of a persistent cyclic process in the DNA damage.

References

(1) Sedman, R. M.; Beaumont, J.; McDonald, T. A.; Reynolds, S.; Krowech, G.; Howd, R. *J. Environ. Sci. Health, Part C* **2006**, *24*, 155–182.

(2) Vincent, J. B. *Sports Med.* **2003**, *33*, 213–230.

(3) O'Brien, P.; Kortenkamp, A. *Environ. Health Perspect.* **1994**, *102*, 3–10.

(4) (a) Yasuji, H.; Kiyoko, N.; Sadahiro, K.; Tamio, M. *J. Health Sci.* **1999**, *45*, 329–332. (b) Yasuji, H.; Kiyoko, N.; Sadahiro, K.; Tamio, M. *J. Health Sci.* **2000**, *46*, 75–80.

(5) (a) Bose, R. N.; Fonkeng, B. S.; Moghaddas, S.; Stroup, D. *Nucleic Acids Res.* **1998**, *26*, 1588–1596. (b) Bose, R. N.; Moghaddas, S.; Mazzer, P. A.; Dudones, L. P.; Joudah, L.; Stroup, D. *Nuleic Acids Res.* **1999**, *27*, 2219–2226. (c) Joudah, L.; Moghaddas, S.; Bose, R. N. *Chem. Commun.* **2002**, 1742–1743. (d) Mazzer, P. A.; Maurmann, L.; Bose, R. N. *J. Inorg. Biochem.* **2007**, *101*, 44–55.

(6) Hoffman, K. A. *Chem. Ber.* **1906**, *39*, 3181.

(7) House, D. A.; Garner, C. S. *Inorg. Chem.* **1966**, *5*, 840–844.

(8) (a) Ghosh, S. K.; Laali, K.; Gould, E. S *Inorg. Chem.* **1988**, *27*, 4224–4228. (b) Ghosh, S. K.; Gould, E. S. *Inorg. Chem.* **1989**, *28*, 1538–1542.

(9) Stomberg, R. *Nature* **1965**, *207*, 76.

(10) Cage, B.; Leniek, R.; Dalal, N. *J. Appl. Phys.* **2000**, *87*, 610–612.

(11) Typical reaction mixtures consisted of **I** (0.25−2.0 mM) and GSH (4.0−60.0 mM) in phosphate buffer (0.10 M, pH 7.0) at ambient temperature (∼22 °C).

(12) O'Brien, P.; Pratt, J.; Swanson, F. J.; Thornton, P.; Wang, G. *Inorg. Chim. Acta* **1990**, *169*, 265–269.

(13) Experimental: Compound **I** was prepared by the method of House and Garner.[7] EPR experiments were performed on a Bruker EMX instrument, and *g* values and hyperfine splitting were determined with reference to DPPH. Typical data acquisition parameters were as follows: frequency window, 3540 ± 100 G; acquisition time, 100 s; modulation frequency, 100 kHz; modulation amplitude, 1.000 G; attenuation, 10 dB. Mass spectrometric measurements were carried out on a Thermo Finnegan LCQ Advantage instrument in positive polarity mode with a capillary temperature of 200 °C, a source voltage of 4.0 kV, and a capillary voltage of 42 V. ^{31}P NMR experiments were performed on a Bruker 500 MHz instrument in 10% D_2O in bis−tris buffer. At the end of the reaction, the mixture was treated with EDTA (10 mM) to bind Cr(III).

(14) Abbreviations: DEPMPO, 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

(15) Kortenkamp, A.; Casadevall, M.; Faux, S. P.; Jenner, A.; Shayer, R. O. J.; Woodbridge, N.; O'Brien, P. *Arch. Biochem. Biophys.* **1996**, *329*, 199–207.

(16) Shi, S.; Dalal, N. S. *Environ. Health Perspect.* **1994**, *102*, 231–236.

(17) Shi, X.; Dalal, N. S. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 137–142.

(18) Goodgame, D. M. L.; Joy, A. M. *J. Inorg. Biochem.* **1986**, *26*, 219–224.

(19) Aiyar, J.; Berkovits, H. J.; Floyd, R. A.; Wetterhahn, K. E. *Environ. Health Perspect.* **1991**, *92*, 53–62.

(20) Bose, R. N.; Moghaddas, S.; Gelerinter, E. *Inorg. Chem.* **1992**, *31*, 1987–1994.

(21) Luo, H.; Lu, Y.; Mao, Y.; Shi, X.; Dalal, N. S. *J. Inorg. Biochem.* **1996**, *64*, 25–35.

JA102173V