Activation of Molecular Oxygen during the Reactions of Chromium(VI/V/IV) with Biological Reductants: Implications for Chromium-Induced Genotoxicities[1]

Peter A. Lay* and Aviva Levina

Contribution from the School of Chemistry, University of Sydney, New South Wales 2006, Australia

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Abstract: The first systematic study on the roles of O_2 in the reactions of Cr(VI/V/IV) with major intracellular reductants, cysteine (**1**), glutathione (**2**), and ascorbic acid (**3**) as well as with vitamin E analogue Trolox (**4**), has been performed. The reactions of **1**−**3** with Cr(VI) (aqueous buffer solutions, pH = 4.5−7.5, 25 °C) led to a slow O_2 consumption (measured by a Clark oxygen electrode). The reactions of **1**−**3** with the relatively stable Cr(V) and Cr(IV) 2-ethyl-2-hydroxybutanoato complexes under the same conditions were accompanied by fast O_2 consumption. The O_2 consumption during the reactions of Cr(VI/V/IV) with **1**−**3** did not lead to a significant accumulation of H_2O_2 (determined with catalase). No significant O_2 consumption was detected for the reactions of Cr(VI/V/IV) with **4**. To reveal the mechanisms of O_2 activation, the kinetics of the Cr(V/IV) reactions with **1**−**4** at pH 4.5 and 7.5 were studied by stopped-flow UV−visible spectrophotometry; and the kinetic data were processed by the global analysis method. The stoichiometries and products of these reactions were studied by UV−visible, CD, and EPR spectroscopies. The proposed mechanism of O_2 activation includes oxidations of **1**−**4** by Cr(V/IV) to produce organic radical intermediates, which then react with O_2 in chain processes. No evidence was found for the direct activation of O_2 by the Cr compounds. Implications of the proposed mechanism to the DNA damage induced by the Cr(VI) + reductant + O_2 systems have been discussed.

Introduction

Chromium compounds are among the most important occupational carcinogens found in industry.[2] The ability of Cr(VI) to oxidize biomolecules with the formation of Cr(III) complexes is believed to be responsible for the mutagenicity and carcinogenicity of Cr(VI).[3] However, neither Cr(VI) nor Cr(III) alone cause appreciable DNA damage (which is a necessary but not sufficient first step en route to cancer).[3] Wetterhahn and co-workers[4] first proposed that reactive Cr(V) and Cr(IV) intermediates, formed during the course of Cr(VI) reactions with intracellular reductants, may be the DNA-damaging species. Indeed, treatments of isolated DNA with Cr(VI) in the presence of reductants (such as AsA or GSH) led to various kinds of DNA damage, including single strand breaks, and formation of Cr(III)−DNA complexes and DNA−protein cross-links.[3] The abilities of the relatively stable Cr(V) and Cr(IV) 2-hydroxycarboxylato complexes to cleave DNA in vitro and to cause mutations in both bacterial and mammalian cells have been first established by Lay and co-workers.[5] Recent results of Shi et al.[6] show that Cr(V) complexes are generated in living organisms exposed to Cr(VI), which supports the hypothesis about the role of Cr(V) (and possibly Cr(IV)) intermediates in Cr(VI)-induced genotoxicities.

Experimental evidence is mounting for a role of molecular oxygen in Cr-induced genotoxicities. Sugden et al.[7] found that Cr(VI)-induced mutations in certain strains of *Salmonella* are observed only in the presence of O_2. Kortenkamp and co-workers[8] have shown that the absence of O_2 (or the presence of catalase) strongly reduces the in vitro DNA damage induced by the Cr(VI) + AsA and Cr(VI) + GSH systems. These authors initially suggested that hydroxyl radicals formed in O_2-dependent Fenton-like reactions were the ultimate active species in the abovementioned systems.[9] However, further studies have shown that the nature of the DNA damage observed is

(1) Abbreviations: AsA = *L*-ascorbic acid; Cys = *L*-cysteine; DHAA = *L*-dehydroascorbic acid; DMPO = dimethyl-1-pyrroline *N*-oxide; DMSO = dimethyl sulfoxide; ehbaH_2 = 2-ethyl-2-hydroxybutanoic acid; GSH = glutathione (γ-glutamylcysteinylglycine); HEPES = *N*-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid]; Red = reductant; SOD = superoxide dismutase; Trolox = 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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inconsistent with the action of •OH.[3b,8,10] The extent of DNA damage was correlated to the presence of both Cr(V) intermediates (observed by EPR spectroscopy) and O_2, which led to suggestion that the possible DNA-damaging species are Cr(V/IV) peroxo or superoxo complexes.[3b,8,11] Recently, Zhang and Lay[12] reported the EPR spectroscopic evidence for the formation of Cr(V) peroxo and peroxo−ascorbato complexes during the Cr(VI) reduction by AsA in aerated aqueous media. Maximal concentrations of Cr(V) peroxo species corresponded[12] with the conditions of maximal DNA damage in vitro by the Cr(VI) + AsA system.[3]

Reported evidence for the O_2 activation in the Cr(VI) + Red reactions under biologically relevant conditions includes (i) epoxidation of olefins by the Cr(VI) + GSH + O_2 system[13] and (ii) O_2 consumption during the reactions of Cr(VI) with GSH[10a] as well as with a less common intracellular reductant, α-lipoic acid.[14] To our knowledge, no other examples of O_2 consumption during the reductions of Cr(VI/V/IV) were reported, although this is one of the most general and convenient methods for the studies of O_2 activation mechanisms.[15]

Because of the extensive speculation in the literature as to the role of O_2 activation in in vitro (and by implication in vivo) Cr-induced genotoxicities,[3] we have undertaken systematic studies of the effects of O_2 on biologically relevant Cr redox chemistry. The current work presents the first study of O_2 consumption during the reactions of Cr(VI/V/IV) with major biological reductants: Cys, GSH, and AsA[3] as well as with Trolox, a water-soluble analogue of vitamin E and a standard antioxidant in biochemical studies.[16] To reveal the likely mechanisms of O_2 activation, global kinetic analyses[17,18] of the Cr(V/IV) reactions with the abovementioned reductants were performed.

Experimental Section

Caution. *As(III) and Cr(VI) compounds are human carcinogens,*[2,19] *and Cr(V) complexes are mutagenic and potentially carcinogenic.*[5] *Contact with skin and inhalation must be avoided.*

Reagents. The following commercial reagents of analytical grade were used for the preparation of the reaction solutions: As_2O_3, As_2O_5, *L*-dehydroascorbic acid, 5,5-dimethyl-1-pyrroline *N*-oxide, dimethyl sulfoxide, 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), glutathione (reduced form), 2-ethyl-2-hydroxybutanoic acid and (*R*)-(+)-Trolox (all Aldrich); *L*-ascorbic acid from BDH Biochemicals; *L*-cysteine, HEPES hemisodium salt, catalase (EC 1.11.1.6, 2900 units/mg protein) and superoxide dismutase (EC 1.15.1.1, 4950 units/mg protein), (all Sigma); DNA sodium salt (from fish sperm; ICN Biomedicals); $Na_2CrO_4 \cdot 4H_2O$, $NaH_2PO_4 \cdot H_2O$, CH_3COONa, H_2O_2 (30% aqueous solution), $NaClO_4 \cdot H_2O$, NaOH, and $HClO_4$ (all Merck). The Cr(V) complexes, $Na[Cr^VO(ehba)_2] \cdot H_2O$ and $K_3[Cr^V(O_2)_4]$, were synthesized by known methods,[20,21] and their purities were confirmed by UV−visible and EPR spectroscopies. Water was purified by the Milli-Q technique. All buffer solutions were stirred overnight with ∼10 g L^{-1} of Chelex 100 chelating resin (Bio-Rad, analytical grade). The purified buffers were assessed for the traces of catalytic metals using Buettner's ascorbate method.[22] The achieved purity of the buffers corresponded to <0.5 μM Fe.

Dissolved Oxygen Measurements. A Clark-type oxygen microelectrode with a working volume of 1−5 mL and a response time <1 s (Rank Brothers Ltd, Cambridgeshire, UK), connected to a MacLab/2e recorder, was used to follow the changes in O_2 concentrations in the reaction solutions. The time between measurements was 1.5 s, and the results were independent of the stirring rate in the range 10−20 s^{-1} (15 s^{-1} was used in most experiments). The electrode was calibrated as percent of air saturation; 0% corresponds to the solutions saturated with O_2-free Ar. Unless stated otherwise, the dissolved O_2 measurements were carried out at 25 ± 0.1 °C, under atmospheric pressure of air, in solutions containing 1.0 M $NaClO_4$. Under these conditions, saturation with air corresponds to $[O_2] = 0.178$ mM.[23] Typical conditions for the O_2 consumption measurements were $[Cr]_0 = 0.20$ mM; $[Red]_0 = 5.0$ mM (Red = Cys, GSH, AsA, or Trolox); 0.10 M buffer (acetate, pH = 4.50 ± 0.05 or HEPES, pH = 7.50 ± 0.05; the pH values were measured after demetalation of the buffers). The reactions of Cr(VI/V/IV) with DNA were carried out at $[Cr]_0 = 0.20$ mM; $[DNA]_0 = 2.0$ g L^{-1}; 37 ± 0.1 °C; in 0.10 M phosphate (pH 7.0) or 0.050 M acetate (pH 3.8)[24] buffers in the absence of $NaClO_4$. In a typical experiment, 3.0 mL of buffer solution was allowed to thermostat and equilibrate for 15 min, and then the solution was isolated from air and 5−50 μL of concentrated Cr and Red solutions were injected. Control experiments included the O_2 consumption measurements for the Cr(VI/V/IV) + buffer and Red + buffer systems. Reductant solutions were prepared daily and kept under Ar. Stock solutions of Cr(V) ($Na[Cr^VO(ehba)_2]$) in DMSO were stable for several weeks (checked by UV−visible spectroscopy).[25] Stock solutions of Cr(IV) (20 mM) were prepared ∼30 s before use in the reactions of 20 mM Cr(VI) with 100 mM As(III) in the presence of 1.0 M $ehbaH_2$ at pH 3.0; the concentrations of Cr(IV) were checked by UV−visible spectroscopy.[26,27]

Evolution of O_2 after the addition of catalase was used to assess the formation of H_2O_2 in the Cr(VI/V/IV) + Red + O_2 reactions.[28] The experimental procedure is illustrated by Figure 1. Addition of Cr(V) to a solution of AsA caused a fast O_2 consumption (point 2).

(10) Observation of the characteristic EPR spectra of DMPO-•OH adducts is often reported as the evidence for the formation of •OH radicals in the Cr(VI) + Red + O_2 + DMPO systems, for example: (a) Liu, K. J.; Shi, X.; Dalal, N. S. *Biochem. Biophys. Res. Commun*. **1997**, *235*, 54−58. However, analogous EPR signals can be observed due to the direct reactions of DMPO with Cr in high oxidation states: (b) Judd, R. J. Ph.D. Thesis, University of Sydney, 1992. (c) Sugden, K. D.; Wetterhahn, K. E. *Inorg. Chem*. **1996**, *35*, 651−657.

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(24) The reactions of Cr(V) and Cr(IV) ehba complexes with plasmid DNA in 0.050 M acetate buffer, pH 3.8, 37 °C led to the extensive DNA cleavage.[5a,b]

(25) Stability of $[Cr^VO(ehba)_2]^-$ in DMSO solution is described in the following: Judd, R. J.; Hambley, T. W.; Lay, P. A. *J. Chem. Soc., Dalton Trans*. **1989**, 2205−2210. The additions of DMSO (≤5% vol.) did not significantly affect the results of the O_2 consumption experiments.

(26) Generation, stability and UV−visible spectra of Cr(IV)−ehba complexes are described in the following: (a) Ghosh, M. C.; Gould, E. S. *Inorg. Chem*. **1991**, *30*, 491−494. (b) Codd, R.; Lay, P. A.; Levina, A. *Inorg. Chem*. **1997**, *36*, 5440−5448.

(27) Due to the method of Cr(IV) preparation, the solutions for the typical Cr(IV) + Red reactions contained 0.80 mM As(III), 0.20 mM As(V), and 10 mM ehba. Preliminary experiments have shown that the addition of As(III) (≤5 mM), As(V) (≤0.5 mM) and ehba (≤20 mM) did not significantly affect the results of the O_2 consumption experiments.

(28) Preliminary experiments have shown that the activity of catalase (assessed by the rates of O_2 evolution after the addition of H_2O_2) did not change in the presence of 1.0 mM of Cr(VI), Cr(V), or Cr(IV) (pH = 7.0−7.5).

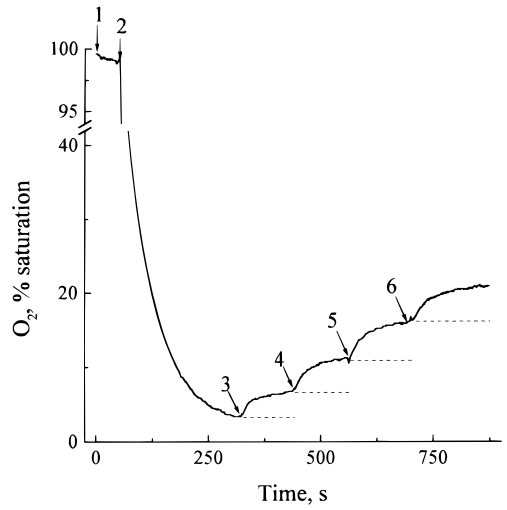

Figure 1. Test for the formation of H_2O_2 in the reaction of 1.0 mM Cr(V) with 1.0 mM AsA in air-saturated solution (0.10 M HEPES buffer; pH = 7.5; 1.0 M $NaClO_4$; 25 °C; $[O_2]_0 = 0.18$ mM). Point 1: buffer + AsA; point 2: Cr(V) added; point 3: 25 mg L^{-1} catalase added; and points 4−6: 17 μM H_2O_2 added.

When the O_2 consumption was practically finished, catalase was added (point 3). This led to some O_2 evolution due to the decomposition of H_2O_2; the overall reaction was[29]

$$O_2 \xrightarrow[-R]{+RH_2} H_2O_2 \xrightarrow{\text{catalase}} H_2O + 0.5O_2 \qquad (1)$$

Calibration of $[H_2O_2]$ was performed by sequential additions of H_2O_2 solution, that was standardized by iodometric titration[30] (points 4−6 in Figure 1). The molar amounts of evolved O_2 corresponded to 1/2 of added H_2O_2 (Figure 1, eq 1).

Kinetic Studies. The Cr(V/IV) + Red reactions were followed under conditions that corresponded to those used for the O_2 consumption experiments (in air-saturated solutions). A SX-17 MV stopped-flow spectrophotometer (Applied Photophysics Ltd., Leatherhead, UK) with a diode-array detector was used. Typically, 250 time-dependent spectra (logarithmic timebase, integration time 2.56 ms, deadtime ~2 ms, λ = 350−750 nm, resolution ~1 nm) were collected in 500 s. In typical stopped-flow experiments, the solutions of Red:$[Red]_0 = 10$ mM, 0.20 M buffer, pH 4.6 or 7.6 (changed to 4.5 or 7.5 after mixing with the acidic solutions of Cr(V/IV), see below), 1.0 M $NaClO_4$, were mixed in a 1:1 ratio with the solutions of 0.40 mM Cr(V) or Cr(IV) in 1.0 M $NaClO_4$. The solutions of Cr(V) ($Na[Cr^VO(ehba)_2]$) were acidified with $HClO_4$ to pH 3.0.[31] The solutions of Cr(IV) were prepared by the reactions of 0.40 mM Cr(VI) with 2.0 mM As(III) in the presence of 20 mM $ehbaH_2$, pH 3.0.[26] To minimize the influence of Cr(V/IV) decomposition, the solutions of Cr(V) and Cr(IV) were prepared ~1 min before use. Analogous experiments were performed in Ar-saturated solutions, using a SX-17 MV anaerobic accessory. Since Cr compounds are known to be light-sensitive,[32] the possible influences of UV radiation on their reactions were checked by (i) a comparison of kinetic data obtained with the diode-array detector (*i.e.*, using a white light beam, 200−750 nm) with those obtained from a monochromator (502 nm) with photomultiplier detection and (ii) a variation of the slit width (0.5−2 mm). Most of the studied reactions (with the exception of the Cr(V) decomposition at pH 4.5 in the absence of Red) did not show any significant light dependence, probably because they were much faster than the possible light-induced processes. Processing of the kinetic data was performed by a global analysis method using Pro-Kineticist software,[33] as described previously.[18]

Stoichiometry Studies. Reactions of 0.20 mM Cr(V/IV) with 0.050−0.80 mM Red were performed in Ar-saturated solutions at 21 ± 1 °C (other conditions corresponded to those of the O_2 consumption experiments). After completion of the reaction, 0.20 M NaOH was added to achieve a rapid disproportionation of unreacted Cr(V/IV) to Cr(VI) and Cr(III).[26b] The UV−visible spectra were then recorded on a HP 8452A diode-array spectrophotometer and the reaction stoichiometries were determined from (i) the disappearance of Cr(VI) absorbance at 372 nm ($\epsilon^{372} = 4.81 \times 10^3$ M^{-1} cm^{-1})[34] and (ii) the appearance of the absorbance due to unreacted Red at 200−350 nm. The ratio $\Delta[Red]/\Delta[O_2]$ was determined for the reaction of 1.0 mM Cr(V) with 2.5 mM Cys (0.10 M HEPES buffer, pH 7.5, 25 °C, reaction time 5 min). The $\Delta[Red]$ value was determined as a difference in amounts of Cys (measured spectrophotometrically with Ellman's reagent; $\lambda_{max} = 412$ nm, $\epsilon_{max} = 1.4 \times 10^4$ M^{-1} cm^{-1})[35] remaining after the reactions with Cr(V) in Ar- and air-saturated solutions. The $\Delta[O_2]$ value was estimated from O_2 consumption during the Cr(V) + Cys reaction in air-saturated solution ($[O_2]_0 = 0.18$ mM). Control experiments in the absence of Cr(V) showed that the decrease in [Cys] due to its autoxidation was insignificant over the time scale of the reaction.

Circular Dichroism (CD) Spectroscopy. The CD spectra of the Cr(III) products obtained from the reactions of 5.0 mM Cr(VI/V/IV) with 125 mM Red (reaction time ~3 min for Cr(V/IV) and ~3 h for Cr(VI); 21 ± 1 °C; other conditions correspond to the O_2 consumption experiments) were recorded on a Jasco 710 spectropolarimeter. Typical instrument settings were λ = 350−650 nm; scan rate 500 nm/min; response time 1.25 s, and number of scans, 5. Noise reduction in the resulting spectra was performed by a Fourier transform procedure in Origin software.[36]

EPR Spectroscopy. The X-band EPR spectra were recorded on a Bruker EMX spectrometer, equipped with an internal NMR gaussmeter and a microwave frequency counter. The spectra were taken at 21 ± 1 °C, using a flat quartz cell. Typical instrument settings were center field, 3500 G; sweep width, 200 G; resolution, 1024 points; microwave frequency, ~9.66 GHz; microwave power, 2 mW; modulation frequency, 100 kHz; modulation amplitude, 0.27 G; time constant, 0.64 ms; sweep time, 5.24 s; number of scans, 20; receiver gain, (1−10) × 10^4. The spectra were processed with WIN-EPR software.[37]

The presented data are the averaged results of 2−3 parallel experiments; the experimental errors for all of the used methods did not exceed 10%.

Results

Studies of O_2 Consumption. Shown in Figure 2 are typical O_2 consumption kinetic curves for the reactions of Cr(VI/V/IV) with Cys, GSH, AsA, or Trolox (0.10 M HEPES buffer, pH = 7.5).[38] The Cr(VI) + Cys reaction is characterized by a significant O_2 consumption with a pronounced induction period (curve 1 at Figure 2a). The Cr(V/IV) + Cys reactions exhibit fast O_2 consumption during the first ~10 s, followed by slower consumption for ~100 s (curves 1 in Figure 2b,c). Similar features, except for much smaller amounts of consumed O_2, are characteristic for Cr(VI/V/IV) reactions with AsA (curves

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(38) The kinetic curves of O_2 consumption (Figures 2, 3, S1, S3, S4) were obtained by subtraction of background O_2 consumption (5.0 mM Red in the corresponding buffer) from the data for the 0.20 mM Cr(VI/V/IV) + 5.0 mM Red systems (in all cases, the background consumption during the time of measurement was ≤10% O_2). Such a subtraction is valid, since the reactions were carried out with large excesses of Red. No significant O_2 consumptions were observed for the Cr(VI/V/IV) + buffer systems.

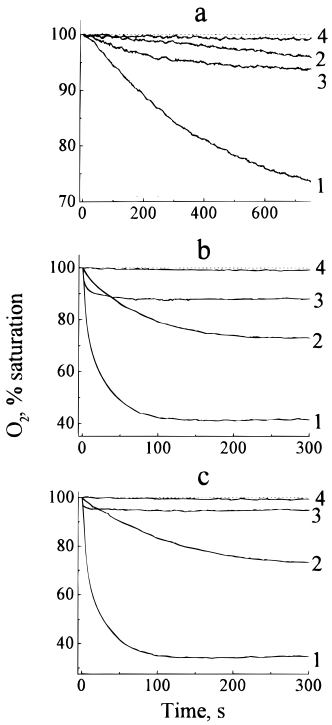


Figure 2. Typical O_2 consumption kinetic curves for the reactions of (a) Cr(VI), (b) Cr(V), or (c) Cr(IV) with Cys (curves 1), GSH (curves 2), AsA (curves 3), or Trolox (curves 4). Conditions: $[Cr]_0 = 0.20$ mM; $[Red]_0 = 5.0$ mM; 0.10 M HEPES buffer; pH = 7.5; 1.0 M $NaClO_4$; 25 °C; $[O_2]_0 = 0.18$ mM.

3 in Figure 2). However, two significant differences between the reactions of Cys and AsA were observed. First, the Cr(V) + AsA reaction leads to ~3 times greater amount of consumed O_2 than the Cr(IV) + AsA reaction, and the slow stage of O_2 consumption is practically absent for the latter reaction (an enlarged view is given in Figure S1, Supporting Information). By contrast, both the amounts of O_2 and the consumption kinetics are similar for the reactions of Cr(V) or Cr(IV) with Cys (Figure 2). Second, the amount of consumed O_2 in the Cr(V) + Cys reaction increases with an increase in $[Cys]_0$ (at constant $[Cr(V)]_0$, Figure S2a in Supporting Information), while the amount of O_2 consumed in the Cr(V) + AsA reaction passes through a maximum with increasing $[AsA]_0$ (Figure S2b), and the maximal O_2 consumption corresponds to the stoichiometric AsA/Cr(V) ratio of ~0.7 (see the stoichiometry results below). Consumption of O_2 in the reactions of Cr(VI/V/IV) with GSH is slower than in the analogous reactions of Cys and AsA (curves 2 in Figure 2). The reactions of Cr(VI/V/IV) with Trolox did not lead to significant O_2 consumption (curves 4 in Figure 2). Similar observations were made for analogous Cr(VI/V/IV) + Red reactions in 0.10 M acetate buffers, pH 4.5 (Figure S3 in Supporting Information). Significant differences for the reactions at pH 4.5 included (i) much smaller O_2 consumption rates in the Cr(VI) + Red reactions and (ii) the appearance of induction periods in the kinetic curves of O_2 consumption for the reactions of Cr(V) (but not Cr(IV)) with Cys and GSH (Figure S3).

To determine the influences of buffers on the O_2 consumption, the Cr(VI/V/IV) + Red reactions were studied in 0.10 M phosphate buffers (pH 7.5) as well as in self-buffered systems (Red = Cys, pH 7.5). The influences of excess ehba ligand were studied by performing the reactions in the presence of 0.10 M ehba (pH 7.5 and 4.5). The kinetics of O_2 consumption were not significantly affected by the nature of buffer, while the

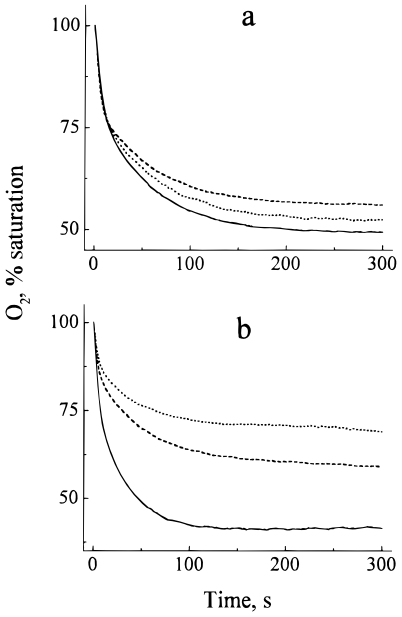


Figure 3. Influence of radical scavengers on O_2 consumption during the reactions of 0.20 mM Cr(V) with 5.0 mM Cys in air-saturated solutions (25 °C). (a) 0.10 M phosphate buffer, pH = 7.0, without $NaClO_4$; no additions (solid line); 50 mg L^{-1} catalase (dashed line); 50 mg L^{-1} SOD (dotted line). (b) 0.10 M HEPES buffer; pH = 7.5; 1.0 M $NaClO_4$; no additions (solid line); 50 mM DMPO (dashed line); 100 mM DMPO (dotted line).

presence of 0.10 M ehba caused some decrease in O_2 consumption rates for the reactions of Red with Cr(V/IV) (but not with Cr(VI)). Typical results of these studies are shown in Figure S4, Supporting Information.

The influences of radical scavengers (catalase, SOD, or DMPO)[39] on O_2 consumption were studied for the Cr(V) + Cys reaction at pH = 7.0−7.5 (Figure 3). The presence of catalase or SOD caused a decrease in the amounts of O_2 consumed in the slow stage, with the effect of catalase being more significant (Figure 3a).[40] Changes in the concentrations of either of the enzymes (25−100 mg L^{-1}) did not lead to significant changes in O_2 consumption. By contrast, the addition of 10−100 mM DMPO caused a concentration-dependent decrease in the amounts of O_2 consumed in the first stage of reaction; typical results are given in Figure 3b.

No significant O_2 consumption was detected for the reactions of Cr(VI/V/IV) with DNA at pH 3.8 or 7.0, 37 °C.

Formation of H_2O_2 During the Cr(VI/V/IV) + Red + O_2 Reactions. Many studies have suggested a crucial role for H_2O_2 in Cr-induced in vitro DNA damage;[9,12,41] therefore, it was important to establish whether O_2 consumption by the Cr(VI/V/IV) + Red systems led to accumulation of H_2O_2. The results of $[H_2O_2]$ determinations with catalase (Figure 1, eq 1) showed that ≤10% of the consumed O_2 was converted to H_2O_2 (rows 1−15 in Table 1). Low yields of H_2O_2 were observed either in the presence of excess Red or in excess Cr(V) (rows 2−3 and 11−12 in Table 1; see the stoichiometry results below). The accumulation of H_2O_2 was significantly increased by the

(39) Preliminary kinetic experiments have shown that the rates of Cr(V) reactions with catalase, SOD, or DMPO under the studied conditions were insignificant in comparison with those for the Cr(V) + Cys reaction.

(40) The use of 0.10 M phosphate buffer, pH 7.0, in the absence of $NaClO_4$, led to maximal effects of catalase and SOD. Similar, but smaller, effects of the both enzymes were observed in 0.10 M HEPES buffer, pH 7.5, 1.0 M $NaClO_4$.

(41) Tsou, T.-C.; Yang, J.-L. *Chem. Biol. Interact.* **1996**, *102*, 133−153.

Table 1. Formation of H_2O_2 during the Cr(VI/V/IV) + Red Reactions

conditions[a]	O_2 consumed, μM	H_2O_2 formed, μM
1.0 mM Cr(VI) + 5.0 mM Cys[b]	1.1×10^2	4.2
1.0 mM Cr(V) + 5.0 mM Cys	1.6×10^2	4.2
1.0 mM Cr(V) + 1.0 mM Cys	33	0
1.0 mM Cr(IV) + 5.0 mM Cys	1.6×10^2	3.0
1.0 mM Cr(V) + 5.0 mM Cys[c]	1.6×10^2	3.4
1.0 mM Cr(V) + 5.0 mM Cys[c,d]	1.6×10^2	13
10.0 mM Cys[e]	37	0
1.0 mM Cr(V) + 5.0 mM GSH	92	3.2
1.0 mM Cr(IV) + 5.0 mM GSH	59	2.4
10.0 mM GSH[e]	39	0
1.0 mM Cr(V) + 5.0 mM AsA	1.1×10^2	11
1.0 mM Cr(V) + 0.50 mM AsA	1.7×10^2	8.8
1.0 mM Cr(IV) + 5.0 mM AsA	26	0
1.0 mM Cr(V) + 1.0 mM AsA[c]	1.7×10^2	9.2
1.0 mM Cr(V) + 1.0 mM AsA[c,d]	1.7×10^2	14
10.0 mM AsA[e]	71	34

[a] The reaction conditions, unless stated otherwise, are as follows: 0.10 M HEPES buffer, pH 7.5; 1.0 M $NaClO_4$; 25 °C; reaction time 5 min. [b] Reaction time 30 min. [c] 0.10 M phosphate buffer, pH 7.0, without $NaClO_4$. [d] Reaction in the presence of 50 mg L^{-1} SOD. [e] Autoxidation in 0.10 M HEPES buffer, pH 8.0, 1.0 M $NaClO_4$.

presence of SOD (rows 5−6 and 14−15 in Table 1). Autoxidation of AsA (in the absence of Cr) led to significant accumulation of H_2O_2 (yield ∼50% according to eq 1; row 16 in Table 1); by contrast, autoxidations of Cys and GSH did not produce measurable amounts of H_2O_2 (rows 7 and 10 in Table 1).

Stoichiometry Studies. Typical UV−visible spectral changes, which were used to determine the stoichiometries of the Cr(V/IV) + Red reactions, are shown in Figure 4. The stoichiometries found (column 5 in Table 2) are as follows: (i) 1 mol of Cys or GSH reduces 1 mol of Cr(IV) or 0.5 mol of Cr(V) to Cr(III); (ii) 1 mol of AsA or Trolox reduces 2 mol of Cr(IV) to Cr(III); (iii) 2 mol of AsA reduce 3 mol of Cr(V) to Cr(III); and (iv) Trolox reacts with Cr(V) in a molar ratio close to 1:1.

Comparison of [Cys] values after completion of the reaction of excess Cys with Cr(V) in Ar- and air-saturated solutions, in connection with the O_2 consumption measurements (see the determination of $\Delta[Red]/\Delta[O_2]$ in Experimental Section), showed that the consumption of 1.0 mol O_2 during this reaction caused the additional oxidation of 2.0 ± 0.5 mol Cys.

Product Studies. As all of the reductants used in this work are optically active, the compositions of Cr(III) products formed in the reactions of Cr(VI/V/IV) with excess Red at pH 7.5 and 4.5 were studied by CD spectroscopy.[42] A summary of the spectra is given in Figure S5, Supporting Information. In all cases, the Cr(III) products possessed optical activity, which shows the presence of Red or their oxidation products in the coordination spheres of the resulting Cr(III) complexes. However, the CD spectra for the products of the Cr(VI) + Red reactions (in the absence of ehba ligand) are different from those for the reactions of Cr(V)−ehba and Cr(IV)−ehba complexes with Red (Figure S5). It suggests that in the two last cases the products include mixed Cr(III)−ehba−Lig complexes (Lig = Red or its oxidation product).

Kinetic Studies. Time-dependent UV−visible spectra for the reactions of Cr(V/IV) with large excesses of Red at both pH 7.5 and 4.5 were fitted by sequences of two or three pseudo-first-order reactions; the optimized rate constants are given in Table 2.[43] Deviations between the experimental absorbance values and those estimated from the kinetic schemes did not exceed the spectrophotometer noise level; typical experimental and estimated kinetic curves are shown in Figure 5. The estimated spectra of the reacting species in reactions 1−20 (Table 2) are given in Figures S6−S10, Supporting Information; significant spectral features are summarized in Table 3. A comparison of the kinetics for the Cr(V/IV) + Red reactions (1−16 in Table 2) with those for the decompositions of Cr(V/IV)−ehba complexes (17−20 in Table 2) was performed.[44] In most cases (with the exceptions of the Cr(V/IV) reactions with Cys or GSH at pH 7.5), the decompositions of Cr(V/IV)−ehba complexes were much slower than their reactions with Red.

A comparison of the kinetics for the Cr(V/IV) + Red reactions in Ar- and air-saturated solutions was performed for the reactions of 1.0 mM Cr(V) or Cr(IV) with 5.0 mM Cys at

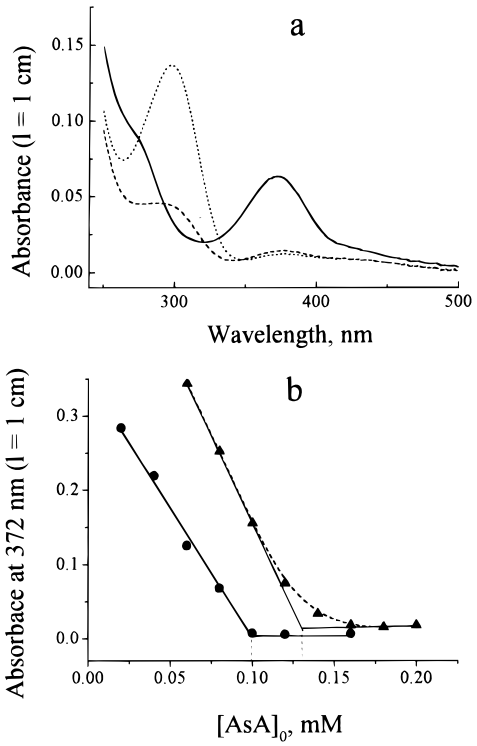


Figure 4. Typical results of stoichiometry studies. (a) UV−visible spectra of the solutions after the reactions of 0.20 mM Cr(V) with AsA (0.10 M acetate buffer, pH 4.5, 1.0 M $NaClO_4$, 21 °C, Ar-saturated solutions); $[AsA]_0$ = 0.12 mM (solid line), 0.14 mM (dashed line), and 0.16 mM (dotted line). After completion of the reactions (reaction time 10 min), and 15 min before taking the spectra, 0.20 mM NaOH was added. Absorbance maximum at 372 nm corresponds to $[CrO_4]^{2-}$; maximum at 297 nm corresponds to ascorbate(2-) ion. (b) Dependency of absorbance at 372 nm on $[AsA]_0$ for the Cr(IV) + AsA (circles) and Cr(V) + AsA (triangles) reactions at pH 4.5; other conditions as in (a).

(42) The ratio $[Red]_0/[Cr]_0 = 25$, used in the CD spectroscopic studies, was the same as for most of the O_2 consumption and kinetic experiments (see Experimental Section); however, the absolute values of $[Red]_0$ and $[Cr]_0$ were higher in order to obtain satisfactory signal-noise ratios in the CD spectra. Similar spectra were obtained with lower concentrations: $[Cr]_0$ = 1.0 mM.; $[Red]_0$ = 25 mM.

(43) Kinetics of the reactions of $[Cr^VO(ehba)_2]^-$ with Cys and AsA were studied previously at pH = 2−4 in the presence of excess ehba. At low $[Red]_0/[Cr(V)]_0$ ratios, these reactions possessed autocatalytic behavior: (a) Ghosh, S. K.; Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1987**, *26*, 3722−3727. (b) Ghosh, S. K.; Bose, R. N.; Gould, E. S. *Inorg. Chem.* **1987**, *26*, 2684−2687. However, autocatalysis was not observed under the conditions used in this work.

(44) The decompositions of Cr(V) and Cr(IV) are complex processes, including parallel second- and first-order reactions.[26,31] The simplest kinetic schemes, applied here (Table 2), do not reflect true mechanisms of these reactions. The kinetic data (17−20 in Table 2) are given only for comparison with the reactions in the presence of Red (1−16 in Table 2).

Table 2. Stoichiometries and Kinetics of the Reactions Cr(V/IV) + Red[a]

no.	Red	Ox	pH[b]	stoichiometry[c]	kinetic scheme[d]	rate constants,[e] s^{-1}
1	Cys	Cr(IV)	7.5	*f*	A	0.63; 6.4×10^{-3}
2	Cys	Cr(IV)	4.5	1.0	B	81; 3.7; 0.12
3	Cys	Cr(V)	7.5	2.0	B	13; 2.1; 4.3×10^{-3}
4	Cys	Cr(V)	4.5	2.0	A	1.7; 6.8×10^{-2}
5	GSH	Cr(IV)	7.5	*f*	A	0.69; 1.9×10^{-2}
6	GSH	Cr(IV)	4.5	1.0	B	80; 0.14; 1.5×10^{-2}
7	GSH	Cr(V)	7.5	*f*	B	1.3; 2.7×10^{-2}; 3.3×10^{-3}
8	GSH	Cr(V)	4.5	2.0	B	0.21; 8.3×10^{-2}; 1.3×10^{-2}
9	AsA	Cr(IV)	7.5	0.50	B	1.3×10^{2}; 12; 1.4×10^{-2}
10	AsA	Cr(IV)	4.5	0.50	A	1.2×10^{2}; 1.1
11	AsA	Cr(V)	7.5	0.65	B	65; 3.7; 8.8×10^{-3}
12	AsA	Cr(V)	4.5	0.65	A	80; 34
13	Trolox	Cr(IV)	7.5	0.50	B	6.2; 3.2; 7.7×10^{-2}
14	Trolox	Cr(IV)	4.5	0.50	B	1.2×10^{2}; 3.8; 5.3×10^{-3}
15	Trolox	Cr(V)	7.5	*f*	B	6.3; 1.7; 6.1×10^{-2}
16	Trolox	Cr(V)	4.5	0.90	B	6.5; 1.5; 2.0×10^{-2}
17		Cr(IV)	7.5		C	0.60; 13[g]
18		Cr(IV)	4.5		C	78; 15[g]
19		Cr(V)	7.5		D	3.0×10^{-2}
20		Cr(V)	4.5		D	4.2×10^{-3} [h]

[a] Reaction kinetics were followed by stopped-flow UV−visible spectrophotometry with a diode-array detector (see Experimental Section). Conditions: $[Cr]_0 = 0.20$ mM; $[Red]_0 = 5.0$ mM; 1.0 M $NaClO_4$; 25 °C; air-saturated solutions. [b] For pH 7.5: 0.10 M HEPES buffer; for pH 4.5: 0.10 M acetate buffer. [c] Amount of Red (mol), which completely reduces 1.0 mol of Cr(V/IV) to Cr(III); error ±0.05. [d] Designations of the kinetic schemes (*S* = initial substance; *I*, *I*′ = intermediates; *P* = product): A = $S \rightarrow I \rightarrow P$; B = $S \rightarrow I \rightarrow I' \rightarrow P$; C = $S \rightarrow I$, 2 $I \rightarrow P$ and D = $S \rightarrow P$. [e] Pseudo-first-order rate constants for the stages 1−3 in the kinetic schemes, respectively. [f] The stoichiometry could not be determined as the rate of the redox reaction was comparable with that for the decomposition of Cr(V/IV). [g] Second-order rate constants, $M^{-1}\ s^{-1}$. [h] This reaction was significantly catalyzed by UV light.

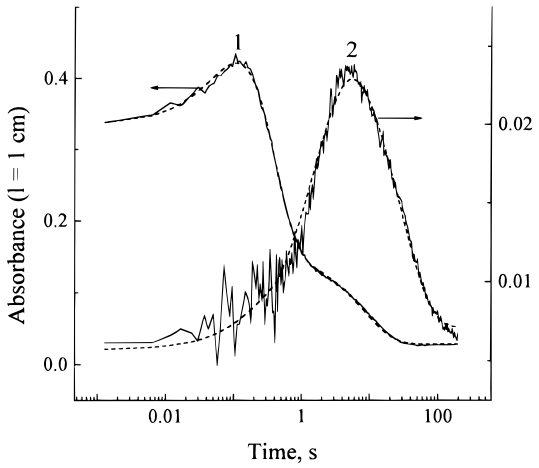


Figure 5. Typical experimental (solid lines) and estimated (dashed lines; see the kinetic schemes and rate constants in Table 2) kinetic curves for the Cr(V/IV) + Red reactions: (1) Cr(IV) + Trolox, pH 7.5, 435 nm and (2) Cr(V) + Cys, pH 4.5, 620 nm. The reaction conditions correspond to Table 2.

pH 4.5 or 7.5 as well as for the reactions of 1.0 mM Cr(V) with 1.0 mM AsA at pH 4.5 or 7.5. In air-saturated solutions, these conditions led to substantial O_2 consumption (≥0.15 mM). However, neither significant differences in the rate constants of Cr(V/IV) reduction nor any evidence for the formation of new absorbing species was revealed in the presence of O_2.

The reactions of Cr(V/IV) with DHAA at pH 7.5 and 4.5 were studied in order to explain the unusual stoichiometries of the Cr(V) + AsA reactions. DHAA was oxidized by both Cr(V) and Cr(IV); however, the reaction rates were low in comparison with the corresponding reactions of AsA (Table S1 in Supporting Information).

EPR Spectroscopic Studies. The aim of these studies was to determine whether relatively stable Cr(V/IV) peroxo or superoxo complexes are formed in the Cr(V/IV) + Red + O_2 systems.[11,12] The reaction mixtures after the fast reactions of 0.50−1.5 mM of Cys, GSH, AsA, or Trolox (70−90% of stoichiometric amount, see Table 2) with 1.0 mM $[Cr^{V}O(ehba)_2]^-$ at pH 4.5 or 7.5 in air-saturated solutions possessed only the characteristic EPR signal of this Cr(V) complex ($g_{iso} = 1.9783$, $A_{iso} = 17.1 \times 10^{-4}\ cm^{-1}$).[20] Analogous results were obtained for the reactions of excess Cr(IV)−ehba complexes with Red (in this case, Cr(V) is formed due to the disproportionation of unreacted Cr(IV)).[26b] Thus, the O_2 consumption by the Cr(V/IV) + Red systems did not lead to the formation of measurable amounts of relatively stable Cr(V) complexes with O_2-derived ligands. In addition, the stabilities of 1.0 mM $[Cr^{V}(O_2)_4]^{3-}$ in buffer solutions (0.10 M HEPES, pH 7.5 or 0.10 M acetate, pH 4.5) were checked. At both pH values, the EPR signals of the Cr(V) peroxo complex disappear in < 3 min (the complex is stable in 0.20 M NaOH solutions, $g_{iso} = 1.9723$, $A_{iso} = 18.4 \times 10^{-4}\ cm^{-1}$).[45]

Discussion

Extensive studies have been performed on the mechanisms of genotoxicities caused by the Cr(VI) + Red (mainly GSH or AsA) systems.[3] The difficulty with mechanistic studies on the Cr(VI) + Red reactions resides in the often very low steady-state concentrations of the intermediates.[18] For this reason, studies on the reactions of Red with the relatively stable Cr(V) and Cr(IV) complexes can provide crucial information about the intermediates in, and the mechanisms of, Cr(VI) + Red reactions.[46] On the other hand, the O_2 activation mechanisms during the Cr(VI) + Red reactions (Figures 2a and S3a) are difficult to delineate, because the O_2 consumption rates are relatively low and comparable to those due to the autoxidations

(45) Dalal, N. S.; Millar, J. M.; Jagadeesh, M. S.; Seehra, M. S. *J. Chem. Phys.* **1981**, *74*, 1916−1923.

(46) The use of the relatively stable Cr(V) and Cr(IV) complexes mimics intracellular conditions, where Cr(V/IV) species are likely to be stabilized by complexation with a variety of ligands, including carbohydrates and 2-hydroxycarboxylates (Barr-David, G.; Bramley, R.; Brumby, S.; Charara, M.; Codd, R.; Farrell, R. P.; Hanson G. R.; Irwin, J. A.; Ji, J.-Y.; Lay, P. A. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 1207−1216).

Table 3. UV−Visible Spectral Characteristics and Assignment of the Reacting Species in the Reactions Cr(V/IV) + Red[a]

reactions and species[b]	spectral features (350−750 nm)[c]	assignment[d]
1(*S*); 5(*S*); 9(*S*); 13(*S*); 17(*S*)	350 ((1.2−1.4) × 10^3); 465 max (1.8 × 10^3)	mixture of $[Cr^{IV}O(ehbaH)_2]^0$, $[Cr^{IV}O(OH_2)_2(ehba)]^0$, and $[Cr^{IV}O(ehba)(ehbaH)]^-$
2(*S*); 6(*S*); 14(*S*);[e] 18(*S*)	350 ((1.5−1.7) × 10^3); 510 max (1.2 × 10^3)	
10(*S*);[f] 2(*I*); 6(*I*); 14(*I*);[e] 17(*I*);[g] 18(*I*)	350 ((1.0−1.5) × 10^3); 500 sh (3 × 10^2)	
3(*S*); 4(*S*); 7(*S*); 8(*S*); 12(*S*); 15(*S*); 16(*S*); 19(*S*); 20(*S*)	350 sh (1.2 × 10^3); 510 sh (1.6 × 10^2); 740 sh (40)[h]	$[Cr^{V}O(ehba)_2]^-$
2(*I*); 6(*I*′)	350 (1.4 × 10^3); 500 max (1.6 × 10^2)	Cr(IV)−ehba−Red[i]
4(*I*)	350 (1.6 × 10^3); 650 max (1.5 × 10^2)	Cr(V)−ehba−Red[i]
8(*I*)	350 (1.5 × 10^3); 650 sh (1 × 10^2)	
11(*S*); 12(*I*)	350 ((1.5−1.8) × 10^3); 510 sh ((2.5−3.5) × 10^2)	
13(*I*); 15(*I*)	350 ((2.0−2.5) × 10^3); 435 max ((2.5−3.0) × 10^3); 500 sh (1.5 × 10^2); 650 sh (1.2 × 10^2)	Cr(V)−ehba−Trolox[i] and Trolox anion radical
16(*I*)	350 (1.7 × 10^3); 435 sh (6 × 10^2); 500 sh (1.5 × 10^2); 650 sh (1.21 × 10^2)	
13(*I*); 15(*I*)[f]	350 ((1.0−1.7) × 10^3); 435 max ((6−8) × 10^2); 650 max (1.2 × 10^2)	
9(*I*); 11(*I*)	360 sh ((0.7−1.2) × 10^3); 480 max (50);[h] 590 max (50)[h]	AsA anion radical and Cr(III)
1(*I*); 3(*I*′); 5(*P*); 7(*I*′);[j] 7(*P*); 9(*I*′); 10(*I*); 11(*I*′); 13(*P*);[k] 14(*I*′);[k] 14(*P*);[k] 15(*P*);[k] 16(*I*′);[k] 16(*P*);[k] 17(*P*); 18(*P*); 19(*P*); 20(*P*)	350 sh ((2−7) × 10^2) or 372 max ((2−25) × 10^2); 430−480 max (50);[h] 540−590 max (50)[h]	($[HCrO_4]^-$ or $[CrO_4]^{2-}$) and Cr(III)
1(*P*); 2(*P*); 3(*P*); 4(*P*); 6(*P*); 8(*P*); 9(*P*); 10(*P*); 11(*P*); 12(*P*)	430−480 max (50);[h] 540−590 max (50)[h]	Cr(III)

[a] Full spectra of the reacting species are given in Figures S6−S10, Supporting Information. [b] Numbers of the reactions correspond to those in Table 2; designations of the reacting species (given in parentheses) correspond to note "d" in Table 2. [c] Max = maximum; sh = shoulder. Values of ϵ (M^{-1} cm^{-1}) are given in parentheses. [d] See Discussion. [e] Some absorbance due to the Trolox anion radical is also present. [f] Some absorbance due to Cr(III) is also present. [g] Some absorbance due to Cr(III) and Cr(V) complexes is present. [h] Error in determination of these ϵ values was ∼50% due to the very low absorbances. [i] Mixtures of the complexes. [j] Some absorbance due to Cr(V) is also present. [k] The spectra for the reactions of Trolox (13−16) possess some absorbance at 350−450 nm even at prolonged reaction times. This was attributed to the Trolox oxidation products.

Scheme 1. Ligand Exchange Equilibria of Cr(V) and Cr(IV) Complexes

$$[Cr^{IV}O(ehbaH)_2]^0 \rightleftharpoons [Cr^{IV}O(ehba)(ehbaH)]^- + H^+ \quad (2)$$

$$[Cr^{IV}O(ehbaH)_2]^0 + 2\,H_2O \rightleftharpoons [Cr^{IV}O(OH_2)_2(ehba)]^0 + ehbaH_2 \quad (3)$$

$$[Cr^{IV}O(ehbaH)_2]^0 + LigH_2 \rightleftharpoons [Cr^{IV}O(ehbaH)(LigH)]^0 + ehbaH_2 \quad (4)$$

$$[Cr^{V}O(ehba)_2]^- + LigH_2 \rightleftharpoons [Cr^{V}O(ehba)(Lig)]^- + ehbaH_2 \quad (5)$$

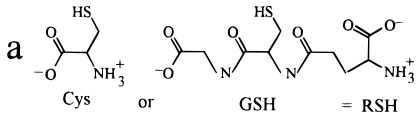
ehbaH$_2$ =  ; LigH$_2$ = Cys, GSH, AsA or Trolox

of Red. Comparative studies (Figures 2, S3, S4) showed fast initial O_2 consumption in the reactions of Cr(V) or Cr(IV) with Red, in contrast to induction periods in the reactions of Cr(VI). Thus, it is likely that the activation of O_2 during the Cr(VI) + Red reactions is connected with the formation of Cr(V/IV) intermediates; therefore, investigating likely mechanisms of O_2 consumption during the Cr(V/IV) + Red reactions will provide insights into the genotoxicities induced by the Cr(VI) + Red + O_2 systems.

Mechanisms of the Cr(V/IV) + Red Reactions. The mechanisms (Schemes 1 and 2) are proposed on the basis of kinetic, stoichiometry, and product studies (Tables 2 and 3, Figures 4 and 5, S5−S10). The pH range used (4.5−7.5) corresponds to that of intracellular reactions of Cr compounds.[26b] Comparisons of kinetic data at pH 4.5 and 7.5 are also important for the mechanistic studies, because Cr(V)−ehba and Cr(IV)−ehba complexes decompose at much slower rates at pH 4.5.[26b,31]

The spectra of the initial species in the Cr(IV) + Red reactions at pH 4.5 and 7.5 (estimated from global analysis of the kinetic data) are characteristic for bis-ligated Cr(IV) oxo complexes (strong absorbance at 400−600 nm; Table 3 and Figures S6−S9).[26b] However, the spectra at pH 7.5 (λ_{max} = 465 nm) are different from those at pH 4.5 (λ_{max} = 510 nm). This difference, which was also observed for the decompositions of Cr(IV) at pH 4.5 and 7.5 in the absence of Red (Table 3 and Figure S10), was assigned to the partial deprotonation of the initial Cr(IV)−ehba complex at pH 7.5 (eq 2 in Scheme 1).[47] The very fast

Scheme 2. Reactions of Cr(V/IV) with Red in the Absence of O_2

a 
Cys or GSH = RSH

$$RSH \rightleftharpoons RS^- + H^+ \quad (6)$$

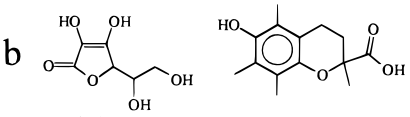
$$Cr(V) + RS^- \longrightarrow Cr(IV) + RS^{\bullet} \quad (7)$$

$$Cr(IV) + RS^- \longrightarrow Cr(III) + RS^{\bullet} \quad (8)$$

$$RS^{\bullet} + RS^- \rightleftharpoons RSSR^{\bullet-} \quad (9)$$

$$Cr(V) + RSSR^{\bullet-} \longrightarrow Cr(IV) + RSSR \quad (10)$$

$$Cr(IV) + RSSR^{\bullet-} \longrightarrow Cr(III) + RSSR \quad (11)$$

b 
AsA or Trolox = RH$_2$

$$RH_2 \rightleftharpoons RH^- + H^+ \quad (12)$$

$$Cr(V) + RH^- \longrightarrow Cr(IV) + R^{\bullet-} + H^+ \quad (13)$$

$$Cr(IV) + RH^- \longrightarrow Cr(III) + R^{\bullet-} + H^+ \quad (14)$$

$$Cr(V) + R^{\bullet-} \longrightarrow Cr(IV) + R + H^+ \quad (15)$$

$$Cr(IV) + R^{\bullet-} \longrightarrow Cr(III) + R + H^+ \quad (16)$$

$$2\,R^{\bullet-} + H^+ \rightleftharpoons R + RH^- \quad (17)$$

$$Cr(V) + R^{\bullet-} \rightleftharpoons Cr(V)\text{–}R^{\bullet-} \longrightarrow Cr(IV)\text{–}R \longrightarrow Cr(III)\text{–}R^{+} \longrightarrow Cr(III) + R^{\bullet}_{ox} + H^+ \quad (18)$$

$$R^{\bullet}_{ox} \longrightarrow \text{further oxidation products} \quad (19)$$

initial decrease of absorbance at 400−600 nm, observed for all of the Cr(IV) reactions at pH 4.5, was assigned to an equilibrium

between bis- and mono-chelated Cr(IV)−ehba complexes (eq 3).[48] Indeed, the rate constants for this stage of the reaction (∼80 s^{-1}, Table 2) were practically independent of the absence or presence of Red (Cys or GSH). For the reactions of Cr(IV) with AsA or Trolox at pH 4.5, the values of the rate constants for the initial stages were even higher (∼120 s^{-1}, Table 2) due to the parallel fast reductions of Cr(IV). For the Cr(V) reactions at both pH values, the spectra of the initial species correspond to that of $[Cr^{V}O(ehba)_2]^-$ (λ_{max} = 510 and 740 nm, Table 3).[20] The spectral changes, attributed to the formation of the mixed-ligand Cr(V) and Cr(IV) complexes (eqs 4 and 5), are enumerated in Table 3; however, this evidence is not definitive due to the absence of the literature data on the UV−visible spectra of such complexes.[49] More informative was the presence of Red ligands in the resulting Cr(III) complexes, as shown by CD spectroscopy (Figure S5). As Cr(III) complexes are kinetically inert on the time scale of the redox reactions,[50] the compositions of the Cr(III) products from the fast Cr(V/IV) + Red reactions show unambiguously that the ligand-exchange reactions 3−5 take place prior to the redox processes. The presence of reactions 2−5 highlights the complexity of the Cr(V/IV) + Red processes, since different complexes of Cr(V) and Cr(IV) can be reduced in parallel reactions.[51] Therefore, the general schemes for the further reactions are given in Scheme 2.

For AsA or Trolox, the formation of the corresponding anion radicals (eqs 13 and 14 in Scheme 2) in the reactions with Cr(V) or Cr(IV) at pH 7.5 was observed in the time-dependent spectra (λ_{max} = 360 nm for AsA[52] and 435 nm for Trolox;[53] Table 3, Figures S8 and S9).[54] The spectral changes due to the formation of the anion radicals were less evident at pH 4.5, probably because of the acid-catalyzed decomposition of these species (eq 17).[52,53] By contrast, the formation of thiyl radicals in the reactions of Cys and GSH (eqs 7 and 8) could not be directly observed in the studied wavelength range.[55] However, the formation and further reactions of such radicals, leading to the disulfide products (eqs 9−11), are generally accepted for the reactions of thiols with a variety of oxidants.[55] The mechanism (eqs 6−11) is consistent with the observed stoichiometries of Cr(V/IV) reactions with Cys and GSH (Table 2).

The stoichiometry of the Cr(V) + AsA reactions at both pH 4.5 and 7.5 (Table 2) suggests that the oxidation of AsA does not stop with the formation of DHAA (the product of two-electron oxidation, usually considered as the stable oxidation product for AsA).[56] However, this unusual stoichiometry cannot be due to the fast reaction of Cr(V) with the initially formed DHAA, since the oxidation of DHAA under these conditions is relatively slow (Table S1). The proposed mechanism, leading to products that are different from DHAA in the Cr(V) + AsA reaction, is shown in eqs 18 and 19 (Scheme 2). It includes the formation of Cr(V) complex with the relatively stable[52] ascorbate anion radical, followed by an electron transfer. The resultant Cr(IV)−DHAA complex undergoes fast intramolecular electron transfer (due to the high redox potential of Cr(IV)),[57] leading to Cr(III) and carbon-based radicals.[58] Similar processes take place, although to much smaller extent, in the Cr(V) + Trolox reaction (see the stoichiometry in Table 2).

Mechanisms of O_2 Consumption During the Cr(V/IV) + Red Reactions. To suggest possible mechanisms of O_2 consumption, the kinetics of the Cr(V/IV) + Red reactions (observed by stopped-flow spectrophotometry) were compared with those of O_2 consumption; typical comparisons are shown in Figure 6. For the Cr(V) + Cys reaction at pH 7.5, the initial fast stage of O_2 consumption followed the formation of the intermediate *I′* (Figure 6a). The spectral features of *I′* (Table 3, Figure S6) suggest that it is due mainly to Cr(III) species (characterized by weak absorbance maxima at ∼420 and ∼580 nm),[59] with a small amount of $[CrO_4]^{2-}$ present (λ_{max} = 372 nm; [Cr(VI)] = ∼5% $[Cr(V)]_0$).[34] Chromium(VI) is formed in the disproportionation of Cr(V),[31] parallel to its reduction by Cys. The last slow stage of the Cr(V) + Cys reaction at pH 7.5 (*I′* → *P*; Table 2) is the reduction of the remaining Cr(VI) by excess Cys, as the final product *P* possesses only the characteristic absorbance maxima of Cr(III) (Table 3). This last stage of the redox reaction was much slower than the second stage of O_2 consumption (Figure 6a). Furthermore, a control experiment has shown that the reaction of such small amounts of Cr(VI) with Cys does not cause any significant O_2 consumption. Thus, the second (and the most significant) stage of O_2 consumption occurs independently of the Cr(V) + Cys reaction and is, therefore, related to the reactions of organic intermediates, formed during the oxidation of Cys by Cr(V). Comparisons of the kinetics of the other Cr(V/IV) + Red reactions (Red = Cys, GSH, or AsA) with the corresponding O_2 consumption data lead to similar conclusions. The exceptions were the reactions of Cr(V) with Cys and GSH at pH 4.5, where induction periods for O_2 consumption were apparent (Figure S3) and the

(47) Protolytic reactions of Cr(IV)−ehba complexes were assumed in previous works,[26b] however, application of the stopped-flow technique in the current work allowed to obtain definitive evidence for their existence. The equilibrium (eq 2) is probably achieved within a deadtime of the stopped-flow experiment.

(48) This reaction is due to the mixing of the initial Cr(IV) solution, containing 20 mM ehba, with the buffer solution, which does not contain ehba (see Experimental Section). The estimated spectra of the species *S* and *I* (Table 3, Figures S5−S10) correspond to the equilibrium mixtures of mono- and bis-ehba Cr(IV) complexes at [ehba] = 20 and 10 mM, respectively.[26b]

(49) EPR-spectroscopic evidence for the formation of Cr(V) complexes with Red or their oxidation products during the Cr(VI) + Red reactions have been reported: (a) complexes with Cys or GSH (Kitagawa, S.; Seki, H.; Kametani, F.; Sakurai, H. *Inorg. Chim. Acta* **1988**, *152*, 251−255); (b) complexes with AsA;[12] and (c) complexes with Trolox oxidation product, 2-hydroxy-2-methyl-4-(2,5,6-trimethyl-2,4-dioxo-2,5-cyclohexadienyl)butanoic acid (Dalla-Pozza, A.-M. B.Sc. Honours Thesis, University of Sydney, 1996).

(50) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; John Wiley & Sons: New York, 1988; p 687.

(51) Since all the reductants used possess a number of potential donor groups (see Scheme 2), such mixtures of Cr(V/IV) species may be very complex.

(52) Bielski, B. H. J. In *Ascorbic Acid: Chemistry, Metabolism and Uses*; Seib, P. A., Tobbert, B. M., Eds.; Advances in Chemistry Series, American Chemical Society: New York, 1982; Vol. 200, pp 81−99.

(53) Thomas, M. J.; Bielski, B. D. J. *J. Am. Chem. Soc.* **1989**, *111*, 3315−3319.

(54) The estimated extinction coefficients at 360 and 435 nm for the reacting species containing AsA and Trolox anion radicals, respectively (Table 3), are lower than the literature values for the corresponding anion radicals.[52,53] This is because the applied kinetic schemes (Table 2) did not take into account the second-order disproportionation reactions of the anion-radicals (eq 17 in Scheme 2).

(55) Asmus K.-D. *Methods Enzymol*. **1990**, *186*, 168−180.

(56) Further slow oxidation of DHAA leads to degradation products, such as 2,3-diketogulonic acid: Davies, M. B.; Austin, J.; Partridge, D. A. *Vitamin C: Its Chemistry and Biochemistry*; The Royal Society of Chemistry: Cambridge, U.K., 1991; pp 66−73, 115−146.

(57) Bose, R. N.; Fonkeng, B.; Barr-David, G.; Farrell, R. P.; Judd, R. J.; Lay, P. A.; Sangster, D. F. *J. Am. Chem. Soc.* **1996**, *118*, 7139−7144.

(58) This reaction (eq 18) may be related to the formation of carbon-based radicals during the Cr(VI) + AsA reactions, observed in EPR experiments with the spin trap DMPO (Stearns, D. M.; Wetterhahn, K. E. *Chem. Res. Toxicol*. **1994**, *7*, 219−230). Preliminary EPR experiments in the presence of DMPO led to similar EPR signals in the reactions of Cr(VI) or Cr(V) with AsA (pH 7.5); however, no EPR signals were observed for the Cr(IV) + AsA + DMPO reactions (Levina, A., unpublished results).

(59) Two weak absorbance maxima in visible range (400−450 nm and 550−600 nm) are characteristic for octahedral Cr(III) complexes in aqueous solutions (Larkworthy, L. F.; Nolan, K. B.; O'Brien, P. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon Press: Oxford, U.K., 1987; Vol. 3, pp 699−969).

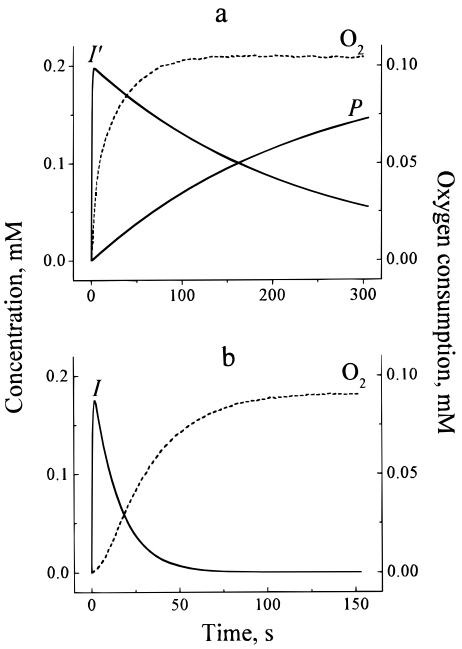


Figure 6. Comparison of the kinetics of the Cr(V) + Cys reaction (observed by stopped-flow spectrophotometry) with the kinetics of O_2 consumption during this reaction. Reaction conditions correspond to Table 2: (a) pH = 7.5 (no. 3 in Table 2); and (b) pH = 4.5 (no. 4 in Table 2). Solid lines are the estimated concentrations of the reaction intermediates *I* or *I′* and the products *P* (see Tables 2 and 3). Dashed lines are the amounts of consumed O_2.

Scheme 3. Reactions Leading to O_2 Consumption

a Cys or GSH = RSH

$$RS^{\bullet} + O_2 \longrightarrow RSOO^{\bullet} \quad (20)$$

$$RSOO^{\bullet} + RSH \longrightarrow RSOOH + RS^{\bullet} \quad (21)$$

$$RSOOH \longrightarrow \text{further oxidation products} \quad (22)$$

$$RSSR^{\bullet-} + O_2 \longrightarrow RSSR + O_2^{\bullet-} \quad (23)$$

$$RSH + O_2^{\bullet-} \longrightarrow RSO^{\bullet} + OH^- \quad (24)$$

$$RSO^{\bullet} + RSH \longrightarrow RSOH + RS^{\bullet} \quad (25)$$

$$RSOH + RSH \longrightarrow RSSR + H_2O \quad (26)$$

$$RSH + O_2^{\bullet-} + H^+ \longrightarrow RS^{\bullet} + H_2O_2 \quad (27)$$

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (28)$$

b AsA = RH_2

$$R^{\bullet-} + O_2 \longrightarrow R + O_2^{\bullet-} \quad (29)$$

$$RH_2 + O_2^{\bullet-} \longrightarrow R^{\bullet-} + H_2O_2 \quad (30)$$

$$R_{ox}^{\bullet} + O_2 \longrightarrow R_{ox}OO^{\bullet} \quad (31)$$

$$R_{ox}OO^{\bullet} + RH^- \longrightarrow R_{ox}OOH + R^{\bullet-} \quad (32)$$

$$R_{ox}OOH \longrightarrow \text{further oxidation products} \quad (33)$$

maximal rates of O_2 consumption approximately corresponded to the maximal concentrations of the intermediates *I* (Figure 6b). The UV−visible spectra of *I* were attributed to the mixed Cr(V)−ehba−Red complexes (Table 3).

Likely reactions leading to O_2 consumption are outlined in Scheme 3. The formation of thioperoxyl radicals ($RSOO^{\bullet}$, eq 20) is known to be a reason for O_2 consumption during the reactions of thiols, including Cys and GSH, with one-electron oxidants.[55] Reaction 20 can lead to the further autoxidation of RSH in a radical chain process (an example of a chain propagation reaction is given by eq 21).[60] An alternative path for O_2 consumption in the reactions of Cys or GSH with Cr(V/IV) is given by eqs 23−26.[61] Notably, both paths of O_2 consumption (eqs 20−22 and 23−26) do not lead to accumulation of H_2O_2, which is in agreement with the very low yields of H_2O_2 detected during the Cr(VI/V/IV) + RSH + O_2 reactions (Table 1) and the absence of Cr(V) peroxo species in the EPR spectra. Possible minor processes, leading to accumulation of H_2O_2, are shown by eqs 27 and 28.[61] An increase of H_2O_2 accumulation in the presence of SOD (rows 5 and 6 in Table 1) is due to the catalysis of the superoxide dismutation, eq 28,[62] which supports the role of superoxide anion in the mechanism of O_2 consumption (eqs 23−28). The proposed mechanism (eqs 20−28) is supported by the results of experiments in the presence of radical scavengers (Figure 3). A decrease in the initial O_2 consumption in the presence of DMPO (an effective scavenger of $RS^{\bullet}$)[60] shows that O_2 is consumed due to the formation of thyil radicals. The influence of SOD is due to the catalysis of reaction 28, but catalase accelerates the decomposition of the peroxo products, including H_2O_2 and RSOOH.[29] Thus, the initial O_2 consumption in the Cr(V/IV) + RSH + O_2 systems is due to the reactions of Cr(V/IV) with RSH, leading to $RS^{\bullet}$ radicals, which then rapidly react with O_2 (eqs 7, 8, and 20 in Schemes 2 and 3). The second (slower) stage of O_2 consumption is due to the chain reactions of the organic radical intermediates (eqs 21−27). The observed two stages in O_2 consumption are similar to those previously reported for the reactions of Cys and GSH with peroxonitrite.[63] The induction periods in the O_2 consumption curves for the Cr(V) + RSH reactions at pH 4.5 (Figure 6b) confirm the assumption that Cr(V)−ehba−RSH complexes are formed prior to the generation of $RS^{\bullet}$ radicals (Schemes 1 and 2).

Reactions 29 and 30 (Scheme 3), leading to the formation of superoxo and peroxo species, are considered as the main source of O_2 consumption during the metal-catalyzed autoxidations of AsA.[52] However, the assumption about the dominance of reactions 29 and 30 in the Cr(V/IV) + AsA + O_2 system is in contradiction with the observed low yields of H_2O_2, either in the absence or presence of SOD (Table 1). Furthermore, eqs 29 and 30 cannot explain the differences in O_2 consumption for the reactions of AsA with Cr(V) or Cr(IV) (Figures 2, S1, and S3), as the anion radicals are formed in comparable amounts in the reactions of AsA with both of the oxidants (Table 3, Figure S8). The alternative mechanism of fast O_2 consumption during the Cr(V) + AsA reactions is represented by eqs 18 and 31 (Schemes 2 and 3). The slower stage of O_2 consumption in the Cr(V) + AsA systems may be caused by the slow chain reactions of the relatively stable organic radicals, represented by eq 32. The small O_2 consumption in the Cr(IV) + AsA reactions can be explained by eqs 29 and 30 as well as by the presence of some Cr(V) in the stock solutions of Cr(IV) due to its fast disproportionation.[26b] The processes leading to the O_2 consumption during the Cr(V/IV) + AsA reactions require the presence of ascorbate anion radical and are, therefore, competing with its disproportionation (eq 17). This explains the observed maximum in the dependency of the amount of O_2 consumed on $[AsA]_0$ (Figure S2b). In the case of Trolox, the high

(60) Karoui, H.; Hogg, N.; Joseph, J.; Kalyanaraman, B. *Arch. Biochem. Biophys*. **1996**, *330*, 115−124.
(61) Winterbourn, C. C.; Metodiewa, D. *Arch. Biochem. Biophys*. **1994**, *314*, 284−290.
(62) Brunori, M.; Rotilio, G. *Methods Enzymol*. **1984**, *105*, 22−35.
(63) Quijano, C.; Alvarez, B.; Gatti, R. M.; Augusto, O.; Radi, R. *Biochem. J*. **1997**, *322*, 167−173.

efficiency of the disproportionation reaction 17,[53] combined with the low reactivity of the Trolox anion radical toward Cr(V) and O_2, leads to negligible O_2 consumption (Figures 2 and S3).

Two alternatives to the proposed mechanism (Schemes 1−3) were considered. Kortenkamp et al.[3b,8] and Lefebvre and Pézerat[64] suggested that O_2 activation during the Cr(VI) + Red reactions was connected with the formation of Cr(V/IV) peroxo or superoxo complexes. Perez-Benito and co-workers[65] suggested that the Cr(VI) + RSH reactions in neutral aqueous solutions produced Cr(II) intermediates, which then rapidly react with O_2.[66] Both possibilities were examined for the Cr(V/IV) + Red reactions. The kinetic studies of the Cr(V/IV) + Red reactions, performed in Ar-saturated or air-saturated solutions, did not reveal either significant changes in kinetics or the formation of new intermediates in the presence of O_2.[67] The results of EPR-spectroscopic studies showed no evidence for the formation of the relatively stable Cr(V) peroxo or superoxo complexes during the Cr(V/IV) + Red + O_2 reactions. Formation of relatively stable Cr(II) intermediates in the Cr(V/IV) + Red systems is inconsistent with the stoichiometry results, obtained from Ar-saturated solutions (Table 2). Therefore, if Cr(V/IV) peroxo or superoxo complexes or Cr(II) intermediates are formed during the Cr(V/IV) + Red reactions, they should be unstable and exist at low steady-state concentrations. However, a comparison of the O_2 consumption kinetics with that of the redox processes shows that in most cases the redox reactions are practically complete before most of the O_2 is consumed (Figure 6a). Furthermore, if one assumes that O_2 is activated in the reactions with Cr compounds, rather than with organic radicals, it becomes difficult to explain the absence of O_2 consumption in the reactions of Trolox, as this compound reduces both Cr(V) and Cr(IV) with the rates comparable to those of the corresponding reactions with Cys, GSH, or AsA (Table 2). Thus, the current work shows that O_2 consumption during the Cr(V/IV) + Red processes is caused by reactions of organic radical intermediates; Cr(V/IV) species act as the initiators of radical chain reactions.

Implications to the Roles of O_2 in Cr-Induced Genotoxicities. The lack of significant H_2O_2 accumulation as a result of O_2 consumption during the Cr(VI/V/IV) + Red reactions (Table 1) supports the suggestion of Kortenkamp et al.[3b,8] that Fenton-like reactions (eq 34) do not play a significant role in the systems Cr(VI) + Red + O_2 in the absence of added H_2O_2:

$$H_2O_2 + Cr^{(n-1)+} \rightarrow Cr^{n+} + {}^{\bullet}OH + OH^- \qquad (34)$$

The absence of evidence for the formation of Cr(V/IV) peroxo or superoxo complexes in the Cr(V/IV) + Red + O_2 systems does not disprove the potential role of such complexes (existing at low steady-state concentrations) in genotoxic effects of the Cr(VI) + Red + O_2 systems. Appreciable concentrations of Cr(V) peroxo and peroxo−ascorbato complexes are generated during the Cr(VI) + AsA + O_2 reaction in neutral aqueous solutions, if H_2O_2 is allowed to accumulate during the autoxidation of AsA in solution, prior to its mixing with Cr(VI).[12] These peroxo complexes are formed by the reaction of H_2O_2 with Cr(V) intermediates of the Cr(VI) + AsA reaction. The low yields of H_2O_2, produced in the fast O_2 consumption caused by the Cr(V/IV) + AsA reactions, contrast with the significant yield of H_2O_2 in the relatively slow O_2 consumption due to the AsA autoxidation (Table 1) and may be indicative of negligible amounts of Cr(IV/V) peroxo complexes being produced in the former reactions. In agreement with previous work,[61] little or no H_2O_2 is formed during the autoxidation of Cys or GSH (Table 1), and no evidence was found for the formation of Cr(V) peroxo species in the Cr(VI) + RSH + O_2 systems (RSH = Cys or GSH).[68] Therefore, though the formation of Cr(V) peroxo species is the most likely reason for the in vitro DNA damage by the Cr(VI) + AsA + O_2 system,[12] similar assumptions cannot be applied to the Cr(VI) + RSH + O_2 systems. This is consistent with the observations that the in vitro DNA damage induced by intermediates in the Cr(VI) + AsA reaction is more susceptible to inhibition by catalase than that caused by intermediates in the Cr(VI) + GSH reaction.[8,9] Furthermore, Cr(V) peroxo species are unlikely to be important for Cr(VI)-induced genotoxicities in vivo, since such species are detected only in the presence of significant equilibrium concentrations of H_2O_2,[11b,12] which is excluded in biological systems due to the presence of scavenging enzymes.[3b]

The Cr(V/IV) species, formed during the reactions of Cr(VI) with intracellular reductants (Cys, GSH, or AsA) in the presence of biological stabilizing ligands, such as carbohydrates and 2-hydroxycarboxylates,[6,46] can act as the initiators of chain reactions involving organic radicals and O_2 (Schemes 2 and 3). These processes may be responsible for the experimentally observed[69] formation of carbon-based radicals and enhanced level of lipid peroxidation in living organisms exposed to Cr(VI). The formation of active oxygen species, including lipid peroxides can, in turn, cause the reoxidation of Cr(III) complexes,[70] accumulated in Cr(VI)-exposed cells,[71] leading to highly reactive Cr(V/IV) species.

The role of Cr(V/IV) species as the initiators of radical chain reactions in the presence of Red and O_2 (Schemes 2 and 3) does not exclude the possibility of direct DNA oxidation by these species. Indeed, the actions of Cr(V/IV) in acetate buffer at pH 3.8 are known to cause extensive strand breaks in isolated DNA;[5] however, no significant O_2 consumption by these systems was observed (see Results). Furthermore, rapid one-electron reductions of Cr(V/IV) by Red (Table 2, Scheme 2) lead to the possibility of similar (although much slower)[72] reactions with DNA. These reactions will produce DNA radicals or anion radicals, which then can be further oxidized by O_2.[73] Similar mechanisms have been proposed by Sugden and Wetterhahn[74] for the O_2-dependent oxidation of nucleotides by $[Cr^{V}O(ehba)_2]^-$. The other possible mechanism for direct

(64) Lefebvre, Y.; Pézerat, H. *Chem. Res. Toxicol*. **1992**, *5*, 461−463.

(65) Perez-Benito, J. F.; Arias, C.; Lamrhari, D. *New J. Chem*. **1994**, *18*, 663−666.

(66) Formation of Cr(II) intermediates is well documented for the reactions of Cr(VI) with alcohols in strongly acidic media: Scott, S. L.; Bakac, A.; Espenson, J. H. *J. Am. Chem. Soc*. **1992**, *114*, 4205−4213.

(67) This is in agreement with the recent data (O'Brien, P.; Woodbridge, N. *Polyhedron* **1997**, *16*, 2081−2086), showing no O_2 dependence for the kinetics of the Cr(VI) + AsA reaction (observed spectrophotometrically by the decay of Cr(VI)) in demetalated buffers. The inhibition of this reaction by O_2, reported earlier (Dixon, D. A.; Sadler, N. P.; Dasgupta, T. P. *J. Chem. Soc., Dalton Trans*. **1993**, 3489−3495), was probably due to the significant autoxidation of AsA in unpurified buffers.[12.]

(68) Zhang, L. Ph.D. Thesis, University of Sydney, 1998.

(69) Kadiiska, M. B.; Xiang, Q.-H.; Mason, R. P. *Chem. Res. Toxicol*. **1994**, *7*, 800−805, and references therein.

(70) Bakac, A.; Wang, W.-D. *J. Am. Chem. Soc*. **1996**, *118*, 10325−10326.

(71) Dillon, C. T.; Lay, P. A.; Cholewa, M.; Legge, G. J. F.; Bonin, A. M.; Collins, T. J.; Kostka, K. L.; Shea-McCarthy, G. *Chem. Res. Toxicol*. **1997**, *10*, 533−535.

(72) The additions of 2 g L^{-1} of DNA did not cause significant changes in the rates of Cr(V) or Cr(IV) decompositons ($[Cr]_0$ = 0.20 mM, 0.10 M phosphate buffer, pH 7.0, without $NaClO_4$, 37 °C).

(73) These reactions are not expected to lead to significant O_2 consumption because of the relatively low rates of DNA oxidation by Cr(V/IV) complexes.[73.]

(74) (a) Sugden, K. D.; Wetterhahn, K. E. *J. Am. Chem. Soc*. **1996**, *118*, 10811−10818. (b) Sugden, K. D.; Wetterhahn, K. E. *Chem. Res. Toxicol*. **1977**, *10*, 1397−1406.

interactions of Cr(V/IV) intermediates with DNA includes the formation of Cr(V/IV)−DNA−Red complexes, which are then converted to the corresponding Cr(III) complexes. Evidence for the formation of such Cr(III)−DNA−Red complexes have been reported for the living cells exposed to Cr(VI) as well as for the reactions of isolated DNA with Cr(VI) in the presence of GSH or Cys.[3]

Thus, the formation of organic radical intermediates during the Cr(VI/V/IV) + Red reactions, leading to O_2 activation (Schemes 2 and 3), is one of the possible mechanisms, contributing to Cr(VI)-induced genotoxicities. The alternative mechanisms include direct interactions of DNA with the Cr(V/IV) intermediates of the Cr(VI) + Red reactions.[3−5,74] These two types of mechanisms are not mutually exclusive, and both require further investigation in terms of their possible involvement in Cr-related carcinogenicities.

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Supporting Information Available: Table of rate constants for the reaction Cr(V/IV) + DHAA and figures showing typical O_2 consumption kinetic curves; dependences of O_2 consumption on $[Red]_0$; CD spectra of the Cr(III) products for the Cr(VI/V/IV) + Red reactions; and estimated spectra of the reacting species (global kinetic analysis data) for the Cr(V/IV) + Red reactions (11 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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