Reactions of Chromium(VI/V/IV) with Bis(*O*-ethyl-L-cysteinato-*N*,*S*)zinc(II): A Model for the Action of Carcinogenic Chromium on Zinc-Finger Proteins[1]

Aviva Levina, Angela M. Bailey, Guillaume Champion,† and Peter A. Lay*

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

Received December 16, 1999

Abstract: The reactions of Cr(VI/V/IV) with a model thiolato complex $[Zn(SR)_2]$ (RSH = *O*-ethyl-L-cysteine), resembling a tetrahedral (2*S*,2*N*) Zn(II) binding site in zinc-finger proteins, have been studied in comparison with those of the free ligand. The stability of $[Zn(SR)_2]$ in aqueous solutions with pH 6−11 (25 °C, Ar-saturated) has been established by CD spectroscopy. Stoichiometries and products of the reactions of $[Zn(SR)_2]$ or RSH with $[Cr^{VI}O_4]^{2-}$ or $[Cr^{V}O(ehba)_2]^-$ (ehba = 2-ethyl-2-hydroxybutanoato(2−)) at pH 6.5−8.5 (25 °C, Ar) were studied by UV−vis and CD spectroscopies and by electrospray mass spectrometry. Disulfide (RSSR) is the only detectable oxidation product of both $[Zn(SR)_2]$ and RSH. In the case of RSH, relatively stable Cr(III) complexes, $[Cr^{III}(SR)_3]^0$ and $[Cr^{III}(SR)_2]^+$, are also formed and subsequently hydrolyzed to $[Cr^{III}(Cys)_2]^-$. This is the first observation of a Cr(III)-facilitated hydrolysis of a cysteine ester. Ascorbate promotes the oxidation of $[Zn(SR)_2]$ by $[Cr^{VI}O_4]^{2-}$; this reaction leads to formation of RSSR and of the Cr(III)−thiolato complexes. Kinetics of the reactions of $[Zn(SR)_2]$ or RSH with $[Cr^{VI}O_4]^{2-}$, $[Cr^{V}O(ehba)_2]^-$, or $[Cr^{IV}O(qa)(qaH)]^-$ (qa = quinato(2−) = (1*R*,3*R*,4*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylato(2−)) at pH 7.40 (25 °C, Ar) were studied by conventional and stopped-flow UV−vis spectrophotometry and by global kinetic analysis. Complexes of Cr(V) and Cr(IV) rapidly react with both the reductants, and reactions of Cr(V) pass through the Cr(IV) intermediates. By contrast with the reaction with RSH, the reaction of $[Cr^{V}O(ehba)_2]^-$ with $[Zn(SR)_2]$ is not accompanied by a significant O_2 consumption (measured by an oxygen electrode), suggesting the ability of $[Zn(SR)_2]$ to inhibit free radical reactions, similar to that of zinc metallothioneins. The mechanism of $[Zn(SR)_2]$ oxidation by Cr(VI/V/IV), including intramolecular formation of a disulfide bond, has been proposed. Implications of the results to the Cr(VI)-induced carcinogenesis and to the biological activity of Cr(III) are discussed.

Introduction

Chromium(VI) is an established carcinogen and one of the major chemical occupational hazards.[2] Mutagenicity of Cr(VI) in cell assays is well-known, but mechanisms of this action are still a matter of debate.[3] At the current state of knowledge, the following chemical properties of Cr are thought to be responsible for its mutagenicity and carcinogenicity: (i) tetrahedral $[Cr^{VI}O_4]^{2-}$ ions (resembling $[SO_4]^{2-}$ and $[HPO_4]^{2-}$) easily permeate the cells through anion channels,[4] while insoluble Cr(VI) particles enter the cells by phagocytosis;[5] (ii) inside the cells or while passing through cell membranes, Cr(VI) is reduced by a variety of compounds, such as ascorbate, glutathione, NADH, and tocopherols,[6] causing the formation of the highly reactive Cr(V/IV) and free radical intermediates, which are capable of inducing oxidative damage to DNA and proteins;[4−6] (iii) Cr(V/IV) can exist in the cells as relatively long-lived species, stabilized by biological ligands such as 2-hydroxycarboxylates and 1,2-diolates;[7] (iv) kinetically inert Cr(III) complexes[8] of DNA and proteins are formed as the final products of Cr(VI) intracellular reductions, leading to the lesions that may disrupt functioning of the biomolecules.[9] Numerous studies have shown the abilities of the Cr(VI) + reductant systems and of the model Cr(V/IV) complexes to induce various forms of potentially carcinogenic DNA damage, including strand breaks, abasic sites, and DNA−protein or DNA−DNA cross-links.[10,11]

† On leave from Ecole Normale Supérieure de Lyon, 69007 Lyon, France.

(1) Abbreviations: Cys = L-cysteinato(2−); DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EDTA = ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; $ehbaH_2$ = 2-ethyl-2-hydroxybutanoic acid; ESMS = electrospray mass spectrometry; HEPES = 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; qaH_2 = D-(−)-quinic acid = (1*R*,3*R*,4*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid; RSH = *O*-ethyl-L-cysteine; Tris = tris(hydroxymethyl)aminomethane.

(2) *IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 49. Chromium, Nickel and Welding*; International Agency on the Research of Cancer: Lyon, France, 1990.

(3) De Flora, S.; Camoirano, A.; Bagnasco, M.; Zanacchi, P. In *Handbook of Metal−Ligand Interactions in Biological Fluids*; Berthon, G., Ed.; Marcel Dekker: New York, 1995; Vol. 2, pp 1020−1036.

(4) Connett, P. H.; Wetterhahn, K. E. *Struct. Bonding (Berlin)* **1983**, *54*, 93−124.

(5) Singh, J.; Carlisle, D. L.; Pritchard, D. E.; Patierno, S. R. *Oncol. Rep.* **1998**, *5*, 1307−1318 and references therein.

(6) Cieślak-Golonka, M. *Polyhedron* **1996**, *15*, 3667−3689 and references therein.

(7) Codd, R.; Lay, P. A. *J. Am. Chem. Soc.* **1999**, *121*, 7864−7876 and references therein.

(8) 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.

(9) (a) Zhitkovich, A.; Voitkun, V.; Kluz, T.; Costa, M. *Environ. Health Perspect.* **1998**, *106* (Suppl. 4), 969−974. (b) Bridgewater, L. C.; Manning, F. C. R.; Patierno, S. *Mol. Carcinog.* **1998**, *23*, 201−206 and references therein.

10.1021/ja9944047 CCC: $19.00
Published on Web 06/16/2000

Another possible mechanism of Cr genotoxicity, namely, the Cr(VI)-induced damage of nuclear transcription factors, has received much less attention. Disruption of transcription factor functions, induced by transition-metal ions, can block the normal DNA replication,[12] or prevent the repair of damaged DNA sites.[13] Zinc-finger proteins form the largest family of transcription factors; some of them, such as p53, are thought to be critically important in carcinogenesis.[14] Biological activity of zinc-finger proteins is dependent on the formation of Zn(II)−S bonds with the protein cysteine residues, and oxidative damage of these bonds leads to the loss of protein−DNA binding ability.[15] There are several conflicting reports on suppression[16] or activation[17] of the DNA binding abilities of different transcription factors in the presence of Cr(VI/V/IV). Our preliminary studies have shown a decrease in DNA binding ability of a zinc-finger protein, GATA-1, treated by a model Cr(V) complex.[18] The chemistry of Cr(VI/V/IV) interactions with zinc-finger proteins remains unknown due to the absence of model studies and to the general lack of knowledge on the reactivities of Zn(II)−S bonds.[19]

In this work, we studied the reactions of Cr(VI/V/IV) with a simple model complex, bis(*O*-ethyl-L-cysteinato-*N*,*S*)zinc(II) ($[Zn(SR)_2]$), which mimics a typical tetrahedral (2Cys, 2His) binding site of Zn(II) in zinc-finger proteins, in comparison with the corresponding reactions of the free ligand (RSH). The relatively stable complexes, $[Cr^{V}O(ehba)_2]^-$ and $[Cr^{IV}O(qa)(qaH)]^-$, used in this work are models of stabilized Cr(V/IV) species which are likely to form intracellularly.[7] The results demonstrate the ability of Cr(VI/V/IV) to cause Zn(II) release with the formation of S−S bonds, and to a lesser extent, of Cr(III)−S bonds upon the reaction with $[Zn(SR)_2]$. The ability of $[Zn(SR)_2]$ to inhibit the formation of reactive oxygen species, resembling that of zinc metallothioneins,[20] has also been established. In addition, hydrolysis of RSH has been observed to be facilitated by binding to Cr(III); this reaction may be relevant to Cr(III)−peptide interactions in vivo.

Experimental Section

Caution. *Cr(VI) and As(III) compounds are human carcinogens,*[2,21] *and Cr(V/IV) complexes are mutagenic and potentially carcinogenic.*[22] *Contact with skin and inhalation must be avoided.*

Reagents. The following commercial reagents of analytical or higher purity grade were used without purification: L-cysteine, *O*-ethyl-L-cysteine hydrochloride, DMF, DMSO, 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), 2-ethyl-2-hydroxybutanoic acid, As_2O_3, NaOH, and $Na_2CrO_4{\cdot}4H_2O$ (all Aldrich); CH_3COOH, $HClO_4$, HCl (35% aqueous solution), NH_3 (32% aqueous solution), Na_2EDTA, and KBr (all Merck); $Zn(SO_4)_2{\cdot}7H_2O$ (BDH Chemicals); L-ascorbic acid (BDH Biochemicals); D-(−)-quinic acid (ICN Biomedicals); Tris and Chelex 100 (Bio-Rad); HEPES (Research Organics); *sym*-diphenylcarbazide (Fluka). Water was purified by the Milli-Q technique. Zinc(II) complexes, $[Zn(SR)_2]$ and $Na_2[Zn(Cys)_2]{\cdot}6H_2O$, were synthesized by literature methods,[23] and their purities were confirmed by FTIR spectroscopy and by determination of [Zn] and [thiol]. Chromium complexes, $Na[Cr^{V}O(ehba)_2]{\cdot}H_2O$ and $Na[Cr^{III}(Cys)_2]{\cdot}2H_2O$, were synthesized by literature methods,[24,25] and their purities were confirmed by FTIR spectroscopy (Cr(V) and Cr(III)), UV−vis and EPR spectroscopies (Cr(V)), or determination of [Cr] and [thiol] (Cr(III)).

General Methods. Stock solutions of the buffers (0.50 M HEPES + NaOH, pH 7.40, or 1.0 M CH_3COOH + NH_3, pH 7.4) were treated by Chelex 100 chelating resin (the pH values were measured after the treatment using an Activon 210 ionometer with an AEP 321 combined glass/calomel electrode), and stored at 4 °C. Concentrations of the catalytic metals (Fe(III) and Cu(II)) in the purified buffers, determined by Buettner's ascorbate method,[26] were <0.5 μM. Stock solutions of the reagents, 0.20 M RSH·HCl in H_2O, 0.10 M ascorbic acid in H_2O, 0.10 M $[Zn(SR)_2]$ in DMF, and 0.10 M $Na[Cr^{V}O(ehba)_2]$ in DMF, were prepared daily; the former two solutions were stored under Ar.[27] Equivalent amounts of NaOH were added to the reaction solutions to neutralize RSH·HCl and ascorbic acid. The reaction solutions were prepared within ∼10 min of use and were saturated with high-purity Ar (BOC gases), except for the O_2 consumption experiments, where air-saturated solutions were used. Residual $[O_2]$ in Ar-saturated solutions were <0.01 mM. Measurements of residual O_2 as well as O_2 consumption studies were performed using a Clark-type oxygen electrode (Rank Brothers), as described previously.[28] All experiments were carried out at 25 ± 0.1 °C, using a Grant LTD 6G, Thermomix 1419, or HP 89090A thermostat. In the stoichiometry and product studies, the reaction mixtures were passed through 0.2 μm cellulose membrane filters (Minisart RC) prior to the analyses to remove insoluble products. The UV−vis spectroscopic measurements were carried out using a Hewlett-Packard HP 8452A diode-array spectrophotometer. The EPR spectra (X-band) were recorded on a Bruker EMX spectrometer, with experimental settings described previously.[28] The FTIR spectra (in KBr matrix) were recorded using the diffuse reflectance technique on a BioRad FTS-40 spectrometer. Determinations of [Zn] and [Cr] were performed by C_2H_2/air flame AAS (0.10 M HCl matrix), using a Varian SpecAA-800 spectrometer.

(10) (a) Kortenkamp, A.; Casadevall, M.; Da Cruz Fresco, P.; Shayer, R. O. J. *NATO ASI Ser., Ser. 2* **1997**, *26*, 15−34. (b) Stearns, D. M.; Wetterhahn, K. E. *NATO ASI Ser., Ser. 2* **1997**, *26*, 55−72 and references therein.

(11) Levina, A.; Barr-David, G.; Codd, R.; Lay, P. A.; Dixon, N. E.; Hammershøi, A.; Hendry, P. *Chem. Res. Toxicol.* **1999**, *12*, 371−381 and references therein.

(12) Louie, A. Y.; Meade, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6663−6668 and references therein.

(13) Hartwig, A. *Toxicol. Lett.* **1998**, *102−103*, 235−239.

(14) (a) Klug, A. *J. Mol. Biol.* **1999**, *293*, 215−218. (b) Green, A.; Parker, M.; Conte, D.; Sarkar, B. *J. Trace Elem. Exp. Med.* **1998**, *11*, 103−118. (c) Méplan, C.; Mann, K.; Hainaut, P. *J. Biol. Chem.* **1999**, *274*, 31663−31670 and references therein.

(15) (a) Casadevall, M.; Sarkar, B. *J. Inorg. Biochem.* **1998**, *71*, 147−152. (b) Fojta, M.; Kubicarova, T.; Vojtesek, B.; Palecek, E. *J. Biol. Chem.* **1999**, *274*, 25749-25755. (c) Park, J. S.; Wang, M.; Park, S. J.; Lee, S. H. *J. Biol. Chem.* **1999**, *274*, 29075−29080.

(16) Shumilla, J. A.; Wetterhahn, K. E.; Barchowsky, A. *Arch. Biochem. Biophys.* **1998**, *349*, 356−362.

(17) (a) Ye, J. P.; Zhang, X.; Young, H. A.; Mao, Y.; Shi, X. *Carcinogenesis* **1995**, *16*, 2401−2405. (b) Shi, X. L.; Ding, M.; Ye, J. P.; Wang, S. W.; Leonard, S. S.; Zang, L. Y.; Castranova, V.; Vallyathan, V.; Chiu, A.; Dalal, N.; Liu, K. J. *J. Inorg. Biochem.* **1999**, *75*, 37−44. (c) Kaltreider, R. C.; Pesce, C. A.; Ihnat, M. A.; Lariviere, J. P.; Hamilton, J. W. *Mol. Carcinog.* **1999**, *25*, 219−229.

(18) (a) O'Connell, A. M. B.Sc. (Hons.) Thesis, University of Sydney, 1997. (b) Bailey, A. M.; Crossley, M.; Lay, P. A. Unpublished results.

(19) (a) Kuehn, C. G.; Isied, S. S. *Prog. Inorg. Chem.* **1980**, *27*, 153−221. (b) Brand, U.; Rombach, M.; Vahrenkamp, H. *J. Chem. Soc., Chem. Commun.* **1998**, 2717−2718 and references therein.

(20) (a) Elgohary, W. G.; Sidhu, S.; Krezoski, S. O.; Petering, D. H.; Byrnes, R. W. *Chem.-Biol. Interact.* **1998**, *115*, 85−107. (b) Cai, L.; Tsiapalis, G.; Cherian, M. G. *Chem.-Biol. Interact.* **1998**, *115*, 141−151.

(21) Leonard, A.; Lauwerys, R. R. *Mutat. Res.* **1980**, *75*, 49−62 and references therein.

(22) Dillon, C. T.; Lay, P. A.; Bonin, A. M.; Cholewa, M.; Legge, G. J. F.; Collins, T. J.; Kostka, K. L. *Chem. Res. Toxicol.* **1998**, *11*, 119−129 and references therein.

(23) Synthesis and X-ray crystal structures of the Zn(II) complexes are described in Bell, P.; Sheldrick, W. S. *Z. Naturforsch.* **1984**, *39b*, 1732−1737.

(24) Synthesis of the Cr(V) complex is described in Krumpolc, M.; Roček, J. *J. Am. Chem. Soc.* **1979**, *101*, 3206−3209. Its X-ray crystal structure is described in Judd, R. J.; Hambley, T. W.; Lay, P. A. *J. Chem. Soc., Dalton Trans.* **1989**, 2205−2210.

(25) Synthesis and X-ray crystal structure of the Cr(III) complex are described in De Meester, P.; Hodgson, D. J.; Freeman, H. C.; Moore, C. J. *Inorg. Chem.* **1977**, *16*, 1494−1498.

(26) Buettner, G. R. *Methods Enzymol.* **1990**, *186*, 125−127.

(27) The use of the stock solutions of $[Zn(SR)_2]$ and $Na[Cr^{V}O(ehba)_2]$ in DMF was convenient due to their high solubility and stability in this solvent. Amounts of DMF in the reaction mixtures were ≤3% (v/v). In control experiments, the absence of DMF (with all the stock solutions freshly prepared in water) did not significantly affect the results of stoichiometric and kinetic studies.

(28) Lay, P. A.; Levina, A. *J. Am. Chem. Soc.* **1998**, *120*, 6704−6714.

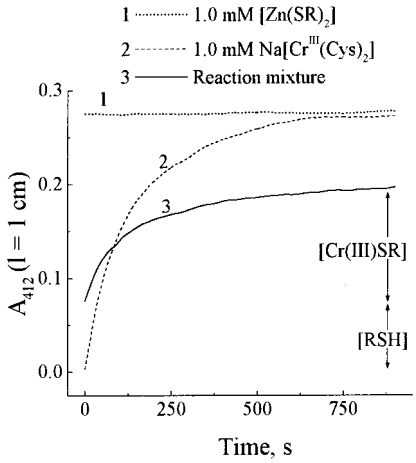


Figure 1. Typical results of the time-dependent determination of thiol groups with Ellman's reagent (see the Experimental Section for the procedure). Reaction mixture: $[Cr^{VI}O_4]^{2-}$ (1.0 mM) + ascorbate (2.0 mM) + RSH (2.0 mM), 10 mM NH_4OAc buffer, pH 6.7 → 7.7, reaction time before addition of reagent 1 h, 25 °C.

Determination of [Cr(VI)] was performed by a modified diphenylcarbazide method.[29] A solution of diphenylcarbazide in DMSO (10 μL, 40 mM) and the reaction mixture (10 μL, containing 0.01−1.0 mM Cr(VI)) were added to 0.98 mL of 0.10 M HCl, and the absorbance at 540 nm ($\epsilon = (4.2 \pm 0.1) \times 10^4$ M^{-1} cm^{-1}) was measured after 2 min against the blank containing HCl and diphenylcarbazide solutions. Calibrations were performed by a standard additions method to exclude the influence of the other components of the reaction mixtures. As the described method is known to be sensitive to Cr(V),[30] the absence of measurable amounts (>0.5 μM) of Cr(V) in the reaction mixtures was established by EPR spectroscopy (at the time when [Cr(VI)] was measured, all the unreacted Cr(V) was disproportionated to Cr(VI) and Cr(III)).[31]

Determination of Thiol Groups. Ellman's reagent[32] (10 μL, 0.10 M, in DMSO) and the reaction mixture (10 μL, 0.02−4.0 mM of thiol groups) were added to the buffer solution (0.98 mL, 0.10 M Tris + 1.0 mM Na_2EDTA, pH 8.0) and the absorbance changes at 412 nm were followed for 15 min at 25 °C against the blank containing the buffer solution and Ellman's reagent (Figure 1). In control experiments, no significant changes of A_{412} with time were observed for the reactions of RSH, $[Zn(SR)_2]$, or $Na_2[Zn(Cys)_2]$ (line 1 in Figure 1). For the reaction of $[Cr^{III}(Cys)_2]^-$ with Ellman's reagent, A_{412} increased over 15 min from nearly zero (within the instrumental error of 0.005 AU) to the value corresponding to two molar equivalents of thiol groups (line 2 in Figure 1), presumably due to the slow release of thiol groups from the Cr(III) complex. This kinetic difference was used to distinguish between free (or Zn(II)-bound) and Cr(III)-bound thiol groups in the reaction mixtures (line 3 in Figure 1). Calibrations ($\epsilon = (1.35 \pm 0.03) \times 10^4$ M^{-1} cm^{-1}) were performed by a standard additions method.

CD Spectroscopy. The spectra were recorded on a JASCO 710 spectropolarimeter; the instrumental settings were as follows: (i) for the stability studies of $[Zn(SR)_2]$, $\lambda = 200-300$ nm ($\Delta\lambda = 0.2$ nm); scan rate, 100 nm/min; response time, 0.25 s; (ii) for the studies of Cr(III) products, $\lambda = 300-700$ nm ($\Delta\lambda = 1$ nm); scan rate, 500 nm/min; response time, 0.125 s. In both cases, the spectra were averaged over five scans. Noise reduction in the resulting spectra was performed by a fast Fourier transform procedure in Origin software.[33]

Electrospray Mass Spectrometry. The ESMS analyses were performed using a Finnigan LSQ mass spectrometer; typical experimental settings were as follows: sheath gas (N_2) pressure, 60 psi; spray voltage, 5.0 kV; capillary temperature, 200 °C; capillary voltage, 19 V; tube lens offset, 25 V; *m*/*z* range, 100−2000. Analyzed solutions (~5 μL of an undiluted reaction mixture in 10 mM NH_4OAc buffer) were injected into a flow of 50% (v/v) MeOH/H_2O (flow rate 0.20 mL min^{-1}). Acquired spectra were the averages of 10 scans (scan time 10 ms). Simulations of the mass spectra were performed using IsoPro software.[34]

Kinetic Studies. Most of the kinetic data were acquired on an Applied Photophysics SX-17 MV stopped-flow spectrophotometer with a diode-array detector (deadtime ~2 ms, integration time 2.56 ms, $\lambda = 340-740$ nm, resolution ~1 nm); typically, 200 or 250 spectra were collected within 20 or 200 s (logarithmic timebase). All measurements were performed under an Ar atmosphere using an SX-17 MV anaerobic accessory. In a typical reaction of Cr(V), a solution of $Na[Cr^VO(ehba)_2]$ in H_2O (0.40 mM, acidified with $HClO_4$ to pH 3.5 to minimize decomposition)[35] was mixed in a 1:1 ratio with a solution containing $[Zn(SR)_2]$ (4.0 mM) and HEPES (0.20 M, pH 7.40). Chromium(IV) complexes were generated using a SX-17 MV sequential-mixing accessory. In a typical reaction of Cr(IV), a solution of Na_2CrO_4 (0.80 mM) was mixed in a 1:1 ratio with a solution containing 20 mM As(III) and 0.40 M qaH_2/qaH (pH 3.30).[36] After 1 s, the resulting solution was mixed in a 1:1 ratio with a solution containing $[Zn(SR)_2]$ (4.0 mM) and HEPES (0.20 M, pH 8.90, changed to pH 7.40 after the mixing). Slow reactions of Cr(VI) with $[Zn(SR)_2]$ were followed using the HP8452 A spectrophotometer; 60 spectra (300−800 nm) were collected with 15 s intervals (integration time 0.2 s). The time-dependent spectra from both spectrophotometers were processed by a global kinetic analysis method,[37] using Pro-Kineticist software,[38] as described previously.[28,31,39]

All reported results were reproduced in at least two independent experimental series, using different sets of stock solutions. Typical deviations between the results of parallel experiments were 5−10% for the stoichiometry and kinetic studies, 10−20% for CD spectroscopy, and 25−30% for ESMS.

Results

Stability Studies of $[Zn(SR)_2]$ in Aqueous Solutions. Both dissolution of $[Zn(SR)_2]$ in water (pH 6.8) and mixing of Zn(II) (one molar equivalent) with RSH (two molar equivalents) at pH 6.5−8.5 led to identical (within 10% experimental error) CD spectra, which are clearly distinct from those of RSH as shown by a negative ellipticity with $\lambda_{min} = 230$ nm, $[\theta] = -2.5 \times 10^4$ deg cm^2 $dmol^{-1}$, and $\Delta\epsilon = -7.4$ cm^2 $mmol^{-1}$ (lines 1 and 2 in Figure 2). This feature, which was not changed in the presence of buffers (NH_4OAc, phosphate, or HEPES), is remarkably similar to the reported CD spectra of zinc-finger peptides.[40] Significant changes in the CD spectra of $[Zn(SR)_2]$ due to acid hydrolysis were observed at pH ≤ 6.0, while complete hydrolysis was achieved at pH ≤ 5.0 ([Zn] = 1.0 mM, reaction time <1 min at 25 °C, lines 3 and 4 in Figure 2). This is in agreement with the results of potentiometric studies of the Zn(II)−RSH systems.[41] Reaction of $[Zn(SR)_2]$ (1.0 mM) with a NaOH solution (~1 mM; pH 11) did not lead to significant changes in the CD spectra over 5 min (25 °C); however, a prolonged reaction (12 h at 25 °C) led to the hydrolytic cleavage

(29) *Standard Methods for the Examination of Water and Wastewater*, 16th ed.; American Public Health Association: Washington, DC, 1985; Section 312B, pp 201−204.
(30) Eckert, J. M.; Judd, R. J.; Lay, P. A.; Symons, A. D. *Anal. Chim. Acta* **1991**, *255*, 31−33.
(31) Levina, A.; Lay, P. A.; Dixon, N. E. *Inorg. Chem.* **2000**, *39*, 385−395.
(32) Ellman, G. L. *Arch. Biochem. Biophys*. **1959**, *82*, 70−77.
(33) *Origin. Technical Graphics and Data Analysis for Windows*, Version 4.1; Microcal Software Inc.: Northampton, MA, 1996.
(34) Senko, M. IsoPro 3.0, Sunnyvale, CA, 1998.
(35) Krumpolc, M.; Roček, J. *Inorg. Chem.* **1985**, *24*, 617−621.
(36) Codd, R.; Lay, P. A.; Levina, A. *Inorg. Chem.* **1997**, *36*, 5440−5448.
(37) (a) Maeder, M.; Zuberbühler, A. D. *Anal. Chem.* **1990**, *62*, 2220−2224. (b) Beechem, J. M. *Methods Enzymol.* **1992**, *210*, 37−55. (c) Henry, E. R.; Hofrichter, J. *Methods Enzymol.* **1992**, *210*, 129−192.
(38) *Pro-Kineticist, Version 4.10*; Applied Photophysics Ltd.: Leatherhead, U.K., 1996.
(39) Lay, P. A.; Levina, A. *Inorg. Chem.* **1996**, *35*, 7709−7717.
(40) Posewitz, M. C.; Wilcox, D. E. *Chem. Res. Toxicol.* **1995**, *8*, 1020−1028 and references therein.
(41) Gockel, P.; Vahrenkamp, H.; Zuberbühler, A. D. *Helv. Chim. Acta* **1993**, *76*, 511−520.

Table 1. Summary of the Results of Stoichiometry and Product Studies

reaction[a]	time, h	stoichiometry[b]	$Cr^{III}SR$,[c] %	main products (found by ESMS)[d]
Cr(VI) + RSH	2−12	3.0 ± 0.2	14−21	RSSR, $[Cr^{III}(SR)_2]^+$, $[Cr^{III}(SR)_3]^0$, $[Cr^{III}(SR)_2(Cys)]^-$,[f] $[Cr^{III}(Cys)_2]^-$[f]
Cr(VI) + $[Zn(SR)_2]$	72	3.0 ± 0.1	0	RSSR[g]
Cr(V) + RSH	0.5	2.0 ± 0.1	0−10[e]	RSSR, $[Cr^{III}(ehba)(ehbaH)_2]^-$, $[Cr^{III}(SR)_2]^+$,[e] $[Cr^{III}(SR)_3]^0$[e]
Cr(V) + $[Zn(SR)_2]$	0.5	1.9 ± 0.1	0	RSSR, $[Zn(SR)(RSSR)]^+$, $[Zn(SR)(ehba)_2]^-$
Cr(VI) + ascorbate + RSH	1	3.2	32−38	RSSR, DHAA, $[Cr^{III}(SR)_2]^+$, $[Cr^{III}(SR)_3]^0$, $[Cr^{III}(SR)_2(Cys)]^-$[f]
Cr(VI) + ascorbate + $[Zn(SR)_2]$	1	3.0 ± 0.1	3.5−7.5	RSSR, DHAA

[a] Cr(VI) = $[Cr^{VI}O_4]^{2-}$; Cr(V) = $[Cr^{V}O(ehba)_2]^-$; see the text for the reaction conditions and Tables S1 and S2 for the experimental details. [b] Amount of thiol groups (mol) consumed for the reduction of 1 mol of Cr(VI/V) to Cr(III); determined from the values of [Cr(VI)] and [RSH] ([$Zn^{II}SR$]) (Table S1). [c] Ratio of Cr(III)-bound thiol groups formed in the reaction (determined with Ellman's reagent, Figure 1) to the initial amount of thiol groups. [d] Reactions in 10 mM NH_4OAc; DHAA = dehydroascorbic acid. [e] Detectable amounts of Cr(III)−RSH species were found only in the presence of excess RSH. [f] These products are formed due to the hydrolysis of the initial Cr(III)−RSH species. [g] About 100% of the formed Cr(III) and of the Zn(II) released from $[Zn(SR)_2]$ were found in the precipitate (presumably as hydroxo complexes) at the time of analysis (determined by AAS).

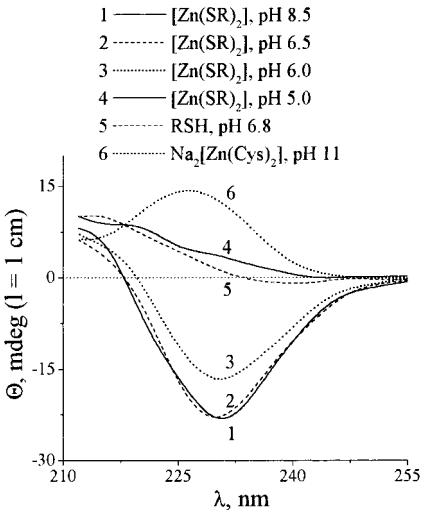


Figure 2. Typical results of $[Zn(SR)_2]$ stability studies and control experiments (CD spectra). Solutions of $[Zn(SR)_2]$ or $Na_2[Zn(Cys)_2]$ (1.0 mM) or RSH (2.0 mM) in 10 mM NH_4OAc buffers were diluted with water 10 times for the spectroscopy.

of the ligands with the formation of $[Zn(Cys)_2]^{2-}$ (identified by comparison with the CD spectrum of the authentic compound, line 6 in Figure 2). This is in agreement with the literature data on alkaline hydrolysis of Zn(II)-bound cysteine esters.[42]

Thus, the CD spectroscopic studies showed the stability of $[Zn(SR)_2]$ in aqueous solutions (pH 6−11) in the absence of O_2. In the following work, the stability of $[Zn(SR)_2]$ under the conditions used for stoichiometric and kinetic studies (in the absence of oxidants) was confirmed by CD and ESMS.

Stoichiometry and Product Studies (UV−Vis and CD Spectroscopies). In stoichiometry experiments (Tables 1 and S1, Supporting Information), $[Cr^{V}O(ehba)_2]^-$ (1.0 mM), $[Cr^{VI}O_4]^{2-}$ (1.0 mM), or $[Cr^{VI}O_4]^{2-}$ (1.0 mM) + ascorbate (1.0−2.0 mM) was used as the oxidant, and the reductant was RSH (1.5−4.0 mM) or $[Zn(SR)_2]$ (0.75−2.0 mM). The reaction mixtures were analyzed when the reaction rates became negligible (determined from the absence of significant UV−vis absorbance changes at 300−800 nm during 30 min). All the reactions were repeated in two buffer systems: (i) 0.10 M HEPES (pH 7.40 ± 0.05); (ii) 10 mM NH_4OAc, the pH values were changed over the 6.5−8.5 range (Table S1). The former buffer allowed a comparison of the different reactions under constant pH conditions, while the latter allowed a direct comparison with the ESMS results (10 mM NH_4OAc was the optimal medium for ESMS). No qualitative differences in stoichiometry and products between the reactions in the two buffer systems were apparent (Table S1).

The $[Cr^{V}O(ehba)_2]^-$ + RSH, $[Cr^{V}O(ehba)_2]^-$ + $[Zn(SR)_2]$ and $[Cr^{VI}O_4]^{2-}$ + RSH reactions were relatively fast (seconds or minutes time scale at 25 °C); while the $[Cr^{VI}O_4]^{2-}$ + $[Zn(SR)_2]$ reaction was much slower (hours or days time scale at 25 °C). Determinations of [Cr(VI)] and [thiol] after the completion of the reactions led to the expected stoichiometries of 2.0 ± 0.1 or 3.0 ± 0.2 for the reactions of $[Cr^{V}O(ehba)_2]^-$ or $[Cr^{VI}O_4]^{2-}$, respectively (Table 1); i.e., the thiol acts as a one-electron reductant in conversion of Cr(V) or Cr(VI) to Cr(III).[28] Time-dependent determinations with Ellman's reagent (Figure 1) revealed the formation of Cr(III)−thiol species in the reactions of $[Cr^{VI}O_4]^{2-}$ with RSH (with either reagent in excess), as well as in the reactions of $[Cr^{V}O(ehba)_2]^-$ with excess RSH (Table 1). By contrast, no detectable amounts of Cr(III)−thiol products were observed for the $[Cr^{V}O(ehba)_2]^-$ (excess) + RSH, $[Cr^{V}O(ehba)_2]^-$ + $[Zn(SR)_2]$, or $[Cr^{VI}O_4]^{2-}$ + $[Zn(SR)_2]$ reactions (Table 1). Formation of Cr(III)−thiol species, determined with Ellman's reagent, corresponded to the formation of optically active Cr(III) species, revealed by CD spectroscopy ($\lambda_{max} \approx 550$ nm, $\lambda_{min} \approx 435$ nm, line 1 in Figure 3a). Intensities of the CD signals correlated well with the $[Cr^{III}SR]$ values, determined with Ellman's reagent (Figure S1, Supporting Information).

The reactions of $[Zn(SR)_2]$ or RSH with the $[Cr^{VI}O_4]^{2-}$ + ascorbate system were designed to mimic biological conditions, where highly oxidizing species are formed during the reactions of Cr(VI) with intracellular reductants, predominantly ascorbate.[43] In the $[Cr^{VI}O_4]^{2-}$ + ascorbate + RSH systems, both reductants react with Cr(VI) at comparable rates, leading to the formation of significant amounts of Cr(III)−thiol species (Table 1). More importantly, the relatively fast $[Cr^{VI}O_4]^{2-}$ + ascorbate reaction is able to accelerate the slow $[Cr^{VI}O_4]^{2-}$ + $[Zn(SR)_2]$ reaction. The reactions of $[Cr^{VI}O_4]^{2-}$ with ascorbate in the presence of $[Zn(SR)_2]$ led to the oxidation of significant amounts of thiol groups and to the reduction of additional Cr(VI) (Table S1). The uncatalyzed $[Cr^{VI}O_4]^{2-}$ + $[Zn(SR)_2]$ reaction is probably negligible under these conditions, as [Cr(VI)] is low due to the rapid reaction with ascorbate.[43] However, the most obvious evidence for the ascorbate-promoted reaction of $[Zn(SR)_2]$ with $[Cr^{VI}O_4]^{2-}$ was the formation of Cr(III)−thiol species, detected both by Ellman's reaction (Table 1) and by CD spectroscopy (line 1 in Figure 3b). The CD spectrum of the reaction $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ product was

(42) (a) Yatsimirskii, K. B.; Tikhonova, L. P. *Zh. Neorg. Khim.* **1967**, *12*, 417−423; *Russ. J. Inorg. Chem. (Engl. Transl.)* **1967**, *12*, 215−218. (b) Hay, R. W.; Porter, L. J. *J. Chem. Soc., Chem. Commun.* **1967**, 653−654.

(43) (a) Stearns, D. M.; Wetterhahn, K. E. *Chem. Res. Toxicol.* **1994**, *7*, 9−230. (b) Zhang, L.; Lay, P. A. *J. Am. Chem. Soc.* **1996**, *118*, 12624−12637.

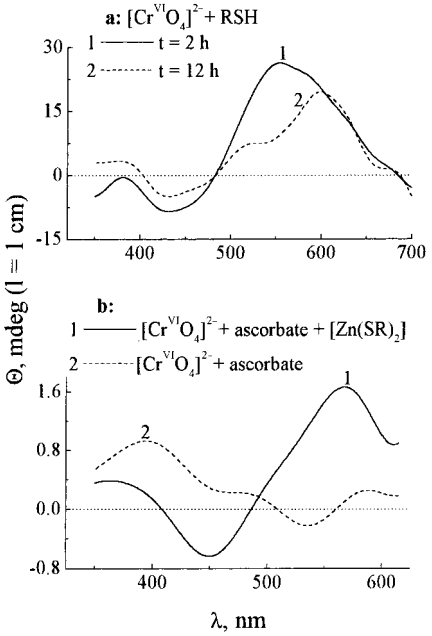


Figure 3. Typical CD spectra of the reaction mixtures. (a) Reaction of $[Cr^{VI}O_4]^{2-}$ (1.0 mM) with RSH (4.0 mM) in 10 mM NH_4OAc buffer, pH 6.8 → 8.5, 25 °C; spectra are taken immediately after completion of the reaction (2 h, line 1) or after 12 h (line 2). (b) Reaction of $[Cr^{VI}O_4]^{2-}$ (1.0 mM) with ascorbate (2.0 mM) in the presence (line 1) or absence (line 2) of 1.0 mM $[Zn(SR)_2]$; 10 mM NH_4OAc buffer, pH 6.5 → 8.0, reaction time 1 h, 25 °C.

similar, though not identical, to that of the $[Cr^{VI}O_4]^{2-}$ + RSH reaction, and very different from that of the $[Cr^{VI}O_4]^{2-}$ + ascorbate reaction (Figure 3). No Cr(III)−thiol species were formed under similar conditions in the reactions of $[Zn(SR)_2]$ either with $[Cr^{VI}O_4]^{2-}$ (in the absence of ascorbate) or with the Cr(III) products of the $[Cr^{VI}O_4]^{2-}$ + ascorbate reaction (established by CD spectroscopy and Ellman's reaction, Table S1).

Product Studies by Mass Spectrometry. Typical results of ESMS analyses of the reaction mixtures (in 10 mM NH_4OAc buffers under the conditions corresponding to those of the stoichiometry studies) are shown in Figure 4, and a list of all significant (≥2 times the noise level and reproduced in parallel experiments) ESMS signals and their relative abundancies is given in Table S2, Supporting Information. Assignment of the signals (Tables 1 and S2) was performed from their *m*/*z* values and isotopic distribution features (typical examples are shown in Figure S2, Supporting Information). In all of the studied systems, no significant signals with *m*/*z* > 1000 were detected. Reactions of RSH (detected as $RSH{\cdot}H^+$, *m*/*z* = +150) with $[Cr^{V}O(ehba)_2]^-$, $[Cr^{VI}O_4]^{2-}$, or $[Cr^{VI}O_4]^{2-}$ + ascorbate resulted in the formation of the corresponding disulfide ($RSSR{\cdot}H^+$, *m*/*z* = +297) as the only detectable oxidation product, as well as of $[Cr^{III}(SR)_2]^+$ (*m*/*z* = +348) and $[Cr^{III}(SR)_3]^0$ species (the latter formed a number of adducts with both cations and anions under ESMS conditions, Figure 4a and Table S2). However, after 12 h (pH 8.5, 25 °C), both of the initially formed Cr(III) complexes were converted into $[Cr^{III}(Cys)_2]^-$ (*m*/*z* = −290; confirmed by the observation of the characteristic CD spectrum of this species, line 2 in Figure 3a).[39] Thus, binding to Cr(III) effectively promotes the hydrolysis of RSH. Under similar conditions, no significant hydrolysis of Zn(II)-bound RSH was observed by ESMS (Table S2) or CD spectroscopy (Figure 2). In agreement with the results of UV−vis and CD spectroscopies (Table 1), no detectable amounts of Cr(III)−RSH complexes were formed in the $[Cr^{VI}O_4]^{2-}$ + $[Zn(SR)_2]$, $[Cr^{V}O(ehba)_2]^-$ + $[Zn(SR)_2]$, or $[Cr^{V}O(ehba)_2]^-$ (excess) + RSH reactions (Table S2).

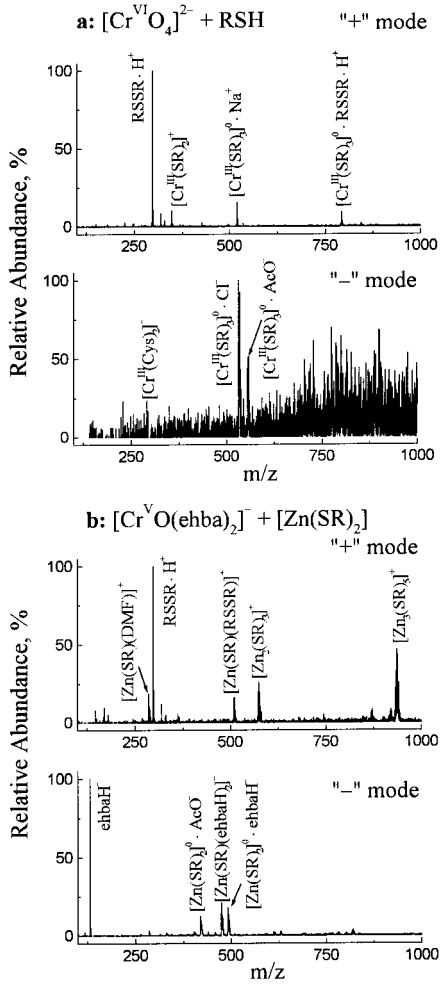


Figure 4. Typical ESMS of the reaction mixtures (the lists of all significant signals and their assignments are given in Table S2). (a) Reaction of $[Cr^{VI}O_4]^{2-}$ (1.0 mM) with CysOEt (4.0 mM) in 10 mM NH_4OAc buffer, pH 6.8 → 8.5, reaction time 2 h, 25 °C. (b) Reaction of $[Cr^{V}O(ehba)_2]^-$ (1.0 mM) with $[Zn(SR)_2]$ (1.5 mM) in 10 mM NH_4OAc buffer, pH 7.4 → 6.7, reaction time 0.5 h, 25 °C.

Dehydroascorbate was the only detected oxidation product of ascorbate; no Cr(III) complexes with ascorbate or dehydroascorbate were detected in the products of the $[Cr^{VI}O_4]^{2-}$ + ascorbate + RSH or $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ reactions (Table S2).

No Cr(III)−RSH species were observed by ESMS in the products of $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ reactions despite the detection of such species by Ellman's reaction and by CD spectroscopy (Table 1, Figure 3b). The possible reasons are (i) low concentrations of Cr(III)−RSH species formed in the $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ reactions (Table 1), thus their signals could not be distinguished from the noise in ESMS, and (ii) the formation of mixed RSH−Cr(III)−ascorbate (dehydroascorbate) complexes, which are not stable under ESMS conditions. The latter suggestion is supported by the shifts in the λ_{max} and λ_{min} values in the CD spectra of the $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ reaction products in comparison with those of the $[Cr^{VI}O_4]^{2-}$ + RSH reaction (lines 1 in Figure 3a,b) and by the failure to detect Cr(III)−ascorbate (dehydroascorbate) species by ESMS.

The neutral $[Zn(SR)_2]$ complex was detected by ESMS in the form of ion adducts (e.g., $[Zn(SR)_2]^0{\cdot}AcO^-$, *m*/*z* = −419) or clusters (e.g., $[Zn_2(SR)_3]^+$, *m*/*z* = +572, or $[Zn_3(SR)_5]^+$, *m*/*z* = +932, Figure 4b). The clusters were probably formed upon the removal of solvent under ESMS conditions, as their relative

Table 2. Pseudo-First-Order Rate Constants for the Reactions of Cr(VI/V/IV) with Buffer Alone, $[Zn(SR)_2]$, or RSH[a]

		reductant	
oxidant	buffer	$[Zn(SR)_2]$	RSH
$[Cr^{VI}O_4]^{2-}$	$< 10^{-6}$ [d]	9.4×10^{-5} [d]	7.4×10^{-3}
$[Cr^{V}O(ehba)_2]^{-}$	2.5×10^{-2} [e]	9.1×10^{-2}	3.0
$[Cr^{V}O(ehba)_2]^{-}$ [b]	4.3×10^{-3} [e]	3.5×10^{-1}	6.7
$[Cr^{IV}O(qa)(qaH)]^{-}$ [c]	4.0×10^{-3} [e]	2.0×10^{-1}	1.3

[a] Observed rate constants (s^{-1}) for the reaction step where the main amount of the initial Cr(VI/V/IV) is reduced (determined from global kinetic analyses of time-dependent UV−vis spectra, Figures S4−S14 and Table S3). These data are the averaged results of two independent experimental series; relative deviations between the results of parallel experiments did not exceed 10%. Reaction conditions: $[Cr]_0 = 0.20$ mM; $[Zn(SR)_2]_0 = 2.0$ mM or [RSH] = 4.0 mM; 0.10 M HEPES buffer, pH 7.40; 25 °C. [b] Reaction in the presence of 0.10 M ehbaH. [c] Cr(IV) was generated with a yield of ∼40% in the $[Cr^{VI}O_4]^{2-}$ + As(III) + qaH_2 reaction[36] (sequential stopped-flow mixing); reaction in the presence of 0.10 M qaH. [d] Rate constants were estimated from the decays of [Cr(VI)] (determined with diphenylcarbazide, Figure S3). [e] These rate constants correspond to the decay of Cr(V/IV) due to the parallel disproportionation and oxidation of the ligand.[31,36] Kinetics of these reactions were satisfactorily described by the pseudo-first-order rate law (Figures S5, S8, and S12).[31]

concentrations were dependent on capillary temperature and voltage (no such dependencies were found for the mononuclear Zn(II) or Cr(III) species). The reactions of $[Zn(SR)_2]$ with $[Cr^{V}O(ehba)_2]^{-}$, $[Cr^{VI}O_4]^{2-}$ or $[Cr^{VI}O_4]^{2-}$ + ascorbate led to the formation of RSSR as the only detected oxidation product. A Zn(II) complex with the oxidized ligand ($[Zn(SR)(RSSR)]^{+}$, $m/z = +508$) was detected in the reaction of $[Zn(SR)_2]$ with $[Cr^{V}O(ehba)_2]^{-}$, but not with $[Cr^{VI}O_4]^{2-}$ or $[Cr^{VI}O_4]^{2-}$ + ascorbate (Figure 4b, Table S2).

Kinetic Studies. The kinetic experiments were performed in 0.10 M HEPES buffers (pH 7.40 ± 0.05; 25 °C) in the presence of large excesses of reductants ($[Cr]_0 = 0.10-0.20$ mM; $[RSH]_0 = 2.0-4.0$ mM or $[Zn(SR)_2]_0 = 1.0-2.0$ mM). The results of global kinetic analyses of time-dependent UV−vis spectra are presented in Figures S3−S14, Supporting Information. A summary of the observed rate constants is given in Table 2 (the full list is in Table S3, Supporting Information), and typical kinetic curves are shown in Figure 5. A slow reaction of $[Cr^{VI}O_4]^{2-}$ with $[Zn(SR)_2]$ was observed in HEPES buffer, while the buffer itself did not react with Cr(VI) under the studied conditions (Figure 5a). The kinetic analysis of this reaction was complicated by the formation of a precipitate (Figure S3); thus, the rate constants were determined from [Cr(VI)] measurements with diphenylcarbazide (Table 2). The reactions of $[Zn(SR)_2]$ or RSH with $[Cr^{V}O(ehba)_2]^{-}$ were significantly faster than the disproportionation of the Cr(V) complex in HEPES buffer, and led to the formation of an intermediate with $\lambda_{max} \approx 630$ nm, corresponding to monochelated Cr(IV)−ehba complexes (Figures 5b and S5−S7).[28,31,36] Similar reactions in the presence of 0.10 M ehhaH led to the formation of an intermediate with $\lambda_{max} \approx 460$ nm, corresponding to $[Cr^{IV}O(ehba)(ehbaH)]^{-}$ (Figures 5c and S8−S10).[28,31,36] Thus, Cr(IV)−ehba complexes are formed and subsequently reduced to Cr(III) during the reactions of $[Cr^{V}O(ehba)_2]^{-}$ with $[Zn(SR)_2]$ or RSH at pH 7.40. The reactions of $[Zn(SR)_2]$ or RSH with Cr(IV)−ehba complexes, generated by the $[Cr^{VI}O_4]^{2-}$ + As(III) + $ehbaH_2$ reaction,[44] were also studied; however, the disproportionation rate of the Cr(IV)−ehba species at pH 7.40 was comparable with the rates of their reactions with the reductants (Figure S11). Therefore, the reactions of Cr(IV) were studied using the $[Cr^{IV}O(qa)(qaH)]^{-}$ complex, which is much more stable in neutral aqueous solutions than the Cr(IV)−ehba complexes.[36] Generation of $[Cr^{IV}O(qa)(qaH)]^{-}$ by the $[Cr^{VI}O_4]^{2-}$ + As(III) + qaH_2 reaction was not quantitative under the studied conditions (yield ∼40%, determined from the UV−vis spectra), but the reactions of Cr(IV) were easily observed from the decrease of its specific absorbance with $\lambda_{max} = 490$ nm (Figures 5d and S12−S14).[36] Variations of [As(III)] (2.5−10 mM) did not affect the kinetics of $[Cr^{IV}O(qa)(qaH)]^{-}$ reactions with RSH or $[Zn(SR)_2]$.

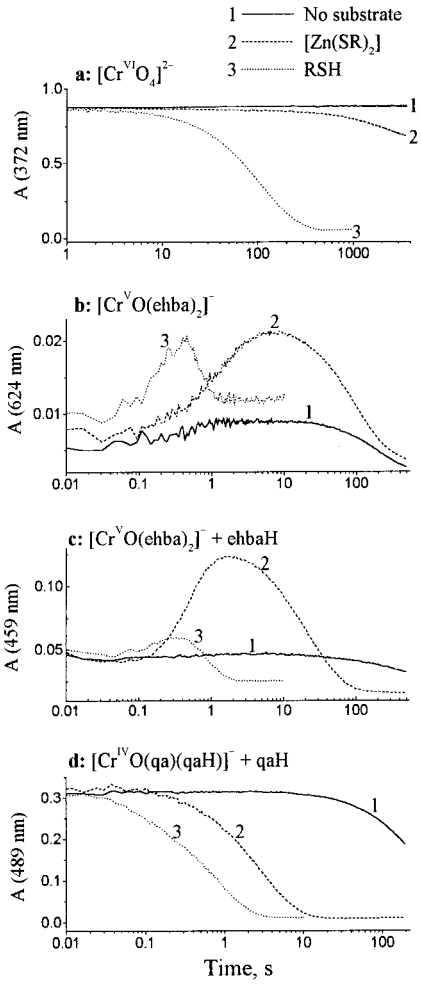


Figure 5. Characteristic kinetic curves (path lengths 1.0 cm) for the reactions of Cr(VI/V/IV) ($[Cr]_0 = 0.20$ mM) in 0.10 M HEPES buffers (pH 7.40, 25 °C) in the absence or presence of thiols (2.0 mM $[Zn(SR)_2]$ or 4.0 mM RSH).

In summary, the kinetic experiments (Table 2, Figure 5) showed that the reactions of $[CrO_4]^{2-}$, $[Cr^{V}O(ehba)_2]^{-}$, or $[Cr^{IV}O(qa)(qaH)]^{-}$ with $[Zn(SR)_2]$ were significantly faster than those with the buffer, but were much slower than those with RSH (under similar concentration, pH, and temperature conditions). The complexity of the studied reactions is evident from (i) the formation of numerous intermediates revealed by global kinetic analyses (Figures S4−S14) and (ii) the observed reaction orders of ≤0 with respect to the substrates (at $[Zn(SR)_2]_0 = 1.0-2.0$ mM or [RSH] = 2.0−4.0 mM) for all the reactions except $[Cr^{VI}O_4]^{2-}$ + RSH (Table S3). Detailed kinetic analyses of these reactions were beyond the scope of this work.

Oxygen Consumption Studies. Under the conditions corresponding to those of the kinetic studies (but in air-saturated solutions), the $[Cr^{VI}O_4]^{2-}$ + RSH reaction led to a relatively slow O_2 consumption, while the $[Cr^{V}O(ehba)_2]^{-}$ + RSH reaction caused a fast O_2 consumption followed by a slower stage (Figure 6a). These results are similar to those for the Cr(VI/V) + cys-

(44) Ghosh, M. C.; Gould, E. S. *Inorg. Chem.* **1991**, *30*, 491−494.

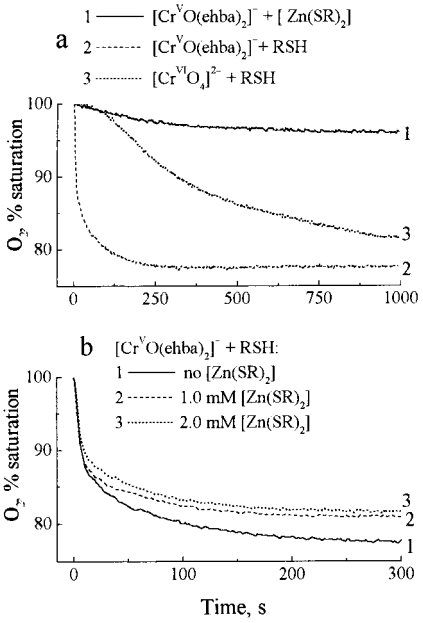


Figure 6. Typical results of O_2 consumption studies (0.10 M HEPES buffers, pH 7.40, air-saturated, 25 °C) for (a) the reactions of Cr(VI/V) ([Cr]$_0$ = 0.20 mM) with [Zn(SR)$_2$] (2.0 mM) or RSH (4.0 mM) and (b) the reactions of $[Cr^{V}O(ehba)_2]^-$ (0.20 mM) with RSH (4.0 mM) in the absence or presence of [Zn(SR)$_2$]. Background O_2 consumptions due to RSH were subtracted;[28] O_2 consumptions due to [Zn(SR)$_2$] were negligibly low under these conditions.

teine reactions.[28] By contrast, the $[Cr^{V}O(ehba)_2]^- + [Zn(SR)_2]$ reaction, while leading to a relatively fast reduction of Cr(V) (Table 2), is accompanied by a very slow O_2 consumption (Figure 6a). Moreover, additions of [Zn(SR)$_2$] to the $[Cr^{V}O(ehba)_2]^-$ + RSH systems led to a concentration-dependent decrease in the amounts of O_2, consumed in the fast stage (Figure 6b).

Discussion

Mechanisms of Cr(VI/V/IV) Reactions with RSH or [Zn(SR)$_2$]. Reductions of Cr(VI) to Cr(III) proceed through sequences of two- and/or one-electron steps with the formation of Cr(V/IV) intermediates.[45] The mechanisms of these reactions are often difficult to delineate due to the very low steady-state concentrations of the intermediates.[39] Application of relatively stable Cr(V/IV) complexes helps to understand the mechanisms of Cr(VI) interactions with different types of reductants.[28,46] Both RSH and [Zn(SR)$_2$] effectively reduce $[Cr^{IV}O(qa)(qaH)]^-$ to Cr(III) (Table 2, Figure 5d). The reductions of $[Cr^{V}O(ehba)_2]^-$ to Cr(III) with RSH or [Zn(SR)$_2$] proceed through the Cr(IV)−ehba intermediates (Figure 5b,c). Thus, it is likely that the mechanisms of Cr(VI/V/IV) reductions to Cr(III) with RSH or [Zn(SR)$_2$] in neutral aqueous solutions involve a series of one-electron steps. Scheme 1 (eqs 1−3) presents a generally accepted mechanism of thiol reactions with one-electron oxidants in the absence of O_2.[28,47] The validity of this mechanism for the studied processes is supported by the stoichiometries of Cr(VI/V) reactions with RSH or [Zn(SR)$_2$] and by the formation of RSSR as the only detectable oxidation product (Table 1).

One-electron oxidations of thiols are accompanied by O_2 consumption due to the formation of thiyl radicals, which readily react with O_2 (eqs 4 and 5 in Scheme 1).[28,47] A minor route for O_2 consumption in these reactions is connected to the formation of superoxo radicals (eq 6 in Scheme 1).[28,48] From the comparison of the rates of Cr(VI/V) reactions with RSH or [Zn(SR)$_2$] (Table 2) with the rates of O_2 consumption in Cr(VI/V) reactions with RSH (Figure 6a), it would be expected that the $[Cr^{V}O(ehba)_2]^- + [Zn(SR)_2]$ reaction would lead to a significant O_2 consumption (slower than that for the $[Cr^{V}O(ehba)_2]^- + RSH$ reaction, but faster than that for the $[Cr^{VI}O_4]^{2-} + RSH$ reaction). The absence of such a consumption (Figure 6a) suggests that, in this reaction, RS$^\bullet$ is not released into the reaction solution. A possible mechanism of intramolecular formation of S−S bonds during the one-electron oxidation of [Zn(SR)$_2$] is presented in Scheme 2. A minor O_2 consumption during the $[Cr^{V}O(ehba)_2]^- + [Zn(SR)_2]$ reaction (Figure 6a) can be attributed to the reaction of the formed anion radical (eqs 8 and 6 in Schemes 2 and 1).

[Zn(SR)$_2$] as a Model of Zinc Metallothioneins. Regulatory functions of zinc metallothioneins in the cells are thought to be based on their easy oxidation with the formation of intramolecular S−S bonds within the Zn(II)−Cys clusters, leading to the release of Zn(II).[49] In this work, the first evidence has been obtained for a similar mechanism of Zn−S bond oxidation (including intramolecular formation of a S−S bond) in a simple mononuclear Zn(II)−thiolate complex (Scheme 2).

Scheme 1. Proposed Mechanism for the Reduction of Cr(VI/V/IV) by RSH in the Presence or Absence of O_2[28,47,48]

$$RS^- + Cr^{(n+1)+} \longrightarrow RS^\bullet + Cr^{n+} \quad (1)$$

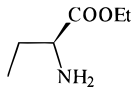
$$RS^- + RS^\bullet \longrightarrow RSSR^{\bullet-} \quad (2)$$

$$RSSR^{\bullet-} + Cr^{(n+1)+} \longrightarrow RSSR + Cr^{n+} \quad (3)$$

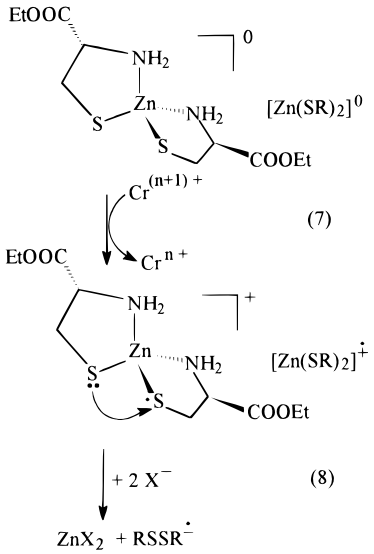
$$RS^\bullet + O_2 \longrightarrow RSOO^\bullet \quad (4)$$

$$RSOO^\bullet \longrightarrow \text{oxidation products} \quad (5)$$

$$RSSR^{\bullet-} + O_2 \longrightarrow RSSR + O_2^{\bullet-} \quad (6)$$

n = 5, 4, 3; R = 

Scheme 2. Proposed Mechanism for the Intramolecular Formation of the Disulfide Bond during the Oxidation of [Zn(SR)$_2$]



(45) Mitewa, M.; Bontchev, P. R. *Coord. Chem. Rev.* **1985**, *61*, 241−272 and references therein.

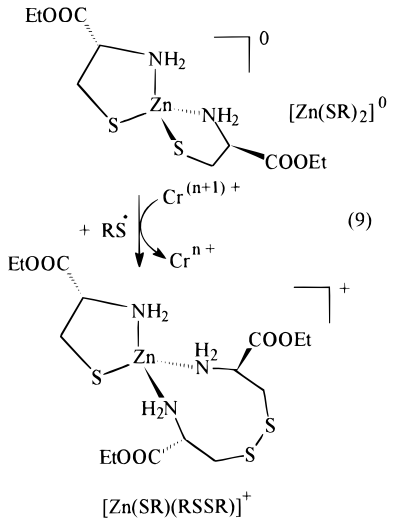
(46) Farrell, R. P.; Lay, P. A. *Comments Inorg. Chem.* **1992**, *13*, 133−175.

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

(48) Winterbourn, C. C.; Metodieva, D. *Arch. Biochem. Biophys.* **1994**, *314*, 284−290.

(49) Maret, W.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3478−3482.

Scheme 3. Proposed Mechanism for the Thiyl Radical Trapping during the Oxidation of $[Zn(SR)_2]$



The decrease in O_2 amounts consumed in the fast stage during the $[Cr^{V}O(ehba)_2]^- + RSH$ reaction in the presence of $[Zn(SR)_2]$ (Figure 6b) is probably due to the ability of $[Zn(SR)_2]$ to react with RS• (a similar effect was previously observed for 5,5-dimethyl-1-pyrroline *N*-oxide, which is a known scavenger of thiyl radicals).[28] The proposed mechanism of this reaction (Scheme 3) is in agreement with the formation of $[Zn(SR)(RSSR)]^+$ species, detected by ESMS in the $[Cr^{V}O(ehba)_2]^- + [Zn(SR)_2]$ systems (Figure 4b, Table 1). Zinc(II) complexes with unusually large (7−10-membered) chelate rinds, similar to the proposed structure of $[Zn(SR)(RSSR)]^+$ (Scheme 3), are known.[50] Zinc(II) complexes of metallothioneins[20] and synthetic polynuclear Zn(II)−thiolates[51] are known to scavenge free radicals, although no mechanism of this action has been proposed. On the basis of the research presented here, Schemes 2 and 3 depict likely mechanisms by which Zn(II)−thiolato species act as the inhibitors of radical processes. Free radicals can either serve as one-electron oxidants in the reactions similar to eq 7 (Scheme 2), or be directly trapped (as RS• in eq 9, Scheme 3).

$[Zn(SR)_2]$ as a Model of Zinc-Finger Proteins. Our choice of $[Zn(SR)_2]$ for modeling of Cr(VI/V/IV) reactions with Zn(II)−S bonds in biological systems was determined by the following: (i) a tetrahedral (2*S*,2*N*) coordination site,[23] resembling that of many zinc-finger proteins;[12−15] (ii) a convenient synthesis;[23] (iii) a significant solubility in water (∼5 mM at 25 °C); (iv) a stability at physiological pH values (Figure 2).[52] The negative ellipticities at 210−240 nm in the CD spectra of zinc-finger peptides were previously attributed to the formation of peptide secondary structures.[40] However, a similar CD spectrum of a model complex, $[Zn(SR)_2]$ (Figure 2), allows an assignment of these features to the Zn(II)−S charge-transfer band.[53]

Stoichiometric and kinetic studies (Tables 1 and 2, Figure 5a) have shown that the direct reactions of Cr(VI) with Zn(II)−S bonds are unlikely to be important under the physiological conditions due to their low rates in comparison with those of Cr(VI) reductions by cysteine or ascorbate.[39,43] However, the promotion of the $[Cr^{VI}O_4]^{2-} + [Zn(SR)_2]$ reaction by ascorbate may serve as a model for an important route of Cr(VI)-induced oxidative damage of zinc-finger proteins. Highly reactive species formed during the reduction of Cr(VI) by ascorbate include Cr(V), Cr(IV), and carbon-based radicals.[43] Formation of the Cr(III)−RSH species (Table 1 and Figure 3b) points to the participation of Cr(V/IV) intermediates in the $[Cr^{VI}O_4]^{2-}$ + ascorbate + $[Zn(SR)_2]$ reaction. The ability of Cr(V/IV) to oxidize $[Zn(SR)_2]$ has been confirmed using the relatively stable and biologically relevant[7] complexes, $[Cr^{V}O(ehba)_2]^-$ and $[Cr^{IV}O(qa)(qaH)]^-$ (Figure 5). Formation of RSSR during the reactions of Cr(VI/V) with $[Zn(SR)_2]$ (Table 1) is in agreement with the formation of disulfides during the reactions of zinc-finger peptides with one-electron oxidants (such as nitroso compounds or Cu(II)).[54] The lack of any significant reaction with O_2, and the implication that intramolecular coupling of the RS• radicals occurs (Scheme 2), provides further evidence for the relevance of $[Zn(SR)_2]$ as a model of Zn(II)−S binding sites of proteins.[49]

Catalysis and Inhibition of Thiol Oxidation by Cr(VI) in the Presence of Zn(II). Our kinetic studies (Table 2, Figure 5a) have shown a decrease by ∼2 orders of magnitude of the $[Cr^{VI}O_4]^{2-} + [Zn(SR)_2]$ reaction rates in comparison with those of the $[Cr^{VI}O_4]^{2-} + RSH$ reaction (HEPES buffer, pH 7.40, 25 °C). On the other hand, Perez-Benito and co-workers[55] have shown that the reductions of Cr(VI) by the thiols containing a free carboxylato group (e.g., cysteine or glutathione) were accelerated in the presence of Zn(II) (acetate buffer, pH < 6, 25 °C). This acceleration was explained by the formation of Zn(II) complexes with carboxylato groups of the substrate and acetate (while the thiol group of the substrate remains protonated and unbound to Zn(II)), which increases the reactivity of the thiol group toward Cr(VI).[55] Indeed, Zn(II) is known to form complexes with the deprotonated thiol groups of cysteine or glutathione only at pH > 7.[41,56] Thus, Zn(II) can either catalyze or inhibit the reactions of Cr(VI) with thiols, dependent on the nature of substrate and on the pH value and the buffer conditions.

Hydrolysis of Cr(III)-Bound Cysteine Ester. It is known that the metal ions with the high affinities to the thiol group (Ni(II), Cd(II), Zn(II), Pb(II), Hg(II)) promote the alkaline hydrolysis of cysteine esters.[42] In the current work, it was found that Cr(III) is an effective promotor of RSH hydrolysis in a slightly basic medium (pH 8.5, 25 °C). By contrast, significant hydrolysis of Zn(II)-bound RSH was observed only at pH ≥ 11 (25 °C). A possible mechanism of the Cr(III)-assisted hydrolysis of RSH is presented in Scheme 4. Two main Cr(III)−RSH complexes, $[Cr^{III}(SR)_3]^0$ and $[Cr^{III}(SR)_2]^+$ (detected by ESMS, Figure 4a and Table 1), exist in solutions in the presence of excess RSH (eq 10 in Scheme 4). The latter species is easily converted to the thermodynamically more stable[25,39] $[Cr^{III}(Cys)_2]^-$ complex (eq 11 in Scheme 4); this reaction is driven by the high affinity of Cr(III) for carboxylato donors.[8] Complexes of Cr(III) with cysteine-rich peptides are known to act as cofactors in the interaction of insulin molecules with cell

(50) (a) Gockel, P.; Vahrenkamp, H. *Chem. Ber.* **1996**, *129*, 1243−1249. (b) Ranganathan, S.; Jayaraman, N.; Roy, R.; Madhusudana, K. P. *Tetrahedron Lett.* **1993**, *34*, 7801−7804.

(51) Guo, S.; Ding, E.; Chen, H.; Yin, Y.; Li, X. *Polyhedron* **1999**, *18*, 735−740 and references therein.

(52) The complex $[Zn(Cys)_2]^{2-}$ was also studied as a possible model of zinc-finger proteins. However, CD spectroscopy and ESMS have shown that this complex undergoes a significant acid hydrolysis with the formation of free cysteine at pH 7.4 (25 °C). Therefore, interpretation of the stoichiometries and kinetics of the $[Zn(Cys)_2]^{2-}$ reactions with Cr(VI/V/IV) at these conditions was difficult: Champion, G.; Bailey, A. M.; Levina, A.; Lay, P. A. Unpublished results.

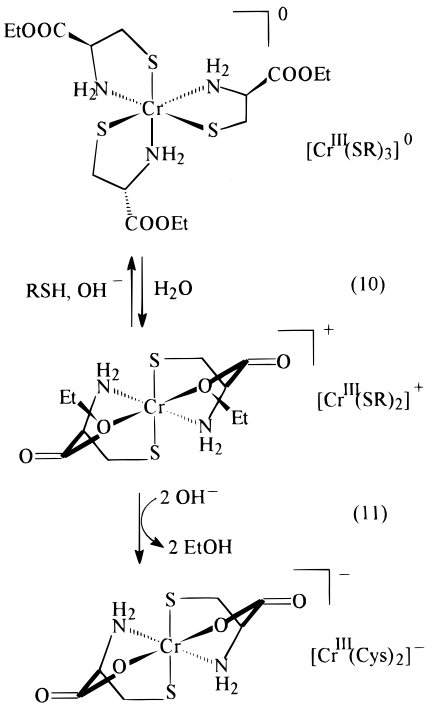
(53) Payne, J. C.; Ter Horst, M. A.; Godwin, H. A. *J. Am. Chem. Soc.* **1999**, *121*, 6850−6855.

(54) Yu, X.; Hathout, Y.; Fenselau, C.; Sowder, R. C.; Henderson, L. E.; Rice, W. G.; Mendeleyev, J.; Kun, E. *Chem. Res. Toxicol.* **1995**, *8*, 586−590.

(55) (a) Perez-Benito, J. F.; Lamrhari, D.; Arias, C. *Can. J. Chem.* **1994**, *72*, 1637−1644. (b) Perez-Benito, J. F.; Saiz, N.; Amat, E. *J. Mol. Catal., A* **1998**, *135*, 1−10.

(56) Díaz-Cruz, M. S.; Mendieta, J.; Monjonell, A.; Tauler, R.; Esteban, M. *J. Inorg. Biochem.* **1998**, *70*, 91−98.

Scheme 4. Proposed Mechanism for the Hydrolysis of Cr(III)-Bound RSH



membrane receptors.[57] Reactions similar to eqs 10 and 11 (Scheme 4) may contribute to the biological activity of Cr(III)−peptide complexes by providing the required geometries of the insulin−Cr(III)−peptide−receptor adducts.

Conclusion

Studies of the model systems have shown the susceptibility of Zn(II)−S bonds to the oxidation by Cr(VI/V/IV) species at physiological pH values. Similar reactions in the organisms exposed to Cr(VI) may cause the oxidative damage of Zn(II)-dependent transcription factors (zinc-finger proteins), leading to the disruption of normal DNA replication, and contribute to the Cr(VI)-induced carcinogenesis.

Acknowledgment. The financial support of this work by an Australian Research Council (ARC) grant (to P.A.L.) and ARC RIEFP grants for the EPR, CD, and stopped-flow spectrometers is gratefully acknowledged. We thank Dr. Merlin Crossley (Department of Biochemistry, University of Sydney) for helpful discussions.

Supporting Information Available: (i) Figures showing the correlation between [Cr^{III}SR] values and the intensities of CD signals, typical experimental and simulated signals in ESMS, and the results of global kinetic analyses and (ii) tables showing the detailed results of stoichiometry, product, and kinetic studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA9944047

(57) (a) Davis, C. M.; Sumrall, K. H.; Vincent, J. B. *Biochemistry* **1996**, *35*, 12963−12969. (b) Davis, C. M.; Vincent, J. B. *Arch. Biochem. Biophys.* **1997**, *339*, 335−343.