Chromium(VI) Forms Thiolate Complexes with γ-Glutamylcysteine, *N*-Acetylcysteine, Cysteine, and the Methyl Ester of *N*-Acetylcysteine†

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Received December 21, 1994⊗

Reaction of potassium dichromate with γ-glutamylcysteine, *N*-acetylcysteine, and cysteine in aqueous solution resulted in the formation of 1:1 complexes of Cr(VI) with the cysteinyl thiolate ligand. The brownish red Cr(VI)−amino acid/peptide complexes exhibited differential stability in aqueous solutions at 4 °C and ionic strength = 1.5 M, decreasing in stability in the order: γ-glutamylcysteine > *N*-acetylcysteine > cysteine. ^{1}H, ^{13}C, and ^{17}O NMR studies showed that the amino acids act as monodentate ligands and bind to Cr(VI) through the cysteinyl thiolate group, forming RS−$Cr^{VI}O_3^-$ complexes. No evidence was obtained for involvement of any other possible ligating groups, *e.g.*, amine or carboxylate, of the amino acid/peptide in binding to Cr(VI). EPR studies showed that chromium(V) species at $g = 1.973-4$ were formed upon reaction of potassium dichromate with γ-glutamylcysteine and *N*-acetylcysteine. Reaction of potassium dichromate or sodium dichromate with *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine in *N*,*N*-dimethylformamide (DMF) also led to the formation of RS−$Cr^{VI}O_3^-$ complexes as determined by UV/vis, IR, and ^{1}H NMR spectroscopy. Thus, an early step in the reaction of Cr(VI) with cysteine and cysteine derviatives in aqueous and DMF solutions involves the formation of RS−CrO_3^- complexes. The Cr(VI)−thiolate complexes are more stable in DMF than in aqueous solution, and their stability towards reduction in aqueous solution follows the order cysteine < *N*-acetylcysteine < γ-glutamylcysteine < glutathione.

Introduction

Chromium(VI) has been proven to be an extremely toxic and carcinogenic substance.[1] The uptake-reduction model was proposed[2] as a means of explaining the differential carcinogenicity of chromium(VI) as compared to chromium(III). In this model, Cr(VI), which exists in the tetrahedral form of chromate, CrO_4^{2-}, under physiological conditions, is able to pass through the cell membrane via general anion transport channels.[2] Cr(III), which exists in an octahedral form under physiological conditions, is unable to utilize cellular transport systems, and therefore is taken up much more slowly. According to this model, once inside the cell, Cr(VI) is reduced to Cr(III) by various cellular reductants including glutathione (GSH) and ascorbate. The intermediates produced by intracellular reduction, such as reactive Cr(VI), Cr(V), and Cr(IV) species, along with the final Cr(III) product, may be the ultimate carcinogenic forms of chromium, leading to Cr-DNA adducts, DNA cross links and DNA strand breaks.[3]

The reduction of Cr(VI) by thiols, a process crucial to the uptake-reduction model, has been studied by several investigators. McAuley and Olatunji studied the kinetics of Cr(VI) oxidation of GSH, β-mercaptoethylamine (BME), and penicillamine under acidic conditions.[4] They postulated that a transient Cr(VI)−thioester for GSH, BME, and penicillamine of the form RS−CrO_3^- (eq 1) was involved in the reaction mechanism.[4,5]

$$RSH + [HOCrO_3]^- \rightarrow [RS\text{-}CrO_3]^- + H_2O \qquad (1)$$

However, they further stated that before such a mechanism could be confirmed, more evidence was necessary on the nature of Cr(VI) intermediates.

Since that time a great deal of evidence has accumulated which supports the theory that Cr(VI) forms short-lived intermediates with thiol reductants.[3,6−9] Connett and Wetterhahn's[8] studies of the reaction of Cr(VI) with cellular thiols and Kwong and Pennington's[9] study of the reaction of Cr(VI) with cysteine under physiological pH conditions supported McAuley and Olatunji's mechanism[4,5] for Cr(VI)−thioester formation as an intermediate step in Cr(VI) reduction. A general mechanism for the reduction of Cr(VI) by thiols was proposed[8] which initially involves the formation of a Cr(VI)−thioester intermediate in a first step identical to that in McAuley and Olatunji's mechanism.[4,5] We have recently shown that potassium dichromate reacts with GSH (eq 2) at the sulfhydryl group

$$Cr_2O_7^{2-} + GSH \rightarrow [GS\text{-}CrO_3]^- + HCrO_4^- \qquad (2)$$

forming a glutathionate-CrO_3^- complex, the first structurally characterized Cr(VI)−thiolate complex of biological relevance.[6] The complex was not isolated due to its instability, but was characterized by ^{1}H, ^{13}C, ^{17}O NMR and UV/vis spectroscopy

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† Abbreviations: BME, β-mercaptoethylamine; Cys, cysteine; DMF, *N*,*N*-dimethylformamide; DTT, dithiothreitol; GSH, glutathione; GSSG, oxidized glutathione; TMS, tetramethylsilane; TSP, 3-(trimethylsilyl)propionic acid.

⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1995.

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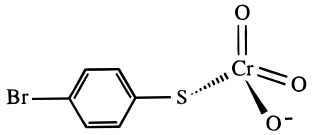
in aqueous solution. A subsequent Raman spectroscopic study confirmed that glutathione binds to chromium(VI) through the cysteinyl thiolate forming $GSCrO_3^-$.[7]

While researchers interested in the biological significance of Cr(VI) reduction have concentrated on cellular thiols and utilized aqueous conditions, the work of Mazurek *et al.*[10,11] has focused on the industrial significance of Cr(VI) reduction, using smaller, non-biological thiols in non-aqueous solvents. The reaction of Cr(VI) with alkane- and arenethiols in *N,N*-dimethylformamide (DMF), as in aqueous solution, resulted in the oxidation of thiols to the corresponding disulfides and the reduction of Cr(VI) to Cr(III).[12] NMR, UV/vis, and IR studies revealed that the first step in the reduction of Cr(VI) by thiols in DMF involved the substitution of a thiolate, RS^-, for the hydroxyl group in hydrogen chromate, $HOCrO_3^-$, and the formation of a Cr(VI)−thioester which was subsequently reduced by additional thiol.[10,12] Mazurek *et al.* have synthesized and crystallized several Cr(VI)−thioesters of small alkyl and aryl thiols, and have structurally characterized tetraphenylarsonium (*p*-bromophenyl)thiolato)chromate(VI), $[Ph_4As][p\text{-}BrPhS{-}CrO_3]$, *via* X-ray crystallography.[11]



[*p*-BrPhS-CrO_3]$^-$

In order to determine whether the reaction of dichromate with glutathione, which gives a Cr(VI)−thiolate complex, is representative of a general class of reactions applicable to other biological thiols, we set out to investigate the reaction of cysteine and cysteine derivatives with dichromate (eq 3). We studied

$$Cr_2O_7^{2-} + RSH \rightarrow [RS\text{-}CrO_3]^- + HCrO_4^- \qquad (3)$$

the formation of Cr(VI)−thiolate complexes from the reaction of $Na_2Cr_2O_7{\cdot}2H_2O$ and $K_2Cr_2O_7$ with γ-glutamylcysteine, *N*-acetylcysteine, and cysteine in aqueous solution, and *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine in DMF. Although it was not possible to isolate any of the Cr(VI)−thiolate complexes as solids due to their instability, their formation was established using UV/vis, IR, and NMR spectroscopy. We found that the biological thiols used in this study, even γ-glutamylcysteine with multiple amine, amide, and carboxylate coordination sites, bind to Cr(VI) through a chromium−sulfur bond only, forming $RS{-}CrO_3^-$ complexes.[6,7] These studies confirm that an early step in the reaction of Cr(VI) with thiols in aqueous solution and in DMF involves the formation of Cr(VI)−thiolate complexes.

Experimental Section

Materials. Carboxypeptidase A, cellulose, dithiothreitol (DTT), oxidized glutathione (GSSG), oxidized and reduced L-cysteine and *N*-acetyl-L-cysteine (Sigma grade) were obtained from Sigma Chemical Co., St. Louis, MO. Sodium dichromate ($Na_2Cr_2O_7{\cdot}2H_2O$), potassium dichromate ($K_2Cr_2O_7$), DMF (ACS grade, <0.15% water), ammonium hydroxide (NH_4OH, ACS grade), and potassium chloride (KCl) were obtained from Fisher Chemical Co., Fair Lawn, NJ. DMF (HPLC grade or anhydrous, 99+%), ammonium formate (NH_4O_2CH, 97%), deuterated nitric acid (DNO_3, 65 wt % solution in D_2O, 99+ atom %), deuterated sodium hydroxide (NaOD), and 99.8% deuterium oxide (D_2O) were obtained from Aldrich Chemical Co., Milwaukee, WI. Dowex G-50 anion exchange resin was obtained from Bio Rad Laboratories, Richmond, CA. Deuterated DMF (DMF-d_7, 99.5 atom %) was obtained from Cambridge Isotope Laboratories, Woburn, MA. ^{17}O-labeled water (10.8 atom %) was purchased from Icon Services, Summit, NJ.

Syntheses. γ-Glutamylcysteine. A modification of the literature procedure[13] was used for the synthesis of γ-glutamylcysteine. GSSG (5.0 g) was reacted with 5000 units of carboxypetidase A in 250 mL of H_2O in a shaking water bath at 37 °C for 2 weeks. Initially, 2000 units of the enzyme was added to the solution and the pH was adjusted to 7.4 with concentrated NH_3(aq). After 3 days, an additional 2000 units of enzyme was added, and the pH was adjusted again. A final 1000 units of enzyme was added after 1 week. The solution turned cloudy, and 10 g of cellulose was added. The resultant suspension was suction filtered with a no. 1 Whatman Filter twice and then charged onto a Dowex G-50 anion exchange column and repeatedly rinsed with ~3 L of deionized water. The product was eluted from the column by the addition of ~250 mL of 4 M ammonium formate. The eluant was collected and was extensively lyophilized to remove the ammonium formate. Most of the oxidized product was reduced by the addition of 5x DTT followed by several ethyl acetate extractions. An aliquot of the oxidized product was saved for comparative purposes with the reduced product.

Methyl Ester of *N*-acetylcysteine. The esterification of *N*-acetylcysteine was accomplished via a modification of the general procedure outlined in Jones and Wigfield.[14] The crystalline solid was characterized as the methyl ester of *N*-acetylcysteine by ^1H NMR spectroscopy (in $CDCl_3$); the ^1H NMR data obtained were in excellent agreement with that reported by Jones and Wigfield.[14]

Methods. NMR Spectroscopy. NMR spectra were obtained on Varian XL-300 and Varian Unity 300 spectrometers.

I. Aqueous Samples. As previously described for aqueous NMR spectroscopic studies, solutions were made up with the ionic strength (*I*) adjusted to 1.5 M with KCl and measurements were made at 4 ± 1 °C.[6] The final concentrations after mixing were 0.080 M Cr(VI) (0.040 M $K_2Cr_2O_7$) and 0.040 M thiol. When Cr(VI) was mixed with cysteine, *N*-acetylcysteine or γ-glutamylcysteine, the solution turned color from orange to brownish red. The pH* (measured pH uncorrected for deuterium isotope effect) of the reaction mixture increased from 5.0 to 6.3−6.4 over a 65 min period. ^1H and ^{13}C chemical shifts are reported relative to an external tetramethylsilane (TMS) reference. Reference spectra, except for the cysteine reaction, were obtained with a 10 mm coaxial tube which contained 10% TMS in CCl_4 in the outer tube.[15] Spectra for the cysteine reaction were obtained using a 5 mm coaxial tube and referenced to 10% TMS in CCl_4 in the inner tube.[16] Standard Varian software was used for data aquisition and processing. ^{17}O NMR spectra were acquired at a frequency of 40.662 MHz as previously described.[6] Total collection time per spectrum was approximately 12 min. ^{13}C NMR spectra of the carbon skeleton and the carbonyl region of the Cr(VI)−thiolate complexes were acquired at 75.429 MHz with broad band proton decoupling. Spectra of the carbon skeleton were obtained as previously described.[6] Spectra of the carbonyl region were acquired with a 10−15° flip angle and a sweep width of 700−1200 Hz. Nearly 1000 transients were accumulated, which took approximately 15 min.

II. Non-Aqueous Samples. Formation of *N*-acetylcysteinate−Cr(VI) and methyl *N*-acetylcysteinate−Cr(VI) complexes in DMF-d_7 at

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(15) Under these conditions, the signal of the methyl protons of internal TSP is ~0.3 ppm upfield of external TMS; in addition, the ^{13}C signal of internal dioxane is 66.1 ppm relative to external TMS.
(16) When *tert*-butyl alcohol was added as an internal reference, there was virtually no change in location of the peaks of the cysteine protons. Under these conditions, the peak due to the *tert*-butyl alcohol methyl protons was 0.96 ppm downfield from external TMS. See Taddei, F.; Pratt, L. *J. Chem. Soc.*, **1964**, 1553.
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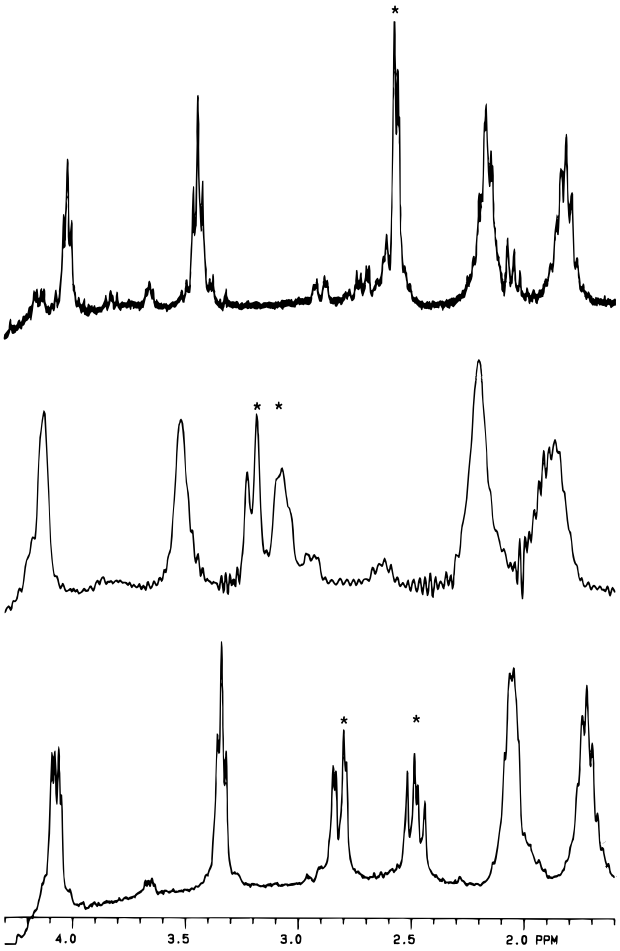


Figure 1. Proton NMR spectra of γ-glutamylcysteine, the γ-glutamylcysteinate-CrO_3^- complex and γ-glutamylcystine at 277 K in D_2O, pH* = 6.2, I = 1.5 M. Top: γ-Glutamylcysteine (0.040 M). Middle: Reaction mixture of potassium dichromate (0.040 M) and γ-glutamylcysteine (0.040 M). Bottom: γ-Glutamylcystine (0.020 M). An asterisk denotes cysteinyl methylene protons.

room temperature was monitored using ^{1}H NMR spectroscopy. The final concentrations of the reactants were 0.050 M Cr(VI) (0.025 M $K_2Cr_2O_7$) and 0.050 M *N*-acetylcysteine after mixing. ^{1}H NMR spectra were acquired with the following parameters: sweep width = 2506.3 Hz, acquisition time = 1.379 s, relaxation time = 2.50 or 3.0 s, and number of scans = 32. Proton chemical shifts are reported relative to an internal TMS reference. The reaction of the methyl ester of *N*-acetylcysteine (0.0338 M) with $K_2Cr_2O_7$ (0.0178 M) was followed similarly, with some modification of the acquisition parameters: sweep width = 2146.8 Hz, acquisition time = 1.192 s, and relaxation time = 2.4 s.

UV/Vis Spectroscopic Studies. The reaction of *N*-acetylcysteine (0.0333 M) with $Na_2Cr_2O_7 \cdot 2H_2O$ (0.0147 M, 0.0293 M Cr(VI)) in DMF at room temperature was followed by UV/vis spectroscopy. Every 7−10 min for a total of 70 min, a 0.10 mL aliquot of the reaction mixture was diluted to 4.0 mL with DMF in order to achieve an absorbance <1, and was analyzed using a Pekin-Elmer Lambda 9 UV/Vis/NIR spectrophotometer over the range of 300−500 nm. The reaction of the methyl ester of *N*-acetylcysteine (3.33 mM) with $Na_2Cr_2O_7 \cdot 2H_2O$ (0.98 mM, 1.96 mM Cr(VI)) in DMF at room temperature was monitored by diluting a 0.20 mL aliquot of the reaction mixture to 1.0 mL every 7−15 min for a total of >100 min and measuring the UV/vis spectrum.

IR Spectroscopic Studies. The reactions of *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine (0.10 M final concentration) with $K_2Cr_2O_7$ (0.044 M final concentration, 0.088 M Cr(VI)) in DMF were monitored at room temperature using a Perkin-Elmer M1605 FTIR spectrophotometer. IR spectra were taken of the components before mixing and then of the reaction mixture, over a period of 2 h. KBr and NaCl cells with 0.2 mm pathlength were used for IR measurements, and subtraction spectra were calculated against a DMF reference spectrum.

Table 1. ^{1}H and ^{13}C Chemical Shifts for γ-Glutamylcysteine, γ-Glutamylcysteinate−CrO_3^- Complex, and γ-Glutamylcystine

		γ-glutamyl cysteine	γ-glutamyl cysteine−Cr(VI)	γ-glutamyl cystine
		^{1}H NMR (δ, ppm)[a]		
cys:	CH_2	2.57[b]	3.07, 3.20	2.47, 2.82[c]
	CH	4.02[b]	4.15	4.07[c]
γ-glu:	γ-CH_2	2.16[d]	2.25	2.05[e]
	β-CH_2	1.83[d]	1.85	1.72[e]
	α-CH	3.44[d]	3.52	3.34[e]
		^{13}C NMR (δ, ppm)[a]		
cys:	CH_2	25.7	37.8	39.0
	CH	56.5	56.5	65.5
	COO	176.9	177.1	177.3
γ-glu:	γ-CH_2	31.1	31.3	31.1
	β-CH_2	26.0	26.1	26.2
	α-CH	53.8	54.0	53.8
	CONH	174.6	174.5	174.8
	COO	174.5	174.4	174.6

[a] NMR data obtained under the following conditions: pH* = 6.20 ± 0.05 at 277 K. Shifts referenced to TMS at 0.00 ppm. ^{1}H shifts are within ± 0.02 ppm; ^{13}C shifts are within 0.1 ppm. [b] Coupling constants for cysteinyl spin system: J_{gem} = −14.1 Hz, J_{AX} = 6.4 Hz, and J_{AM} = 4.7 Hz. [c] Coupling constants for cysteinyl spin system: J_{gem} = −14.1 Hz, J_{AX} = 9.4 Hz, and J_{AM} = 4.0 Hz. [d] Coupling constants for glutamyl spin system: J_{AM} = 6.4 Hz, J_{MX} = 6.7 Hz, and J_{AX} = 2.0 Hz. [e] Coupling constants for glutamyl spin system: J_{AM} = 6.3 Hz, J_{MX} = 7.5 Hz, and J_{AX} = 2.0 Hz.

EPR Spectroscopic Studies. EPR spectra for reactions of Cr(VI) with *N*-acetylcysteine and γ-glutamylcysteine in aqueous solution under the same conditions used for the NMR spectra (see above) were acquired on a Bruker ESP 300 equipped with a homemade frequency detector and an Er 4111T VT temperature controller set at 4 °C. Spectra were acquired at a frequency of 9.418 GHz with a center field of 3400−3420 G, a sweep width of 75−200 G, a modulation amplitude of 1.0 G, and microwave power of 20 mW.

Results

I. Aqueous Solution Studies. ^{1}H, ^{13}C, and ^{17}O NMR Spectroscopy for the Reaction of γ-Glutamylcysteine with $K_2Cr_2O_7$. In order to determine the binding site of Cr(VI) on the γ-glutamylcysteine ligand, the pH_0* = 5.0, I = 1.5 M, 0.5:1.0 γ-glutamylcysteine:Cr(VI) reaction was monitored by ^{1}H NMR spectroscopy at 4 °C. Upon addition of Cr(VI), the spectra showed several new peaks (Figure 1, Table 1), which were attributed to the formation of a γ-glutamylcysteine thiolate complex with Cr(VI) and the oxidized peptide, γ-glutamylcystine. No reduced γ-glutamylcysteine was observed in the proton spectra after addition of Cr(VI). The peaks showed no significant changes in chemical shift over a 60 min time period. The peaks of the diastereotopic protons of the cysteinyl methylene protons shifted 0.50−0.63 ppm downfield from their position in the uncomplexed thiol, and split into two distinct resonances upon addition of Cr(VI). The peak of the cysteinyl methyne proton also shifted 0.13 ppm downfield from its uncomplexed position. No significant changes in chemical shift were observed for the peaks of the glutamyl residue protons upon addition of Cr(VI); however, all fine structure of the peaks was lost, presumably due to the hindered rotation of the glutamyl residue. These results are consistent with the formation of a monodentate γ-glutamylcysteinate−Cr(VI) complex. Peaks due to the cysteinyl residue of oxidized thiol, γ-glutamylcystine, were also observed for the diastereotopic methylene and the methyne protons. Peaks of the glutamyl residue of γ-glutamylcystine (in the reaction mixture) were not discerned due to overlap with the glutamyl peaks of the Cr(VI)−thiolate complex.

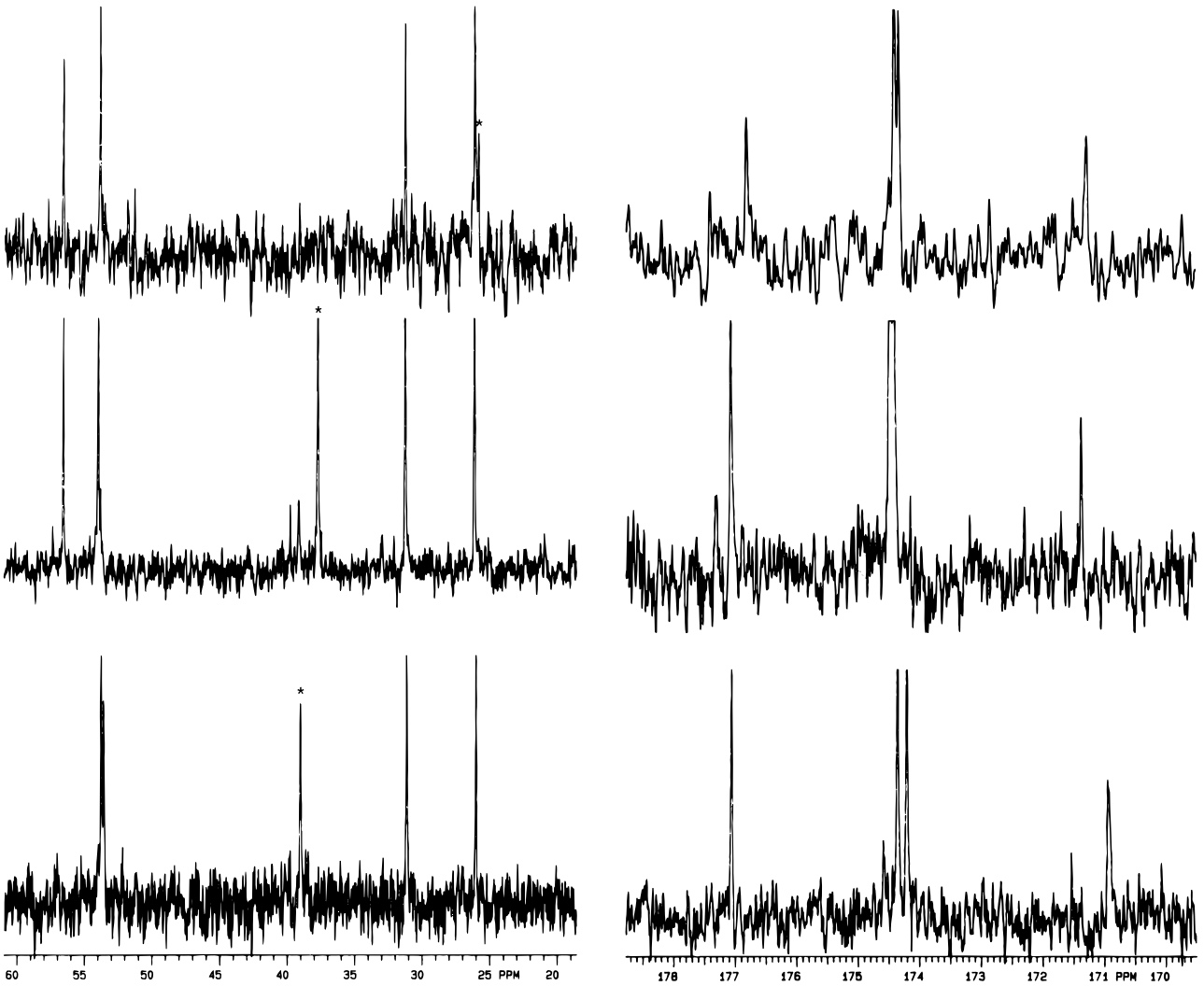


Figure 2. ^{13}C NMR spectra of (A) the carbon skeleton region and (B) the carbonyl region of γ-glutamylcysteine, the γ-glutamylcysteinate$-CrO_3^-$ complex and γ-glutamylcystine at 277 K in D_2O, pH* = 6.2, and I = 1.5 M. Top: γ-Glutamylcysteine (0.040 M). Middle: Reaction mixture of potassium dichromate (0.040 M) and γ-glutamylcysteine (0.040 M). Bottom: γ-glutamylcystine (0.020 M). An asterisk denotes cysteinyl methylene carbons.

The reaction mixture was studied by ^{13}C NMR spectroscopy at 4 °C to confirm the hypothesis that γ-glutamylcysteine binds to chromium(VI) solely through a Cr−S bond. Only the peaks due to the cysteinyl methylene and methyne carbons, which are adjacent to the thiolate group, showed a significant change in chemical shift. The cysteinyl methylene peak shifted 12.2 ppm downfield while the methyne peak showed a 0.2 ppm upfield shift from its uncomplexed position (Figure 2A, Table 1). In addition, the cysteinyl methylene and methyne carbon peaks due to the oxidized thiol, γ-glutamylcystine were observed. The cysteinyl carboxylate carbon of the dipeptide ligand exhibited a peak which was shifted 0.2 ppm downfield from its uncomplexed position (Figure 2B, Table 1). While the peak of the cysteinyl carbonyl carbon of γ-glutamylcystine was observed, the peaks of the glutamyl carbons showed no significant change in their chemical shifts and overlapped with those of the Cr(VI)−thiolate complex. Thus, it is unlikely that either the carboxylate or the carbonyl oxygens are involved in binding to the metal center. The small shifts observed in the location of these peaks were presumably due to the increase in the pH* of the reaction mixture.

Since the pH* of the γ-glutamylcysteine/$K_2Cr_2O_7$ reaction mixture (thiol:Cr(VI) = 0.5:1.0, pH_0* = 5.0) increased to 6.3 over a 70 min period, the change in ^1H and ^{13}C chemical shifts as a function of pH* was determined for both reduced and oxidized γ-glutamylcysteine. The proton spectra of the γ-glutamylcysteine (Figure 1, Table 1) showed little change in chemical shift with pH* from 4.0 to 9.0; however, all of the signals shifted upfield by 0.1−0.2 ppm between pH* 9.0 and 9.6. Similar results were observed for the ^{13}C spectra of the carbon skeleton of γ-glutamylcysteine (Figure 2A, Table 1), which showed only very slight changes for all peaks from pH* 4.0 to 9.6. The carbonyl region showed relatively small changes from pH* 4.0 to 8.7; however, the glutamyl carboxylate peak shifted 2.1 ppm downfield at pH* 9.6 and the cysteinyl carboxylate and carbonyl peaks increasingly shifted downfield as pH* increased above 8.7. The ^1H and ^{13}C NMR spectra of the oxidized species, γ-glutamylcystine (Figures 1 and 2, Table 1), also showed slight changes in chemical shift from pH* 4.0 to 9.3; however, from pH* 9.3 to 11.5, several peaks showed significant change in chemical shift. These results are consistent with deprotonation of the thiol and amine groups of γ-glutamylcysteine at pH* $\sim$ 9−10 and the amine group of γ-glutamylcystine at pH* $\sim$ 10−11. Thus, the small changes in pH* from 5.0 to 6.3 upon reaction of $K_2Cr_2O_7$ with γ-glutamylcysteine cannot account for the large changes in chemical shifts observed for the ^1H and ^{13}C cysteinyl methylene peaks, which instead are consistent with formation of a monodentate Cr(VI)−thiolate complex.

In order to determine the electronic and structural properties of the oxo ligands of the Cr(VI) upon complexation with γ-glutamylcysteine, the reaction of the thiol with $K_2Cr_2O_7$ (thiol: Cr(VI) = 0.5:1.0, pH_0*= 5.0, I = 1.5 M, 4 °C) was studied by ^{17}O NMR spectroscopy. ^{17}O NMR spectra (Figure 3) of the reaction of γ-glutamylcysteine with dichromate revealed a new peak at 1127 ppm, which was ~12 ppm downfield of the terminal oxygens of dichromate. In addition, peaks due to both the terminal (1115 ppm) and bridging oxygens (327 ppm) of dichromate and the oxygens of chromate/hydrogen chromate (830−845 ppm) were still observed. The line width of the new ^{17}O peak (220−260 Hz) assigned to the γ-glutamylcysteinate−Cr(VI) complex was considerably broader than the peak due to the terminal oxygens of dichromate (~30 Hz) and narrower than the chromate/hydrogen chromate oxygens (~400−600 Hz). The line width of the oxygen peak of chromate/hydrogen chromate in the reaction solution decreased from ~600 to ~400 Hz over the course of the reaction, presumably due to the increase in pH* from 5.0 to 6.3 (chromate oxygen line width = 13 Hz at pH* = 13.0). The line width of the peak of the oxo ligands in the Cr(VI)−thiolate complex was not dependent on pH within the narrow pH range observed. The fact that the new peak of the Cr(VI)−thiolate complex is within 12 ppm of the terminal oxygens of dichromate indicates that the oxygens on the metal center in the Cr(VI)−thiolate complex are in a similar electronic environment, *e.g.*, $-Cr^{VI}O_3^-$, and is consistent with the formation of a γ-glutamylcysteinate−CrO_3^- complex.

The stability of the γ-glutamylcysteinate−CrO_3^- complex was determined from integration of the ^1H and ^{17}O NMR spectra as a function of time. The cysteinyl methylene proton signals of the complex decreased ~25% at 60 min after mixing, while the cysteinyl methylene signals of γ-glutamylcystine had increased correspondingly. The integration measurements also showed that the γ-glutamylcysteinate−CrO_3^- complex was the predominant species (> 50%) in the reaction mixture, even at 60 min after mixing. Integration of the ^{17}O peak assigned to γ-glutamylcysteinate−CrO_3^- showed that the intensity decreased by ~50% and correspondingly the peak of the chromate oxygens increased by ~70% at 60 min after mixing the thiol and dichromate. However, the increase in pH over the course of the reaction may also be partly responsible for the increase in the chromate signal. These results indicate that the γ-glutamylcysteinate−CrO_3^- complex is fairly stable under these conditions.

NMR Spectroscopy for the Reaction of *N*-Acetylcysteine and Cysteine with $K_2Cr_2O_7$. In order to determine whether RS−CrO_3^- complexes would form with other cysteine derivatives, the reactions of $K_2Cr_2O_7$ with *N*-acetylcysteine and with cysteine were monitored by ^1H NMR spectroscopy under the same conditions used for the analogous reaction with γ-glutamylcysteine above. Upon addition of Cr(VI) to *N*-acetylcysteine, the spectra showed several new peaks (Table 2), none of which showed a significant change in chemical shift over 70 min. No peaks could be attributed to uncomplexed *N*-acetylcysteine. The spectra showed the formation of the *N*-acetylcysteinate−Cr(VI) complex by the appearance of two new diastereotopic cysteinyl methylene signals which were shifted 0.38−0.58 ppm downfield from the signal of uncomplexed *N*-acetylcysteine (Table 2). In addition, peaks due to the oxidized thiol, *N*-acetylcystine, were observed. Data obtained from the ^{13}C NMR spectra of the reaction showing a 11.9 ppm downfield shift of the methylene carbon were in agreement with the ^1H NMR data and indicate the formation of a monodentate Cr(VI)−thiolate complex (Table 2). ^{17}O NMR studies of the reaction of *N*-acetylcysteine with dichromate yielded a new signal at 1128 ppm (Figure 3) in addition to the peaks of dichromate and chromate/hydrogen chromate, indicating the formation of the *N*-acetylcysteinate−CrO_3^- complex.

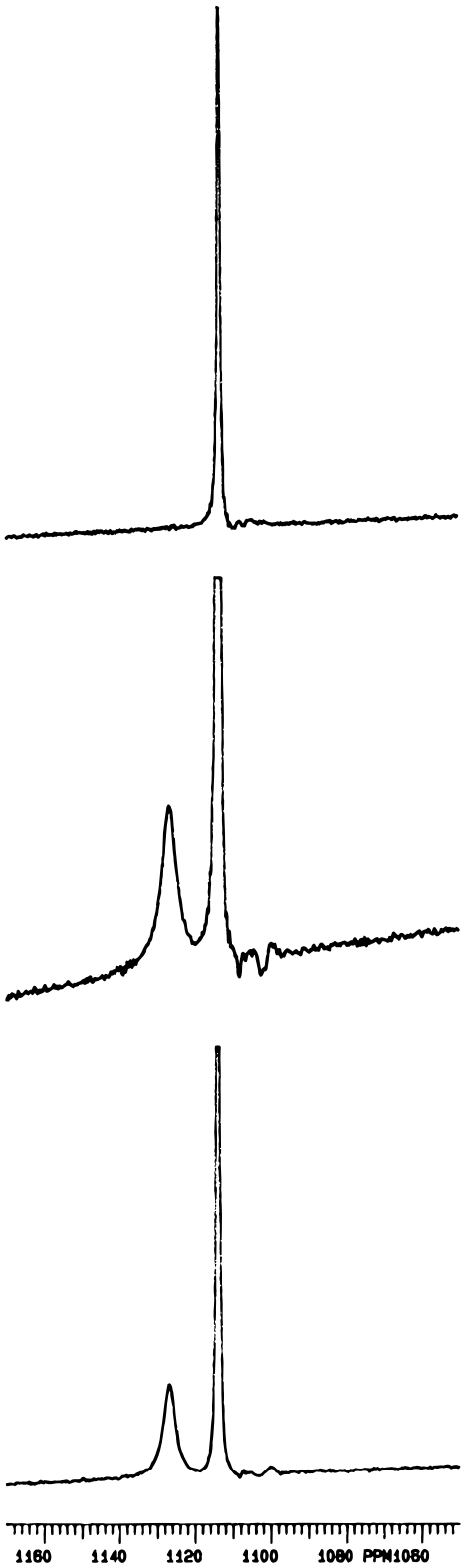


Figure 3. ^{17}O NMR of the terminal oxygens of dichromate and the oxygens of the γ-glutamylcysteinate−CrO_3^- and *N*-acetylcysteinate−CrO_3^- complexes. Top: Potassium dichromate (0.040 M). Middle: Reaction mixture of potassium dichromate (0.040 M) and γ-glutamylcysteine (0.040 M). Bottom: Reaction mixture of potassium dichromate (0.040 M) and *N*-acetylcysteine (0.040 M). Conditions: 277 K in 95% H_2O/5% D_2O enriched ~10% in ^{17}O, pH = 6.2, I = 1.5 M.

The ^1H NMR spectrum of the reaction of cysteine with $K_2Cr_2O_7$ exhibited new peaks which were attributed to the

Table 2. ^{1}H and ^{13}C NMR Data for *N*-Acetylcysteine (*N*-Ac-Cys), *N*-Acetylcysteinate−CrO_3^- Complex and *N*-Acetylcystine, and ^{1}H NMR Chemical Shifts for Cysteine (Cys), Cysteinate−CrO_3^- and Cystine

	N-Ac-Cys	*N*-Ac-Cys−CrO_3^-	*N*-Ac-cystine	Cys	Cys−CrO_3^-	cystine
			^{1}H NMR (δ, ppm)[a]			
CH_2	2.70	3.08, 3.28	2.64, 2.98	2.78	3.27, 3.18	3.25, 2.94
CH	4.13	4.15	4.18	3.73	3.79	3.75
CH_3	1.87	1.78	1.82			
			^{13}C NMR (δ, ppm)[a]			
CH_2	25.9	37.8	39.4−40.0			
CH	56.7	56.7	54.0			
CH_3	21.7	21.9	22.7			
CON	173.3	173.0	173.4			
COO	176.6	176.2	177.0			

[a] The NMR data was collected under the following conditions: pH* = 6.20 ± 0.05 at 277 K. All chemical shifts referenced to an external reference, 10% TMS in CCl_4, at 0.00 ppm. ^{1}H shifts are within ± 0.02 ppm. ^{13}C shifts are within 0.1 ppm.

cysteinate−CrO_3^- complex and cystine (Table 2). The cysteinyl methylene signals of the Cr(VI)−thiolate complex were shifted 0.40−0.49 ppm downfield from the signal of uncomplexed cysteine. The spectrum of the reaction at 15 min after mixing exhibited broadening of the peaks, presumably due to the presence of paramagnetic reduced Cr species.

The stabilities the *N*-acetylcysteinate−CrO_3^- and cysteinate−CrO_3^- complexes were determined from integration of the NMR spectra over time. The peaks of the *N*-acetylcysteinate−CrO_3^- complex had decayed by ∼33% 60 min after mixing, and correspondingly, the peaks of *N*-acetylcystine had increased by ∼50%, as measured by the integration of the cysteinyl methylene proton peaks of both species. The integration of these peaks also showed that the *N*-acetylcysteinate−CrO_3^- complex was the predominant species in the reaction mixture, even 70 min after mixing. Integration of the ^{17}O peak assigned to the *N*-acetylcysteinate−CrO_3^- complex showed a ∼75% decrease at 60 min after mixing, while the peak of the chromate oxygens increased by ∼100%, which is largely attributed to the rise in pH during the course of the reaction. In contrast, integration of the methyne peaks showed that the cysteinate−CrO_3^- complex underwent ∼30% decay to the oxidized thiol within 15 min and decay to cystine was complete within ∼40 min after mixing. These results indicate that the cysteinate−CrO_3^- complex is much less stable than the γ-glutamylcysteinate−CrO_3^- and *N*-acetylcysteine−CrO_3^- complexes.

EPR Spectroscopy for the Reaction of γ-Glutamylcysteine and *N*-Acetylcysteine with $K_2Cr_2O_7$. Although the formation of disulfides in the reactions of γ-glutamylcysteine and *N*-acetylcysteine with $K_2Cr_2O_7$ indicated an overall two-electron reduction pathway, EPR studies were performed to detect the possible formation of Cr(V) species. Numerous studies[6,18−20] have previously shown the formation of Cr(V) species upon the reaction of Cr(VI) with thiols. The EPR spectra obtained of the γ-glutamylcysteine/dichromate reaction showed a weak isotropic signal at $g = 1.973$, ($\Delta H = 1.4$ G) that decayed to ∼20% of its original intensity after 60 min. EPR spectroscopy of the *N*-acetylcysteine:Cr(VI) reaction mixture also showed a weak signal at $g = 1.974$, ($\Delta H = 1.7$ G), which was no longer detectable after 50 min. Under these conditions (thiol:Cr(VI) = 0.5:1.0, pH_0* = 5.0, $I = 1.5$ M, 4 °C) it appears that one-electron reduction to Cr(V) is only a minor pathway.

II. Non-Aqueous Solution Studies. UV/vis, IR and NMR spectroscopic experiments were carried out on the reaction of $Na_2Cr_2O_7 \cdot 2H_2O$ or $K_2Cr_2O_7$ with *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine in DMF in order to characterize the Cr(VI) complexes of these biological thiols in non-aqueous solution and to compare the results with previous studies of the reactions of $Na_2Cr_2O_7$ with alkane- and arenethiols in DMF.[10]

Table 3. UV/Vis Data for Chromium(VI) Species in DMF and Aqueous Solution

complex	λ_{max} (nm)	ϵ, (M^{-1} cm^{-1})[a]	solvent	ref
$Cr_2O_7^{2-}$	374	2250	DMF	10
	374	2160	DMF	this work
CrO_4^{2-}	372	3950	DMF	10
$HOCrO_3^-$	355	1620	DMF	10
PhS−CrO_3^-	420	2080	DMF	10
n-BuS−CrO_3^-	416	1890	DMF	10
[*N*-Ac-Cys]−CrO_3^-	410	1740	DMF	this work
[Methyl *N*-Ac-Cys]−CrO_3^-	408	1740	DMF	this work
penicillamine−CrO_3^-	420	1228	water	4
GS−CrO_3^-	425	1290	water	4

[a] All ϵ's are per chromium.

UV/Vis Spectroscopy for the Reaction of *N*-Acetylcysteine and the Methyl Ester of *N*-Acetylcysteine with $Na_2Cr_2O_7$. The UV/vis spectrum for $Na_2Cr_2O_7 \cdot 2H_2O$ in DMF exhibited a peak at $\lambda_{max} = 374$ nm ($\epsilon = 2160$ M^{-1} cm^{-1}; lit.[10] $\epsilon = 2250$ M^{-1} cm^{-1}) which initially shifted to 353 nm, the absorption maximum of hydrogen chromate, upon addition of *N*-acetylcysteine at room temperature (Table 3).[10] The $HCrO_4^-$ peak decreased in absorbance over the course of the first 70 min of the reaction, and simultaneously, a new peak assigned to the *N*-acetylcysteinate−CrO_3^- thiolate complex emerged at 410 nm, reached a maximum after 65 min, and persisted for at least an additional 35 min. The extinction coefficient determined from averaging the peak intensities at 410 nm after 60−65 min was 1740 ± 90 M^{-1} cm^{-1}. The reaction of methyl *N*-acetylcysteinate with $Na_2Cr_2O_7 \cdot 2H_2O$ resulted in a new peak at 408 nm ($\epsilon = 1740 \pm 60$ M^{-1} cm^{-1}) which was assigned to the methyl *N*-acetylcysteinate−CrO_3^- complex (Table 3). The absorption peak at 408 nm attained maximum intensity at approximately 50 min after mixing and remained stable up to 100 min after mixing. The λ_{max} and ϵ values for both the methyl *N*-acetylcysteinate−Cr(VI) and *N*-acetylcysteinate−Cr(VI) complexes are consistent with those reported in the literature for RS−CrO_3^- complexes (Table 3).[4,10]

Infrared Spectroscopy for the Reaction of *N*-Acetylcysteine and the Methyl Ester of *N*-Acetylcysteine with $K_2Cr_2O_7$. IR analysis was focused on the Cr−O region of 700−1000 cm^{-1}, and the sulfhydryl (S−H) region at 2200−2700 cm^{-1} in order to gain more information regarding the identity of the Cr(VI) complexes formed upon reaction of *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine with $K_2Cr_2O_7$ in DMF at

(18) Goodgame, D. M. L.; Joy, A. M. *J. Inorg. Biochem.*, **1986**, *26*, 219−224.
(19) Aiyar, J.; Borges, K. M.; Floyd, R. A.; Wetterhahn, K. E. *Toxicol. Environ. Chem.* **1989**, *22*, 135−145.
(20) Borges, K. M.; Boswell, J. S.; Liebross, R. H.; Wetterhahn, K. E. *Carcinogenesis* **1991**, *12*, 551−561.

room temperature. The IR spectrum of $K_2Cr_2O_7$ in DMF exhibited three characteristic stretches in the region 700−1000 cm^{-1}: 936 cm^{-1} for the asymmetric Cr−O stretch, 874 cm^{-1} for the symmetric Cr−O stretch, and 780 cm^{-1} for the asymmetric stretch of the μ-oxo (Cr−O−Cr) bond. The reaction of *N*-acetylcysteine with $K_2Cr_2O_7$ showed the disappearance of the dichromate μ-oxo stretch at 780 cm^{-1} and 10−16 cm^{-1} increases of the Cr−O stretches 10 min after mixing. The broad S−H stretch[21] at 2541−2545 cm^{-1} also disappeared. The reaction of methyl *N*-acetylcysteine ester with $K_2Cr_2O_7$ in DMF produced similar results. The IR data are consistent with the formation of *N*-acetylcysteinate−CrO_3^- and methyl *N*-acetylcysteinate−CrO_3^- thiolate complexes in DMF.

^{1}H NMR Spectroscopy for the Reaction of *N*-Acetylcysteine and the Methyl Ester of *N*-Acetylcysteine with $K_2Cr_2O_7$. In order to stabilize the Cr(VI)−thiolate complexes and follow their formation by NMR spectroscopy, the reactions of *N*-acetylcysteine and methyl *N*-acetylcysteine ester with $K_2Cr_2O_7$ in DMF-d_7 were run at ∼1:1 thiol:Cr(VI) at room temperature. Ten minutes after the addition of *N*-acetylcysteine to $K_2Cr_2O_7$, the ^{1}H NMR spectrum showed that the sulfhydryl proton had disappeared and new peaks were observed. There was virtually no change in the spectrum after 90 min from the start of the reaction. The ^{1}H NMR spectrum showed some unreacted *N*-acetylcysteine and minor peaks attributable to the oxidation of *N*-acetylcysteine to the disulfide, *N*-acetylcystine. The multiplet associated with the cysteinyl methylene protons shifted 0.30 ppm downfield from the uncomplexed thiol, similar to the shift observed for the aqueous reaction of *N*-acetylcysteine with $K_2Cr_2O_7$. The ^{1}H NMR spectra for the reaction of methyl-*N*-acetylcysteine with $K_2Cr_2O_7$ in DMF-d_7 were similar to those for the analogous reaction of *N*-acetylcysteine with $K_2Cr_2O_7$. The largest changes in chemical shift occurred with the cysteinyl methylene protons, which are closest to the coordination site with the metal center, supporting the conclusion that *N*-acetylcysteinate−CrO_3^- and methyl *N*-acetylcysteinate−CrO_3^- have monodentate thiolate ligands in DMF as well as aqueous solutions.

Discussion

Previous work has shown that glutathione reacts with dichromate to form a 1:1 GS−CrO_3^- thiolate complex, in which the glutathionate ligand is bound to the metal center solely *via* a Cr−S bond.[6,7] In the current study, we report that this mode of binding to Cr(VI) applies to other biological thiols, including γ-glutamylcysteine, *N*-acetylcysteine, cysteine, and the methyl ester of *N*-acetylcysteine and occurs in both aqueous and DMF solutions. These studies are indicative of a general class of reaction of thiols with dichromate, and are consistent with earlier studies[4,6,8−10] on the reaction of alkane- and arenethiols and other thiols with dichromate. However, our findings suggest that the mechanism for the thiol/dichromate reaction in aqueous solution follows a different pathway from that proposed by Mazurek and coworkers[10] for the reaction in non-aqueous solution (DMF). Apparently in aqueous solutions only one Cr(VI) moiety per $Cr_2O_7^{2-}$ forms a thiolate complex with the added thiol; the other Cr(VI) species is released as hydrogen chromate, $HCrO_4^-$, in equilibrium with the starting material and chromate CrO_4^{2-} (Scheme 1). This was evidenced by our ^{17}O NMR studies

Scheme 1

$$Cr_2O_7^{2-} + RSH \rightarrow [RS\text{-}CrO_3]^- + HCrO_4^- \quad (3)$$

$$2\ HCrO_4^- \rightleftharpoons Cr_2O_7^{2-} + H_2O \quad (4)$$

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+ \quad (5)$$

which showed that in an aqueous solution containing 1:1 mixture of γ-glutamylcysteine or *N* -acetylcysteine and dichromate, some chromate, hydrogen chromate, and dichromate were still present over 1 h after mixing, even though no reduced thiol was observed in the corresponding ^{1}H and ^{13}C NMR spectra. In this mechanism, the initial step involves an attack by the added thiol, through the sulfhydryl group, at one of the Cr(VI) centers of the dichromate species. This leads to displacement of a hydrogen chromate anion and the formation of the Cr(VI)−thiolate complex. However, Mazurek *et al.* suggested that the formation of Cr(VI)−thioesters, from the reaction of $Na_2Cr_2O_7$ with alkane- and arenethiols in DMF, follows a biphasic pathway.[10] The initial phase involves hydrolysis of dichromate to $HCrO_4^-$, in a reaction where the added thiol acts as an acid catalyst.[10] In the second phase, the resulting $[HO-CrO_3]^-$ reacts with the added thiol producing the Cr(VI)−thioester complex (Scheme 2).[10] In this mechanism, the thiol sulfur atom

Scheme 2

$$Cr_2O_7^{2-} + H_2O \xrightarrow{\text{"RSH"}} 2\ [HO\text{-}CrO_3]^- \quad (6)$$

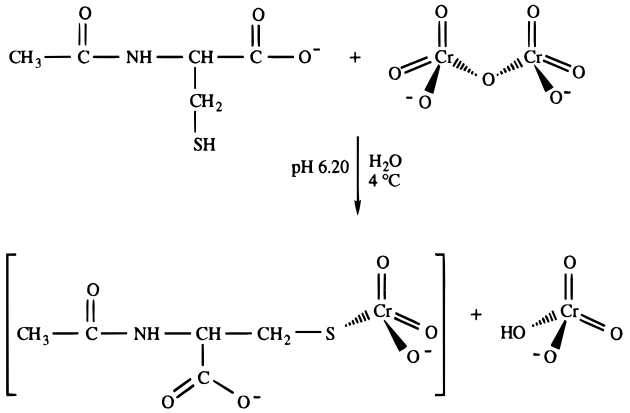
$$2\ [HO\text{-}CrO_3]^- + 2\ RSH \rightarrow 2\ [RS\text{-}CrO_3]^- + 2\ H_2O \quad (7)$$

apparently does not interact with chromium before the hydrolysis of dichromate (eq 6). Our studies with *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine support Mazurek *et al.*'s proposal[10] that reactions of dichromate with thiols in DMF follow Scheme 2. It is possible that the aqueous reactions follow the mechanism in Scheme 1 because $HCrO_4^-$ is a better leaving group in aqueous solution than in DMF, with H_2O providing better solvation for the resulting anions. High ionic strength conditions allow for a successful thiol attack at dichromate in aqueous solution, which leads to the formation of the Cr(VI)−thioester and $HCrO_4^-$ anions. However, once the well-solvated $HCrO_4^-$ is formed, the hydroxyl group is not as readily displaced by an attacking thiol. Instead, $HCrO_4^-$ undergoes equilibration reactions to $Cr_2O_7^{2-}$ and CrO_4^{2-}, as shown in Scheme 1 above and confirmed by the ^{17}O NMR data. Scheme 3 depicts the reaction of *N*-acetylcysteine with $Cr_2O_7^{2-}$ in aqueous solution at 4 °C.

The relative stablities of the Cr(VI)−thiolate complexes in aqueous solution clearly show that as the side chains of the thiolate ligand become larger and more sterically hindered, the complex is more stable with respect to reduction. Although the majority of decay appears to involve two-electron reduction producing oxidized thiols in the form of disulfides, a minor pathway may involve one-electron reduction of Cr(VI) by thiol or Cr(IV), since Cr(V) was detected in reactions with γ-glutamylcysteine and *N*-acetylcysteine. Comparison of these Cr(V) signals with those previously observed for reactions of GSH with dichromate under similar conditions[6] suggests that the $g = 1.973-4$ signals observed for γ-glutamylcysteine:Cr(VI) and *N*-acetylcysteine:Cr(VI) reaction mixtures are due to a square pyramidal Cr(V) species, with a Cr═O bond, two singly coordinated oxo or aquo ligands, and a cysteine moeity bound

(21) Weast, J., Ed. *CRC Handbook of Chemistry and Physics;* CRC Press: Boca Raton, FL, 1984; p 65.

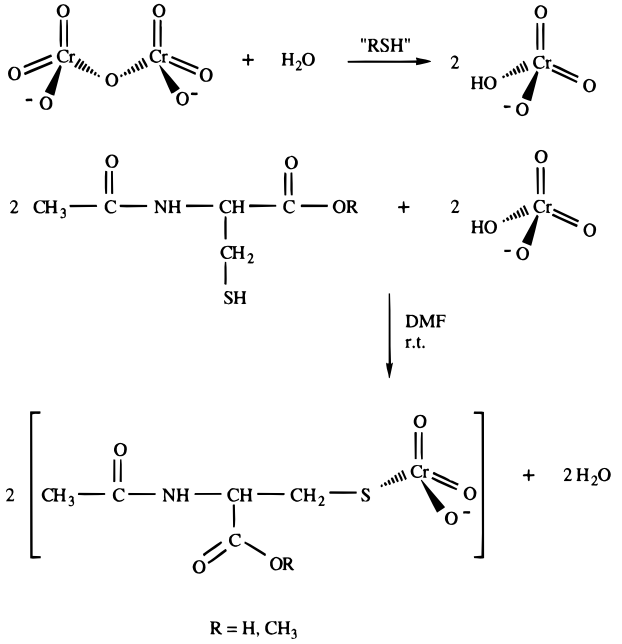
Scheme 3



through the cysteinyl carboxylate and thiolate. In terms of the predominant two-electron reduction, the ^{1}H NMR spectra for the reaction of GSH and dichromate in aqueous solution showed very little formation of oxidized glutathione (GSSG) over a 1 h time period,[6] yet spectra for the reaction of *N*-acetylcysteine and dichromate showed significant amounts of oxidized thiol upon mixing. The *N*-acetylcysteinate$-CrO_3^-$ and cysteinate$-CrO_3^-$ complexes were not as stable as the glutathionate$-CrO_3^-$ complex. The integration of the chemical shifts for the *N*-acetylcysteine:Cr(VI) reaction mixture showed a 33% decay of the *N*-acetylcysteinate$-CrO_3^-$ complex over a 1 h period, and a marked increase of *N*-acetylcystine. A more rapid decay was observed for the cysteinate$-CrO_3^-$ complex which underwent ∼100% decay over the same period of time. The γ-glutamylcysteinate$-CrO_3^-$ complex exhibited less decay, ∼25%, after 1 h from formation. However, the GS$-CrO_3^-$ exhibited considerably less decay, below 20% after 1 h, and the amount of GSSG formed did not increase significantly with time.[6] Thus, it appears that the stability of the RSCrO_3^- complexes towards reduction in aqueous solution follows the order cysteine < *N*-acetylcysteine < γ-glutamylcysteine < glutathione. However, the Cr(VI)−thiolate complexes appear to be more stable in DMF than in aqueous solution. The aqueous work with *N*-acetylcysteinate$-CrO_3^-$ showed that the complex forms within a few minutes and degrades substantially in less than one hour; however, in DMF the same complex forms over a 60 min time span, and shows little sign of degradation through 2 h of reaction.

UV/Vis, IR and ^{1}H NMR spectra for the reaction of dichromate with *N*-acetylcysteine (Scheme 4) in DMF were indicative of the formation of the corresponding Cr(VI)−thioester, and were consistent with the results reported by Mazurek *et al.* for Cr(VI)−thioesters of alkane- and arenethiols.[10] The ^{1}H NMR spectra for *N*-acetylcysteine and the ester of methyl *N*-acetylcysteine exhibited characteristic downfield shifts of ∼0.3 ppm in the methylene proton signals upon formation of the corresponding Cr(VI)−thioesters. These changes in chemical shifts are similar to those observed upon formation of Cr(VI)−thioesters from the aqueous reactions of γ-glutamylcysteine, *N*-acetylcysteine, and cysteine with dichromate, and are in agreement with the literature values reported for GS$-CrO_3^-$ and *n*-BuS$-CrO_3^-$.[6,10] The signals of the β-methylene cysteinyl protons immediately adjacent to the sulfhydryl showed significant downfield shifts upon binding of the thiol to Cr(VI); however, the other proton signals showed much smaller changes in chemical shifts, decreasing with distance from the thiol group. ^{17}O NMR studies of the reaction of γ-glutamylcysteine and *N*-acetylcysteine with dichromate showed new signals at 1127−1128 ppm which were shifted 12−

Scheme 4



13 ppm downfield from the terminal oxygens of dichromate. These results are quite similar to those of the previously characterized glutathionate$-CrO_3^-$ complex, in which the oxygens of the $-Cr^{VI}O_3^-$ moiety exhibited a signal at 1133 ppm.[6] The downfield shift observed upon replacement of one oxygen on Cr(VI) by a thiolate ligand is consistent with Filowitz *et al.*'s[22] observation that ^{17}O chemical shifts were largely a function of electronic environment and that downfield shifts correlate with increased π-bonding of oxygens to the metal center. The line widths of the new ^{17}O peaks (220−260 Hz) were quite similar to the line width previously observed for the oxygen peak of the $-Cr^{VI}O_3^-$ moiety in GS$-CrO_3^-$ (180−200 Hz)[6] which was attributed to the lower symmetry of the electric field gradient for the Cr(VI)−thiolate complex compared to dichromate or chromate.[22,23] These results are consistent with monodentate binding of the thiols to Cr(VI) forming RS$-CrO_3^-$ complexes having three equivalent Cr−O bonds, similar to that found by X-ray crystal structure analysis of the ((*p*-bromophenyl)thiolato)chromate(VI) salt, [Ph_4As][*p*-BrPhSCrO_3].[11]

The reaction of *N*-acetylcysteine and the methyl ester of *N*-acetylcysteine with sodium dichromate in DMF displayed shifts in the UV/vis spectra associated with hydrogen chromate/dichromate at 350/374 nm to 408−410 nm (ϵ = 1740 M^{-1} cm^{-1}). These are similar to peak shifts from 374 nm to 410−420 nm (ϵ = 1800−2100 M^{-1} cm^{-1}) reported for Cr(VI)−thioester formation upon reaction of alkanethiols and arenethiols, *e.g.*, *n*-BuSH, *t*-BuSH, PhSH, and *p*-BrPhSH, with $Na_2Cr_2O_7$ in DMF.[10] Cr(VI)−thioester intermediates have also been characterized in aqueous kinetic studies by a shift in absorption maximum for hydrogen chromate at 350 nm to 420-430 nm with ϵ = 1228 M^{-1} cm^{-1} for penicillamine and 1328 M^{-1} cm^{-1} for BME.[4] This shift in the ligand-to-metal charge transfer band to longer wavelength is presumably caused by the cleavage of a Cr−O bond and formation of a Cr−S bond.

These Cr(VI)−thioesters were also characterized by IR spectroscopy which showed the disappearance of the μ-oxo peak

(22) Besecker, C. J.; Klemperer,W. G.; Maltbie, D. J.; Wright, D. A. *Inorg. Chem.* **1985**, *24*, 1027−1032.
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at 780 cm^{-1} and a 10−16 cm^{-1} increase in the symmetric and asymmetric Cr−O stretches of dichromate upon the formation of both Cr(VI)−thioesters of the biological thiols in the current study and the alkane- and arenethiols in earlier studies.[10] The IR data in DMF are consistent with the ^{17}O NMR data in aqueous solution indicating increased π-bonding of oxygens to the metal center in the RS−CrO_3^- complexes. Previous studies have utilized Raman spectroscopy to monitor the reaction of GSH with $K_2Cr_2O_7$ in aqueous solution leading to the formation of GS−CrO_3^-.[7] The formation of the GS−CrO_3^- complex was indicated by the disappearance of the S−H band 2526 cm^{-1} and the appearance of two new peaks at 889 and 432 cm^{-1}, the later of which was attributed to the Cr−S bond.[7]

Our results, as well as those of others,[7,10,11] indicate that Cr(VI) binds to thiols solely through the thiolate moiety, even in the presence of potential carboxylate and amine coordinating groups. Cr(VI)−thiolate complexes might be considered unusual because Cr(VI) is considered a *hard* acid while sulfur is a *soft* base.[24] X-ray crystal structure analysis revealed that Cr(VI) preferred coordination to the *hard* carboxylate oxygen *vs* the pyridinium nitrogen in the nicotinate−CrO_3^- complex formed upon reaction of chromium trioxide with nicotinic acid.[25] Cavaleiro *et al.*[26] reported that other metals with an electronic configuration similar to Cr(VI), *i.e.*, Mo(VI) and W(VI), form 1:1 complexes with cysteine, $[MoO_3(Cys)]^{2-}$ and $[WO_3(Cys)]^{2-}$, in which cysteine acts as a tridentate ligand, utilizing its thiolate, amino, and carboxylate groups in coordinating the metal center. More recently, Yamasaki and Shibahara[27] synthesized several dimeric tungsten(V)−cysteine complexes, $[W(O)(\mu\text{-}S)(Cys)]_2^{2-}$. $K_2[W(O)(\mu\text{-}S)(Cys)]_2 \cdot 5H_2O$ was structurally characterized and the cysteine ligand on each tungsten atom was found to adopt a tridentate binding mode through its thiolate, amino, and carboxylate groups.[27] Sakurai *et al.* have shown by X-ray crystallography that substituted cysteine ligands, such as the methyl ester of cysteine, bind to vanadium(IV) through both the thiolate and amino groups forming the vanadyl complex $[V(O)(Me\text{-}Cys)_2]$.[28] In all these studies,[26−28] the cysteine moiety exhibits a multidentate binding mode to the corresponding metal center, in sharp contrast to the monodentate binding mode exhibited by the thiolate ligands in the Cr(VI)−thioester complexes. However, cysteine also binds to Hg(II), which is considered a *soft* acid,[24] solely through the thiolate group in complexes with the methylmercury and phenylmercury(II).[29,30] Other modes of complexation for cysteine involve binding of the thiolate group to more than one metal center. Jensen and coworkers[31] prepared and structurally characterized the dinuclear platinum(II)−*N*-acetylcysteinate complex $[Pt(\mu\text{-Ac-Cys})(bpy)]_2$, (bpy = 2,2′-bipyridine), in which the two metal centers are bridged by the two deprotonated sulfhydryl groups of the *N*-acetylcysteine ligands. Our studies have not determined the mode of binding of Cr(VI) to protein cysteine moieties or to small biological thiols *in vivo*, and thus the formation of Cr(VI)−thioester complexes with multidentate or bridging thiolate ligands *in vivo* cannot be ruled out. However, monodentate thiolate binding in the form of RS−CrO_3^- appears to be the most likely mode for interaction.

Conclusion

The reaction of the biological thiols γ-glutamylcysteine, *N*-acetylcysteine, cysteine, and the methyl ester of *N*-acetylcysteine with dichromate, leads to the formation of 1:1 Cr(VI)−thioester complexes in which the thiolate ligand adopts a monodentate binding mode to metal center *via* a Cr−S bond and the Cr(VI) is also coordinated to three equivalent oxo ligands. The resulting RS−CrO_3^- complexes form both in aqueous and organic solvents, but the Cr(VI)−thiolate complexes exhibit considerably greater stability in DMF in comparison to aqueous solution. Currently, our goal is to further characterize these Cr(VI)−thiolate complexes in order to gain better understanding of their reactivity in biological systems and potential role in chromium(VI)-induced genotoxicity. Investigating the biological activity of these complexes is significant as previous studies[32−35] have shown that the reaction of Cr(VI) with intracellular reductants is a prerequisite for genotoxicity of chromium.

Acknowledgment. This work was supported by PHS Grant Nos. CA34869 from the National Cancer Institute and ES07167 from the National Institute of Environmental Health Sciences. The EPR spectrometer was purchased with funding from National Science Foundation Grant No. CHE-8701406. The NMR spectrometer was purchased with funding from National Science Foundation Grant no. CHE-9213480.

Supporting Information Available: Figures showing proton and ^{13}C NMR spectra of *N*-acetylcysteine, the *N*-acetylcysteinate−CrO_3^- complex and *N*-acetylcystine, UV/vis spectra showing the time evolution for the formation of the methyl *N*-acetylcysteinate−CrO_3^- complex in DMF and tables of IR data for the Cr(VI)−thioesters in DMF, and ^1H NMR data for *N*-acetylcysteine, the *N*-acetylcysteinate−CrO_3^- complex, methyl *N*-acetylcysteine, and the methyl *N*-acetylcysteinate−CrO_3^- complex in DMF (7 pages). Ordering information is given on any current masthead page.

IC941452D

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