solution has been rarely investigated. A vast amount of literature is available for the metal ion catalysis in transacylation reactions.[1] Catalysis by binuclear metal ions, however, has not been reported. The present results indicate that the participation of the binuclear species could be a general phenomenon when the geometry of the transition state is appropriate.

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Reaction of Chromium(VI) with Thiols: pH Dependence of Chromium(VI) Thio Ester Formation

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Abstract: The rates of ligand substitution reactions of chromium(VI) with cysteamine, cysteine, cysteine ethyl ester, homocysteine, 3-mercaptopropionic acid, *N*-acetylcysteine, and thioglycolic acid which result in the formation of chromium(VI) thio esters have been studied over a wide range of pH. The apparent second-order rate constants (k_1) for the formation of the chromium(VI) thio esters varied dramatically with pH. Analysis of the pH dependence of the rate constants revealed that (a) in the pH range of 5-10, the reaction of chromium(VI) with cysteine ethyl ester and cysteamine could be described by reaction of the chromium(VI) and aminothiol species having two of the three possible groups (oxo of chromium(VI), amine, or sulfhydryl) protonated, (b) in the pH range of 3-10, the reaction of chromium(VI) with cysteine and homocysteine could be described by the reaction of chromium(VI) and aminothiocarboxylic acid species having two, three, and four of the four possible groups (oxo of chromium(VI), amine, sulfhydryl, and carboxylate) protonated, (c) in the pH range of 2-10, the reaction of chromium(VI) with 3-mercaptopropionic acid and *N*-acetylcysteine could be described by the reaction of chromium(VI) and thiocarboxylic acid species having one, two, and three of the three possible groups (oxo of chromium(VI), sulfhydryl and carboxylate) protonated, and (d) in the pH range of 2-10, the reaction of chromium(VI) with thiolglycolic acid could be described by the reaction of chromium(VI) and thiocarboxylic acid species having two and three of the three possible groups protonated. Linear plots of log k_1 vs. pK_a of the thiol were obtained only for reaction of chromate or hydrogen chromate with species having the sulfhydryl group protonated. In these cases, log k_1 was inversely correlated with the thiol pK_a. Reaction of the thiols with hydrogen chromate was 10-100 times slower than with chromate. Protonation of carboxylate groups resulted in a ~1000 times increase in the apparent second-order rate constant for the reaction of chromate with the aminothiocarboxylic acids and thiocarboxylic acids. The rate of reaction of thioglycolic acid with chromium(VI) was anomalously high when compared to the other thiols and suggests a possible chelate effect or a more favorable geometry for proton transfer from -COOH to -CrOH. Our results indicate that ligand substitution reaction of chromium(VI) with thiols involves attack of chromate or hydrogen chromate by the protonated thiol with rate-determining proton transfer from the sulfhydryl to the chromium(VI).

The formation of chromium(VI) esters during the reaction of chromium(VI) with organic and inorganic acids has been well-documented.[2-5] Most studies of chromium(VI)-thiol reactions have been carried out under acidic conditions. Under these conditions, transient orange species ($\lambda_{max} \sim 420$-440 nm) have been observed and assigned as chromium(VI) thio esters on the basis of spectral features (the red shift in absorbance maximum compared to hydrogen chromate and large extinction coefficients).[6] Chromium(VI) thio esters have been detected upon reaction of chromium(VI) with cysteine,[7] penicillamine, glutathione, cysteamine,[6] thiourea,[8] hydrogen thiocyanate,[9] and hydrogen thiosulfate[10] under acidic conditions (eq 1). Although chromium(VI)

$$RSH + HCrO_4^- \rightleftharpoons RSCrO_3^- + H_2O \qquad (1)$$

is considered a *hard* acid and sulfur a *soft* base,[11] the equilibrium constants for the formation of chromium(VI) thio esters are several orders of magnitude greater than chromium(VI) oxy esters.[6]

Our kinetic studies of the reaction of glutathione and other thiols with chromium(VI) at pH 7.4[12] and Kwong and Pennington's study of the reaction of cysteine with chromium(VI) at pH 7.0[13] showed that a general mechanism for the reaction of chromium(VI) with thiols involves the formation of a chromium(VI) thio ester (eq 2) followed by either a redox reaction involving a second molecule of thiol (eq 3) or a unimolecular redox reaction of the thio ester which leads to Cr(V) and a radical (eq 4) or Cr(IV) in the case of dithiols capable of forming an intramolecular disulfide bond (eq 5). We have obtained spectral and kinetic evidence at pH 7.4 for the formation of chromium(VI) thio esters upon the reaction of chromium(VI) with glutathione, cysteine ethyl

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$$RSH + Cr^{VI} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RSCr^{VI} \quad (2)$$

$$RSCr^{VI} + RSH \xrightarrow{k_2} Cr^{IV} + RSSR \quad (3)$$

$$RSCr^{VI} \xrightarrow{k_3} Cr^{V} + RS^{\bullet}? \quad (4)$$

$$RSCr^{VI} \xrightarrow{k_4} Cr^{IV} + R{-}S \quad (5)$$

(SH; S)

ester, cysteine, cysteamine, coenzyme M, homocysteine, *N*-acetylcysteine, coenzyme A, mercaptoethanol, and thioglycolate; however, no chromium(VI) thio esters were detected upon reaction of chromium(VI) with penicillamine, dithiothreitol, 2,3-dimercaptosuccinate, thiolactate, and thiomalate.[12] Brønsted plots show that the second-order rate constants, k_1, for the formation of the chromium(VI) thio ester are inversely related to the pK_a's of the thiol groups. We suggested that the mechanism for the ligand substitution reaction (eq 2) involved the attack of the un-ionized thiol on chromate, CrO_4^{2-}.[12]

Although the reactions of chromium(VI) with penicillamine,[14] cysteine,[12,13] glutathione,[12,15] and other thiols[12] have been studied near neutral pH, there have been no detailed analyses of the pH dependence of these reactions at pH > 3. In order to probe the mechanism of substitution reactions of chromium(VI) with thiol ligands, we have now examined the kinetics of chromium(VI) thio ester formation with seven thiols over a wide range of pH. The aminothiols, cysteine ethyl ester and cysteamine were examined over the pH range 5–10, the aminothiocarboxylic acids, cysteine and homocysteine, were examined over the pH range of 3–10, and the thiocarboxylic acids, thioglycolic acid, 3-mercaptopropionic acid, and *N*-acetylcysteine, were examined over the pH range of 2–10. Analysis of the resulting k_1 vs. pH profiles revealed that only certain ionized forms of the thiols and chromium(VI) react in this pH range.

Experimental Procedures

Materials. Potassium dichromate (1000 ppm Atomic Absorbance Reference Solution) was purchased from Fisher Scientific Co., Pittsburgh, PA. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Determination of Chromium(VI) and Thiol Concentrations. The concentration of chromium(VI) was determined by measuring the absorbance at 372 nm in 0.1 M KOH (ϵ_{372} 4810 M^{-1} cm^{-1}).[16] The concentration of the thiols was measured at 412 nm after reaction with Ellman's reagent (ϵ_{412} 13 700 M^{-1} cm^{-1}).[17]

Determination of the pK_a of Hydrogen Chromate and the pK_a's of the Thiols. The pK_a of hydrogen chromate at 25 °C, 0.5 M KCl, was determined spectrophotometrically at 370 nm.[18] The macroscopic and microscopic ionization constants of the thiols were determined at 25 °C and 0.5 M KCl, under anaerobic conditions. The macroscopic ionization constants for the thiol groups of cysteamine, *N*-acetylcysteine, thioglycolic acid, and 3-mercaptopropionic acid were determined spectrophotometrically at 230 nm.[19] The macroscopic ionization constants for the amino groups and carboxylate groups on these thiols were calculated from potentiometric data obtained by using a Fisher Model 390 automatic titrator by the Gran plot technique.[20] The microscopic ionization constants for the thiol and amino groups of cysteine, homocysteine, and cysteine ethyl ester were calculated from computer fits of spectrophotometric data at 230 nm[19] with use of the program FIND.KAS.[21]

Kinetics of Chromium(VI) Reduction. The reduction of chromium(VI) (3.7×10^{-5} M) was monitored by following the absorbance of chromium(VI) at λ_{max} (350–372 nm depending on pH). In each case, the reactions were examined at 25 °C at an ionic strength adjusted to 0.5 M with KCl. The pH of the reaction mixtures was adjusted with small additions of HCl or NaOH, and in most cases, the excess of thiol (0.0001–0.51 M) was sufficient to act as its own buffer over the time period of kinetic data collection. In some cases, the reactions were run in 0.1 M Tris–HCl. The values for the apparent second-order rate constants for the formation of the chromium(VI) thio esters, k_{1app}, were obtained from the absorbance data by methods previously described.[12] For each set of thiol–chromium(VI) reactions, the k_{1app} vs. pH profile was obtained and analyzed as described below.

Determination of Micro Rate Constants from the k_{1app} vs. pH Profiles. The simple rate expression for chromium(VI) thio ester formation is given by eq 6. However, at any particular pH, the thiol is present as several different species depending on the state of protonation of the COOH, NH_2, and SH functional groups and of the chromium(VI) (CrO_4^{2-} or $HCrO_4^-$). Thus, eq 6 may be rewritten as the linear com-

$$\text{rate} = k_1[\text{thiol}][\text{chromium(VI)}] \quad (6)$$

bination of a whole series of thiol–chromium(VI) combinations multiplied by what we will term the "micro" rate constants k_{1A1}, k_{1B1}, etc., as illustrated for cysteine and homocysteine in eq 7.

$$\begin{aligned}\text{rate} = {} & k_{1A1}[^-S\text{-}R(COO^-)\text{-}NH_2][CrO_4^{2-}] + \\ & k_{1B1}[HS\text{-}R(COO^-)\text{-}NH_2][CrO_4^{2-}] + \\ & k_{1B2}[^-S\text{-}R(COO^-)\text{-}NH_3^+][CrO_4^{2-}] + \\ & k_{1B3}[^-S\text{-}R(COO^-)\text{-}NH_2][HCrO_4^-] + \\ & k_{1B4}[^-S\text{-}R(COOH)\text{-}NH_2][CrO_4^{2-}] + \\ & k_{1C1}[HS\text{-}R(COO^-)\text{-}NH_3^+][CrO_4^{2-}] + \\ & k_{1C2}[^-S\text{-}R(COO^-)\text{-}NH_3^+][HCrO_4^-] + \\ & k_{1C3}[HS\text{-}R(COO^-)\text{-}NH_2][HCrO_4^-] + \\ & k_{1C4}[^-S\text{-}R(COOH)\text{-}NH_3^+][CrO_4^{2-}] + \\ & k_{1C5}[HS\text{-}R(COOH)\text{-}NH_2][CrO_4^{2-}] + \\ & k_{1C6}[^-S\text{-}R(COOH)\text{-}NH_2][HCrO_4^-] + \\ & k_{1D1}[HS\text{-}R(COOH)\text{-}NH_3^+][CrO_4^{2-}] + \\ & k_{1D2}[HS\text{-}R(COO^-)\text{-}NH_3^+][HCrO_4^-] + \\ & k_{1D3}[^-S\text{-}R(COOH)\text{-}NH_3^+][HCrO_4^-] + \\ & k_{1D4}[HS\text{-}R(COOH)\text{-}NH_2][HCrO_4^-] + \\ & k_{1E1}[HS\text{-}R(COOH)\text{-}NH_3^+][HCrO_4^-] + \\ & k_{1E2}[HS\text{-}R(COO^-)\text{-}NH_3^+][HCrO_4^-][H^+] + \\ & k_{1E3}[^-S\text{-}R(COOH)\text{-}NH_3^+][HCrO_4^-][H^+] + \\ & k_{1F1}[HS\text{-}R(COOH)\text{-}NH_3^+][HCrO_4^-][H^+] \end{aligned} \quad (7)$$

The computer program 3.PROT[22] which uses an iterative technique to fit the data to eq 7 was used to determine the relative contribution of each species combination to the k_{1app} vs. pH profile and, hence, the "micro" rate constants for the various possibilities. In the case of the aminothiols and thiocarboxylic acids which have only two ionizable groups, the computer program 2.PROT[22] was used. The computer programs require the input of the macroscopic or microscopic ionization constants for the various ionizing groups on the thiols (see Table I) and the acid dissociation constant for the hydrogen chromate ion (pK_a = 5.98).[12]

Results

pK_a Values of the Thiols. The pK_a's for the sulfhydryl, amino, and carboxylate groups of the thiols were determined at 25 °C and 0.5 M ionic strength. Table I summarizes the macroscopic or microscopic ionization constants determined for the thiols under these conditions. Macroscopic ionization constants were obtained for cysteamine, thioglycolic acid, 3-mercaptopropionic acid, and *N*-acetylcysteine, since there was a large difference in the pK_a's of the ionizing groups. Microscopic ionization constants were obtained for cysteine, homocysteine, and cysteine ethyl ester, whose ionization scheme is presented in eq 8. The values obtained agree

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(22) (a) Connett, P. H.; Hampton, T. H. 2.PROT, 1983, Dartmouth College, Hanover, NH. (b) Connett, P. H.; Hampton, T. H. 3.PROT, 1985, Dartmouth College, Hanover, NH. These programs are nonlinear least-squares curve-fitting programs designed to extract micro rate constants from observed k_{1app} vs. pH data. Given that $k_1 = k_{1A1}f(x) + k_{1B1}f(y) + k_{1B2}f(z)$, etc., the PROT programs search for parameters k_{1A1}, k_{1B1}, k_{1B2}, etc., such that k_1 is as close as possible to the experimental value expressed as a function of pH. $F(x)$, $f(y)$, $f(z)$, etc., are functions which define the proportion of the reacting species in a particular state of ionization and, therefore, are entirely defined by the pK_a's of the ionizing groups and the pH.

Table I. Macroscopic and Microscopic pK_a Values for the Thiols Used in the Kinetic Studies with Chromium(VI)

thiol	pK_A	pK_B	pK_C	pK_D	pK(COOH)	T, °C	I, M	ref
cysteine	8.4	8.85	10.05	9.6		25	0.5	this study
					1.96	20	0.01	30, 33
	8.21	8.65	10.00	9.56		30	1.0	31
	8.53	8.86	10.36	10.03		23	0.2	19
homocysteine	9.27	9.30	10.16	10.13		25	0.5	this study
					2.22			32
	9.02	9.04	9.71	9.69		30	1.0	31
cysteine ethyl ester	7.58	6.96	8.45	9.07		25	0.5	this study
	7.3	6.76	8.33	8.87		30	1.0	31
	7.45	6.77	8.41	9.09		23	0.2	19
cysteamine	8.37[a]	10.44[b]				25	0.5	this study
	8.22[a]					30	1.0	31
	8.35[a]					23	0.2	19
	8.27[a]	10.53[b]				25	0.0	33
3-mercaptopropionic acid	10.15[a]					25	0.5	this study
					4.34	25	0.0	34
N-acetylcysteine	9.55[a]				3.21	25	0.5	this study
	9.52[a]					30	0.0	33
thioglycolic acid	10.07[a]				3.67	25	0.5	this study
	10.55[a]				3.6	25	0.0	33
					3.67	25	0.0	34
	9.82[a]					30	1.0	31

[a] pK_a (SH/S^-). [b] pK_a (NH_3^+/NH_2).

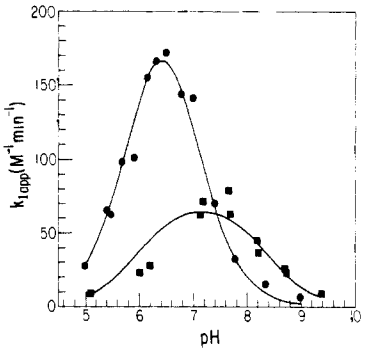
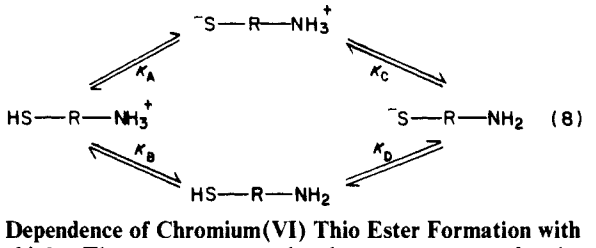


Figure 1. pH dependence of the apparent second-order rate constants, k_{1app}, for the formation of chromium(VI) thio esters with cysteine ethyl ester (●) and cysteamine (■) at 25 °C and I = 0.5 M. Initial [Cr(VI)] = 3.7×10^{-5} M and [thiol] = 0.001–0.22 M. Curves represent best fits of the data which were obtained with any member of class C by using the program 2.PROT.[22] Micro rate constants calculated on the bases of these best fits are listed in Table II.

well with literature values (also given in Table I) obtained under slightly different conditions.



pH Dependence of Chromium(VI) Thio Ester Formation with Aminothiols. The apparent second-order rate constants for the formation of chromium(VI) thio esters with cysteine ethyl ester and cysteamine were determined over the pH range 5–10 (Figure 1). The possible combinations of different chromium(VI) and thiol species which could react to give the chromium(VI) thio ester are summarized in Table II, together with the calculated rate constant that each combination would require if it was solely responsible for the observed kinetic data. There are five classes of possible reacting species which vary in the degree of protonation of the chromium(VI) and the thiol. The various classes are designated by A–E in Table II. The expected pH profiles generated by the program PLT2OUT[23] for each combination of chromium(VI) and cysteine ethyl ester species are shown in Figure 2. It is clear that the proton-dependent pathway E has little or no contribution to the reaction at pH > 5. The best fit of the data for both of the aminothiols (Figure 1) was obtained with class C, which represents some combination of chromium(VI) and thiol having two groups protonated, i.e., CrO_4^{2-} reacting with $HS-R-NH_3^+$ (C1), $HCrO_4^-$ reacting with $^-S-R-NH_3^+$ (C2), or $HCrO_4^-$

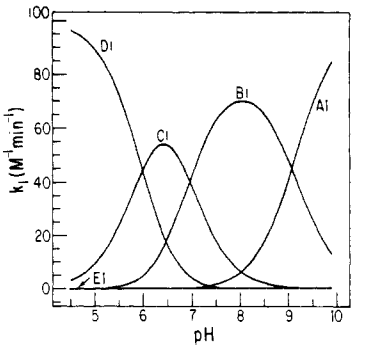


Figure 2. Theoretical plots of the pH dependence of the rate constants for the reaction of various species of chromium(VI) and cysteine ethyl ester to form the chromium(VI) thio ester. The curves were generated by using the program PLT2OUT[23] and represent results obtained by setting the micro rate constant for the desired combination of species to 100 M^{-1} min^{-1} and setting all others to zero.

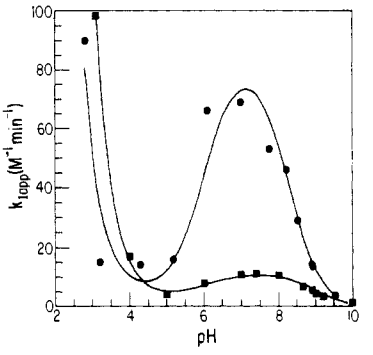


Figure 3. pH dependence of the apparent second-order rate constants, k_{1app}, for the formation of chromium(VI) thio esters with cysteine (●) and homocysteine (■) at 25 °C and I = 0.5 M. Initial [Cr(VI)] = 3.7×10^{-5} M and [thiol] = 0.0005–0.36 M. Curves were generated by using the program 3.PROT[22] and represent best fits of the data which were obtained by combining any member of class C and any member of class D with any member of class E. Micro rate constants calculated on the bases of these best fits are listed in Table II.

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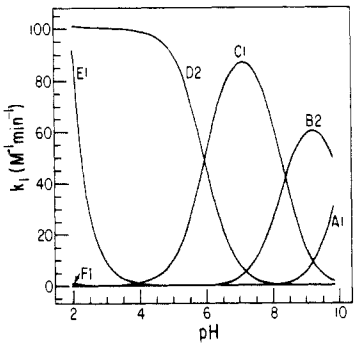

Figure 4. Theoretical plots of the pH dependence of the rate constants for the reaction of various species of chromium(VI) and cysteine to form the chromium(VI) thio ester. The curves were generated by using the program PLT3OUT[23] and represent results obtained by setting the micro rate constant for the desired combination of species to 100 M^{-1} min^{-1} and setting all others to zero.

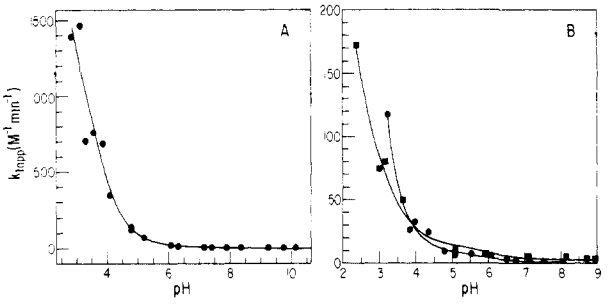

Figure 5. pH dependence of the apparent second-order rate constants, k_{1app}, for the formation of chromium(VI) thio esters with (A) thioglycolic acid and (B) 3-mercaptopropionic acid (●) and *N*-acetylcysteine (■) at 25 °C and I = 0.5 M. Initial [Cr(VI)] = 3.7 × 10^{-5} M and [thiol] = 0.001–0.514 M. Curves were generated by using the program 2.PROT[22] and represent best fits of the data which were obtained by combining any member of class C (except for thioglycolic acid), any member of class D, and any member of class E with any member of class F. Micro rate constants calculated on the basis of these best fits are listed in Table II.

reacting with HS–R–NH_2 (C3).

pH Dependence of Chromium(VI) Thio Ester Formation with Aminothiocarboxylic Acids. The apparent second-order rate constants for the formation of chromium(VI) thio esters with cysteine and homocysteine were determined over the pH range 3–10 (Figure 3). There are six possible classes of reacting species (A–F in Table II) which vary in the degree of protonation of the chromium(VI) and the thiol. The calculated rate constants for the various species combinations which give rise to the computer fits shown in Figure 3 are summarized in Table II. The theoretical plots for the reaction of various species of chromium(VI) and cysteine obtained by using the program PLT3OUT[23] are shown in Figure 4. The best fit of the pH profiles shown in Figure 3 required contributions from class C [CrO_4^{2-} reacting with HS–R(COO^-)–NH_3^+ (C1), $HCrO_4^-$ reacting with $^-$S–R(COO^-)–NH_3^+ (C2), $HCrO_4^-$ reacting with HS–R(COO^-)–NH_2 (C3), CrO_4^{2-} reacting with $^-$S–R(COOH)–NH_3^+ (C4), CrO_4^{2-} reacting with HS–R(COOH)–NH_2 (C5), or $HCrO_4^-$ reacting with $^-$S–R(COOH)–NH_2 (C6)], class D [CrO_4^{2-} reacting with HS–R–(COOH)–NH_3^+ (D1), $HCrO_4^-$ reacting with HS–R(COO^-)–NH_3^+ (D2), $HCrO_4^-$ reacting with $^-$S–R(COOH)–NH_3^+ (D3), or $HCrO_4^-$ reacting with HS–R(COOH)–NH_2 (D4)], and class E [$HCrO_4^-$ reacting with HS–R(COOH)–NH_3^+ (E1), $HCrO_4^-$ reacting with HS–R(COO^-)–NH_3^+ + H^+ (E2), or $HCrO_4^-$ reacting with $^-$S–R(COOH)–NH_3^+ + H^+ (E3)]. Reactions of $HCrO_4$– with $^-$S–R(COOH)–NH_2 (C6) and $^-$S–R(COOH)–NH_3^+ + H^+ (E3) are considered unlikely on the basis of the large calculated rate constants.

pH Dependence of Chromium(VI) Thio Ester Formation with Thiocarboxylic Acids. The apparent second-order rate constants for the formation of chromium(VI) thio esters with thioglycolic acid, 3-mercaptopropionic acid, and *N*-acetylcysteine were determined over the pH range 2–10 (Figure 5). There are five possible classes of reacting species (B–F in Table II). The different species combinations which can give rise to the computer fits shown in Figure 5, together with the calculated rate constants, are summarized in Table II. The theoretical plots for the various combinations of species for the chromium(VI)–thioglycolic acid reaction obtained by using the program PLT2OUT[23] are shown in Figure 6. Although in all cases the dominant contribution to the reaction of the thiocarboxylic acids with chromium(VI) was the proton-dependent path F ($HCrO_4^-$ reacting with HS–R–COOH + H^+), the best fit of the pH profiles shown in Figure 5 required contributions from other classes. In the case of thioglycolic acid, the best fit was obtained by combining any member of class D [CrO_4^{2-} reacting with HS–R–COOH (D1), $HCrO_4^-$ reacting with HS–R–COO^- (D2), or $^-$S–R–COOH (D3)] with class E [$HCrO_4^-$ reacting with HS–R–COOH (E1), $HCrO_4^-$ reacting with HS–R–COO^- + H^+ (E2), or $HCrO_4^-$ reacting with $^-$S–R–COOH + H^+ (E3)], and class F. In the case of 3-mercaptopropionic acid and *N*-acetylcysteine, the best fit was obtained by combining any member of class C [CrO_4^{2-} reacting with HS–R–COO^- (C1), $HCrO_4^-$ reacting with $^-$S–R–COO^- (C2), or CrO_4^{2-} reacting with $^-$S–R–COOH (C4)] and any member from class D with both class E and class F.

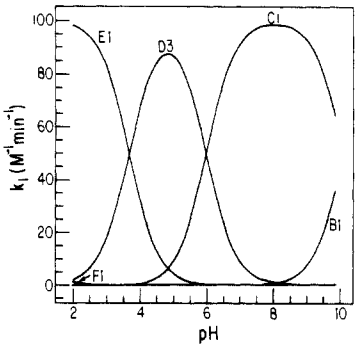

Figure 6. Theoretical plots of the pH dependence of the rate constants for the reaction of various species of chromium(VI) and thioglycolic acid to form the chromium(VI) thio ester. The curves were generated by using the program PLT2OUT[23] and represent results obtained by setting the micro rate constant for the desired combination of species to 100 M^{-1} min^{-1} and setting all others to zero.

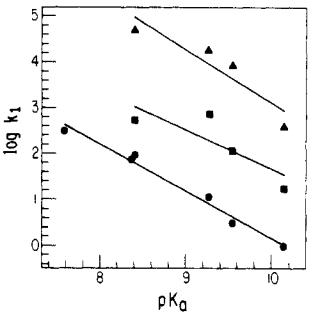

Figure 7. Plot of the log of the micro rate constants for the reaction of CrO_4^{2-} with HS–R–NH_3^+, HS–R(COO^-)–NH_3^+, or HS–R–COO^-, k_{1C1} (●), and for the reaction of CrO_4^{2-}, k_{1D1} (▲), or $HCrO_4^-$, k_{1E1} (■), with HS–R(COOH)–NH_3^+ or HS–R–COOH, vs. pK_a of the sulfhydryl group. For the log k_{1C1} vs. pK_a plot, the slope = –1.02 and r = –0.992. For the log k_{1D1} vs. pK_a plot, the slope = –1.16 and r = –0.919. For the log k_{1E1} vs. pK_a plot, the slope = –0.853 and r = –0.834.

Dependence of the Rate Constant for Chromium(VI) Thio Ester Formation on the pK_a of the Thiol. A plot of log k_{1C1} vs. pK_a of the thiol (Figure 7) showed an inverse linear relationship. Similar plots of log k_{1C2} or log k_{1C3} vs. pK_a for the thiol or amine groups did not produce linear fits (data not shown). A plot of log k_{1C4} vs. pK_a of the thiol showed an inverse linear relationship (slope = –1.50, r = –0.915). Plots of log k_{1D1} and log k_{1E1} vs. the pK_a of the thiol (Figure 7) showed inverse linear relationships. Log plots of the rate constants for other members of class D and E showed that there was no correlation with thiol pK_a. In all cases,

Table II. Second-Order "Micro" Rate Constants for the Formation of Chromium(VI) Thio Esters Calculated from the Best Fits of the k_{1app} vs. pH Profiles. Since All Members of a Class Produce the Same Fit, Individual "Micro" Rate Constants Were Calculated by Setting Other Members of the Same Class to Zero

			k_1, M^{-1} min^{-1} [a,b]						k_1, M^{-1} min^{-1} [a,b]		
	Cr^{VI} species	aminothiol species	cysteamine	cysteine ethyl ester	aminothiocarboxylic acid species	cysteine	homocysteine	thiocarboxylic acid species	thioglycolic acid	3-mercaptopropionic acid	*N*-acetylcysteine
A1	CrO_4^{2-}	^-S-R-NH_2	0	0	^-S-R(COO^-)-NH_2	0	0				
B1	CrO_4^{2-}	HS-R-NH_2	0	0	HS-R(COO^-)-NH_2	0	0				
B2	CrO_4^{2-}	^-S-R-NH_3^+	0	0	^-S-R(COO^-)-NH_3^+	0	0	^-S-R-COO^-	0	0	0
B3	$HCrO_4^-$	^-S-R-NH_2	0	0	^-S-R(COO^-)-NH_2	0	0				
B4	CrO_4^{2-}				^-S-R(COOH)-NH_2	0	0				
C1	CrO_4^{2-}	HS-R-NH_3^+	73	3.09×10^2	HS-R(COO^-)-NH_3^+	84	11	HS-R-COO^-	0	0.94	3.0
C2	$HCrO_4^-$	^-S-R-NH_3^+	1.78×10^4	1.23×10^4	^-S-R(COO^-)-NH_3^+	2.21×10^4	2.15×10^4	^-S-R-COO^-	0	1.39×10^4	1.11×10^4
C3	$HCrO_4^-$	HS-R-NH_2	2.40×10^6	2.95×10^5	HS-R(COO^-)NH_2	6.22×10^8	2.30×10^8				
C4	CrO_4^{2-}				^-S-R(COOH)NH_3^+	2.26×10^8	1.20×10^8	S-R-COOH	0	6.06×10^5	6.55×10^6
C5	CrO_4^{2-}				HS-R(COOH)NH_2	6.37×10^8	1.27×10^8				
C6	$HCrO_4^-$				^-S-R(COOH)NH_2	2.26×10^{12}	1.8×10^{12}				
D1	CrO_4^{2-}				HS-R(COOH)-NH_3^+	5.03×10^4	1.90×10^4	HS-R-COOH	9.35×10^3	3.73×10^2	8.5×10^3
D2	$HCrO_4^-$	HS-R-NH_3^+	0	0	HS-R(COO^-)-NH_3^+	4.8	3.3	HS-R-COO^-	46	8.5	14.6
D3	$HCrO_4^-$				^-S-R(COOH)-NH_3^+	1.3×10^7	3.7×10^7	^-S-R-COOH	1.25×10^7	5.52×10^6	3.17×10^7
D4	$HCrO_4^-$				HS-R(COOH)-NH_2	3.7×10^7	3.9×10^7				
E1	$HCrO_4^-$				HS-R(COOH)-NH_3^+	5.31×10^2	7.16×10^2	HS-R-COOH	1.33×10^3	17.2	1.09×10^2
E2	$HCrO_4^-$	HS-R-NH_3^+ + H^+	0	0	HS-R(COO^-)-NH_3^+ + H^+	4.84×10^4 [d]	1.19×10^5 [d]	HS-R-COO^- + H^+	6.22×10^6 [d]	3.76×10^5 [d]	1.77×10^5 [d]
E3	$HCrO_4^-$				^-S-R(COOH)-NH_3^+ + H^+	1.33×10^{11} [d]	1.33×10^{12} [d]	^-S-R-COOH + H^+	1.56×10^{13} [d]	2.43×10^{11} [d]	3.87×10^{11} [d]
F1	$HCrO_4^-$				HS-R(COOH)-NH_3^+ + H^+	0	0	HS-R-COOH + H^+	2.52×10^5 [d]	1.83×10^5 [d]	2.05×10^4 [d]

[a] Calculated by using 2.PROT.[22] [b] The error associated with these values is ≤15%. [c] Calculated by using 3.PROT.[22] [d] Third-order rate constant, M^2 min^{-1}.

the rate constant for thioglycolic acid was anomalously high when compared to the other thiols and was therefore excluded from the calculation of the slopes.

Discussion

We have shown that over a wide range of pH, the reaction of chromium(VI) with thiols to form chromium(VI) thio esters requires attack by thiols having a protonated sulfhydryl group on chromium(VI). Of the nine different species combinations considered for their possible involvement in the formation of the chromium(VI) thio ester in the reaction between chromium(VI) and the aminothiols, the six combinations represented by classes A, B, D, and E were eliminated on the basis of the observed k_1 vs. pH profile. The data are consistent with the three possible combinations in class C, i.e., reaction of CrO_4^{2-} with HS-R-NH_3^+ and reaction of $HCrO_4^-$ with $^-$S-R-NH_3^+ or HS-R-NH_2. On the basis of the k_1 vs. pH profile alone, we cannot distinguish among these three possibilities. However, best fits of the data for the reaction of chromium(VI) with both the aminothiocarboxylic acids and the thiocarboxylic acids (except thioglycolic acid) also required the inclusion of class C. In addition, these thiols (including thioglycolic acid) required contributions from classes D and E, which involve species with one and two more protonated groups, respectively.

Plots of log k_1 for thiol species (sulfhydryl group protonated, C1, D1, and E1) vs. thiol pK_a showed inverse linear relationships. The dissociative mechanism suggested by Haim[24] for substitution reactions of hydrogen chromate with nucleophiles under strongly acidic conditions predicts a positive correlation of log k_1 with pK_a; however, no such correlation of the rate constants for reaction of chromium(VI) with thiolate species (C2, C4, D3, and E3) with pK_a was observed. These results suggest that only species having the sulfhydryl group protonated attack chromate or hydrogen chromate at a significant rate. The large decrease in the rate constant for chromium(VI) thio ester formation with the pK_a of the thiol (slopes ~ -1) is consistent with RS-H bond breaking in the rate-determining step as postulated previously.[12] These results are also consistent with the rate-determining proton transfer which has been proposed in ligand substitution reactions of hydrogen chromate with hydrogen thiosulfate,[25] hydrogen thiocyanate,[26] hydrogen chromate,[27] dihydrogen phosphate,[28] and dihydrogen phosphite.[29] The rate of the proton-independent pathway for the reaction of hydrogen chromate with penicillamine, glutathione, cysteamine, and cysteine was also found to vary with the acidity of the thiol and was ~20–300 times smaller than the rate of the proton-dependent pathway.[6,7]

The reaction of the thiols containing both sulfhydryl and carboxylate groups protonated with hydrogen chromate was slower (10–100 times) than with chromate (E1 vs. D1). Attack by the sulfhydryl group on chromate is favored, since proton transfer from the sulfhydryl, R-SH, to the more basic sites of chromate, $^-$O-CrO_3^-, is expected to be faster than transfer to the weaker acceptor site of hydrogen chromate, $^-$O-CrO_3H. In all cases, protonation of the carboxylate resulted in a dramatic ($\sim 10^3$ times) increase in the rate of reaction of aminothiocarboxylic acids and thiocarboxylic acids with chromate (D1 *vs.* C1). This implies that proton transfer from the bound carboxylic acid group, –COOH, to –CrOH is more facile than transfer from the less acidic amino group. The rate constants calculated for the reaction of hydrogen chromate with thiols having deprotonated carboxylate groups (D2) showed no correlation with sulfhydryl or carboxylate pK_a's and were much smaller than those for the reaction of chromate with thiols having protonated carboxylate groups (D1); therefore, the

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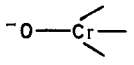
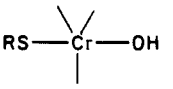
D2 pathway is considered unlikely.

The rates of reaction of chromate and hydrogen chromate with thioglycolic acid were anomalously high when compared to the other thiols examined. One possible explanation for the higher rate observed with thioglycolic acid is a chelation effect. Involvement of both the carboxyylate and thiol groups in the formation of the chromium(VI) thio ester would be favored for thioglycolic acid which could form a five-membered chelate ring with chromium. In contrast, chelation of chromium would not be favored with cysteine, *N*-acetylcysteine, and 3-mercaptopropionic acid which would form six-membered rings or homocysteine which would form a seven-membered ring. Another possible explanation is that with thioglycolic acid, the internal geometry of the transition state is the most favorable for proton transfer from the bound carboxylic acid, -COOH, to -CrOH.

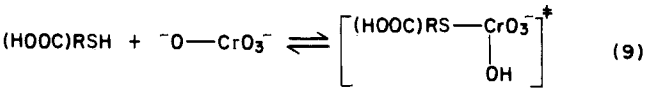
In conclusion, these studies have shown that the ligand substitution reactions of chromium(VI) with thiols which result in the formation of chromium(VI) thio esters involves attack by the protonated thiol on either chromate (eq 9) or hydrogen chromate with proton transfer as the rate-determining step. Protonation of chromate inhibits attack by RSH on



and formation of the five-coordinate transition state,



Loss of water and formation of the chromium(VI) thio ester at high pH (>3) is facilitated by proton transfer from a bound carboxylic acid group to -CrOH (eq 10). Under acidic conditions H_3O^+ facilitates water elimination. In the absence of a proton donor (H_3O^+, -COOH, $-NH_3^+$), the reaction is very slow since hydroxide is the leaving group. The following mechanism is proposed for these reactions:



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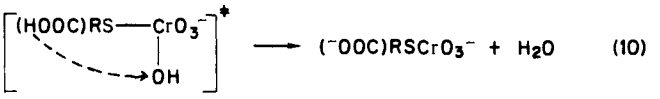

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Registry No. Cr(VI), 18540-29-9; glutathione, 70-18-8; L-cysteine ethyl ester, 3411-58-3; L-cysteine, 52-90-4; cysteamine, 60-23-1; coenzyme M, 45127-11-5; homocysteine, 6027-13-0; *N*-acetyl-L-cysteine, 616-91-1; coenzyme A, 85-61-0; 2-mercaptoethanol, 60-24-2; thioglycolate, 68-11-1.

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Biosynthesis of Estrogens: Aromatization of (19*R*)-, (19*S*)-, and (19*RS*)-[19-^{3}H,^{2}H,^{1}H]-3β-Hydroxyandrost-5-en-17-ones by Human Placental Aromatase†

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Abstract: It is shown, with the use of (19*R*)- and (19*S*)-[19-^{3}H,^{2}H,^{1}H]-3β-hydroxyandrost-5-en-17-ones, that the "first" C-19 hydroxylation by human placental aromatase involves a normal kinetic isotope effect $k_H > k_D > k_T$. The hydroxylation proceeds stereospecifically in the retention mode; i.e., the incoming hydroxyl assumes the orientation of the displaced (isotopic) hydrogen atom.

In the 1940s and 1950s, it was recognized that cholesterol[1] and particularly testosterone and other C_{19} steroids[2] can be metabolized to estrogens. However, the mechanism of the removal of the C-19 methyl group and the mode of aromatization of ring A were not understood. It was Meyer[3] who demonstrated that the first step of the aromatization process involved C-19 hydroxylation of an androgen substrate. Subsequently, it was shown that the extrusion of the 10β-methyl involves three oxidative steps, each of which requires 1 mol of O_2 and 1 mol of NADPH.[4] Whether the aromatization proceeds in discrete stages or is a continuous process occurring on the enzyme surface is not clear and is being debated.[5]

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