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Catalysis by zinc ion in the reactions of carcinogenic chromium(VI) with thiols

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Abstract

The reactions of Cr(VI) with nine biological and nonbiological thiols (RSH) in aqueous acetate buffer (pH 5.60) have been followed by an spectrophotometric method, and a relatively stable intermediate (the thioester $RSCrO_3^-$) has been observed. The rate constants corresponding to the formation (k_1), decomposition (k_{-1}), and redox transformation (k_2) of that intermediate have been obtained in both the absence and presence of zinc ion. This ion acts as a true catalyst for both the formation and decomposition of the observed intermediate. The equilibrium constants for the formation of $RSCrO_3^-$ from Cr(VI) and RSH for the different thiols have been determined. A mechanism is proposed for the formation of $RSCrO_3^-$ in the presence of catalyst according to which the rate-determining step would be the bimolecular reaction between a zinc–thiolate complex and an acetyl–chromate ester, involving an interchange of ligands.

Keywords: Chromium(VI); Chromium(VI)–thioesters; Homogeneous catalysis; Thiols; Zinc ion

1. Introduction

Chromium(VI) is a well-established, potent carcinogen [1,2], but it is believed that it can cause DNA lesions and mutations only after its intracellular reaction with appropriate biological compounds [3–5]. Since under physiological–pH conditions, it is present mainly as the relatively weak oxidant chromate ion, CrO_4^{2-} [6], it can only be metabolized to the mutagenically active, lower oxidation states [7,8] by the action of strong reductants such as L-ascorbate [9–16] or biological thiols [9,13,17–20]. In particular, the tripeptidic thiol glutathione is present in ordinary mammalian cells in a concentration high enough [21] to be thought to play an important role in the mechanism of chromium-induced carcinogenesis [22–29].

Zinc ion, a micronutrient present in most cells and necessary in aerobes for the formation of superoxide dismutase [30], as well as many other enzymes [31], has recently been reported to exert a promoting effect on the reducibility of chromium(VI) by the thiols DL-penicillamine [32] and glutathione [33]. This effect seems to be rather specific of zinc ion, because other divalent transition-metal ions (such as manganese(II)) show no activity on the chromium-(VI)–thiol reactions [33]. We have now studied the effect of zinc ion on the reactions of

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chromium(VI) with nine biological and nonbiological thiols, with a view to clarify one of the possible routes for the intracellular activation of chromium as carcinogen. The determination of three rate constants for each kinetic experiment (a rather unusual situation in chemical kinetics), corresponding to the formation, decomposition, and redox transformation of the relatively stable chromium(VI)–thioesters involved as intermediates in the chromium(VI)–thiol reactions has allowed us to conclude that the role of zinc ion in those reactions is that of acting as a true catalyst for the formation of the transient chromium(VI)–thioesters.

2. Experimental

2.1. Materials

Deionized water was subjected to distillation in an all-glass apparatus and circulated through a Millipore purification system before its use as solvent. Potassium dichromate, zinc sulfate, acetic acid (HAc), and sodium acetate (NaAc) were purchased from Merck. L-cysteine, glutathione, 2-mercaptoethanol, 3-mercaptopropionic acid, mercaptosuccinic acid, DL-penicillamine, thioglycolic acid, thioglycolic acid ethyl ester, and thiolactic acid were purchased from Sigma.

2.2. Kinetic experiments

Thiol was always used in large excess with respect to Cr(VI). The pH and ionic strength were kept constant during the kinetic runs with the aid of an HAc–NaAc buffer. The concentration of zinc ion was moved in a range where it did not appreciably alter the pH and ionic strength of the solutions. The pH values were determined by means of a Crison 2001 pH-meter provided with a combination microelectrode. The reactions were monitored simultaneously at both 370 and 430 nm (wavelengths corresponding to the reactant Cr(VI) and to the transient thioesters, respectively) by means of a diode array Hewlett-Packard 8451A spectrophotometer provided with a thermostated compartment for the standard 1-cm quartz cells. The electronic spectra were recorded with an Hitachi U 2000 UV–Vis spectrophotometer.

2.3. Determination of the rate constants

The Cr(VI)–thiol reactions can be written schematically as:

$$\mathrm{Cr(VI)} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{RSCrO_3^-} \overset{k_2}{\rightarrow} \text{Products} \qquad (1)$$

where $RSCrO_3^-$ is the relatively stable Cr(VI)–thioester intermediate (RSH standing for the corresponding thiol), and k_1, k_{-1}, and k_2 are the first-order rate constants (under a large excess of RSH with respect to Cr(VI)) associated, respectively, to the formation, decomposition, and redox transformation of that intermediate. The latter three processes may not be (and in fact they are not) elementary, but, provided that all the intermediates other than $RSCrO_3^-$ involved in the reactions are present in minute concentrations, the integrated rate equations for the reactant and the relatively stable intermediate are [33–35]:

$$[\mathrm{Cr(VI)}] = \frac{c_0}{\omega_2 - \omega_1}\left[(k_1 + \omega_2)\exp(\omega_1 t) - (k_1 + \omega_1)\exp(\omega_2 t)\right] \qquad (2)$$

$$[\mathrm{RSCrO_3^-}] = \frac{k_1 c_0}{\omega_2 - \omega_1}\left[\exp(\omega_2 t) - \exp(\omega_1 t)\right] \qquad (3)$$

where c_0 is the initial Cr(VI) concentration, and parameters ω_1 and ω_2 are combinations of the three first-order rate constants involved:

$$\omega_1 = -\frac{1}{2}\left\{k_1 + k_{-1} + k_2 + \left[(k_1 + k_{-1} + k_2)^2 - 4k_1k_2\right]^{1/2}\right\} \qquad (4)$$

$$\omega_2 = -\frac{1}{2}\left\{k_1 + k_{-1} + k_2 - \left[(k_1 + k_{-1} + k_2)^2 - 4k_1k_2\right]^{1/2}\right\} \qquad (5)$$

Thus, in each kinetic experiment we had to obtain four fitting parameters (c_0, k_1, k_{-1}, and k_2). Consequently, the experimental errors (both accidental and systematic) were much more pronounced than usual in chemical kinetics, since for most kinetic systems only one (for instance, for first-order reactions [36]) or two (for instance, for consecutive irreversible reactions [5,24] or for autocatalytic reactions [37]) rate constants have to be determined.

The values of [Cr(VI)] and [$RSCrO_3^-$] at time t were obtained from the absorbance readings at 370 and 430 nm, respectively, discounting at the latter wavelength the small contribution caused by the reactant. The data were then fitted by nonlinear least-squares regressions (using an iterative computer program) to the functions:

$$[\mathrm{Cr(VI)}] = A_1\exp(\omega_1 t) + A_2\exp(\omega_2 t) \quad (6)$$

$$[\mathrm{RSCrO_3^-}] = A_3[\exp(\omega_2 t) - \exp(\omega_1 t)] \quad (7)$$

Due to the experimental errors, the value obtained for ω_1 when Eq. (6) was used to fit the experimental data was different from the value obtained when Eq. (7) was used, and the same happened with ω_2, but the discrepancies were slight for most of the thiols.

To obtain the rate constants, one of the possibilities was to use the fitting parameters inferred from the absorbance data at 370 nm alone:

$$k_1 = -\frac{A_1\omega_1 + A_2\omega_2}{A_1 + A_2} \quad (8)$$

$$k_{-1} = -\frac{A_1A_2(\omega_1 - \omega_2)^2}{(A_1 + A_2)(A_1\omega_1 + A_2\omega_2)} \quad (9)$$

$$k_2 = -\frac{(A_1 + A_2)\omega_1\omega_2}{A_1\omega_1 + A_2\omega_2} \quad (10)$$

In addition, although the absorbance data at 430 nm could not be used alone to get the rate constants (because Eq. (7) involves only three fitting parameters, whereas Eq. (6) involves four) they could be used in combination with the absorbance data at 370 nm to get, once obtained the value of k_1 from Eq. (8), some alternative values for the other two rate constants:

$$k_{-1} = -\left(\omega_1 + \omega_2 + k_1 + \frac{\omega_1\omega_2}{k_1}\right) \quad (11)$$

$$k_2 = \frac{\omega_1\omega_2}{k_1} \quad (12)$$

Eqs. (8)–(12) can be deduced from the definitions of the exponential parameters ω_1 and ω_2 (Eqs. (4) and (5), respectively) on one hand and of the preexponential parameters A_1 and A_2 (compare Eq. (6) with Eq. (2)) on the other.

The method based on the combination of the absorbance data at the two wavelengths (Eqs. (8), (11) and (12) with the ω_1 and ω_2 values obtained from Eq. (7)) yielded better results only for three of the thiols studied (mercaptosuccinic acid, DL-penicillamine, and thiolactic acid), whereas for the other six thiols, the method based on the absorbance data at 370 nm alone (Eqs. (8)–(10) with the ω_1 and ω_2 values obtained from Eq. (6)) yielded better results. All the rate constants (and other parameters) given in this article are the averages from two inde-

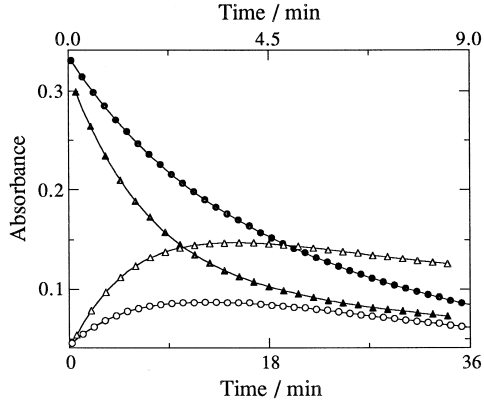


Fig. 1. Absorbance vs. time plots for the reaction of chromium(VI) (1.60×10^{-4} M) with 3-mercaptopropionic acid (5.00×10^{-3} M) in the presence of an HAc (2.00×10^{-2} M)–NaAc (0.200 M) buffer at pH 5.60 and 25.0°C. Wavelengths: 370 (filled points) and 430 (empty points) nm; $[Zn^{2+}] = 0$ (circles, bottom time-scale) and 4.00×10^{-4} M (triangles, top time-scale).

Table 1
Rate constants in the absence of zinc ion[a]

Thiol	k_1^o (10^{-3} s^{-1})	k_{-1}^o (10^{-3} s^{-1})	k_2^o (10^{-3} s^{-1})
L-Cysteine[b]	7.20 ± 0.43	4.10 ± 0.03	1.66 ± 0.01
Glutathione[b]	3.36 ± 0.17	1.48 ± 0.04	0.80 ± 0.09
2-Mercaptoethanol[b]	0.65 ± 0.02	–	2.33 ± 0.11
3-Mercaptopropionic acid[b]	0.94 ± 0.02	0.42 ± 0.03	0.99 ± 0.13
Mercaptosuccinic acid[c]	7.60 ± 0.28	9.17 ± 0.18	9.37 ± 0.09
DL-Penicillamine[c]	3.30 ± 0.08	1.13 ± 0.34	9.62 ± 0.15
Thioglycolic acid[b]	8.98 ± 0.23	3.10 ± 0.10	6.47 ± 0.32
Thioglycolic acid ethyl ester[b]	5.10 ± 0.23	2.18 ± 0.06	2.41 ± 0.11
Thiolactic acid[c]	7.42 ± 0.18	2.02 ± 0.08	13.55 ± 0.01

[a] $[Cr(VI)]_o = 1.60 \times 10^{-4}$ M, $[RSH] = 5.00 \times 10^{-3}$ M, $[HAc] = 2.00 \times 10^{-2}$ M, [NaAc] = 0.200 M, pH 5.60 ± 0.11, and 25.0°C.
[b] From absorbance readings at 370 nm.
[c] From absorbance readings at both 370 and 430 nm.

pendent experimental determinations, and for each thiol, the better set of results corresponding to one of the two methods outlined above was chosen.

3. Results

Typical absorbance vs. time plots at 370 and 430 nm for one of the thiols, in the absence and presence of zinc ion, are shown in Fig. 1. It can be seen that the initial rates (both for the reactant disappearance and the intermediate formation), as well as the maximum concentration of intermediate increased with rising $[Zn^{2+}]$. The fit of the experimental data to biexponential laws (Eqs. (6) and (7)) was excellent.

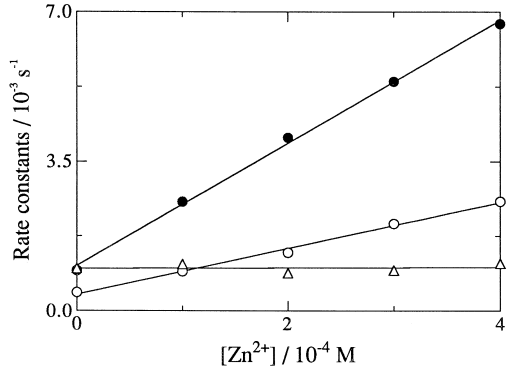


Fig. 2. Dependence of the first-order rate constants k_1 (filled circles), k_{-1} (empty circles), and k_2 (triangles) on the concentration of zinc ion for the reaction of chromium(VI) (1.60×10^{-4} M) with 3-mercaptopropionic acid (5.00×10^{-3} M) in the presence of an HAc (2.00×10^{-2} M)–NaAc (0.200 M) buffer at pH 5.60 and 25.0°C.

The values of the three rate constants (k_1, k_{-1}, and k_2) in the absence of zinc ion are given in Table 1. Both rate constants k_1 and k_{-1} increased linearly with rising $[Zn^{2+}]$, whereas k_2 was almost independent of it (Fig. 2). The slopes of those plots ($k_{1,c}$, $k_{-1,c}$, and $k_{2,c}$) corresponding to each thiol are given in Table 2. The maximum percentages of the total chromium present in the form of the relatively stable intermediate $RSCrO_3^-$ at $[Zn^{2+}] = 0$ and 4.00×10^{-4} M were calculated from the equation:

$$\left[RSCrO_3^-\right]_{max} = \frac{k_1 c_0}{\omega_2 - \omega_1}\left[\left(\frac{\omega_2}{\omega_1}\right)^{\frac{\omega_2}{\omega_1-\omega_2}} - \left(\frac{\omega_2}{\omega_1}\right)^{\frac{\omega_1}{\omega_1-\omega_2}}\right] \quad (13)$$

and they are given in Table 3. For all the thiols studied, the intermediate was more stable in the presence of zinc ion than in its absence.

4. Discussion

The Cr(VI)–RSH reactions do not follow a simple stoichiometry when the initial concentra-

Table 2
Catalytic rate constants[a]

Thiol	$k_{1,c}$ (M^{-1} s^{-1})	$k_{-1,c}$ (M^{-1} s^{-1})	$k_{2,c}$ (M^{-1} s^{-1})
L-Cysteine[b]	14.4 ± 2.9	10.9 ± 1.7	-0.6 ± 0.3
Glutathione[b]	9.6 ± 1.0	5.3 ± 0.4	-0.2 ± 0.2
2-Mercaptoethanol[b]	4.5 ± 0.5	1.9 ± 0.1	-1.7 ± 2.3
3-Mercaptopropionic acid[b]	14.4 ± 0.4	5.4 ± 0.2	0.1 ± 0.3
Mercaptosuccinic acid[c]	46.9 ± 2.1	47.6 ± 3.2	10.2 ± 2.2
DL-Penicillamine[c]	8.9 ± 0.4	6.3 ± 1.3	-0.2 ± 0.8
Thioglycolic acid[b]	94.0 ± 10.3	66.0 ± 3.1	18.9 ± 2.1
Thioglycolic acid ethyl ester[b]	15.0 ± 0.4	7.9 ± 0.6	-0.5 ± 0.2
Thiolactic acid[c]	68.1 ± 1.8	33.8 ± 4.8	3.9 ± 1.3

[a] $[Cr(VI)]_o = 1.60 \times 10^{-4}$ M, $[RSH] = 5.00 \times 10^{-3}$ M, $[Zn^{2+}] = (0–4.00) \times 10^{-4}$ M, $[HAc] = 2.00 \times 10^{-2}$ M, $[NaAc] = 0.200$ M, pH 5.60 ± 0.11, and 25.0°C.
[b] From absorbance readings at 370 nm.
[c] From absorbance readings at both 370 and 430 nm.

tions of oxidant and reductant are similar, but, under a large excess of the latter, the following stoichiometry is accepted [5,38]:

$$2HCrO_4^- + 6RSH + 8H^+ = 2Cr^{3+} + 3RSSR + 8H_2O \quad (14)$$

$RSCrO_3^-$ being involved in the reactions as a relatively stable intermediate [4,39]. The results given in Table 3 indicate that the presence in the medium of zinc ion notably increases the stability of the observed intermediate. However, this effect is consistent with two possible interpretations: (i) Zinc ion is a catalyst for the formation of that intermediate. (ii) Zinc ion is an actual constituent of the intermediate. In the first case, the chemical composition of the observed intermediate would be the same ($RSCrO_3^-$), irrespective of whether zinc ion is absent or present in the medium, whereas in the second case when zinc ion is present, another intermediate (a complex of possible formula $[Zn(RSCrO_3)]^+$) would also be formed.

Table 3
Maximum percentages of the chromium(VI)–thioester intermediates in both the absence and presence of catalyst[a]

Thiol	$(\%)^b_{i,max}$	$(\%)^c_{i,max}$
L-Cysteine[d]	47.9 ± 1.2	51.7 ± 0.3
Glutathione[d]	50.8 ± 0.4	56.6 ± 0.2
2-Mercaptoethanol[d]	17.8 ± 1.8	36.1 ± 3.7
3-Mercaptopropionic acid[d]	31.4 ± 2.6	56.2 ± 1.6
Mercaptosuccinic acid[e]	24.1 ± 0.4	32.6 ± 2.5
DL-Penicillamine[e]	18.5 ± 0.2	26.9 ± 0.3
Thioglycolic acid[d]	37.8 ± 1.4	44.5 ± 0.5
Thioglycolic acid ethyl ester[d]	42.6 ± 0.1	51.1 ± 0.1
Thiolactic acid[e]	24.8 ± 0.4	43.7 ± 2.9

[a] $[Cr(VI)]_o = 1.60 \times 10^{-4}$ M, $[RSH] = 5.00 \times 10^{-3}$ M, $[HAc] = 2.00 \times 10^{-2}$ M, $[NaAc] = 0.200$ M, pH 5.60 ± 0.11, and 25.0°C.
[b] Obtained as: $(\%)_{i,max} = 100\ [RSCrO_3^-]_{max} / c_o$ in the absence of zinc ion.
[c] Obtained as: $(\%)_{i,max} = 100\ [RSCrO_3^-]_{max} / c_o$ at $[Zn^{2+}] = 4.00 \times 10^{-4}$ M.
[d] From absorbance readings at 370 nm.
[e] From absorbance readings at both 370 and 430 nm.

The key to confirm if zinc ion is a true catalyst or not may be given by the k_2 vs. $[Zn^{2+}]$ plots, since the susceptibility of the proposed intermediate $[Zn(RSCrO_3)]^+$ toward a reductive attack by an RSH molecule to form Cr(IV) and RSSR [6] would be expected to be very different from that of $RSCrO_3^-$, so that, if zinc ion is a constituent of the intermediate, the rate constant k_2 should necessarily be much affected by the concentration of that ion. From the $k_{2,c}$ values (the slopes of the k_2 vs. $[Zn^{2+}]$ plots) given in Table 2, it can be concluded that for six of the thiols studied, k_2 was nearly independent of $[Zn^{2+}]$, and that only for three thiols (mercaptosuccinic acid, thioglycolic acid, and thiolactic acid) a clear-cut positive value of $k_{2,c}$ was found. However, even in the latter three cases, the value of $k_{2,c}$ was much smaller than those of both $k_{1,c}$ and $k_{-1,c}$, suggesting

that the positive values of $k_{2,c}$ might be the consequence of systematic experimental errors. In fact, for those three thiols, the Cr(VI)–RSH reactions were considerably faster than for the other six, rendering more difficult the determination of accurate values for the three rate constants from each kinetic experiment.

On the other hand, the electronic spectra of two reaction mixtures, differing only in the absence or presence of zinc ion, were periodically scanned as the reaction advanced. In both cases an isosbestic point situated at the same wavelength (390 nm) was detected during the formation of the observed intermediate. Moreover, the spectra of the intermediate obtained in the absence and in the presence of zinc ion (recorded at the instant when the intermediate concentration reached its maximum value) were practically identical (Fig. 3), showing in both cases a peak at 430 nm, and differing only in the value of the absorbance at that peak as a consequence of $[RSCrO_3^-]_{max}$ being different for the two reaction mixtures.

Thus, from both the kinetic data and the spectral properties it can be inferred that zinc ion is not a constituent of the observed intermediate, and that its role in the Cr(VI)–RSH reactions is that of being a catalyst for the formation of the intermediate, according to the equation:

$$HCrO_4^- + RSH = RSCrO_3^- + H_2O \qquad (15)$$

Since that process is indeed reversible [33], the value of the equilibrium constant for each thiol could be obtained from the corresponding rate constants k_1^o and k_{-1}^o (the values of k_1 and k_{-1}, respectively, in the absence of zinc ion) as:

$$K = \frac{k_1^o}{k_{-1}^o[RSH]} \qquad (16)$$

where it has been taken into consideration that k_1 is actually a pseudo- first-order rate constant directly proportional to the thiol concentration, whereas k_{-1} is independent of it [33]. However, as required by the principle of microscopic reversibility, if a certain species is a catalyst for the forward process of a reversible reaction, it must also be a catalyst for the backward process [40]. This has actually been confirmed by the experiments, given that both rate constants k_1 and k_{-1} increased when $[Zn^{2+}]$ was raised (see Fig. 2 and Table 2). Hence, the equilibrium constant may also be calculated as:

$$K = \frac{k_{1,c}}{k_{-1,c}[RSH]} \qquad (17)$$

since the value of K cannot be affected by the absence (Eq. (16)) or presence (Eq. (17)) of catalyst. The equilibrium constants for the different thiols determined by both methods appear in Table 4. We can see that, for each thiol, the two K values are of the same order of magnitude, although the experimental errors provoked a certain deviation in some cases. Moreover, the K values for the different thiols are rather similar, indicating that the effect (increasing or decreasing) of a particular substituent on the value of the rate constant k_1^o is roughly compensated by a parallel effect on the value of k_{-1}^o. The lowest equilibrium constant obtained was that

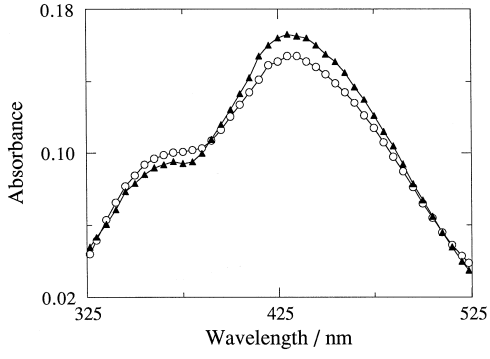


Fig. 3. Detail of the UV–Vis spectra recorded at the instant when the observed intermediate reached its maximum concentration for the reaction mixtures corresponding to the reduction of chromium(VI) (1.60×10^{-4} M) by L-cysteine (5.00×10^{-3} M) in the presence of an HAc (2.00×10^{-2} M)–NaAc (0.200 M) buffer at pH 5.60 and 25.0°C. $[Zn^{2+}]=0$ (circles) and 4.00×10^{-4} M (triangles). The small band at 370 nm corresponds to unreacted free Cr(VI) and the peak at 430 nm corresponds to the observed intermediate.

Table 4
Equilibrium constants for the formation of the Cr(VI)–thioester intermediates[a]

Thiol	K^b (10^2 M^{-1})	K^c (10^2 M^{-1})
L-Cysteine[d]	3.5 ± 0.2	2.7 ± 0.9
Glutathione[d]	4.5 ± 0.3	3.7 ± 0.6
2-Mercaptoethanol[d]	–	4.9 ± 0.8
3-Mercaptopropionic acid[d]	4.5 ± 0.4	5.3 ± 0.3
Mercaptosuccinic acid[e]	1.7 ± 0.1	2.0 ± 0.2
DL-Penicillamine[e]	5.8 ± 1.9	2.8 ± 0.7
Thioglycolic acid[d]	5.8 ± 0.3	2.9 ± 0.5
Thioglycolic acid ethyl ester[d]	4.7 ± 0.3	3.8 ± 0.4
Thiolactic acid[e]	7.3 ± 0.5	4.0 ± 0.7

[a] $[Cr(VI)]_o = 1.60 \times 10^{-4}$ M, $[RSH] = 5.00 \times 10^{-3}$ M, $[Zn^{2+}] = (0–4.00) \times 10^{-4}$ M, $[HAc] = 2.00 \times 10^{-2}$ M, $[NaAc] = 0.200$ M, pH 5.60 ± 0.11, and 25.0°C.
[b] Obtained from Eq. (16).
[c] Obtained from Eq. (17).
[d] From absorbance readings at 370 nm.
[e] From absorbance readings at both 370 and 430 nm.

corresponding to mercaptosuccinic acid, and it is indeed related to the fact that, for this thiol, the value of k^o_{-1} was notably high as compared with those of the other thiols (see Table 1). This might somehow be a consequence of the two carboxyl groups contained in the molecule of this particular thiol.

It seems that acetate ions play an important role in the mechanism corresponding to the reaction pathway catalyzed by zinc ion, because the catalytic effect of the latter on the Cr(VI)–thiol reactions was not observed when either phosphate or citrate buffers were used [32,33]. The experimental data available are consistent with several mechanisms. Basically, there are five alternatives for the rate-determining step (rds): (i) the rds is a reaction between a Zn^{2+}(acetate)(thiolate) complex and Cr(VI); (ii) the rds is a reaction between a Zn^{2+}(acetate)(chromate) complex and RSH; (iii) the rds is a reaction between a Zn^{2+}–thiolate and a Cr(VI)–acetate complexes; (iv) the rds is a reaction between a Zn^{2+}–chromate and an RSH–acetate complexes; and (v) the rds is the decomposition of a Zn^{2+}(acetate)(chromate)(thiolate) combined complex.

Since zinc ion is known to readily accept as ligands both carboxylate and thiolate groups (its participation in many enzymes bonded to amino acid residues containing or not a sulfhydryl group is an important consequence of it [31]), whereas chromium(VI) is known to combine with hydroxyl-containing compounds to form some ester-like condensation species [41] and, in particular, with acetic acid to form an acetyl chromate ion [42], the alternatives (i) and (iii) are more plausible than the others. Moreover, we think that the alternative (iii) might be the correct one, because it seems to be more consistent with the crucial role that acetate ions play in the catalytic reaction pathway. We thus propose for the latter the mechanism:

$$Zn^{2+} + RSH \overset{K_I}{\rightleftarrows} [Zn(RS)]^+ + H^+ \quad (18)$$

$$HCrO_4^- + CH_3CO_2H \overset{K_{II}}{\rightleftarrows} CH_3C(O)OCrO_3^- + H_2O \quad (19)$$

$$[Zn(RS)]^+ + CH_3C(O)OCrO_3^- \underset{k_{-III}}{\overset{k_{III}}{\rightleftarrows}} RSCrO_3^- + [Zn(CH_3CO_2)]^+ \quad (20)$$

$$[Zn(CH_3CO_2)]^+ + H^+ \overset{K_{IV}}{\rightleftarrows} Zn^{2+} + CH_3CO_2H \quad (21)$$

according to which, provided that the interchange of ligands between the zinc–thiolate complex and the acetyl–chromate ester (Eq. (20)) is assumed to be the rds, the following expression can be obtained for the first-order rate constant associated to the formation of the observed intermediate ($RSCrO_3^-$):

$$k_1 = k_1^o + k_{1,c}[Zn^{2+}] \quad (22)$$

with:

$$k_{1,c} = K_I K_{II} k_{III} \frac{[RSH][CH_3CO_2H]}{[H^+]} \quad (23)$$

In addition, according to the principle of microscopic reversibility, the expression corresponding to the first-order rate constant associated to the decomposition of the observed intermediate is:

$$k_{-1} = k^{o}_{-1} + k_{-1,c}[Zn^{2+}] \quad (24)$$

with:

$$k_{-1,c} = \frac{k_{-III}[CH_3CO_2H]}{K_{IV}[H^+]} \quad (25)$$

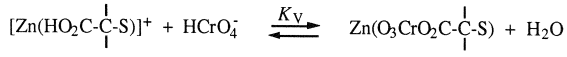
Taking into consideration that the equilibrium constant associated to the rds can be expressed as $K_{III} = k_{III}/k_{-III}$, it follows from Eqs. (17), (23) and (25) that the equilibrium constant for the formation of the observed intermediate is:

$$K = K_I K_{II} K_{III} K_{IV} \quad (26)$$

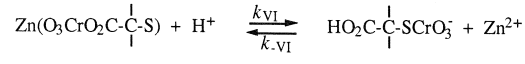
what is indeed coherent with the fact that the stoichiometry corresponding to that formation (Eq. (15)) may be obtained from the addition of the stoichiometries corresponding to Eqs. (18)–(21).

Although the experimental errors associated to the determination of three rate constants from each kinetic experiment are indeed higher than usual, some interesting conclusions may be deduced from the kinetic data given in Tables 1 and 2. The most important inference is that the existence in the RSH molecule of a carboxyl group bonded to the same carbon atom (α position) as the sulfhydryl group (mercaptosuccinic acid, thioglycolic acid, and thiolactic acid) results in the values of the rate constants k^{o}_{1}, $k_{1,c}$, and $k_{-1,c}$ being very high when compared with those corresponding to the other thiols, whereas the rate constants are rather low when the carboxyl and sulfhydryl groups are bonded to different carbon atoms (3-mercaptopropionic acid). This effect is consistent with the results reported by Connett and Wetterhahn [43] for the k^{o}_{1} values corresponding to several thiols, and it does not seem to be caused merely by a change in the electron density of the active functional group (sulfhydryl), since the replacement of the $-CO_2H$ group in the α position by a $-CO_2Et$ group results in a decrease of the rate constants (compare the values corresponding to thioglycolic acid with those for thioglycolic acid ethyl ester), and the decrease is much more marked for both $k_{1,c}$ and $k_{-1,c}$ than for k^{o}_{1}. This suggests that the availability of an acidic hydrogen atom in the surroundings of the active functional group might favour both the formation and the decomposition of the observed intermediate, especially as far as the zinc-catalyzed reaction pathway is concerned.

We thus propose for the thiols containing a carboxyl group in the α position the following alternative mechanism:



(27)



(28)

according to which, previous to the rds (Eq. (28)), hydrogenchromate ion suffers an ester-like combination with the carboxyl group of the thiol (Eq. (27)) instead of the acidic form of the buffer (Eq. (19)). This seems to be consistent with the catalytic effect that the buffer (either acetate, citrate, or phosphate) is known to exert on both the formation and decomposition of the $RSCrO_3^-$ intermediates [32,33], with the advantage that the esterification results now in bringing Cr(VI) into the same molecule containing the sulphur atom, thus making easier the nucleophilic attack of the latter on the chromium atom.

The overall mechanism proposed for these thiols would be a combination of Eqs. (18)–(21) on one hand and of Eqs. (27) and (28) on the other, leading to the following expression for the second-order catalytic rate constants associ-

ated, respectively, with the formation and decomposition of the observed intermediate:

$$k_{1,c} = K_I\left(K_{II}k_{III}\frac{[CH_3CO_2H]}{[H^+]} + K_V k_{VI}\right)[RSH] \quad (29)$$

$$k_{-1,c} = \frac{k_{-III}[CH_3CO_2H]}{K_{IV}[H^+]} + k_{-VI} \quad (30)$$

The additional terms in Eqs. (29) and (30) with respect to Eqs. (23) and (25) would easily explain the high values of both $k_{1,c}$ and $k_{-1,c}$ obtained for mercaptosuccinic acid, thioglycolic acid and thiolactic acid.

Although 2-mercaptoethanol and 3-mercaptopropionic acid have very similar values of k_1^o (Table 1), the values of both $k_{1,c}$ and $k_{-1,c}$ for the latter are much higher than those for the former. Thus, the existence of a carboxyl group in the β position of the RSH molecule seems to be much more favourable for both the formation and decomposition of the observed intermediate through the zinc-catalyzed reaction pathway than the existence of a hydroxyl group in the same position. This suggests that, in the case of 3-mercaptopropionic acid, the RSH molecule might behave as a bidentate ligand with both the thiolate and carboxylate groups bonded to the zinc ion.

The existence of an $-NH_3^+$ group in the β position of the RSH molecule (as in L-cysteine) results in a very strong decrease of both rate constants k_1^o and k_{-1}^o (Table 1) with respect to the values obtained for the parent molecule with a hydrogen atom in the same position (3-mercaptopropionic acid), but the effect of that substituent is much weaker in the case of the catalytic rate constants $k_{1,c}$ and $k_{-1,c}$ (Table 2). This seems to suggest that both the formation and decomposition of the observed intermediate in the absence of zinc ion are disfavoured by electron-withdrawing substituents, what is indeed coherent with the fact that the formation of $RSCrO_3^-$ requires the nucleophilic attack of the sulphur atom of the thiol on the chromium atom, so that a high electron density on the sulphur atom is favourable.

The introduction of two methyl groups into the α position of the RSH molecule (as in DL-penicillamine) results in a strong decrease of the rate constants k_1^o, k_{-1}^o, $k_{1,c}$, and $k_{-1,c}$ (Tables 1 and 2) with respect to the values obtained for the parent molecule with two hydrogen atoms in the same position (L-cysteine). This might be explained by the steric hindrance provoked by the two methyl groups on the solvation of the activated complex associated with the rds corresponding to the noncatalytic and zinc-catalyzed reaction pathways, rather than by electron-density considerations.

However, as far as the redox transformation of the observed intermediate is concerned, the effect of introducing two methyl groups in the α position of the RSH molecule is just the opposite (the value of k_2^o given in Table 1 for DL-penicillamine is much higher than that of L-cysteine). It is known that the chromium atom of the Cr(VI)–thioester intermediate may suffer either a one-electron reduction in an internal redox process:

$$RSCrO_3^- \rightarrow Cr(V) + RS\cdot \quad (31)$$

or else a two-electron reduction by reaction with a second thiol molecule:

$$RSCrO_3^- + RSH \rightarrow Cr(IV) + RSSR \quad (32)$$

Although the two-electron route is clearly predominant under neutral-pH conditions for thiols such as glutathione [6], the high value of k_2^o for DL-penicillamine might indicate that for this particular thiol, the one-electron route has a contribution more important than those corresponding to other similar thiols such as L-cysteine or glutathione. The high values of k_2^o for the three thiols with a carboxyl group in the α position (mercaptosuccinic acid, thioglycolic acid, and thiolactic acid) might indicate that the availability of an acidic hydrogen atom near the metal atom of the thioester favors its reducibil-

ity, probably by transfer of the proton from the carboxyl group to one of the oxygen atoms bonded to the chromium atom of the thioester.

Finally, it should be mentioned that, although the kinetic data obtained in this work do not provide any information on the redox steps leading to the formation of the inorganic reaction product (Cr^{3+}), this would take place by the reductive attack of the thiol on the intermediates Cr(V) and Cr(IV) (formed in Eqs. (31) and (32), respectively), involving probably a complexation step previous to the redox reaction.

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