Kinetics and Mechanism of the Reactions of Superoxochromium(III) Ion with Biological Thiols

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The kinetics of the oxidation of three biological thiols (L-cysteine, glutathione, and DL-penicillamine) to their sulfinic and sulfonic acid derivatives by $CrOO^{2+}$ in aqueous perchloric acid and in the presence of 2-propanol have been studied spectrophotometrically with the aid of the initial-rates method. The kinetic order of the oxidant is 2, whereas that of the reductant is not defined. The acidity of the medium has a slight effect on the initial rates (acid catalysis for both L-cysteine and DL-penicillamine and base catalysis for glutathione). An increase of the ionic strength leads to a rise of the initial rate for both L-cysteine and DL-penicillamine, whereas the initial rate for glutathione is insensitive to the ionic strength. The reactions are inhibited by both 2-propanol and dissolved O_2 and catalyzed by Mn^{2+}, whereas Ce^{3+} has almost no effect on them. At low 2-propanol concentration and in the absence of Mn^{2+} the initial rate vs temperature plots have a minimum at around 20 °C, whereas in the presence of either concentrated 2-propanol or Mn^{2+} the Arrhenius law is fulfilled. A single mechanism is proposed for the three reactions involving a $CrOO^{2+}$/thiol complex, $CrOOH^{2+}$, and CrO^{2+} as intermediates. The bimolecular rate constants for the reactions of the intermediate CrO^{2+} with L-cysteine and DL-penicillamine at 25.0 °C have been obtained (around 10^3 M^{-1} s^{-1} in both cases). Some kinetic data for the decomposition of $CrOO^{2+}$ in the absence of thiol are also given.

Introduction

Chromium(VI) is a strong carcinogen,[1,2] but since it cannot react directly with DNA at physiological pH,[3] it is believed that one or several of the metabolites (either reactive intermediates or the final product Cr(III)) generated from its intracellular reduction must be the actual mutagenic agents.[4] Hence, in an attempt to clarify the mechanism of its carcinogenic action, the reactions of Cr(VI) with different biological reductants (such as L-ascorbic acid,[5,6] hydrogen peroxide,[7,8] or sulfhydryl-containing biomolecules[9,10]) under near-physiological pH conditions have received considerable attention lately, and in many studies, the search for reactive intermediates has been emphasized. In particular, the use of difference UV spectroscopy has allowed the detection of superoxochromium(III) ion ($CrOO^{2+}$) as one of the intermediates involved in the reactions of Cr(VI) with biological thiols.[11] Since $CrOO^{2+}$ is believed to yield free superoxide ion (O_2^-, a species generally considered as extremely dangerous for living organisms[12,13]) by decomposition under neutral-pH conditions,[14] the possibility that $CrOO^{2+}$ might associate with other species (such as Cr(V)[15,16] and Cr(III)[15,17]) in the mechanism involved in the development of the toxic and carcinogenic effects of Cr(VI) should be considered.

In this article, we present the results found in a kinetic study of the reactions of $CrOO^{2+}$ with three biological thiols (L-cysteine, glutathione, and DL-penicillamine, henceforth designated generically as RSH) in aqueous perchloric acid. Although the acidic conditons of our work are not directly relevant to biological studies (except perhaps in the cases of stomach cells or tissues under acute-inflammation conditions[18]), the species $CrOO^{2+}$ seems interesting enough by itself to justify an investigation of its oxidizing properties. The study of the reactivity of that species toward thiols was especially appealing to us in view of the fact that, although $CrOO^{2+}$ is involved as an intermediate in the mechanism of the reactions of Cr(VI) with most organic reductants under aerobic, acidic conditions,[19] much more is currently known on its reactions with inorganic reductants[20−23] than with their organic counterparts.[24]

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Experimental Section

Materials and Methods. The water used as solvent was previously subjected to deionization followed by distillation and circulation through a Millipore system. The chemical reagents were of analytical grade, and they were used as acquired from different sources (Aldrich, Fluka, Merck, and Sigma). The kinetic runs were followed at 246 nm by means of a Hitachi U 2000 UV−vis spectrophotometer provided with a thermostated compartment where the 1 cm path length quartz cells were lodged. Usually, the solution was vigorously stirred to reach air-saturation conditions. In the experiments where the concentration of oxygen had to be decreased or increased, O_2- and Ar-saturated aqueous solutions (from commercial gas bottles and previously washed) were mixed in the desired proportion. The concentration of dissolved O_2 at the beginning of each kinetic experiment was calculated from the known solubilities of this species under O_2 and air atmospheres.[19,25]

Synthesis of Superoxochromium(III) Ion. This species may be prepared either from Cr(III) or from Cr(VI) as starting material. In the first method, Cr^{3+} is reduced to Cr^{2+} by a Zn/Hg amalgam under anaerobic conditions and then injected into an O_2-saturated acidic aqueous solution.[26] In the second method,

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Cr(VI) is reduced by a suitable two-electron organic reductant (we chose 2-propanol, 2-PrOH) in an acidic aqueous solution containing O_2. Scott et al.[19] have reported the formation of $CrOO^{2+}$ as a relatively stable intermediate in the reduction of $HCrO_4^-$ under those conditions. Although higher yields of $CrOO^{2+}$ can be obtained by the first method, it is usually contaminated by a small amount of Cr(IV) (in the form of CrO^{2+}), which shows a very high reactivity toward thiols. Hence, we preferred the second method, since the contaminants present in this case (excess 2-propanol, acetone, and inert Cr(III) complexes) show no reactivity toward either $CrOO^{2+}$ or thiols. Moreover, this method can be more easily implemented than the amalgam method, so that a fresh $CrOO^{2+}$ solution may be prepared right before each kinetic run, thus avoiding the problems related with the aging of the Cr(II) solution used in the first method.

Previous to each kinetic experiment, 20 mL of an air-saturated aqueous solution 1.02×10^{-4} M in Cr(VI) (from potassium dichromate), 0.261 M in 2-propanol, and 1.83 M in $HClO_4$ were left to react in a thermostated volumetric flask, and an aliquot of it was introduced into the spectrophotometer. When the absorbance at 246 nm indicated that the concentration of $CrOO^{2+}$ was at its peak and no Cr(VI) could be detected at 352 nm, the reaction with the thiol was started. To that end, in a typical experiment, 15 mL of the so-prepared superoxide solution was mixed with 10 mL of another thermostated aqueous solution containing the desired concentration of thiol (and, when necessary, other substances); after stirring, an aliquot was introduced into the spectrophotometer to follow the kinetics of the reaction. The method used to prepare the superoxide proved to be both ready and reproducible; when prepared at 25.0 °C, the time necessary to obtain the $CrOO^{2+}$ from Cr(VI) was 6.5 min, and the yield (estimated from the absorbance readings using the value $\epsilon_{245} = 7400$ M^{-1} cm^{-1} for the superoxide[27]) was 28 ± 1%.

Analysis of Products. The organic products of the reactions between $CrOO^{2+}$ and two different reductants (L-cysteine and L-cysteinesulfinic acid) were identified by HPLC. Commercial samples of L-cysteine, L-cysteinesulfinic acid, L-cysteic acid, and L-cystine were used as references. All the samples were subjected to precolumn derivatization of the amino groups with trinitrobenzenesulfonic acid (TNBS).[28−30] The derivatives were separated on a C_{18} reversed-phase column, using as eluent an acetonitrile−aqueous phosphate (10:90, v/v) mixture, the aqueous part consisting of a solution 0.01 M in $NH_4H_2PO_4$ whose pH was previously adjusted to 3.5 with a few drops of 4 M H_3PO_4. The eluent was pumped at a flow rate of 1.0 mL min^{-1}. The HPLC equipment was constituted by a Kontron 414 pump, a Kontron 465 autosampler, a Merck-Hitachi D-2500 integrator, and an Applied Biosystems 1000 S diode-array detector. The column effluent was monitored at two wavelengths (335 and 430 nm). The identification of the TNBS derivatives of the reaction products was based on a comparison of both their corresponding retention times and UV−vis spectra with those obtained for the commercial compounds used as references.

Kinetic Experiments. The absorbance at 246 nm decreased continuously as time elapsed for the decomposition of $CrOO^{2+}$ in the absence of thiol, as well as for its reactions with L-cysteine and DL-penicillamine. However, in the case of the reaction with glutathione the absorbance first decreased, passed through a minimum, and then started to increase slowly. Because of this, the use of an integrated method in the latter case was judged unreliable. Moreover, in some of the experiments the thiol was not in large excess with respect to $CrOO^{2+}$. Therefore, the kinetic data reported in this work were obtained by application of the initial-rates method, and they are the averages of two independent, duplicated determinations. A total number of around 1500 kinetic experiments was performed, and the typical standard deviation from two duplicated initial rates was ±3%.

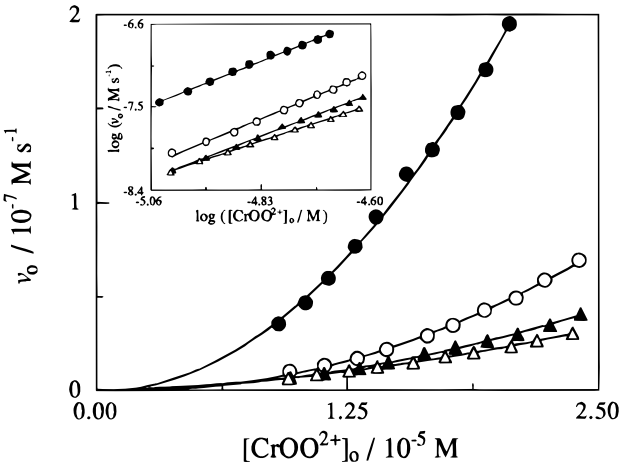

Figure 1. Dependence of the initial rate on the initial concentration of $CrOO^{2+}$ for its decomposition in the absence of thiol (empty triangles, at $[HClO_4] = 1.61$ M, [2-PrOH] = 0.231 M, and $[Mn^{2+}] = 1.30 \times 10^{-3}$ M) and for its reactions with the thiols (at $[RSH]_o = 9.51 \times 10^{-4}$ M, $[HClO_4] = 1.71$ M, [2-PrOH] = 0.245 M, and $[Mn^{2+}] = 0$): L-cysteine (empty circles), glutathione (filled circles), and DL-penicillamine (filled triangles) in the presence of oxygen (2.60×10^{-4} M) at 25.0 °C. Inset: double-logarithmic plots for the four reactions; slopes: 1.71 ± 0.01 (decomposition), 2.16 ± 0.04 (L-cysteine), 2.08 ± 0.04 (glutathione), and 1.99 ± 0.03 (DL-penicillamine).

Results

Reaction Products. The products found from the oxidation of L-cysteine (RSH) by $CrOO^{2+}$ in acidic media were L-cysteinesulfinic acid (RSO_2H) and L-cysteic acid (RSO_3H), but no L-cystine (RSSR) could be detected under the conditions of our experiment. This contrasts with the situation found for other similar reactions, since thiols are oxidized by most oxidants (among them, some closely related to $CrOO^{2+}$, such as Cr(VI),[31] a diperoxochromium(IV) complex,[32] O_2^-,[33] and a superoxocobalt(III) complex[34]) to the corresponding disulfides. However, the case of hydrogen peroxide is special: although under ordinary conditions the RSH compounds are oxidized by H_2O_2 to RSSR,[35] when the RSH molecule is bound to cobalt(III) as a ligand, the oxidation by H_2O_2 leads to RSO_2H,[36−38] whereas when HCO_2H is also present in the medium, the oxidation of RSH by H_2O_2 leads to RSO_3H.[39,40] On the other hand, we have found that under acidic conditions $CrOO^{2+}$ oxidizes L-cysteinesulfinic acid to L-cysteic acid, which is consistent with the fact that RSO_3H is a much more stable oxidation level of the sulfur atom than RSO_2H.[36]

Kinetic Data. Given the instability of $CrOO^{2+}$, it decomposes even if no reductant is present in the medium.[41] Consequently, besides the three $CrOO^{2+}$−RSH reactions, we have also studied the kinetics of the slow decomposition of $CrOO^{2+}$ in the absence of thiol.

The initial rates (v_o) of the four reactions led to upward-concave plots when represented against the initial concentration of oxidant (Figure 1), and the slopes of the log v_o vs log $[CrOO^{2+}]_o$ plots (Figure 1, inset) all were near 2, although for the decomposition reaction the apparent kinetic order found for

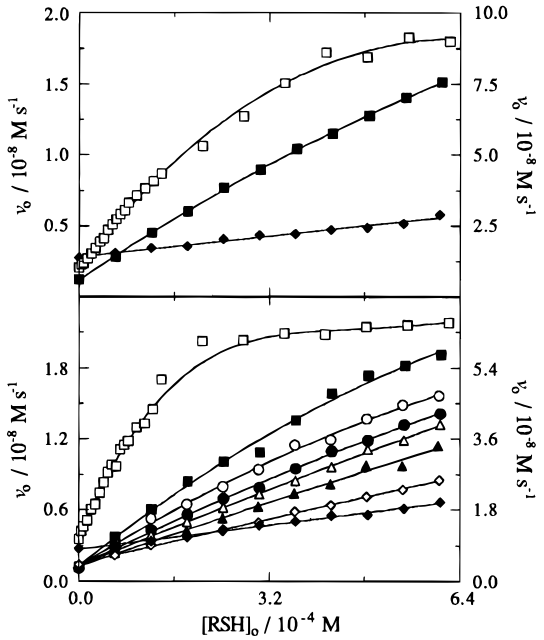

Figure 2. Dependence of the initial rate on the initial concentration of reductant for the reactions of $CrOO^{2+}$ (1.71×10^{-5} M) with DL-penicillamine (up) and L-cysteine (down) at $[HClO_4] = 1.10$ M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C: (empty squares) [2-PrOH] = 0.157 M and $[Mn^{2+}] = 8.86 \times 10^{-4}$ M; (filled squares) [2-PrOH] = 0.157 M and $[Mn^{2+}] = 0$; (empty circles) [2-PrOH] = 0.209 M and $[Mn^{2+}] = 0$; (filled circles) [2-PrOH] = 0.261 M and $[Mn^{2+}] = 0$; (empty triangles) [2-PrOH] = 0.314 M and $[Mn^{2+}] = 0$; (filled triangles) [2-PrOH] = 0.418 M and $[Mn^{2+}] = 0$; (empty diamonds) [2-PrOH] = 0.680 M and $[Mn^{2+}] = 0$; (filled diamonds) [2-PrOH] = 2.77 M and $[Mn^{2+}] = 0$. The initial-rate scales are situated on the left for all the plots except those corresponding to empty squares (to be read on the right).

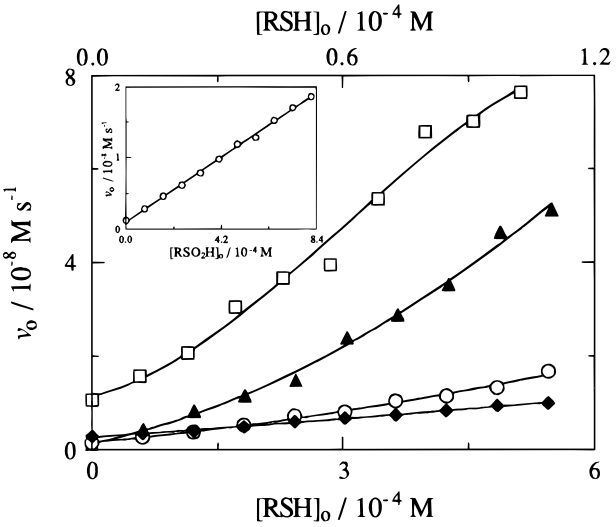

Figure 3. Dependence of the initial rate on the initial concentration of reductant for the reaction of $CrOO^{2+}$ (1.71×10^{-5} M) with glutathione at $[HClO_4] = 1.10$ M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C: (squares) [2-PrOH] = 0.157 M and $[Mn^{2+}] = 8.86 \times 10^{-4}$ M; (triangles) 0.157 M and $[Mn^{2+}] = 0$; (circles) [2-PrOH] = 0.680 M and $[Mn^{2+}] = 0$; (diamonds) [2-PrOH] = 2.77 M and $[Mn^{2+}] = 0$. The $[RSH]_o$ scale is situated on the bottom for all the plots except those corresponding to squares (to be read on the top). Inset: analogous plot for the reaction of $CrOO^{2+}$ with L-cysteinesulfinic acid at [2-PrOH] = 0.157 M and $[Mn^{2+}] = 0$, the other experimental conditions remaining the same as above.

$CrOO^{2+}$ was a little lower, in good agreement with the rate law proposed for the same reaction by Bakac and Espenson.[20]

TABLE 1: Effect of the Acidity of the Medium on the Initial Rates of the $CrOO^{2+}$−RSH Reactions[a]

$[HClO_4]$ (M)[b]	v_o (10^{-8} M s^{-1})		
	L-cysteine	glutathione	DL-penicillamine
1.10	3.18 ± 0.04	7.08 ± 0.04	2.22 ± 0.08
1.28	2.93 ± 0.07	6.96 ± 0.18	2.25 ± 0.08
1.46	3.04 ± 0.05	6.72 ± 0.06	2.46 ± 0.18
1.64	3.21 ± 0.12	6.27 ± 0.05	2.22 ± 0.01
1.83	3.32 ± 0.03	5.88 ± 0.23	2.13 ± 0.10
2.01	3.50 ± 0.03	5.37 ± 0.08	2.22 ± 0.03
2.19	3.46 ± 0.10	5.65 ± 0.08	2.32 ± 0.02
2.38	3.53 ± 0.05	5.53 ± 0.03	2.36 ± 0.05
2.56	3.73 ± 0.01	5.62 ± 0.10	2.51 ± 0.02
2.74	3.84 ± 0.06	5.60 ± 0.24	2.61 ± 0.02

[a] $[CrOO^{2+}]_o = 1.71 \times 10^{-5}$ M, $[RSH]_o = 6.09 \times 10^{-4}$ M, [2-PrOH] = 0.157 M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C. [b] The ionic strength was kept constant with an adequate amount of sodium perchlorate, so that $I = [HClO_4] + [NaClO_4] = 2.74$ M.

TABLE 2: Effect of the Ionic Strength of the Medium on the Initial Rates of the $CrOO^{2+}$−RSH Reactions[a]

$[NaClO_4]$ (M)[b]	v_o (10^{-8} M s^{-1})		
	L-cysteine	glutathione	DL-penicillamine
0.00	1.95 ± 0.13	6.91 ± 0.01	1.65 ± 0.07
0.18	2.10 ± 0.07	7.13 ± 0.32	1.61 ± 0.04
0.37	2.21 ± 0.01	6.82 ± 0.01	1.68 ± 0.03
0.55	2.41 ± 0.14	6.78 ± 0.07	1.76 ± 0.01
0.73	2.40 ± 0.04	6.28 ± 0.82	1.75 ± 0.10
0.91	2.57 ± 0.10	7.01 ± 0.12	1.86 ± 0.11
1.10	2.66 ± 0.08	6.89 ± 0.10	1.99 ± 0.01
1.28	2.91 ± 0.18	6.90 ± 0.07	2.02 ± 0.02
1.46	3.13 ± 0.20	6.85 ± 0.18	2.14 ± 0.03
1.64	3.41 ± 0.01	7.01 ± 0.06	2.32 ± 0.07

[a] $[CrOO^{2+}]_o = 1.71 \times 10^{-5}$ M, $[RSH]_o = 6.09 \times 10^{-4}$ M, $[HClO_4] = 1.10$ M, [2-PrOH] = 0.157 M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C. [b] The values of the ionic strength can be obtained as $I = [NaClO_4] + 1.10$ M.

We have studied the effect of the reducing agent under different concentrations of Mn^{2+} and 2-propanol. For both L-cysteine (Figure 2, down) and DL-penicillamine (Figure 2, up) the v_o vs $[RSH]_o$ plots showed a downward-concave curvature, which was more pronounced as the concentration of Mn^{2+} increased and less pronounced as the concentration of 2-propanol increased, so that at [2-PrOH] = 2.77 M the plots were practically linear. On the contrary, in the case of glutathione (Figure 3), the v_o vs $[RSH]_o$ plots in the absence of Mn^{2+} showed an upward-concave curvature, which was less pronounced as the concentration of 2-propanol increased (again, at [2-PrOH] = 2.77 M the plot was practically linear), whereas in the presence of Mn^{2+} the plot showed both an upward-concave (at low $[RSH]_o$) and a downward-concave (at high $[RSH]_o$) curvature. On the other hand, the reaction between $CrOO^{2+}$ and L-cysteinesulfinic acid (Figure 3, inset) seems to be of first order in the reductant (the v_o vs $[RSH]_o$ plot was linear even at low [2-PrOH]).

The behavior of the reaction of $CrOO^{2+}$ with glutathione was also different from that corresponding to the other two thiols with respect to the effects of both the acidity and the ionic strength of the medium. At constant ionic strength, an increase of $[HClO_4]$ led to a slight increase of v_o in the cases of L-cysteine and DL-penicillamine and a slight decrease in the case of glutathione (Table 1). At constant $[HClO_4]$, the addition of $NaClO_4$ to the solution led to an increase of v_o in the cases of L-cysteine and DL-penicillamine, whereas in the case of glutathione v_o did not change (Table 2).

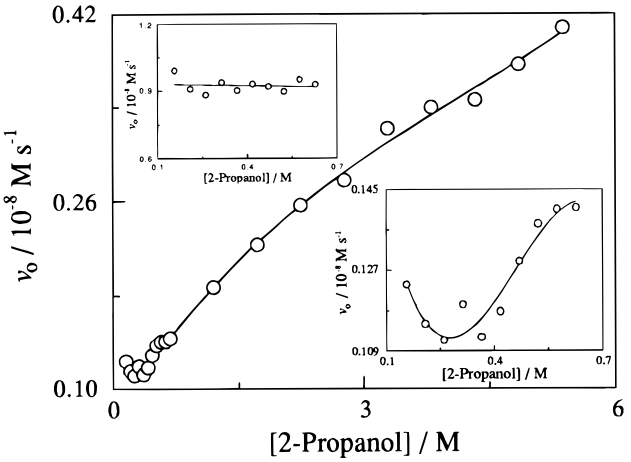

Figure 4. Dependence of the initial rate on the concentration of 2-propanol for the decomposition of $CrOO^{2+}$ (1.71×10^{-5} M) in the absence of thiol at $[HClO_4] = 1.10$ M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C. Right inset: detail of the former plot at low alcohol concentrations. Left inset: effect of 2-propanol on the initial rate of decomposition in the presence of Mn^{2+} (8.86×10^{-4} M), the other experimental conditions remaining the same as above.

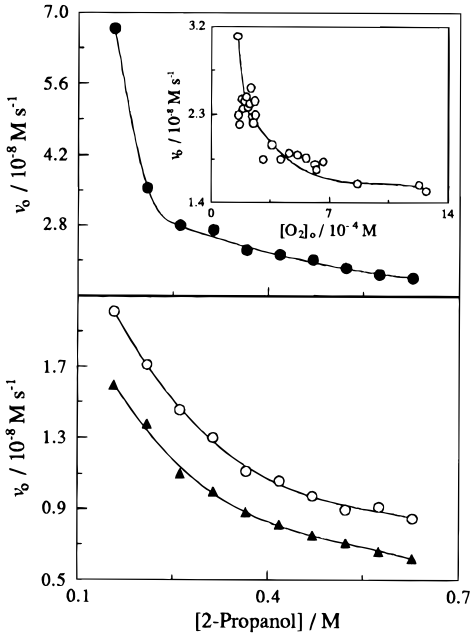

Figure 5. Effect of inhibitors on the initial rate of the reactions of $CrOO^{2+}$ (1.71×10^{-5} M) with thiols (6.09×10^{-4} M) at $[HClO_4] = 1.10$ M and 25.0 °C. Up: effect of 2-propanol on the reaction with glutathione at $[O_2]_o = 2.60 \times 10^{-4}$ M. Down: effect of 2-propanol on the reactions with L-cysteine (circles) and DL-penicillamine (triangles) at $[O_2]_o = 2.60 \times 10^{-4}$ M. Inset: effect of oxygen on the reaction with L-cysteine at [2-PrOH] = 0.157 M.

For the decomposition reaction in the absence of Mn^{2+}, an increase of the alcohol concentration led to a definite increase of v_o at high [2-PrOH] (Figure 4), whereas at low [2-PrOH] a slight decrease of v_o was found (Figure 4, right inset); however, when Mn^{2+} was also present, no effect of [2-PrOH] on v_o could be detected (Figure 4, left inset). The $CrOO^{2+}$−RSH reactions were inhibited both by 2-propanol, the effect being stronger for the case of glutathione (Figure 5 up) than for L-cysteine and DL-penicillamine (Figure 5, down), and by dissolved oxygen (Figure 5, inset).

Manganese(II) and cerium(III) ions are efficient trapping agents for Cr(IV).[42−44] We found that, whereas the decomposition reaction was very sensitive toward both ions (used in the forms of $MnSO_4$ and $Ce(NO_3)_3$, respectively), the $CrOO^{2+}$−RSH reactions were much more sensitive toward Mn^{2+} (Figure 6, up) than toward Ce^{3+} (Figure 6, down). In fact, Mn^{2+} strongly catalyzed the decomposition of $CrOO^{2+}$ as well as its reactions with the three thiols, whereas Ce^{3+} was a potent catalyst for the decomposition, a weak catalyst for the reactions with both glutathione and DL-penicillamine, and a weak inhibitor for the reaction with L-cysteine (Table 3).

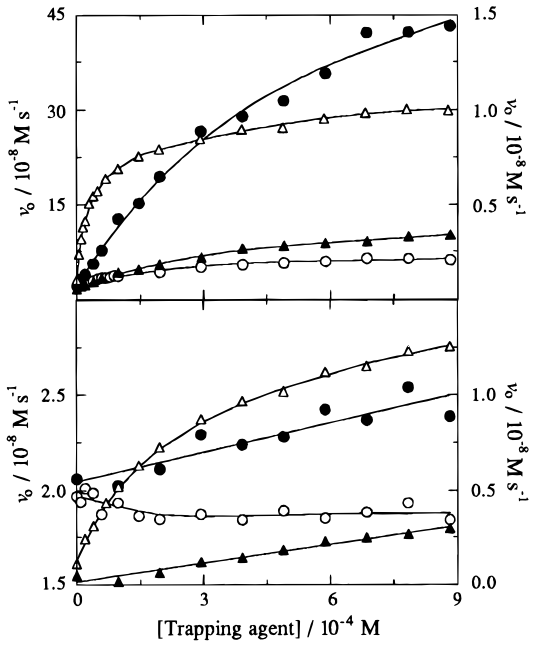

Figure 6. Effect of the Cr(IV)-trapping agents Mn^{2+} (up) and Ce^{3+} (down) on the initial rate of disappearance of $CrOO^{2+}$ (1.71×10^{-5} M) for its decomposition in the absence of thiol (empty triangles) and for its reactions with the thiols L-cysteine (6.09×10^{-4} M, empty circles), glutathione (3.10×10^{-4} M, filled circles), and DL-penicillamine (6.09×10^{-4} M, filled triangles) at $[HClO_4] = 1.10$ M, [2-PrOH] = 0.157 M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C. The initial-rate scales are situated on the left for all the plots except those corresponding to empty triangles (to be read on the right).

TABLE 3: Effect of the Cr(IV)-Trapping Agents[a]

reductant	Mn(II)[b]	Ce(III)[b]
decomposition[c]	9.5 ± 0.4	11.4 ± 0.3
L-cysteine[d]	3.0 ± 0.2	0.94 ± 0.09
glutathione[e]	20.0 ± 1.8	1.16 ± 0.07
DL-penicillamine[d]	6.1 ± 0.3	1.16 ± 0.04

[a] $[CrOO^{2+}]_o = 1.71 \times 10^{-5}$ M, $[HClO_4] = 1.10$ M, [2-PrOH] = 0.157 M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 25.0 °C. [b] Ratio between the initial rate in the presence of 8.82×10^{-4} M Cr(IV)-trapping agent (either Mn^{2+} or Ce^{3+}) and the initial rate in the absence of that agent. [c] $[RSH]_o = 0$. [d] $[RSH]_o = 6.09 \times 10^{-4}$ M. [e] $[RSH]_o = 3.10 \times 10^{-4}$ M.

The effect of temperature on these reactions was rather unusual. In the absence of Cr(IV)-trapping agents, and at low [2-PrOH], only the decomposition of $CrOO^{2+}$ followed the Arrhenius law (Figure 7, inset), whereas for the three $CrOO^{2+}$−RSH reactions the v_o vs T plots in the range 10−45 °C showed both a decreasing and an increasing stretch, with a minimum at around 20 °C (Figure 7). The latter three plots could be fitted by nonlinear least-squares to a double-exponential law (eq 1, where A'_1 and A'_2 include the initial concentrations), so that two apparent activation energies, one negative ($E_{a,1}$) and the other positive ($E_{a,2}$), were obtained for each $CrOO^{2+}$−RSH reaction. However, the v_o vs T plots were uniformly increasing either when Mn^{2+} was present in the solution (Figure 8, up) or when the experiments were done at very high [2-PrOH] (Figure

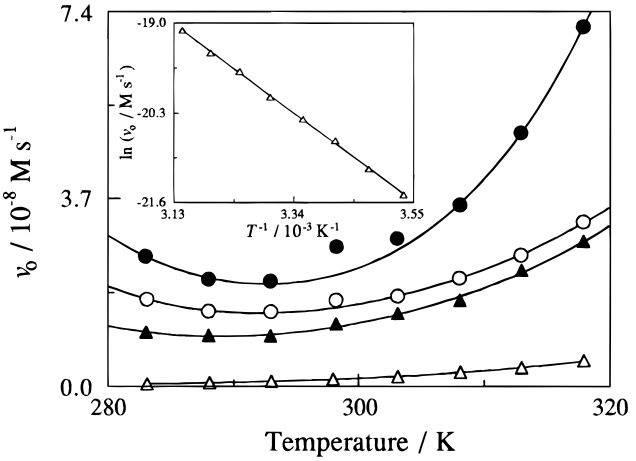

Figure 7. Effect of temperature on the initial rate of disappearance of $CrOO^{2+}$ (1.85×10^{-5} M) for its decomposition in the absence of thiol (empty triangles) and for its reactions with the thiols (3.13×10^{-4} M) L-cysteine (empty circles), glutathione (filled circles), and DL-penicillamine (filled triangles) at $[HClO_4] = 1.10$ M, [2-PrOH] = 0.157 M, and $[O_2]_o = 2.60 \times 10^{-4}$ M. The three upper curves are the best fits to double-exponential Arrhenius laws (eq 1) for the experimental data corresponding to the reactions with L-cysteine ($E_{a,1} = -54 \pm 9$ kJ mol^{-1}, $E_{a,2} = 40 \pm 4$ kJ mol^{-1}), glutathione ($E_{a,1} = -42 \pm 8$ kJ mol^{-1}, $E_{a,2} = 66 \pm 2$ kJ mol^{-1}), and DL-penicillamine ($E_{a,1} = -53 \pm 3$ kJ mol^{-1}, $E_{a,2} = 44 \pm 2$ kJ mol^{-1}). Inset: Arrhenius plot for the decomposition of $CrOO^{2+}$ under the above conditions ($E_a = 51 \pm 1$ kJ mol^{-1}).

8, down), so that good-enough Arrhenius linear plots were obtained for the four reactions (Figure 8, insets).

$$v_o = A'_1 \exp\left(-\frac{E_{a,1}}{RT}\right) + A'_2 \exp\left(-\frac{E_{a,2}}{RT}\right) \qquad (1)$$

An intriguing fact was the finding that, in some of the experiments, the value of v_o corresponding to 25.0 °C (measured up to four times, to exclude the possibility of an accidental error) was notably high when compared with those corresponding to the other temperatures. This peculiar effect was observed for the reactions of $CrOO^{2+}$ with both L-cysteine and glutathione at low [2-PrOH], either in the absence (Figure 7) or in the presence (Figure 8, up) of Mn^{2+}, but it disappeared at high [2-PrOH] (Figure 8, down). On the contrary, this effect was not observed either for the decomposition of $CrOO^{2+}$ or for its reaction with DL-penicillamine under any experimental conditions. When necessary, the point corresponding to 25.0 °C was excluded from the calculations of the activation parameters. The activation enthalpies and entropies associated with the four reactions under the different experimental conditions studied are given in Table 4.

It should be mentioned that at low temperature (10.0 °C) the absorbance vs time plots presented in some of the experiments an unusual profile (Figure 9). At high [2-PrOH] the plots showed a diphasic feature (especially for the case of the reaction with glutathione), with the absorbance decreasing at first very rapidly and then more slowly. On the other hand, in the presence of Mn^{2+} the plots showed a sigmoidal profile (especially for the case of the reaction with DL-penicillamine), so that the rate vs time plots were bell shaped (Figure 9, inset).

Additional Experiments. A few experiments were done to study the effect of dissolved O_2 on the decomposition of $CrOO^{2+}$ in the absence of thiol, the results indicating that O_2 strongly inhibits that reaction at very low $[O_2]_o$, but it has almost no

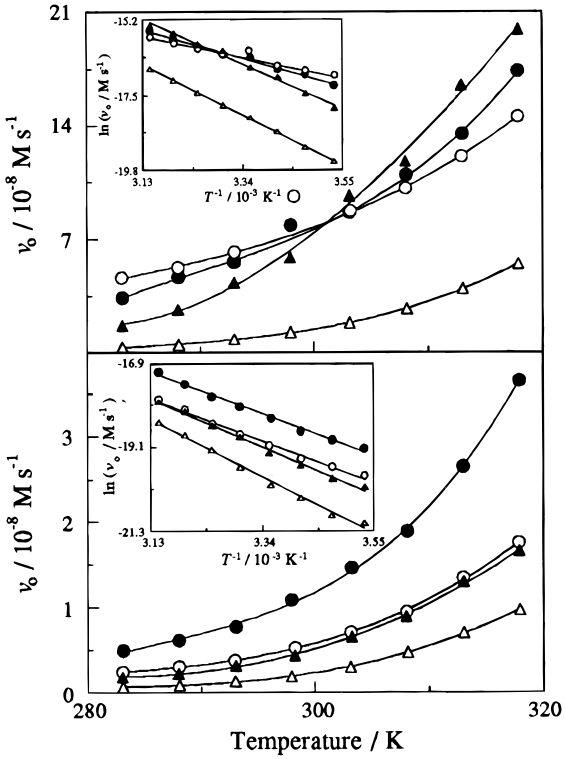

Figure 8. Effect of temperature on the initial rate of disappearance of $CrOO^{2+}$ (1.85×10^{-5} M) for its decomposition in the absence of thiol (empty triangles) and for its reactions with L-cysteine (empty circles), glutathione (filled circles), and DL-penicillamine (filled triangles) at $[HClO_4] = 1.10$ M and $[O_2]_o = 2.60 \times 10^{-4}$ M. Up: $[RSH]_o = 3.13 \times 10^{-4}$ M (for L-cysteine and DL-penicillamine) and 6.03×10^{-5} M (for glutathione), [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 1.77 \times 10^{-3}$ M. Down: $[RSH]_o = 3.13 \times 10^{-4}$ M, [2-PrOH] = 1.20 M, and $[Mn^{2+}] = 0$. Insets: respective Arrhenius plots; the activation energies for the reactions in the presence of Mn^{2+} are 61 ± 1 (decomposition), 25 ± 1 (L-cysteine), 34 ± 1 (glutathione), and 54 ± 2 (DL-penicillamine) kJ mol^{-1}, whereas for the reactions in the absence of that ion the activation energies are 63 ± 1 (decomposition), 43 ± 2 (L-cysteine), 44 ± 2 (glutathione), and 50 ± 2 (DL-penicillamine) kJ mol^{-1}.

effect at high $[O_2]_o$, which is again coherent with the rate law proposed for the decomposition reaction by Bakac and Espenson.[20]

In an attempt to explore the possible interactions between $CrOO^{2+}$ and DNA, some kinetic runs were done in the presence of adenine, but we found no effect of that molecule on either the decomposition of $CrOO^{2+}$ or its reaction with L-cysteine. Hence, the possibility of a direct reaction between $CrOO^{2+}$ and adenine has to be excluded (at least in acidic media).

Some kinetic runs were done to study the possible interference provoked by the Cr(III) present at the beginning of the reactions as a consequence of the method used to synthesize the $CrOO^{2+}$. To that end, some experiments with a variable $[Cr(III)]_o$ (obtained from the reduction of Cr(VI) by 2-propanol in aqueous perchloric acid, allowing the solution to age for several days until all the $CrOO^{2+}$ had been converted into Cr(III)) were performed. We could not find any noticeable effect of Cr(III) on the initial rate of the $CrOO^{2+}$−RSH reactions, which is indeed consistent with the fact that Cr(III) is present in aqueous solution in the form of substitution-inert complexes.[22] The UV−vis spectrum indicated that other chromium species capable of reacting with thiols (Cr(VI), Cr(V), or Cr(IV)) were not present in noticeable amounts as contaminants when the kinetic runs were started. In particular, contamination by Cr(IV) (which may be important when the $CrOO^{2+}$ solution is prepared in the

TABLE 4: Activation Parameters[a]

reductant	$\Delta H^{\circ,\ddagger}$ (kJ mol^{-1})	$\Delta S^{\circ,\ddagger}$ (J K^{-1} mol^{-1})[b]
decomposition[c]	48 ± 1	−71 ± 2
L-cysteine[d,e]	−57 ± 7	−417 ± 26
glutathione[d,e]	−44 ± 8	−364 ± 29
DL-penicillamine[d,e]	−55 ± 3	−413 ± 11
L-cysteine[d,f]	37 ± 3	−93 ± 11
glutathione[d,f]	64 ± 2	−1 ± 7
DL-penicillamine[d,f]	42 ± 2	−77 ± 6
decomposition[g]	60 ± 1	−29 ± 3
L-cysteine[h]	41 ± 2	−84 ± 5
glutathione[h]	41 ± 2	−78 ± 5
DL-penicillamine[h]	47 ± 2	−65 ± 5
decomposition[i]	58 ± 1	−19 ± 2
L-cysteine[j]	22 ± 1	−126 ± 2
glutathione[k]	31 ± 1	−95 ± 3
DL-penicillamine[j]	51 ± 2	−31 ± 7

[a] $[CrOO^{2+}]_o = 1.85 \times 10^{-5}$ M, $[HClO_4] = 1.10$ M, and $[O_2]_o = 2.60 \times 10^{-4}$ M. [b] The activation entropies are associated with the respective pseudo-second-order rate constants ($k_2 = v_o/[CrOO^{2+}]_o^2$) and are referred to the 1 M standard state. [c] $[RSH]_o = 0$, [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 0$. [d] $[RSH]_o = 3.13 \times 10^{-4}$ M, [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 0$. [e] First reaction pathway (eq 1). [f] Second reaction pathway (eq 1). [g] $[RSH]_o = 0$, [2-PrOH] = 1.20 M, and $[Mn^{2+}] = 0$. [h] $[RSH]_o = 3.13 \times 10^{-4}$ M, [2-PrOH] = 1.20 M, and $[Mn^{2+}] = 0$. [i] $[RSH]_o = 0$, [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 1.77 \times 10^{-3}$ M. [j] $[RSH]_o = 3.13 \times 10^{-4}$ M, [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 1.77 \times 10^{-3}$ M. [k] $[RSH]_o = 6.03 \times 10^{-5}$ M, [2-PrOH] = 0.157 M, and $[Mn^{2+}] = 1.77 \times 10^{-3}$ M.

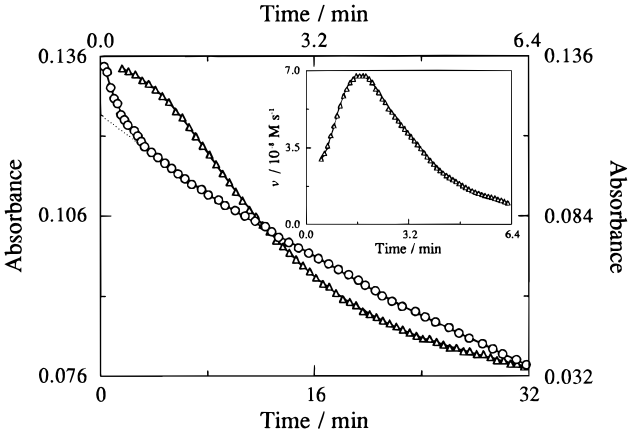

Figure 9. Absorbance vs time plots for two kinetic runs corresponding to the reactions of $CrOO^{2+}$ (1.85×10^{-5} M) with the thiols (3.13×10^{-4} M) glutathione (circles, [2-PrOH] = 1.20 M and $[Mn^{2+}] = 0$) and DL-penicillamine (triangles, [2-PrOH] = 0.157 M and $[Mn^{2+}] = 1.77 \times 10^{-3}$ M) at $[HClO_4] = 1.10$ M, $[O_2]_o = 2.60 \times 10^{-4}$ M, and 10.0 °C. The time and absorbance scales are situated on the bottom and on the left for the plot corresponding to circles, whereas for the plot corresponding to triangles the scales are situated on the top and on the right, respectively. The dotted line is an extrapolation obtained with a third-degree polynomial fit of the absorbance vs time data belonging to the second phase of the kinetic run corresponding to the reaction with glutathione. Inset: rate vs time plot for the kinetic run corresponding to the reaction with DL-penicillamine.

absence of alcohol) can be excluded in view of the high concentration of 2-propanol (0.261 M) used to obtain the superoxide and the value of the bimolecular rate constant reported by Scott et al.[19] for the reduction of Cr(IV) by 2-propanol (12.0 M^{-1} s^{-1}, $t_{1/2} = 0.2$ s).

Given that Cu^{2+} is known to catalyze the dismutation of the superoxide radicals,[45,46] we also studied the possible effect of that metal ion on these kinetic systems. We found that Cu^{2+} (in the form of $CuSO_4$) did not affect the decomposition of $CrOO^{2+}$ in the absence of thiol, whereas in the case of its reaction with L-cysteine we found a very slight catalysis, which is indeed a minor effect if compared with the dramatic catalysis that Cu^{2+} provokes on a parallel reaction, the oxidation of thiols by a superoxocobalt(III) complex.[34] Especially interesting is the finding that the $CrOO^{2+}$−RSH reactions were not inhibited by concentrations of Cu^{2+} up to 1×10^{-4} M (unless incubated with the thiol while the reactants were thermostated, due to the oxidation of RSH to RSSR by O_2 catalyzed by that metal ion[47,48]), since it allows casting aside the mechanisms in which O_2^- plays an active role as either initiator or propagator of a chain reaction.

Discussion

The finding that, under some experimental conditions, two exponential functions are clearly involved in the v_o vs T plots corresponding to the $CrOO^{2+}$−RSH reactions (eq 1) indicates that at least two different reaction pathways are involved in the mechanism.

For the first pathway we propose a direct attack of the thiol on the superoxide (eqs 2−4). The formation of an oxidant−reductant complex is supported by the following findings: (i) Provided that the reaction depicted in eq 2 is exothermic (as happens with the formation of many complexes), the value of ΔH° associated with the equilibrium constant K_I would be negative, which is consistent with the fact that the energy (Figure 7) and enthalpy (Table 4) of activation for this pathway are negative. (ii) Since it can be assumed that the formation of the complex implies a notable increase of order in the system, the value of ΔS° associated with K_I must also be negative, which is consistent with the fact that a negative activation entropy with an unusually high absolute value was obtained for this pathway (Table 4). (iii) The fast initial phase observed in the absorbance vs time plots under some particular experimental conditions (Figure 9) may be interpreted as the time that must elapse for the complex to be in quasi-equilibrium with the reactants. This would be observed only when the reaction was slow enough (at very high [2-PrOH]) and when the value of K_I was high enough (at low temperature). Under all the other experimental conditions the quasi-equilibrium situation would be attained during the process of mixing the reactants.

$$CrOO^{2+} + RSH \overset{K_I}{\rightleftharpoons} [CrOO{-}HSR]^{2+} \quad (2)$$

$$[CrOO{-}HSR]^{2+} + CrOO^{2+} \xrightarrow[\text{slow}]{k_{II}} CrOOH^{2+} + RSO_2^- + Cr^{3+} \quad (3)$$

$$CrOOH^{2+} + RSH \rightarrow CrO^{2+} + RS^\bullet + H_2O \quad (4)$$

Although the kinetic data found in this work do not provide any insight on the nature of the complex formed in eq 2, we might envisage it as a $[RSCrOOH]^{2+}$ complex, with the hydrogen atom bonded to the terminal oxygen atom of the superoxide and the thiyl group bonded to the chromium atom. After the collision of that complex with a second $CrOO^{2+}$ ion, the thiyl group of the former might combine with the superoxide group of the latter to form sulfinate ion, liberating hydroperoxochromium(III) and chromium(III) ions (eq 3).

We believe that the part of the mechanism responsible for the second exponential function in eq 1 starts with the decomposition of the superoxide without the intervention of the thiol (eqs 5−8). According to Bakac et al.,[14] this process takes place by the combination of a unimolecular (eq 5) and a bimolecular (eq 7) pathway.

TABLE 5: Some Relevant Reduction Potentials[a]

redox couple	$E°$(V)	references
$CrOO^{2+}/CrOOH^{2+}$	+1.03	22, 49
$CrOOH^{2+}/CrO^{2+}$	+2.09[b]	22, 49
CrO^{2+}/Cr^{3+}	+1.71[c]	22, 49
$RS^{\bullet}/RSH$	+1.33[d]	50, 51

[a] The values for the chromium redox couples were measured at 25 °C, and the value for the thiyl/thiol couple was measured at 23 ± 2 °C. [b] This value must be considered as a superior limit (the real value might be a little lower). [c] This value must be considered as an inferior limit (the real value might be a little higher). [d] The value is rather insensitive to the nature of the substituent (R).

$$CrOO^{2+} \xrightarrow[\text{slow}]{k_{III}} Cr^{2+} + O_2 \quad (5)$$

$$Cr^{2+} + CrOO^{2+} \xrightarrow{k_{IV}} [CrOOCr]^{4+} \quad (6)$$

$$2CrOO^{2+} \xrightarrow[\text{slow}]{k_{V}} [CrOOCr]^{4+} + O_2 \quad (7)$$

$$[CrOOCr]^{4+} \rightarrow 2CrO^{2+} \quad (8)$$

Figure 7 shows that the values of the initial rate corresponding to the decomposition of the superoxide in the absence of thiol are too low when compared with the values corresponding to the rate-increasing branch of the v_o vs T plots. Thus, the decomposition cannot explain by itself the second exponential function of eq 1. However, when the thiol is present in the medium, it can react with the oxochromium(IV) ion in a one-electron reduction process (eq 9), thus preventing its two-electron reduction by 2-propanol (eq 10),[19,44] and the subsequent regeneration of the superoxide caused by the intervention of dissolved oxygen (eq 11).[19,26] Hence, through eq 9 the thiol exerts an amplification effect on the decomposition of the superoxide.

$$CrO^{2+} + RSH \xrightarrow{k_{VI}} Cr^{3+} + RS^{\bullet} + OH^{-} \quad (9)$$

$$CrO^{2+} + (CH_3)_2CHOH \xrightarrow{k_{VII}} Cr^{2+} + (CH_3)_2CO + H_2O \quad (10)$$

$$Cr^{2+} + O_2 \xrightarrow{k_{VIII}} CrOO^{2+} \quad (11)$$

We have proposed that the reaction of the thiol with either hydroperoxochromium(III) (eq 4) or oxochromium(IV) (eq 9) ions takes place by a direct hydrogen atom transfer, thus involving the formation of thiyl free radicals. On the contrary, we propose (in accordance with the second-order found for $CrOO^{2+}$, Figure 1) that the reaction with the superoxide cannot take place by direct hydrogen-atom transfer from the thiol to a single superoxochromium(III) ion (leading to $CrOOH^{2+}$ and $RS^{\bullet}$), but by the intervention in the process of two $CrOO^{2+}$ ions (eq 3). These assumptions are supported by the values of the reduction potentials given in the literature[22,49−51] (Table 5), since, according to them, the transfer of a hydrogen atom from RSH to either $CrOOH^{2+}$ or CrO^{2+} is thermodynamically favorable (at least under standard conditions), whereas the hydrogen atom transfer to a single $CrOO^{2+}$ ion is unfavorable. The process depicted in eq 3, however, is likely to provide a more favorable route for the reduction of the superoxide, since the relatively stable sulfinate ion (RSO_2^-), instead of the very energetic thiyl free radical ($RS^{\bullet}$), is formed.

Given that we could not detect any disulfide in the reaction product mixture, we propose that most of the thiyl radicals (formed in eqs 4 and 9) migth disappear via oxidation by $CrOO^{2+}$ (eq 12) rather than by dimerization.[52] It should be considered that, albeit the second-order rate constant for the latter process is expected to be higher than the one associated with the former, this disadvantage might be easily overcome by the very high concentration of $CrOO^{2+}$ compared with that of $RS^{\bullet}$.

$$RS^{\bullet} + CrOO^{2+} \rightarrow RSO_2^{-} + Cr^{3+} \quad (12)$$

Manganese(II) ion is a good one-electron reductant for Cr(IV).[42,44] Thus, eq 13 can account for the strong catalytic effect that those ions exert on both the decomposition of $CrOO^{2+}$ and its reactions with thiols (Figure 6, up), since that process avoids the two-electron reduction of Cr(IV) by the alcohol (eq 10) and the subsequent regeneration of the reactant (eq 11).

$$CrO^{2+} + Mn^{2+} + H_2O \xrightarrow{k_{IX}} Cr^{3+} + Mn^{3+} + 2OH^{-} \quad (13)$$

Assuming that the $[CrOO-HSR]^{2+}$ complex is in quasi-equilibrium with the reactants and that all the other intermediates involved are in steady state, the rate law that can be deduced from the proposed mechanism for the reaction in the presence of manganese(II) ion is given by eq 14 (where $[CrOO^{2+}]_T$ is the total superoxide concentration, either free or complexed by the thiol), whereas the rate law for the reaction in the absence of that ion can be obtained as a particular case of eq 14 simply by taking $[Mn^{2+}] = 0$.

$$v = \frac{d[Cr^{3+}]}{dt} = \frac{2K_1k_{II}[CrOO^{2+}]_T^2[RSH]}{1 + 2K_1[RSH]} + \frac{[CrOO^{2+}]_T^2\{2k_{III}k_{IV} + k_{VIII}[O_2](K_1k_{II}[RSH] + 2k_V)\}(2k_{VI}[RSH] + k_{IX}[Mn^{2+}])}{k_{VIII}[O_2](1 + 2K_1[RSH])(k_{VI}[RSH] + k_{VII}[2\text{-PrOH}] + k_{IX}[Mn^{2+}])} \quad (14)$$

Equation 14 is consistent with the profiles found for the v_o vs $[RSH]_o$ plots corresponding to the different thiols. Effectively, eq 14 predicts that the v_o vs $[RSH]_o$ plot consists of a first stretch showing an upward-concave curvature at low thiol concentrations (due to the existence of a quadratic term in [RSH] in the numerator of the second addend of the rate law) followed by another stretch showing a downward-concave curvature at higher thiol concentrations, eventually reaching a saturation limit when the thiol concentration is high enough. Provided that we assume that the value of K_1k_{II} associated with the reaction with glutathione is much higher than those for L-cysteine and DL-penicillamine, the upward-concave stretch will be perceptible only in the former case (Figure 3), whereas in the latter two cases the plots will show only a downward-concave curvature (Figure 2). Moreover, eq 14 also predicts that the downward-concave profile is less pronounced as [2-PrOH] increases (because the term k_{VII}[2-PrOH] in the denominator of the second addend of the rate law becomes more important than the term k_{VI}[RSH]) and, provided that the concentration of alcohol present in the medium is high enough, more pronounced as $[Mn^{2+}]$ increases (because the term $k_{IX}[Mn^{2+}]$ in the numerator of the second addend of the rate law becomes more important than the term $2k_{VI}$[RSH]). It should be noticed that, according to eq 14, manganese(II) ion behaves as a catalyst for the reactions only when the concentration of alcohol is high enough (since then the effect of the term $k_{IX}[Mn^{2+}]$ in the numerator will be more important than the effect of the same term in the denominator). Given that under the experimental conditions of our work manganese(II) ion did behave as a catalyst (Figure 6, up), that condition was actually fulfilled.

In the absence of Mn(II), according to the mechanism proposed, the intermediate Cr(IV) disappears by reacting with either 2-propanol or the thiol. Since the bimolecular rate constant for the Cr(IV)/2-propanol reaction has been reported by Scott et al.[19] ($k_{VIII} = 12.0 \pm 0.4\ M^{-1}\ s^{-1}$), from the study of the competition between the alcohol and the thiol it is possible in principle to obtain the value of the bimolecular rate constant for the Cr(IV)/RSH reaction. A nonlinear least-squares fit of the v_o vs [2-PrOH] experimental data to eq 14 was excellent for both L-cysteine and DL-penicillamine (Figure 5, down), yielding for the rate constant k_{VI} the values $(0.97 \pm 0.05) \times 10^3$ and $(1.09 \pm 0.07) \times 10^3\ M^{-1}\ s^{-1}$, respectively. Thus, the reactivity of Cr(IV) toward those thiols is almost 100 times higher than its reactivity toward 2-propanol, but 100 times lower than its reactivity toward Mn^{2+}.[44] On the contrary, the v_o vs [2-PrOH] experimental data corresponding to glutathione (Figure 5, up) did not fit well to eq 14. The reason for that unfulfillment is that for glutathione the decrease of the initial rate as the alcohol concentration increased was not gradual, as happened with the other two thiols, but very sudden for [2-PrOH] < 0.3 M and much less pronounced at higher concentrations. One of the possible explanations for this behavior might be that in the case of the $CrOO^{2+}$/glutathione reaction the intermediate Cr(IV) is not in steady state (as assumed in the deduction of eq 14) unless the concentration of alcohol in the medium is high enough to compensate for the rapid reduction of $CrOO^{2+}$ to CrO^{2+}.

The effect of the acidity of the medium on the $CrOO^{2+}$/RSH reactions is rather slight, since an increase of 2.5 times in $[HClO_4]$ results in changes in v_o of around 20% (Table 1). The effect for the case of glutathione (base catalysis) is of different sign than for L-cysteine and DL-penicillamine (acid catalysis). The reaction with glutathione differs also from the other two reactions with respect to the effect of the ionic strength (Table 2), as well as with respect to other features of the reaction. This difference is probably caused by the peptidic nature of the glutathione molecule, with more functional groups than the relatively simple amino acids L-cysteine and DL-penicillamine.

The finding that L-cysteic acid is one of the reaction products from the oxidation of L-cysteine by $CrOO^{2+}$ can be easily explained by eq 15. Actually, we have observed (Figure 3, inset) that L-cysteinesulfinic acid is capable of reducing the superoxide and that L-cysteic acid is the major organic product. Although eq 15 is certainly not an elementary step, the mechanism corresponding to this side reaction is not required for the present study, since L-cysteinesulfinate ion is expected to behave as a quasi-stable intermediate (the sulfinic acids derived from L-cysteine and glutathione are relatively stable toward oxidation,[39] which precludes the possibility of RSO_2^- being in steady state). Thus, its reaction with the superoxide will not affect the value of the initial rate ($[RSO_2^-]_o = 0$).

$$2CrOO^{2+} + 3RSO_2^- + 5H^+ = 2Cr^{3+} + 3RSO_3H + H_2O \quad (15)$$

The finding that the initial rate for the decomposition of $CrOO^{2+}$ is affected by the concentration of 2-propanol at $[Mn^{2+}] = 0$, but not when the value of $[Mn^{2+}]$ is high enough (Figure 4), indicates that the alcohol does not react directly with $CrOO^{2+}$ but only with CrO^{2+}.

The inhibition by both 2-propanol and dissolved O_2 (Figure 5) and the catalysis by Mn^{2+} (Figure 6, up) can be taken as strong evidence of CrO^{2+} being involved as an intermediate in the mechanism of the $CrOO^{2+}$/RSH reactions. However, although Ce^{3+} is known to be an efficient trapping agent for Cr(IV) very much like Mn^{2+} (eq 16),[42-44] the effect of Ce^{3+} on the initial rate of the $CrOO^{2+}$/RSH reactions is almost negligible when compared with that of Mn^{2+} (Table 3). A possible explanation would be given by the well-known ability of Ce^{4+} to react with hydroperoxochromium(III) ion regenerating the reactant (eq 17).[14,20,22] Thus, the inhibition caused by eq 17 would compensate for the catalysis caused by eq 16 (similar to that produced by Mn^{2+}).

$$CrO^{2+} + Ce^{3+} + H_2O \rightarrow Cr^{3+} + Ce^{4+} + 2OH^- \quad (16)$$

$$Ce^{4+} + CrOOH^{2+} \rightarrow Ce^{3+} + CrOO^{2+} + H^+ \quad (17)$$

On the contrary, most of the Mn(III) generated when Mn(II) acts as a Cr(IV)-trapping agent might disappear by dismutation (eq 18),[53] instead of by reaction with $CrOOH^{2+}$. The transient colloidal manganese dioxide formed as a product of the latter reaction might explain the autocatalysis observed when the $CrOO^{2+}$/RSH reactions were performed at low temperature and in the presence of Mn^{2+} (Figure 9, inset), since colloidal MnO_2 is known to act as an autocatalyst for many permanganate reactions,[54,55] and the decrease of temperature would favor the adsorption of the reactants on the colloid surface.

$$2Mn^{3+} + 2H_2O = Mn^{2+} + MnO_2 + 4H^+ \quad (18)$$

On the other hand, our experimental results indicate that in the mechanism corresponding to the decomposition of $CrOO^{2+}$ in acidic media and in the absence of thiol CrO^{2+} (but not $CrOOH^{2+}$) is involved as an intermediate, given that the process is strongly catalyzed by both Mn^{2+} and Ce^{3+} (Table 3). However, our results do not exclude the possibility that under less acidic conditions $CrOOH^{2+}$ might also be involved in the mechanism, as proposed by Bakac et al.[14]

Finally, it should be noticed that for the deduction of the rate law (eq 14) we have considered just the steps that we think to be predominant. However, there might also be other minor steps involved (such as the reactions of Cr^{2+} with CrO^{2+} and $[CrOOCr]^{4+}$,[14] for instance). Moreover, it cannot be discarded that another reaction pathway involving as slow step the unimolecular decomposition of the superoxide/thiol complex (eq 19) instead of its reaction with a superoxochromium(III) ion (eq 3) might also have an important contribution to the global reaction.

$$[CrOO{-}HSR]^{2+} \rightarrow Cr^{2+} + RSO_2H \quad (19)$$

Nevertheless, the relatively small effect caused by Ce^{3+} when compared with that caused by Mn^{2+} (Table 3) seems to indicate that $CrOOH^{2+}$ is actually involved as an intermediate in the mechanism of the $CrOO^{2+}$/RSH reactions, and so, it is more consistent with eq 3 than with eq 19.

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Supporting Information Available: Values of the initial rates for the decomposition of superoxochromium(III) ion and for its reactions with L-cysteine, glutathione, and DL-penicillamine at various temperatures in the range 283−318 K under different concentrations of 2-propanol and manganese(II) ion

(3 pages). Ordering information is given on any current masthead page.

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