Mutual catalyst inhibition in the chromium(VI)–copper(II)–hydrogen peroxide reacting system

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Cr(VI) and Cu(II) are both efficient catalysts for the decomposition of H_2O_2. However, in their joint presence, the two catalysts exert a mutual inhibition effect on the catalytic activity of the other, and the study of these inhibition effects has provided important information on the mechanisms of the Cr(VI)/H_2O_2 and Cu(II)/H_2O_2 reactions. The inhibition effect produced by Cr(VI) on the catalytic activity of Cu(II) is the more pronounced (a [Cr(VI)]/[Cu(II)] ratio as low as 2×10^{-4} is enough to provoke a 10% decrease in the decomposition rate), the limit being around 90% inhibition. On the contrary, higher concentrations of Cu(II) are necessary to produce a noticeable inhibition on the catalytic effect of Cr(VI), the limit being 50% inhibition. Besides, Cu(II) prevents the reduction of Cr(VI) to Cr(III) by H_2O_2, so that when [Cu(II)] is high enough the catalyst Cr(VI) is recovered unaltered at the end of the reaction. The inhibition effect produced by Cu(II) on the Cr(VI)/H_2O_2 reaction can be ascribed to its ability to catalyze the dismutation of superoxide free radicals, thus preventing the reduction of Cr(V) to Cr(IV) provoked by them, whereas the inhibition effect produced by Cr(VI) on the Cu(II)/H_2O_2 reaction can be ascribed to its ability to act as an oxidative scavenger for Cu(I), thus preventing its participation as the initiator of a free-radical chain mechanism leading to the decomposition of H_2O_2.

Chromium(VI) is a toxin and a carcinogen[1,2] but cannot react directly with DNA at physiological pH.[3] So, it is believed that the carcinogenic action of Cr(VI) requires its prior intracellular metabolization to more active, mutagenic species.[4,5] Hydrogen peroxide has been singled out as one of the possible promoters of chromium-induced carcinogenesis.[6–10] On its hand, the Cu(II)/H_2O_2 system also causes oxidative DNA damage,[11–13] and its participation, along with the Fe(III)/H_2O_2 system,[14] in important biological processes such as aging and carcinogenesis is suspected.[15] A property that Cr(VI),[16] Cu(II),[17] and Fe(III)[16] have in common is that of catalyzing the decomposition of H_2O_2, and their biologically harmful effects might presumably be related (at least partially) to the involvement in the reaction mechanism of very reactive intermediates such as oxygen-containing free radicals.[18]

Considering that in the Fe(III)/Cu(II)/H_2O_2 combined system it has been known for a long time that each metal catalyst acts as a promoter of the catalytic effect exerted by the other metal on the rate of decomposition of H_2O_2,[19,20] we wondered if the same synergistic effect might also be present in the Cr(VI)/Cu(II)/H_2O_2 combined system. To our surprise, we found exactly the opposite, since in this system each catalyst exerts an inhibition effect on the catalytic activity of the other. We thought that the study of this mutual inhibition effect might reveal some important information on the mechanisms of the Cr(VI)/H_2O_2 and Cu(II)/H_2O_2 reactions, given that, despite extensive studies, the complexity of these reactions is such that a thorough understanding of their respective mechanisms is difficult to attain.[21,22]

Experimental

The catalysts were used as $K_2Cr_2O_7$ and $CuSO_4 \cdot 5H_2O$ (both Merck). The source of H_2O_2 was a 30% solution without stabilizers (Fluka). The solvent was water previously purified by deionization followed by distillation and circulation through a Millipore system. The buffer was a KH_2PO_4–K_2HPO_4 mixture (both Merck). The pH was measured with a Metrohm 605 pH meter provided with a glass-calomel combination electrode. The concentration of CrO_4^{2-} was calculated from the absorbance at 372 nm, measured with a Varian Cary 219 UV-Vis spectrophotometer. The decay of the H_2O_2 concentration was followed by titration with $KMnO_4$ in the presence of H_2SO_4. The peroxide content of the solution was analyzed at 10 different times during the course of the reaction, and the kinetic data were obtained by application of the initial-rates method. This method was necessary, especially in the case of the experiments performed in the presence of Cr(VI), due to the existence of long-lived intermediates.[23]

Results

In the absence of Cr(VI), a plot of the initial rate *vs.* the initial concentration of Cu(II) was definitely upward concave (Fig. 1), indicating that, under the experimental conditions of the present work, the apparent kinetic order of the catalyst in the Cu(II)/H_2O_2 reaction is higher than unity. However, that order is not defined, since a double-logarithmic plot of v_0 *vs.* $[\mathrm{Cu(II)}]_0$ was nonlinear. Moreover, in several other series of experiments, under conditions different from those corresponding to Fig. 1, we obtained v_0 *vs.* $[\mathrm{Cu(II)}]_0$ plots showing a downward concave curvature (apparent order lower than unity). This confirmed that the kinetic order of the catalyst in the Cu(II)/H_2O_2 reaction is not defined. We also observed that the Cu(II)/H_2O_2 reaction presents base catalysis, since an increase of the pH (in aqueous phosphate buffer) led to an increase of the initial rate (experimental results not shown).

In the presence of a constant initial concentration of Cr(VI) as catalyst, the addition to the solution of a variable amount of Cu(II) led to a decrease of the initial rate (Fig. 1). The inhibitor-to-catalyst ratio necessary to decrease by 10% the value of v_0 was $[\mathrm{I}]/[\mathrm{C}] = 5 \times 10^{-2}$, with I = Cu(II) and C = Cr(VI).

Thus, it is interesting to notice that, whereas in the case of the Fe(III)/Cu(II)/H_2O_2 system the decomposition rate in the

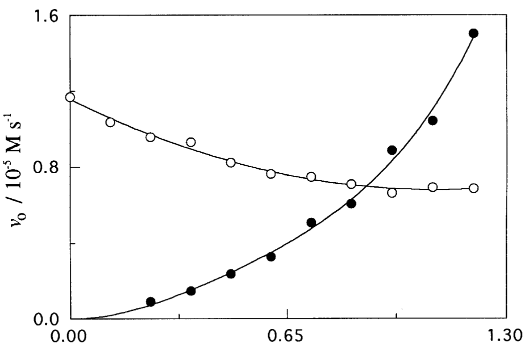

Fig. 1 Effect of Cu(II) on the initial rate of disappearance of H_2O_2 (0.196 M) in the presence of a KH_2PO_4 (0.060 M)–K_2HPO_4 (0.060 M) buffer, at pH 6.71 and 25.0 °C. $[Cr(VI)]_0 = 0$ (filled circles) and 2.56×10^{-4} M (empty circles).

combined presence of Fe(III) and Cu(II) is higher than the sum of the rates corresponding to the presence of each separate catalyst ($v_{Fe\text{-}Cu} > v_{Fe} + v_{Cu}$),[19,20] in the case of the Cr(VI)/Cu(II)/H_2O_2 system, at low values of $[Cu(II)]_0$, the decomposition rate in the combined presence of Cr(VI) and Cu(II) is intermediate between the values of the rate corresponding to the presence of each catalyst separately ($v_{Cu} < v_{Cr\text{-}Cu} < v_{Cr}$). Furthermore, the two curves shown in Fig. 1 cross at a certain point; this means that, at high values of $[Cu(II)]_0$, the decomposition rate in the combined presence of Cr(VI) and Cu(II) is lower than the values of the rate corresponding to the presence of both catalysts considered singly ($v_{Cr\text{-}Cu} < v_{Cr}$ and $v_{Cr\text{-}Cu} < v_{Cu}$). The only possible explanation for this finding is that the inhibition exerted by the catalysts is mutual: Cu(II) is an inhibitor for the Cr(VI)/H_2O_2 reaction and Cr(VI) is an inhibitor for the Cu(II)/H_2O_2 reaction.

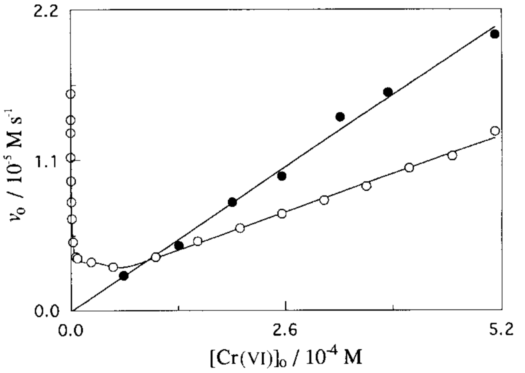

Fig. 2 Effect of Cr(VI) on the initial rate of disappearance of H_2O_2 (0.196 M) in the presence of a KH_2PO_4 (0.060 M)–K_2HPO_4 (0.060 M) buffer, at pH 6.71 and 25.0 °C. $[Cu(II)]_0 = 0$ (filled circles) and 1.20×10^{-4} M (empty circles).

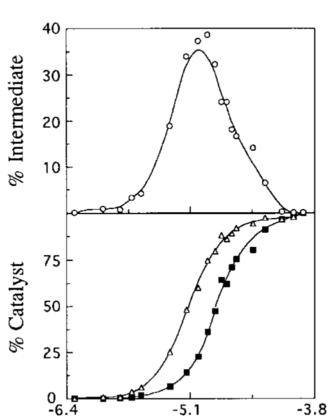

Fig. 3 Effect of Cu(II) on the percentage of total chromium present as catalyst (CrO_4^{2-}, bottom) and as a long-lived intermediate (top) for the decomposition of H_2O_2 (0.196 M) catalyzed by Cr(VI) (2.56×10^{-4} M) in the presence of a KH_2PO_4 (0.060 M)–K_2HPO_4 (0.060 M) buffer, at pH 6.71 and 25.0 °C. Time elapsed from the beginning of the reaction: 2 days (squares and circles) and 9 days (triangles).

In the absence of Cu(II), a plot of the initial rate *vs.* the initial concentration of Cr(VI) was linear (Fig. 2), indicating that the Cr(VI)/H_2O_2 reaction has a first-order dependence on the catalyst concentration. The slope of the plot was $(4.08 \pm 0.16) \times 10^{-2}$ s^{-1}. In the presence of a constant initial concentration of Cu(II) as catalyst, the addition to the solution of a variable amount of Cr(VI) led to a sudden decrease of the initial rate, the inhibitor-to-catalyst ratio necessary to decrease by 10% the value of v_0 now being $[I]/[C] = 2 \times 10^{-4}$, with I = Cr(VI) and C = Cu(II). Thus, at low inhibitor concentrations, the Cu(II)/H_2O_2 reaction was 250 times more sensitive toward inhibition by Cr(VI) than the Cr(VI)/H_2O_2 reaction toward inhibition by Cu(II).

After the initial rate passed through a minimum, a further increase of the initial concentration of Cr(VI) led to a region where v_0 increased linearly with $[Cr(VI)]_0$ (Fig. 2). The slope of this linear plot, corresponding to the Cr(VI)/H_2O_2 reaction inhibited by Cu(II), was $(2.13 \pm 0.06) \times 10^{-2}$ s^{-1}. From the ratio of the slopes of the two linear plots shown in Fig. 2, slope(Cr^{VI})/slope(Cr^{VI} + Cu^{II}) = 1.92 ± 0.13, it follows that the retarding effect provoked by Cu(II) on the Cr(VI)/H_2O_2 reaction has a superior limit at around 50% inhibition. The intercept of the v_0 *vs.* $[Cr(VI)]_0$ linear plot in the combined presence of Cr(VI) and Cu(II) was $(1.7 \pm 0.2) \times 10^{-6}$ M s^{-1}, and it provides the value of the initial rate of the Cu(II)/H_2O_2 reaction once inhibited by high enough concentration of Cr(VI). When compared with the value of the initial rate of the same reaction in the absence of Cr(VI), $v_0 = (1.59 \pm 0.05) \times 10^{-5}$ M s^{-1}, it follows that the retarding effect provoked by Cr(VI) on the Cu(II)/H_2O_2 reaction has a superior limit at around 90% inhibition.

Hence, we can see that neither of the two reactions can be totally suppressed by the other metal acting as an inhibitor, and that, of the two inhibition effects observed in the present work, the one provoked by Cr(VI) on the Cu(II)/H_2O_2 reaction is the more pronounced, both at low concentrations and at high concentrations (superior limit) of inhibitor.

We have attempted to inhibit the Cu(II)/H_2O_2 reaction with other oxidants than Cr(VI). Our results indicate that $NaVO_3$ also inhibits the reaction, whereas $K_3[Fe(CN)_6]$ shows a promoting effect similar to that provoked by Fe^{3+}.[19,20] In the hexacyanoferrate(III)/Cu(II)/hydrogen peroxide combined system we observed the formation of a red-brown precipitate. It might be insoluble $Cu_2[Fe(CN)_6]$ coming from the precipitation of Cu^{2+} with hexacyanoferrate(II) ion, the latter being previously formed from the electron-transfer reduction of hexacyanoferrate(III) ion by the reaction intermediate Cu^+.

On the other hand, we have reported before that, depending on the experimental conditions, Cr(VI) may act both as an oxidizing agent for H_2O_2 and as a true catalyst for its decomposition.[24] When acting as an oxidant, Cr(VI) is reduced to Cr(III), whereas, when acting as a catalyst, it is recovered unaltered at the end of the reaction. The behavior as a catalyst is favored by an increase of pH, or by a decrease of either $[H_2O_2]_0$ or buffer (phosphate) concentration.

In the present work, we have found that, in the Cr(VI)/H_2O_2 reaction, the behavior of the metal as a true catalyst is also favored by addition of Cu(II) to the solution. In a series of experiments at constant $[Cr(VI)]_0$ and variable $[Cu(II)]_0$, we determined the percentages of chromium present in the solution as CrO_4^{2-} from its absorbance at 372 nm. The measurements were taken during the reaction (2 days after the beginning), and repeated at its end (9 days after the beginning). In both cases the percentage of CrO_4^{2-} increased with rising $[\mathrm{Cu(II)}]_0$, but, at intermediate concentrations of Cu(II), the percentage of CrO_4^{2-} recovered at the end was higher than the percentage present in the solution one week before (Fig. 3, bottom). The difference between the two curves corresponds to the percentage of chromium present in the solution 2 days after the beginning as (at least) one long-lived intermediate. This intermediate accumulates in high concentration only at moderate values of $[\mathrm{Cu(II)}]_0$, whereas at both low and high concentrations of inhibitor it behaves as a very reactive, transient intermediate (Fig. 3, top).

Discussion

These findings suggest that the mechanisms involved in the two inhibition effects are very different, and they provide important mechanistic information on the $\mathrm{Cr(VI)}/H_2O_2$ and $\mathrm{Cu(II)}/H_2O_2$ reactions.

Decomposition catalyzed by chromium(VI)

The best explanation we have found for the inhibition caused by Cu(II) on the $\mathrm{Cr(VI)}/H_2O_2$ reaction is based on the property exhibited by Cu(II) of catalyzing the dismutation of superoxide free radicals.[25,26] As we will show, this hypothesis is completely consistent with our experimental findings, and it has given us some clues on the actual mechanism of the $\mathrm{Cr(VI)}/H_2O_2$ reaction. In the absence of Cu(II), the reaction mechanism would consist schematically of the following steps:

$$\mathrm{Cr(VI)} + H_2O_2 \xrightarrow[\text{slow}]{k_\mathrm{I}} \mathrm{Cr(V)} + O_2^{\bullet -} + 2\,H^+ \tag{1}$$

$$\mathrm{Cr(V)} + O_2^{\bullet -} \rightarrow \mathrm{Cr(IV)} + O_2 \tag{2}$$

$$\mathrm{Cr(IV)} + H_2O_2 \xrightarrow[\text{slow}]{H^+} \mathrm{Cr(III)} + O_2^{\bullet -} + 2\,H^+ \tag{3}$$

We have taken into consideration that the pK_a for the hydroperoxyl radical ($HO_2^{\bullet}$) is 4.8 at 25 °C.[25] Therefore, under the experimental conditions of the present work (pH 6.71), the superoxide radicals would be present predominantly in their basic form ($O_2^{\bullet -}$). These radicals would reduce Cr(V) to Cr(IV), thus allowing the reduction of the latter to Cr(III) by a second H_2O_2 molecule. The initial rate of disappearance of H_2O_2 at $[\mathrm{Cu(II)}] = 0$ would be given by:

$$v_0 = k_\mathrm{I}[\mathrm{Cr(VI)}]_0[H_2O_2]_0 \tag{4}$$

However, in the presence of a high enough concentration of Cu(II), the superoxide radicals would dismutate instead of acting as the reducing agent for Cr(V):

$$2\,O_2^{\bullet -} + 2\,H_2O \xrightarrow{Cu^{2+}} H_2O_2 + O_2 + 2\,HO^- \tag{5}$$

Now, the mechanism would be constituted only by eqns. (1) and (5). Since the hydrogen peroxide initially consumed in eqn. (1) is partially regenerated in eqn. (5), the initial rate of disappearance of H_2O_2 at $[\mathrm{Cu(II)}] = \infty$ would be given by:

$$v_0 = \tfrac{1}{2}k_\mathrm{I}[\mathrm{Cr(VI)}]_0[H_2O_2]_0 \tag{6}$$

This equation has been obtained by application of the steady-state approximation to the intermediate $O_2^{\bullet -}$. In fact, superoxide radicals are known to be relatively stable in comparison with other small free radicals (such as $HO^{\bullet}$, for instance). However, in the presence of a high concentration of Cu(II) as a catalyst for their dismutation, the superoxide radicals are expected to be reactive enough to justify the use of this approximation.

Thus, the mechanism proposed here is consistent with the superior limit of 50% inhibition by Cu(II) found for the $\mathrm{Cr(VI)}/H_2O_2$ reaction. It is worth noting that Bakac and Espenson have recently also found a 50% inhibition by Cu(II) in an apparently very different process, the UO_2^{2+}/O_2 reaction in acidic aqueous solution, and the explanation offered by them is also based on the dismutation of superoxide radicals catalyzed by Cu(II).[27]

Therefore, the finding that the $\mathrm{Cr(VI)}/H_2O_2$ reaction is inhibited by Cu(II) may be taken as an indirect proof of the involvement of superoxide radicals as transient intermediates in that reaction. Indirectly, it also implies that Cr(V) must be another intermediate, since a 2-electron reduction of Cr(VI) by H_2O_2 to yield Cr(IV) in the initial redox step would preclude the formation of superoxide radicals and thus would be in disagreement with the experimental results.

The mechanism is also consistent with the finding that Cu(II) prevents the reduction of Cr(VI) to Cr(III) by H_2O_2, since, according to our proposal, one of the steps involved in that process is the reduction of Cr(V) to Cr(IV) by $O_2^{\bullet -}$. That reduction is favored by the fact that Cr(V) and $O_2^{\bullet -}$ are formed together in eqn. (1). This means that for a small interval of time they are confined in the same solvent cage. However, in the absence of Cu(II), an increase of the pH also prevents the reduction of Cr(VI) to Cr(III).[24] This may be explained by the reaction:

$$\mathrm{Cr(IV)} + H_2O_2 \xrightarrow{HO^-} \mathrm{Cr(V)} + HO^{\bullet} + HO^- \tag{7}$$

We are thus suggesting that Cr(IV) may act both as a one-electron oxidant [eqn. (3)] and as a one-electron reductant [eqn. (7)] for H_2O_2. It is logical that these alternative pathways be controlled by the pH, since the protonation of Cr(IV) will result in a decrease of its electron density and thus in an enhancement of its oxidizing power. This strongly suggests that the intermediate Cr(IV) is present in this medium in the form of an easily protonatable oxyanion, such as $[CrO(O_2)_2]^{2-}$, given the tendency of Cr(IV) to form diperoxo complexes.[28]

It should be noticed that the mechanism proposed here for the $\mathrm{Cr(VI)}/H_2O_2$ reaction is only schematic, referring to the different oxidation states of chromium involved but without identification of the actual forms of the different species. A more elaborated mechanism would require considering the formation of several peroxo complexes of Cr(VI), Cr(V), and Cr(IV), with the possible involvement of phosphate ions (coming from the buffer) as ligands.[24]

Decomposition catalyzed by copper(II)

Our experimental results lead to a much more complicated mechanism for the $\mathrm{Cu(II)}/H_2O_2$ reaction. The finding that Cr(VI), even when present at $[\mathrm{Cr(VI)}]_0 \ll [\mathrm{Cu(II)}]_0$ and $[H_2O_2]_0$, is able to provoke an important inhibition on the decomposition of H_2O_2 indicates that its action does not take place by a direct inactivation of either Cu(II) or H_2O_2 and, so, it suggests that Cr(VI) acts as an oxidative scavenger for a very reactive (and very dilute) intermediate, most likely Cu(I). Moreover, we have found that Cr(VI), besides being very efficient as an inhibitor at low concentrations, at higher concentrations can suppress almost completely the $\mathrm{Cu(II)}/H_2O_2$ reaction (the inhibition limit is around 90%). The best explanation for this unexpectedly high degree of inhibition is that the reaction intermediate scavenged by Cr(VI), namely Cu(I), acts in the absence of inhibitor as the initiator of a free-radical chain reaction leading to the decomposition of H_2O_2. In fact, a free-radical chain mechanism has also been proposed by other authors for this reaction.[29,30] The mechanism we envisage is the following:

$$\mathrm{Cu(II)} + H_2O_2 \rightleftarrows CuOOH^+ + H^+ \tag{8}$$

$$CuOOH^+ + H_2O \rightleftarrows \mathrm{Cu(I)} + O_2^{\bullet -} + H_3O^+ \tag{9}$$

$$\mathrm{Cu(I)} + H_2O_2 \rightarrow \mathrm{Cu(II)} + HO^{\bullet} + HO^- \tag{10}$$

$$HO^{\bullet} + H_2O_2 \rightarrow O_2^{\bullet -} + H_3O^+ \tag{11}$$

$$O_2^{\bullet -} + \mathrm{Cu(II)} \rightarrow CuOO^+ \tag{12}$$

$$CuOO^+ \rightarrow \mathrm{Cu(I)} + O_2 \tag{13}$$

$$2\,O_2^{\cdot -} + 2\,H_2O \xrightarrow{Cu^{2+}} H_2O_2 + O_2 + 2\,HO^- \qquad (14)$$

Formation in the first step of hydroperoxide intermediates of the type $CuOOH^+$ is known to occur in the reactions of H_2O_2 with many transition metal ions.[31–33] Decomposition of this intermediate would yield Cu(I), which (in the absence of any organic substrate) is known to react with a second H_2O_2 molecule in a Fenton-like reaction to yield a hydroxyl free radical.[21] The reaction of the latter with a third H_2O_2 molecule to yield a superoxide free radical is thermodynamically favored by the fact that the radical formed as product is a much less energetic species than the radical destroyed as reactant, and it is a much studied, well-known reaction.[34–36] The superoxide radical formed in that reaction may coordinate to Cu(II) to yield superoxocopper(II) ion, $CuOO^+$. There is solid kinetic evidence for the formation of this species from the reaction of Cu(I) with O_2 in aqueous acetonitrile.[37] Although superoxide radicals cannot react directly with the substitutionally-inert Cr(III),[38] they can probably coordinate with the more labile Cu(II) aqua complexes. The unimolecular decomposition of $CuOO^+$ would yield Cu(I) and O_2, in a reaction similar to the one known to take place for the decomposition of $CrOO^{2+}$.[39,40] The Cu(I) formed in eqn. (13) may react again with H_2O_2 in eqn. (10), so that eqns. (10)–(13) constitute the cycle of the free-radical chain mechanism. The chain termination step would occur by dismutation of the superoxide radicals [eqn. (14)].

The inhibition caused by Cr(VI) on the Cu(II)/H_2O_2 reaction would be explained by its ability to act as an oxidative scavenger for the initiator of the chain:

$$\mathrm{Cr(VI)} + Cu^+ \rightarrow \mathrm{Cr(V)} + Cu^{2+} \qquad (15)$$

This reaction is thermodynamically favorable, given the redox potentials involved: E^0 (Cu^{II}/Cu^{I}) = 0.158 V,[41] and E^0 (Cr^{VI}/Cr^{V}) $\geqslant 0.52$ V (estimated from a Frost diagram for chromium),[42] at 25 °C.

Moreover, the mechanism proposed here for the Cu(II)/H_2O_2 reaction accommodates well a number of experimental results from our laboratory, as our finding that the reaction (in aqueous phosphate media) shows base catalysis, since one proton is liberated in the reversible formation of hydroperoxocopper(II) ion [eqn. (8)]. The finding that the reaction has a nondefined kinetic order in Cu(II) might be related to the ability of the latter to catalyze the dismutation of the superoxide radicals. Given the considerable complexity of the mechanism proposed, that ability might lead to a dual situation. Under some particular experimental conditions (for instance, those corresponding to Fig. 1), provided that the rate constant corresponding to the reverse direction of eqn. (9) is high enough, the superoxide-scavenging effect of Cu(II) might benefit the decomposition of H_2O_2 by increasing the number of Cu(I) ions that can escape from the solvent cage that they are sharing with the superoxide radicals [Cu(I) and $O_2^{\cdot -}$ are formed together in eqn. (9)], so that the number of Cu(I) ions available to initiate the chain reaction would also increase. Under these conditions, the apparent kinetic order of Cu(II) would be higher than unity. However, if the depletion of superoxide radicals caused by Cu(II) is too high, a further increase of the concentration of the latter might lead to a situation where the scavenging effect of Cu(II) would disfavor the decomposition rate by its participation in the termination of the chain reaction [eqn. (14)]. Under these conditions, the apparent kinetic order of Cu(II) would be lower than unity. We can thus see that, according to the mechanism proposed here, the complex dependence observed for the reaction rate on the Cu(II) concentration arises from its dual effect as a superoxide scavenger, favoring both the chain-initiation step [eqn. (10)] and the chain-termination step [eqn. (14)].

Our mechanism can also explain some interesting results reported by Tachon, who observed that superoxide dismutase increased the rate of the DNA single-strand cleavage produced by the Cu(II)/H_2O_2 system.[43] Since this type of DNA lesion is likely to be caused by the action of hydroxyl free radicals,[44,45] the addition to the solution of superoxide dismutase will enhance the formation of these lesions by depleting the concentration of superoxide free radicals,[25] resulting consecutively in an increase of the number of Cu(I) ions that can escape from the solvent cage in which they were reversibly formed with superoxide radicals [eqn. (9)], in an increase of the chain-initiation rate [eqn. (10)] and, so, in an increase of the rate of formation of hydroxyl free radicals. Hence, both our and Tachon's results strongly suggest that the rate constant associated with the reverse direction of eqn. (9) is very high.

Nature of the long-lived intermediate

We have also observed the existence of a long-lived intermediate in the Cr(VI)/Cu(II)/H_2O_2 combined system. This intermediate seems to be much more stable at moderate concentrations of Cu(II) than at either low or high values of $[\mathrm{Cu(II)}]_0$ (Fig. 3, top). We think that it might be a diperoxochromium(V) complex [although a tetraperoxochromium(V) complex[46,47] cannot be discarded]. At low $[\mathrm{Cu(II)}]_0$ this intermediate would be reduced to Cr(IV) by superoxide free radicals [eqn. (2)]. On the contrary, when present in high concentration, Cu(II) might catalyze the oxidation of that intermediate to Cr(VI). We propose for the latter reaction the following mechanism:

$$[Cr^{V}O(O_2)_2]^- + \mathrm{Cu(II)} \rightarrow [Cr^{VI}O(O_2)_2Cu^{I}]^+ \qquad (16)$$

$$[Cr^{VI}O(O_2)_2Cu^{I}]^+ + H_2O_2 \rightarrow [Cr^{VI}O(O_2)_2(OH)]^- + \mathrm{Cu(II)} + HO^{\cdot} \qquad (17)$$

Actually, the formula proposed for the peroxo Cr(VI) complex formed in eqn. (17), $[Cr^{VI}O(O_2)_2(OH)]^-$, is consistent with the one known to be applicable to a transient violet intermediate involved in the Cr(VI)/H_2O_2 reaction in neutral or very slightly acidic solutions.[48]

Recent results reported by Kortenkamp and coworkers suggest that superoxo or peroxo complexes involving either Cr(IV) or Cr(V) might be the species responsible for chromium-induced DNA damage.[49] This might be related to their capability to yield highly reactive hydroxyl free radicals, either directly [eqns. (7) and (18)] or, as proposed here, through a Cu(II)-catalyzed reaction pathway [eqns. (16) and (17)].

$$\mathrm{Cr(V)} + H_2O_2 \rightarrow \mathrm{Cr(VI)} + HO^{\cdot} + HO^- \qquad (18)$$

However, it should be noticed that, according to the mechanisms now presented, the finding that Cr(VI) is capable of provoking about a 90% inhibition in the rate of the Cu(II)/H_2O_2 reaction indicates that the rate constant associated with the production of hydroxyl free radicals by the Cu(I)/H_2O_2 reaction [eqn. (10)] must be at least 10 times higher than the rate constant corresponding to the production of hydroxyl free radicals by the Cr(V)/H_2O_2 reaction [eqn. (18)]. Given that the latter might be catalyzed by Cu(II) [eqns. (16) and (17)], that ratio must be considered as an inferior limit [$k(Cu^{I} \rightarrow HO^{\cdot})/k(Cr^{V} \rightarrow HO^{\cdot}) \geqslant 10$]. Again, this is consistent with the redox potentials involved.[41,42] This seems to suggest that, under equal intracellular concentrations, Cu(I) might be a more hazardous species for biological organisms than Cr(V).

On the decomposition catalyzed by iron(III)

Although it was not the objective of the present work, it might be interesting to notice that our results might throw some light on the much studied but still discussed mechanism of the Fe(III)/H_2O_2 reaction. On one hand, it is known that, contrary to the Cr(VI)/Cu(II)/H_2O_2 system, in the Fe(III)/Cu(II)/H_2O_2 combined system there is a mutual catalyst activation.[19,20] On the other hand, Tachon found that, contrary also to the effect produced in the case of the Cu(II)/H_2O_2 system, superoxide dismutase decreased the rate of the DNA single-strand cleavage produced by the Fe(III)/H_2O_2 system.[43]

These results can be easily accommodated in a mechanism similar to the one proposed here for the Cu(II)/H_2O_2 reaction [eqns. (8)–(14)] where the roles of Cu(II) and Cu(I) would respectively be played by Fe(III) and Fe(II). The main difference, however, would be that in the latter case the rate constant associated with the reverse direction of eqn. (9) might be much lower, so that for the mechanism corresponding to the Fe(III)/H_2O_2 system the equivalent of eqn. (9) might be an essentially irreversible reaction. This is indeed in agreement with the fact that the Cu(I) → Cu(II) oxidation by the superoxide radical to yield the hydroperoxide is thermodynamically more favorable than its Fe(II) → Fe(III) counterpart [redox potentials involved: E^0 (Cu^{II}/Cu^{I}) = 0.158 V and E^0 (Fe^{III}/Fe^{II}) = 0.770 V at 25 °C].[41]

Moreover, the opposite effects produced by Cr(VI) (inhibitor) and by Fe(III) (promoter) on the rate of the Cu(II)/H_2O_2 reaction may have a common cause, the ability of both Cr(VI) and Fe(III) to act as oxidative scavengers for the chain initiator Cu(I) (both reactions are thermodynamically favored). However, whereas in the former case the product would be a poor free-radical generator [Cr(V), present as a long-lived intermediate capable of reacting with H_2O_2, eqn. (18), only very slowly], in the latter case the product would be an excellent free-radical generator [Fe(II), present as a very reactive intermediate yielding hydroxyl free radicals by a fast reaction with H_2O_2]. The promoting effect produced by Fe(III) on the Cu(II)/H_2O_2 reaction is easily explained if we take into consideration that Cu(I) would be replaced by Fe(II) as the chain initiator, thus avoiding the difficulty of Cu(I) to escape from the solvent cage shared with the superoxide free radical [Cu(I) is reoxidized by $O_2^{\bullet -}$ much more easily than Fe(II)].

The opposite results found by Tachon for the effect of superoxide dismutase on the Cu(II)/H_2O_2 and Fe(III)/H_2O_2 systems as producers of DNA lesions may also be explained by our hypothesis that eqn. (9) is reversible in the first case and essentially irreversible in the latter. Thus, addition of this enzyme to the Cu(II)/H_2O_2 system would result in an increase of the number of Cu(I) ions that can escape from the solvent cage (shared with superoxide radicals) to initiate the chain reaction and, so, in an increase of the rate of production of hydroxyl free radicals. However, addition of the enzyme to the Fe(III)/H_2O_2 system would result in an increase of the rate of termination of the chain reaction by dismutation of the superoxide radicals and thus in a decrease of the rate of production of hydroxyl free radicals.

Finally, taking some of the results found in the literature along with our own results, it can be concluded that, due to their different abilities to act as generators of extremely reactive hydroxyl free radicals, under equal intracellular concentrations Fe(II) might be a more hazardous species for biological organisms (from the point of view of problems such as premature aging and carcinogenesis) than Cu(I), and the latter more dangerous than Cr(V).

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