On *N*-acetylcysteine. Part II. Oxidation of *N*-acetylcysteine by hydrogen peroxide: kinetic study of the overall process

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The oxidation kinetics of *N*-acetylcysteine (RSH) by hydrogen peroxide has been studied at neutral pH at different concentration ratios from 0.2 to 20 (4×10^{-4} mol $L^{-1} \leq [RSH]_0 \leq 2 \times 10^{-2}$ mol L^{-1}, 10^{-4} mol $L^{-1} \leq [H_2O_2]_0 \leq 10^{-2}$ mol L^{-1}). In all the cases studied, *N*-acetylcystine (RSSR) is the only oxidized product formed. Our kinetic data have focused on the importance of the concentration ratio to reach the stoichiometric oxidation of *N*-acetylcysteine by hydrogen peroxide. Indeed non-stoichiometric oxidation of RSH occurs at relatively low concentration ratios ($R < 2.5$) whereas stoichiometric oxidation is observed when $R > 2.5$. Moreover, it has been shown that in the first minutes of the reaction there is the formation of a complex between RSH and H_2O_2, the stoichiometry of the complex being RSH concentration-dependent for a given R ($R > 2.5$). Reaction mechanisms have been quantitatively established and the k values of each step determined.

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On a étudié la cinétique de l'oxydation de la *N*-acétylcystéine (RSH) par le peroxyde d'hydrogène, à un pH neutre, à divers rapports de concentration allant de 0,2 à 20 (4×10^{-4} mol $L^{-1} \leq [RSH]_0 \leq 2 \times 10^{-2}$ mol L^{-1}, 10^{-4} mol $L^{-1} \leq [H_2O_2]_0 \leq 2 \times 10^{-2}$ mol L^{-1}). Dans tous les cas étudiés, le seul produit formé est la *N*-acétylcystine (RSSR). Nos données cinétiques mettent l'accent sur l'importance du rapport de concentrations nécessaire à l'atteinte de la stoechiométrie. En effet, l'oxydation non stoechiométrique de RSH se produit pour des rapports de concentrations relativement faibles ($R < 2,5$) alors que l'oxydation stoechiométrique est observée lorsque $R > 2,5$. De plus, on a montré que dans les premières minutes de la réaction, il y a formation d'un complexe entre le RSH et le H_2O_2 dont la stoechiométrie, pour une valeur donnée de R ($R > 2,5$), dépend de la concentration du RSH. On a établi quantitativement les mécanismes de réaction et on a déterminé les valeurs de k de chacune des étapes.

[Traduit par la rédaction]

Introduction

The univalent reduction of molecular oxygen results in the formation of reactive oxygen intermediates, so-called reactive oxygen species, that may disrupt cellular function by direct or indirect oxidation of cellular lipids, proteins, and nucleic acids (1–7). It is clear that the cells have developed an extensive collection of antioxidant defence. However, there are situations, both physiological and pathological, in which cells are exposed to an unusually high load of oxidants and free radicals (oxidative stress). Elevated quantities of reactive oxygen species, including H_2O_2, are also produced during the metabolism of certain xenobiotics and are often associated with the initiation of lipid peroxidation and acute toxicity (8, 9). Many authors have implicated reactive oxygen species as important causative agents of aging and of several human diseases. Inactivations of unusual quantities of reactive oxygen species by antioxidant therapy has been proposed by many authors (10–12).

In this framework *N*-acetylcysteine (RSH) has been widely used as an antioxidant in vivo and in vitro. For example, it decreases the toxicity of diquat to hepatocytes (13), protects animals against paracetamol hepathotoxicity (14), and it serves as the antidote of choice for paracetamol overdose in humans (15). It has received attention as an antioxidant for components of cigarette smoke (16, 17) and for treatment of various respiratory diseases (18). Recently, there have been several reports suggesting that *N*-acetylcysteine is cardioprotective against ischemia/reperfusion injury (19, 20) and finally several authors have proposed *N*-acetylcysteine as a therapeutic agent in AIDS (Acquired Immunodeficiency Syndrome) (21–25).

Although it is not clear the mechanisms by which *N*-acetylcysteine might be able to act as an oxidant scavenger in vivo, some authors suggest that the antioxidant properties of RSH come as a result of direct of oxidant species and others propose that RSH acts as a precursor of the natural antioxidant glutathione; or a combination of these two modes of action.

Concerning the antioxidant action of *N*-acetylcysteine towards hydrogen peroxide, it has been shown to react slowly with H_2O_2 (26), and an approximate rate of reaction of *N*-acetylcysteine with H_2O_2 was given by Aruoma et al. (27) (0.85 mol^{-1} L s^{-1}) by following only the loss of *N*-acetylcysteine in the reacting mixture in less than one half-live.

We have therefore investigated the mechanism of oxidation of RSH by H_2O_2 to better understand the process by which RSH might be able to act as a direct oxidant scavenger. The kinetics of this reaction has been studied in neutral aerated aqueous medium at different concentrations: 4×10^{-4} mol $L^{-1} \leq [RSH]_0 \leq 2 \times 10^{-2}$ mol L^{-1}, 10^{-4} mol $L^{-1} \leq [H_2O_2]_0 \leq 10^{-2}$ mol L^{-1}, and at different ratios of $[RSH]_0/[H_2O_2]_0$ (0.2 to 20).

Materials and methods

See ref. 29 for the experimental and theoretical approaches of the *N*-acetylcysteine/H_2O_2 complexation.

Experimental results

Kinetics for the reaction of *N*-acetylcysteine with hydrogen peroxide are functions of the concentration ratios R ($R = [RSH]_0/[H_2O_2]_0$). For a given value of R, they depend on the

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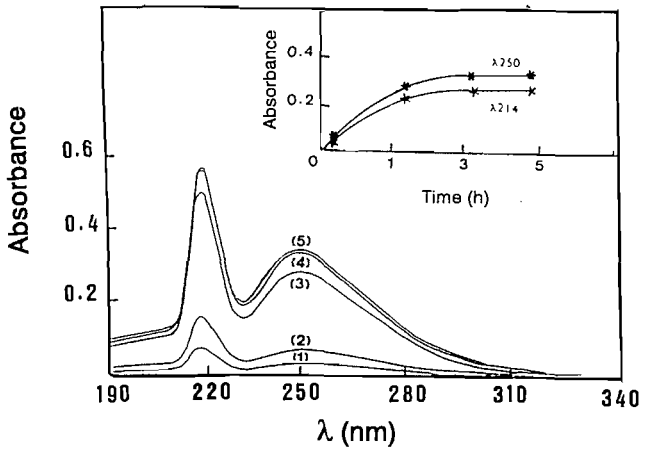

FIG. 1. Differential absorption spectra of the reaction mixture: $R = [RSH]_0/[H_2O_2]_0 = 0.2$, $[RSH]_0 = 2 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-2}$ mol L^{-1}, phosphate buffer 10^{-2} mol L^{-1}, pH 7, $l = 0.875$ cm. Reference: $[RSH]_0 = 4 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 2 \times 10^{-2}$ mol L^{-1} ($l = 0.4375$ cm) (two chambers cell, see ref. 29, Materials and methods section). (*1*) 03 min, (*2*) 0.9 min, (*3*) 1 h 10 min, (*4*) 2 h 10 min, (*5*) 3 h after the start of the reaction. In inset, evolutions of the absorbances as a function of time at λ 250 nm and λ 214 nm.

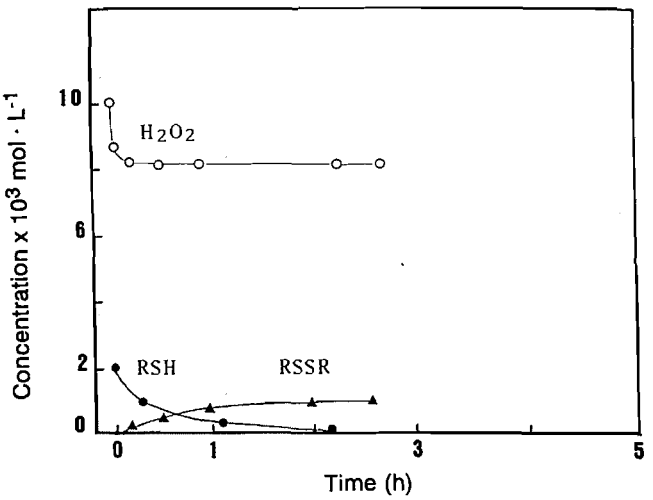

FIG. 2. Kinetic evolution of hydrogen peroxide (○), *N*-acetylcysteine (●), and *N*-acetylcystine (▲) as a function of time. $R = [RSH]_0/[H_2O_2]_0 = 0.2$, $[RSH]_0 = 2 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-2}$ mol L^{-1}, phosphate buffer 10^{-2} mol L^{-1}, pH 7.

concentrations of RSH. Thus we have studied reactions corresponding to $R < 2.5$ (see § I); $R \geq 2.5$ (see § II); and finally for a given R ($R > 2.5$), the effect of the thiol concentration (see § III).

I. Kinetic results for R < 2.5

(a) Differential absorption spectra of the reaction mixture

In Fig. 1, as an example, are reported the differential absorption spectra of a solution containing $[RSH]_0$ 2×10^{-3} mol L^{-1} and $[H_2O_2]_0$ 10^{-2} mol L^{-1} ($R = 0.2$, phosphate buffer 10^{-2} mol L^{-1}, pH 7) at various times after mixing. The differential absorption spectrum (*1*) recorded 3 min after mixing shows two maxima at 214 and 250 nm. These absorptions increase until the end of reaction (spectra (*2*) to (*5*)).

The kinetics at 250 and 214 nm are reported in the inset of Fig. 1. These curves appear to increase rather quickly for an hour, then they grow slowly until the end of reaction. These absorptions have been attributed to RSSR ($\Delta\epsilon_{250} = 330$ mol^{-1} L cm^{-1}). At the end of the reaction, the disulfide concentration is 1×10^{-3} mol L^{-1} which corresponds to the total transformation of RSH into RSSR. Consequently, we can assume that the thiol is stoichiometrically oxidized into RSSR.

(b) Evolution of RSH, H_2O_2, and RSSR

For the same experiment ($[RSH]_0 = 2 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-2}$ mol L^{-1}, $R = 0.2$, phosphate buffer 10^{-2} mol L^{-1}, pH 7) we have reported in Fig. 2, the simultaneous changes in $[H_2O_2]$, [RSH], and [RSSR] as a function of time. Note that the concentration of RSSR has been determined as previously described in I(*a*). As can be seen, the concentration of hydrogen peroxide decreases abruptly, while RSH disappears slowly and RSSR emerges slowly. Indeed, the changes in concentration end in about 2 h for RSH and RSSR whereas for H_2O_2 the change ends in about 30 min after reaction starts. Moreover, the initial drop in H_2O_2 (75% of the total H_2O_2 decay) occurs in less than 2 min. Similar trends have also been observed in $R = 1$ (see ref. 29, Experimental results). This drop has been attributed to the rapid complexation of H_2O_2 by RSH without oxidizing the thiol group in RSH. Thermodynamic equilibrium constant of complexation has been estimated using the method described in ref. 29. In the case of $R = 0.2$ we have found $K = 850$, which is in agreement with the results in ref. 29 (Discussion) ($K = 900 \pm 100$).

At the end of the reaction (~2 h), the final concentrations of RSH, H_2O_2, and RSSR equal to 0, 8×10^{-3} mol L^{-1} and 10^{-3} mol L^{-1}. Hence the amounts of RSH and H_2O_2 consumed are 2×10^{-3} mol L^{-1} and 2×10^{-3} mol L^{-1}. At the end of the reaction, 1 mol of RSH reacts with 1 mol of H_2O_2 to give half a mole of RSSR. The same has been observed for three other experiments ($R = 0.4$, 0.6, 1, see Table 1).

To follow the course of the hydrogen peroxide, we performed the following tests: (*i*) At the end of an experiment ($R = 1$) when no free H_2O_2 was present we have added catalase (10^{-7} mol L^{-1}). We observed the formation of the dioxygen whose concentration equals to the final concentration of RSSR. (*ii*) When we added an excess of RSH at the end of the experiment ($R = 1$) when no free H_2O_2 was present we observed the formation of extra RSSR. (*iii*) We have tested the eventual formation of dioxygen during our kinetic studies ($R = 1$) by Gilson Oxygraph. Any formation of dioxygen observed was due to disproportionation.

Thus, these tests demonstrated the residual oxidizing power of the end product, which might come from the presence of a "bound hydrogen peroxide compound" or from a peroxide bond in the final oxidized product [RSSR...H_2O_2]. It should be noted that these three tests were performed at $R = 1$, since in other cases ($R = 0.2$, 0.4, 0.6) there is always free H_2O_2 remaining in the medium at the end of the reaction. The free H_2O_2 may interfere with the bound hydrogen peroxide.

II. Kinetic results for R > 2.5

(a) Differential absorption spectra of the reaction mixture

As can be seen in Fig. 3 ($[RSH]_0 = 4 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 5 \times 10^{-4}$ mol L^{-1} ($R = 8$, phosphate buffer 10^{-2} mol L^{-1}, pH 7) the differential spectra exhibit two maxima at 214 and 250 nm, showing the formation of RSSR as a function of

TABLE 1. The final balance of the reactions having the concentration ratios R ($R = [RSH]_0/[H_2O_2]_0$) greater than 2.5[a]

$[H_2O_2]_0$ 10^3 mol L^{-1}	$[RSH]_0$ 10^3 mol L^{-1}	$R = [RSH]/[H_2O_2]$	$[RSH]_c$ 10^3 mol L^{-1}	$[H_2O_2]_c$ 10^3 mol L^{-1}	$[RSSR]_f$ 10^3 mol L^{-1}
10	2	0.2	2	2	1
5	2	0.4	2	2	1
1	0.6	0.6	0.6	0.5	0.3
1	1	1	1	1	0.5
1	2.5	2.5	1.73	0.9	0.73
0.8	2.5	3	2	0.75	0.75
0.5	4	8	1	0.5	0.42
0.5	5	10	1	0.5	0.46
0.1	1.5	15	0.18	0.10	0.10
0.125	2.5	20	0.25	0.125	0.12

[a]$[RSH]_c$ = [RSH] consumed, $[H_2O_2]_c$ = $[H_2O_2]$ consumed, and $[RSSR]_f$ = final value of [RSSR].

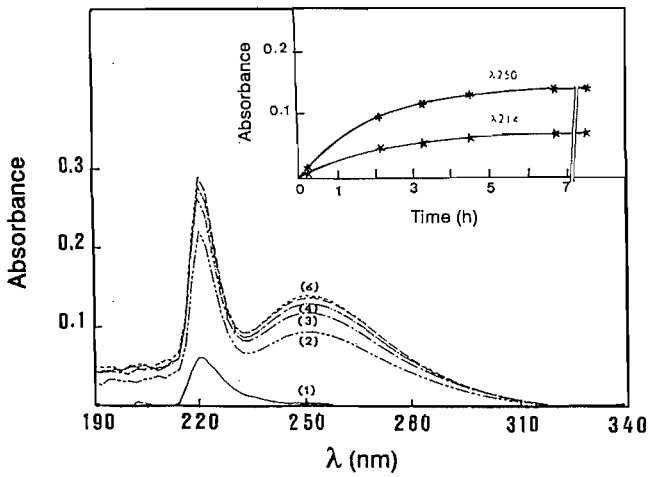

FIG. 3. Differential absorption spectra of the reaction mixture: $R = [RSH]_0/[H_2O_2]_0 = 8$, $[RSH]_0 = 4 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 5 \times 10^{-4}$ mol L^{-1}, phosphate buffer 10^{-2} mol L^{-1}, pH 7, l = 0.875 cm. Reference: $[RSH]_0 = 8 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-3}$ mol L^{-1} (l = 0.4375 cm) (two chambers cell, ref. 29 Materials and methods section). (*1*) 3 min, (*2*) 2 h 10 min, (*3*) 3 h 15 min, (*4*) 4 h 30 mn, (*5*) 5 h 20 min, (*6*) 6 h 40 mn after the start of the reaction. In inset, evolutions of the absorbances as a function of time at λ 250 nm and λ 214 nm.

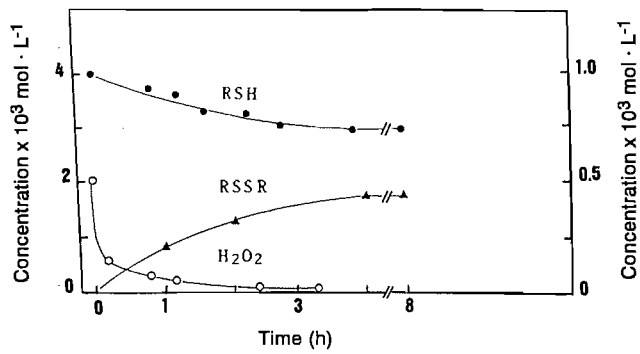

FIG. 4. Kinetic evolution of hydrogen peroxide (○), *N*-acetylcysteine (●), and *N*-acetylcystine (▲) as a function of time. $R = [RSH]_0/[H_2O_2]_0 = 8$, $[RSH]_0 = 4 \times 10^{-3}$ mol L^{-1} (left scale), $[H_2O_2]_0 = 5 \times 10^{-4}$ mol L^{-1} and [RSSR] (right scale), phosphate buffer 10^{-2} mol L^{-1}, pH 7.

time (spectra (*1*)–(*5*)). Figure 3 inset shows the absorbances at 214 and 250 nm. These absorptions increase as a function of time, until they reach a plateau in ~7 h. Notice that these spectra are similar to that observed for $R < 2.5$, meaning that the only oxidized final product is RSSR. At the end of the reaction, the concentration of RSSR is found to be 4.2×10^{-4} mol L^{-1}, showing that 84% of H_2O_2 have oxidized RSH to RSSR.

(b) Changes in [RSH], [H_2O_2], and [RSSR]

For the same experiment ($[RSH]_0 = 4 \times 10^{-3}$ mol L^{-1}, $[H_2O_2]_0 = 5 \times 10^{-4}$ mol L^{-1}, $R = 8$, phosphate buffer 10^{-2} mol L^{-1}, pH 7) it is reported in Fig. 4 the changes in [RSH], $[H_2O_2]$, and [RSSR] with time. We notice the decrease in $[H_2O_2]$ and the slow changes in [RSH], [RSSR], as in the case of $R < 2.5$. The rapid disappearance of H_2O_2 is related to the initial complexation of RSH by H_2O_2. Thermodynamic equilibrium constant of complexation in this case is 875, which is in agreement with the equilibrium constant determined in ref. 29, Discussion section.

At the end of the reaction (~7 h), the final concentrations of RSH, H_2O_2, and RSSR equal to 3×10^{-3}, 0, and 4.2×10^{-4} mol L^{-1}. Thus at the end of the reaction, 2 mol of RSH have reacted with 1 mol of H_2O_2 to give 1 mol of RSSR. The same has been observed for all experiments having concentration ratios of $R > 2.5$. This is the normally accepted stoichiometry when excess thiol reacts with hydrogen peroxide.

III. Effect of different concentrations of RSH on a given R ($R > 2.5$)

We have studied the effect of higher RSH concentrations when R = 10, 15, and 20 ($[RSH]_0 \geq 10^{-2}$ mol L^{-1}). Figure 5 ($[RSH]_0 = 2 \times 10^{-2}$ mol L^{-1}, $[H_2O_2]_0 = 1.33 \times 10^{-3}$ mol L^{-1} (R = 15, phosphate buffer 10^{-2} mol L^{-1}, pH 7) shows the changes in concentrations of H_2O_2, RSH, and RSSR with time. As in previous experiments (see § I and II), there is an initial fast drop in $[H_2O_2]$, and while about 65% of H_2O_2 disappears, RSH does not oxidize into RSSR. Then, the thiol concentration decreases slowly for 4 h while at the same time RSSR is slowly formed and H_2O_2 slowly disappears. The overall reaction shows that 2 mol of RSH react with 1 mol of H_2O_2 to give 1 mol of RSSR, a stoichiometry characteristic of $R > 2.5$. However, complexation of H_2O_2 cannot be explained by 1 mol of H_2O_2 reacting with 1 mol of RSH (reaction [1]). Indeed, with reaction [1], the thermodynamic equilibrium constant for complexation is found to be smaller by one order of magnitude (≈100 instead of 900 ± 100). It is the same observation for the two R's (R = 10, $[RSH]_0 = 10^{-2}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-3}$ mol L^{-1}; and $R = 20$, $[RSH]_0 = 2 \times 10^{-2}$ mol L^{-1}, $[H_2O_2]_0 = 10^{-3}$ mol

TABLE 2. Determination of equilibrium constant for the formation of the complex [RSH...H_2O_2...RSH] : $[C]_{eq}$

$[RSH]_0$ 10^3 mol L^{-1}	$[H_2O_2]_0$ 10^3 mol L^{-1}	R	$[RSH]_{eq}$ 10^3 mol L^{-1}	$[H_2O_2]_{eq}$ 10^3 mol L^{-1}	$[C]_{eq}$ 10^3 mol L^{-1}	K
10	1	10	9	0.5	0.5	111
20	1.33	15	18.2	0.45	0.88	107
20	1	20	19.4	0.32	0.68	110

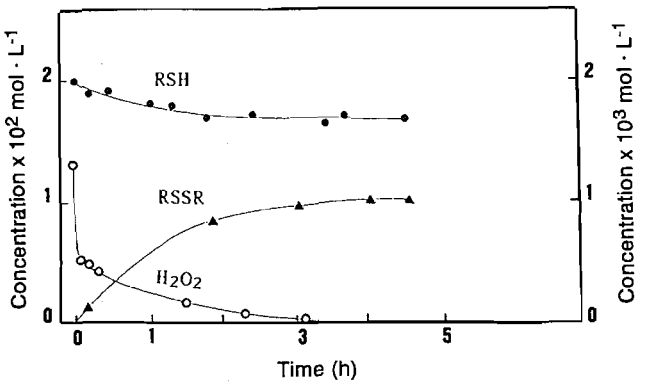

FIG. 5. Kinetic evolution of hydrogen peroxide (○), *N*-acetylcysteine (●), and *N*-acetylcystine (▲) as a function of time. $R = [RSH]_0/[H_2O_2]_0 = 15$, $[RSH]_0 = 2 \times 10^{-2}$ mol L^{-1} (left scale), $[H_2O_2]_0 = 1.33 \times 10^{-3}$ mol L^{-1} and [RSSR] (right scale), phosphate buffer 10^{-2} mol L^{-1}, pH 7.

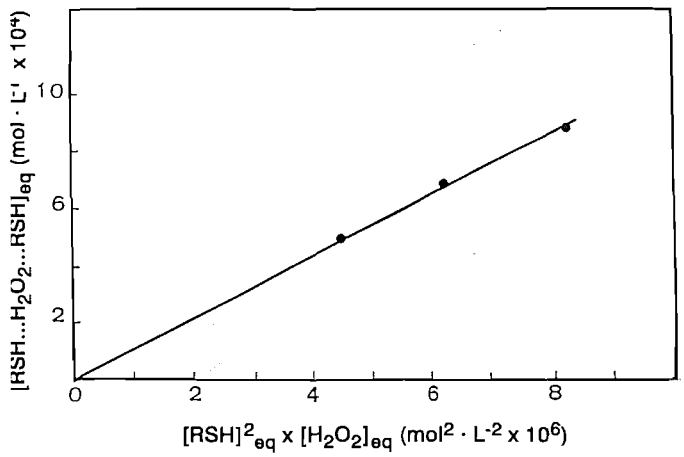

FIG. 6. Chelate concentration at equilibrium $[RSH...H_2O_2...RSH]_{eq}$ as a function of $[RSH]_{eq}^2 \times [H_2O_2]_{eq}$. Phosphate buffer 10^{-2} mol L^{-1}, pH 7.

L^{-1}) for which the equilibrium constants are the same order of magnitude (≈ 100). Hence we propose yet another type of complexation reaction in which 2 mol of RSH complex with 1 mol of H_2O_2 (reaction [2]). This mechanism is compatible with high concentrations of RSH in the medium.

[2] $2\ RSH + H_2O_2 \rightleftarrows [RSH...H_2O_2...RSH]$

Equilibrium constant K_2 is determined by measuring [RSH], $[H_2O_2]$, and [RSH...H_2O_2...RSH] at equilibrium. These values are shown in Table 2. Note that the titanium sulphate method allows one to assay only the free hydrogen peroxide and not the bound hydrogen peroxide in the chelate. Concentration of H_2O_2 at equilibrium, $[H_2O_2]_{eq}$, has been determined by extrapolating to $t = 0$ the subsequent slow decrease in $[H_2O_2]$. The concentration of chelate at equilibrium has been deduced from the difference $[H_2O_2]_0 - [H_2O_2]_{eq}$. Ellman's reagent, which assays the thiol concentration, gives the sum of free *N*-acetylcysteine and its chelated form. Hence the concentration of *N*-acetylcysteine at equilibrium is calculated as the difference between the initial concentration of RSH and two times concentration of the chelate at equilibrium. In Fig. 6 are reported the values of $[RSH...H_2O_2...RSH]_{eq}$ as a function of the product $[RSH]^2_{eq} \times [H_2O_2]_{eq}$. The slope of the straight line is 110 ± 5.

Discussion

We propose the following scheme to interpret our results:

[1] $RSH + H_2O_2 \rightleftarrows [RSH...H_2O_2]$

[2] $RSH + H_2O_2 \rightleftarrows [RSH...H_2O_2...RSH]$

[3] $2\ [RSH...H_2O_2] \rightarrow [RSSR...H_2O_2] + 2H_2O$

[4] $[RSSR...H_2O_2] + 2RSH \rightarrow 2\ RSSR + 2H_2O$

[5] $[RSH...H_2O_2...RSH] \rightarrow RSSR + 2H_2O$

Reaction [1] represents the complexation of H_2O_2 by RSH to yield [RSH...H_2O_2]. This reaction has already been discussed in ref. 29, the preceding paper. The equilibrium constant at low concentrations of RSH ($<10^{-2}$ mol L^{-1}) has been determined. Experimental K_1 is 880 ± 80 (see preceding paper). The overall reaction [2] is the complexation of H_2O_2 by 2 mol of RSH when [RSH] is high ($\geq 10^{-2}$ mol L^{-1}). The equilibrium constant K_2 has been found to equal 110 ± 5. Reaction [3] involves the formation of complexed RSSR from the reaction of [RSH...H_2O_2] with itself. Similar reaction has been proposed for glutathion (28). The formation of RSSR is consistent with the following experimental results: (*i*) at the end of the reaction, when there is no free H_2O_2, the addition of catalase stimulates the formation of oxygen and (*ii*) the addition of an excess of RSH leads to the formation of an extra RSSR equal to bounded H_2O_2 (see Experimental results and discussion in preceding paper). Reaction [3] takes place when a non-stoichiometric process is observed, that is, $R < 2.5$. Reaction [4] represents the action of RSH with the complexed RSSR. This occurs when R is > 2.5 (the concentration of the remaining thiol is less than 10^{-2} mol L^{-1}). Similar reaction has been suggested for glutathion (28). We propose that reaction [5] is an unimolecular decay of the second complex [RSH...H_2O_2...RSH] to give the non-complexed disulfide RSSR.

The corresponding rate constants have been determined by numerical analyses of the kinetic differential equations using an iteration program. This calculation has been performed for three different experimental conditions: (*1*) $R < 2.5$, $[RSH] < 10^{-2}$ mol L^{-1}; (*2*) $R > 2.5$, $[RSH] < 10^{-2}$ mol L^{-1}; (*3*) $R > 2.5$, $[RSH] > 10^{-2}$ mol L^{-2}.

In the first case ($R < 2.5$, $[RSH] < 10^{-2}$ mol L^{-1}) we only take reactions [1], [−1], and [3] into consideration since the oxida-

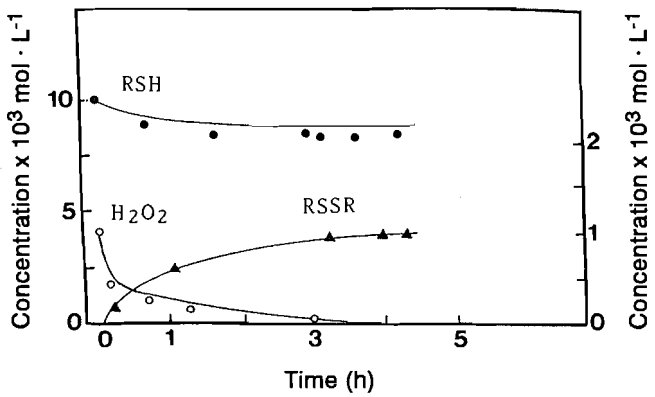

FIG. 7. Comparison between the computed kinetic curves and the experimental points [RSH] + [RSH...H_2O_2] (●), [H_2O_2] (○), [RSSR...H_2O_2] (▲): $R = [RSH]_0/[H_2O_2]_0 = 10$, $[RSH]_0 = 10^{-2}$ mol L^{-1} (left scale), $[H_2O_2]_0 = 10^{-3}$ mol L^{-1} and [RSSR] (right scale), phosphate buffer 10^{-2} mol L^{-1}, pH 7 (points are experimental).

tion of RSH by H_2O_2 is non-stoichiometric, the final product is the chelate [RSSR...H_2O_2]. Using this kinetic scheme we have calculated the three rate constants k_1, k_{-1}, and k_3 (using a known experimental equilibrium constant $K_1 = 880 \pm 80$): $k_1 = 25 \pm 5$ mol^{-1} L s^{-1}, $k_{-1} = 0.03 \pm 0.1$ s^{-1}, $K_1 = 900 \pm 100$, $k_3 = 0.5 \pm 0.2$ mol^{-1} L s^{-1}.

The calculated curves (R = 0.2, 0.4, 0.6, 1) are in perfect agreement with experimental curves, (see as an example for R = 0.2, Fig. 2 where curves are calculated).

Hence the kinetic scheme [1], [−1], and [3] seems to be consistent with the overall oxidation process (reaction I)

[I] $2\ RSH + 2\ H_2O_2 \rightarrow [RSSR...H_2O_2] + 2\ H_2O$

In the second case ($R > 2.5$, [RSH] < 10^{-2} mol L^{-1}) we have determined the rate constant of reaction [4], k_4. Under these conditions, reactions [1], [−1], [3], and [4] are taken into considerations, since the overall oxidation is stoichiometric. Using k_1, k_{-1}, and k_2 reported above, $k_4 = 40 \pm 10$ mol^{-1} L s^{-1} provides the best agreement with all the experimental curves. As an example, see Fig. 4 for R = 8 (the curves are calculated).

Consequently, the kinetic sequence [1], [−1], [3], and [4] is consistent with the overall stoichiometric oxidation process (reaction II).

[II] $2RSH + H_2O_2 \rightarrow RSSR + 2\ H_2O_2$

In the third case ($R > 2.5$, [RSH] > 10^{-2} mol L^{-1}) the rate constants k_2, k_{-2} (given that the experimental equilibrium constant $K_2 = 110 \pm 5$) and k_5 have been determined. Here we used the kinetic scheme [2], [−2], and [5]. The following k values provide the best agreement with the experimental curves (as an example see Fig. 7 for R = 10): $k_2 = 90 \pm 20$ mol^{-2} L^2 s^{-1}; $k_{-2} = 0.9 \pm 0.1$ s^{-1}; $K_2 = 110 \pm 20$; $k_5 = 0.03 \pm 0.01$ s^{-1}.

Thus the kinetic scheme [2], [−2], and [5] is in agreement with the overall stoichiometric oxidation process (reaction II)

[II] $2RSH + H_2O_2 \rightarrow RSSR + 2\ H_2O$

However, it should be noted that the fits are equally good using the full kinetic scheme (reactions [1], [−1], [2], [−2], [3], [4], and [5]) with the same theoretical rate constants used as above.

As to reaction [2], it is obvious that it is not an elementary step. It has been suggested[2] that another way of expressing reaction [2] is

[2′] $[RSH...H_2O_2] + RSH \rightleftarrows [RSH...H_2O_2...RSH]$

Nevertheless, we have no experimental proof to support [2′]. However, we have put the hypothesis to test, since K_2 would be $K_1K_{2'}$. Indeed the fits agree reasonably well with the reaction sequence [1], [−1], [2′], [−2′], [3], [4], and [5]. It should be noted that only k_5 is different by a factor of 3 (0.1 ± 0.05).

Conclusion

Our purpose was to quantitatively determine the mechanism and the kinetic parameters describing the action of hydrogen peroxide with *N*-acetylcysteine in aqueous medium buffered at pH 7. The analysis of the reaction mixture as a function of time, was performed using (*1*) Ellman's reagent to assay the thiol function of RSH and of the eventual complexes [RSH...H_2O_2] and [RSH...H_2O_2...RSH], (*2*) the titanium method to determine free H_2O_2, and (*3*) the differential absorbances of the aqueous solution in the UV region to assay the concentration of the disulfide of *N*-acetylcysteine, RSSR and of the eventual complex [RSSR...H_2O_2]. Thus the overall reaction is known at each stage of the kinetics and particularly at the end of the reaction.

The striking features of the reaction of *N*-acetylcysteine with hydrogen peroxide are summarized as follows. First of all, we have shown the importance of the concentration ratio R ($[RSH]_0/[H_2O_2]_0$) in the kinetic evolution since non-stoichiometric oxidation of RSH occurs when $R < 2.5$ whereas stoichiometric oxidation occurs when $R > 2.5$. Secondly, we have confirmed the formation of a complex between H_2O_2 and RSH in the first minutes of the reaction under all experimental conditions as described in the preceding paper. However, stoichiometry of the complex is dependent on the concentration of RSH at the highest R values ($R > 2.5$).

The rate constants for each step in the reaction mechanism have been calculated and they are in very good agreement with all experimental kinetic data.

The comparison of these results with those obtained with another thiol, the glutathione GSH (28) shows several similarities: (*i*) the formation of a complex between GSH and H_2O_2, [GSH...H_2O_2], with a K value of the same order of magnitude ($K = 2000 \pm 700$ (28)) as in the case of *N*-acetylcysteine ($K = 900 \pm 100$); (*ii*) a non-stoichiometric oxidation process for the lowest values of $R = [GSH]/[H_2O_2]$ (28) as for RSH.

The scavenging of H_2O_2 by RSH and GSH before reacting to give the disulfide certainly contributes to the antioxidant properties of the two biological systems. It is tempting to contemplate if other molecules with similar structures might exhibit similar interesting properties.

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[2]This reaction was suggested by an anonymous referee.

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