

Accumulation and reactions of H₂O₂ during the copper ion catalysed autoxidation of cysteine in alkaline medium

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ACCUMULATION AND REACTIONS OF H_2O_2 DURING THE COPPER ION CATALYSED AUTOXIDATION OF CYSTEINE IN ALKALINE MEDIUM

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Summary

The stoichiometry of the copper catalysed alkaline autoxidation of cysteine has been investigated. Products of this oxidation reaction are cystine, H_2O_2 and H_2O . No oxygen containing sulfur acids are produced as long as cysteine is present in the reaction liquid.

The H_2O_2 generated reacts with cysteine selectively to form cystine and H_2O with $r_{H_2O_2} = k_{H_2O_2} [CyS] [H_2O_2]$ ($k_{H_2O_2} = 0.17 \text{ l mol}^{-1} \text{ s}^{-1}$).

The reaction between H_2O_2 and cysteine is not catalysed by the copper complex as present during the catalytic autoxidation reaction. On the other hand, experiments carried out in the absence of oxygen show a marked catalytic effect ascribed to the presence of a $Cu(I)$ complex.

Accumulation curves of H_2O_2 have been measured during the copper catalysed autoxidation of cysteine. Information about the rate of production of H_2O_2 at the catalytic site has been obtained by making use of these accumulation curves together with the kinetic data obtained for the reaction of H_2O_2 with cysteine. It is concluded that apart from a two electron reduction of dioxygen to H_2O_2 a four electron reduction to H_2O should also be taken into account.

The selectivity of the oxidation reaction justifies a reconsideration of the free thyl radical mechanism proposed in the literature to occur during the copper catalysed autoxidation of cysteine.

Introduction

It is well known that the metal ion catalysed oxidation of thiols (RSH) by molecular oxygen, in aqueous solution is accompanied by the inter-

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mediate formation of hydrogen peroxide, which in turn is converted to water [1, 2]. In some cases disulfide (RSSR) is found to be the main product of oxidation, since the overall stoichiometric relation is 4/1 for the ratio RSH/O₂ according to eqn. (1):



Quite often, accumulation of a part of the hydrogen peroxide formed obscures this simple relation between the total amount of thiol converted and of oxygen consumed. Usually, in those cases little attention is given to support the idea of a selective oxidation of thiol to disulfide [3, 4]. This lack of evidence is rather surprising in the light of the free radical nature of the metal ion catalysed oxidation of thiols in aqueous solution, a mechanism accepted in the literature [5, 6]. Regarding the high reactivity of free thiol radicals in the presence of oxygen, a selective conversion of thiol to disulfide is not obvious.

Apart from this, there is a lack of evidence concerning the mode of reaction of H₂O₂ in solutions containing both thiols and Cu(II) ions. As to the role of copper in the reaction of H₂O₂, serious discrepancies are encountered in the literature. Cavallini *et al.* [1, 7] studied the autoxidation of cysteine catalysed by copper in alkaline medium. They concluded from their experiments that H₂O₂, accumulated during the catalytic reaction, did not react with cysteine. They found that after completion of the oxidation of cysteine decomposition of H₂O₂ occurred. Hanaki *et al.* investigated the copper catalysed autoxidation of cysteine at about pH 7. From experiments performed under anaerobic conditions they inferred that H₂O₂ reacts with cysteine, whereas copper appears to be an effective catalyst. These findings were assumed to be valid also for the aerobic conditions.

In this work the oxidation of cysteine in strongly alkaline medium in the presence of Cu(II) ions has been investigated in more detail. We present a rather simple method to assess the selectivity of the reaction under the condition that accumulation of hydrogen peroxide occurs. The kinetics of the reaction of H₂O₂ with cysteine are determined under the relevant aerobic catalytic conditions. Accumulation curves of H₂O₂ are determined and it is demonstrated that its mode of accumulation can be understood quite well from a knowledge of the kinetics of the reactions involving H₂O₂.

Experimental

Chemicals

L-Cysteine (Merck art. 2838) was used without further purification. An alkaline stock solution was kept free of oxygen. Copper solutions were made from CuSO₄·5H₂O p.a. (Merck art. 2790). Hydrogen peroxide (Brocacef HY 348) was diluted to 0.25 mol l⁻¹ before use. All experiments were carried out on solutions with final concentrations of 0.25 N NaOH (Merck art. 6482) in distilled water.

Equipment

A reaction vessel was connected with a spectrophotometric cell through a liquid recirculation system. This made it possible to record the decay of cysteine (238 nm) and the signal of the Cu(II)-dicysteine complex (330 nm) [1, 7].

The oxygen consumption could be followed by using the conventional Warburg manometric technique.

UV/vis absorption measurements were carried out on a Unicam SP-800 spectrophotometer.

Procedures

The reaction vessel containing 250 ml of the reaction liquid, was kept constant at 23 °C.

Experiments under oxygen atmosphere ($p_{O_2} = 1 \text{ atm}$) were performed with vigorous stirring of the solution to avoid oxygen depletion. The kinetic experiments under N_2 atmosphere were carried out by using oxygen freed solutions of cysteine + NaOH, $CuSO_4$ and H_2O_2 . The experiments were started by adding simultaneously $CuSO_4$ and H_2O_2 to the cysteine solution in the reaction vessel.

A small amount (1 cm^3) of the reaction liquid was taken from the recirculation system to determine the H_2O_2 concentration spectrophotometrically using the titanous chloride method as described by Egerton *et al.* [8].

Results

Characteristics of the copper catalysed autoxidation of cysteine

During the oxidation of cysteine (RSH) by oxygen in strongly alkaline solutions (0.25 mol/l NaOH) progress of the reaction was followed by measuring the amount of oxygen consumed. Typical curves of the oxygen consumption are shown in Fig. 1. The arrows in this figure indicate the point where the characteristic yellow colour attributed to a copper(II)-cysteine complex disappears, which according to Cavallini *et al.* [1] occurs as soon as cysteine is completely converted. The stoichiometric amount of oxygen corresponding to the relation given by eqn. (1) is depicted by the 100% line in Fig. 1. It is obvious that completion of the conversion of cysteine requires an amount of oxygen exceeding that predicted by reaction (1).

Another phenomenon observed during the autoxidation reaction is the accumulation of H_2O_2 . Typical curves are depicted in Fig. 2. The amount of H_2O_2 accumulated progressively increases up to the moment of complete conversion of cysteine. After this moment a rapid loss of H_2O_2 is observed. In Table 1 (column D) the maximum value of this H_2O_2 accumulation is presented as a function of the initial amount of cysteine and copper(II) sulfate.

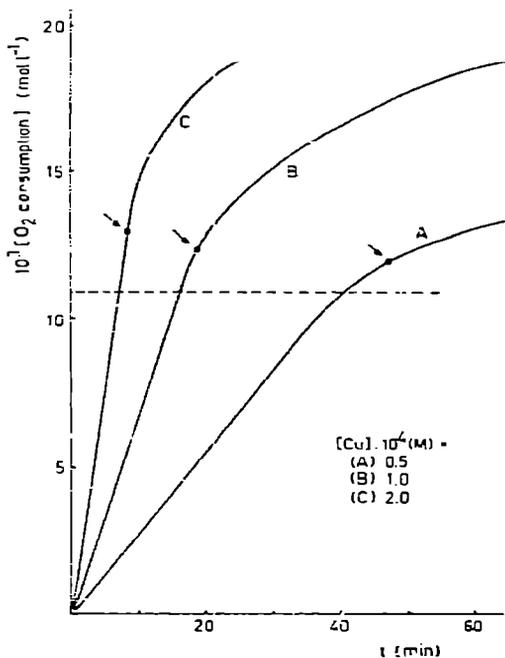
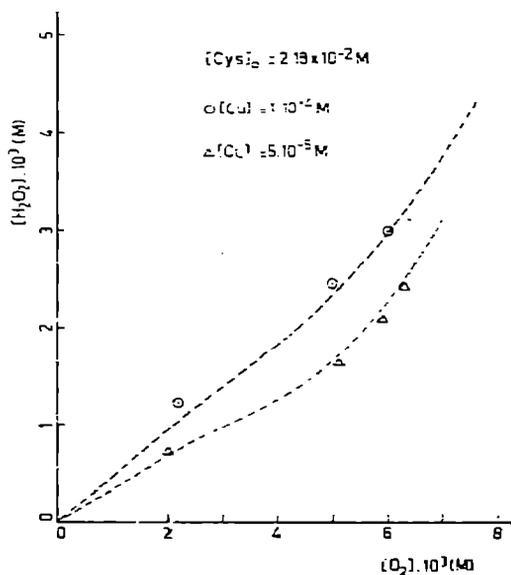


Fig. 1. The oxygen consumption versus time during the Cu catalysed oxidation of $4.35 \times 10^{-2} M$ cysteine for different Cu concentrations. All experiments carried out under 1 atm O_2 pressure at room temperature. $[NaOH] = 0.25 \text{ mol l}^{-1}$. Arrows indicate moment of complete conversion of cysteine (as seen by colour change). Dotted line corresponds to theoretical 100% conversion according to eqn. (1).



(a) (Legend 1 on facing page)

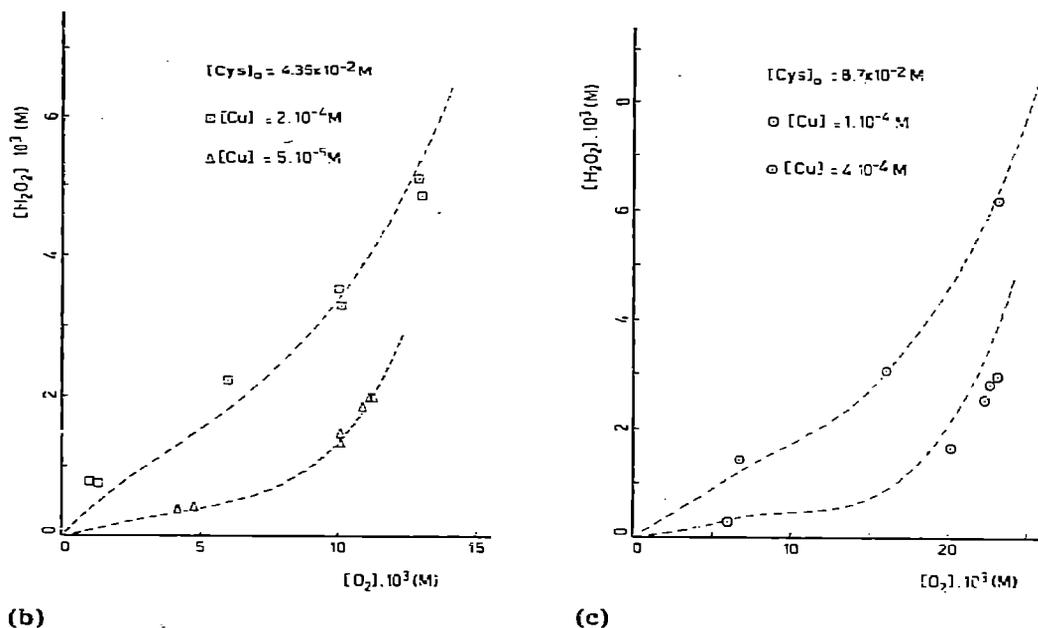


Fig. 2. Accumulation of H_2O_2 vs. O_2 consumption during the Cu catalysed oxidation of cysteine for different Cu concentrations. Dotted curves correspond to the calculated H_2O_2 accumulation according to eqn. (12) using a best fit value $fk = 30.2$ in eqn. (14). The calculation is based on independently obtained data for the H_2O_2 reaction with cysteine.

The reaction paths of hydrogen peroxide

To gain more insight into the accumulation process of H_2O_2 a knowledge of the reaction concerning the production and consumption of H_2O_2 under the actual catalytic conditions is required. The consumption of H_2O_2 in alkaline cysteine solutions has been studied in the absence and presence of Cu ions ($0 - 1.5 \times 10^{-4}$ M). Since the role of copper in these reactions appeared to be influenced strongly by the presence of molecular oxygen, measurements were performed under anaerobic and aerobic conditions, respectively.

Anaerobic conditions

The stoichiometry of the reaction between hydrogen peroxide and cysteine was determined during the reaction from the decay of the concentrations of H_2O_2 and cysteine, both measured spectrophotometrically. It can be seen in Fig. 3 that independently of the amount of copper ions the following relation holds:

$$\Delta[\text{RSH}] = 2\Delta[\text{H}_2\text{O}_2]$$

This relation leads to the following overall reaction:



TABLE 1

Stoichiometric relation between H_2O_2 accumulation and oxygen consumption at the moment of complete conversion of cysteine

A	B	C	D ^a	E ^b	F ^c
$[\text{Cys}]_0 \times 10^3$ (mol l ⁻¹)	$[\text{Cu}]_0 \times 10^4$ (mol l ⁻¹)	$r_{\text{O}_2} \times 10^6$ (mol l ⁻¹ s ⁻¹)	$[\text{H}_2\text{O}_2]_{\text{max}} \times 10^3$ (mol l ⁻¹)	$n_{\text{O}_2} \times 10^3$ (mol l ⁻¹)	$\frac{1}{4}\Delta[\text{Cys}]_{\text{tot}}$ $+\frac{1}{2}[\text{H}_2\text{O}_2]_{\text{max}}$
21.75	0.5	4.0	2.45	6.3	6.7
21.75	1.0	9.7	3.65	7.2	7.3
43.5	0.5	3.3	2.0	11.4	11.9
43.5	0.5	4.7	2.0	11.2	11.9
43.5	1.0	11.0	3.4	13.2	12.6
43.5	1.0	11.7			
43.5	2.0	25.0	5.3	13.5	13.5
43.5	2.0	28.9	4.9	13.2	13.3
43.5	2.0	24.7			
87.0	1.0	11.2	2.95	23.1	23.3
87.0	2.0	29.0	4.35	23.9	23.9
87.0	4.0	63.5	6.5	24.8	25.0
174	2.0	30.2	3.45	44.9	44.2
174	4.0	74.4	4.3	45.7	46.0

^a Accumulated amounts of H_2O_2 .

^b Experimentally obtained amounts of O_2 consumption.

^c Calculated amounts of O_2 consumption using formula (8).

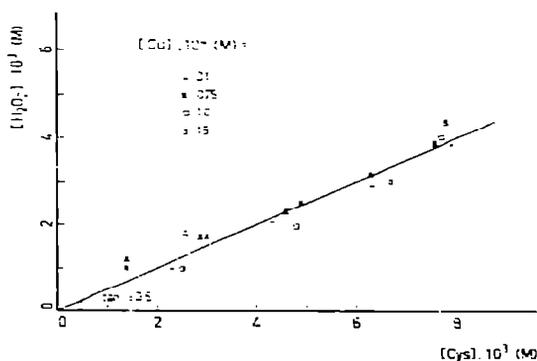


Fig. 3. Stoichiometry of the oxidation reaction of cysteine by H_2O_2 . Measured points have been determined from the decay of the cysteine and H_2O_2 concentrations during the reaction, both measured spectrophotometrically.

In the absence of copper ions the rate of H_2O_2 consumption in strongly alkaline medium was found to be first order in both cysteine and H_2O_2 , thus obeying expression (3):

$$r_{\text{H}_2\text{O}_2} = k_{\text{H}_2\text{O}_2} [\text{RSH}] [\text{H}_2\text{O}_2] \quad (3)$$

with $k_{\text{H}_2\text{O}_2} = 0.17 \text{ l mol}^{-1} \text{ s}^{-1}$.

However, in the presence of copper sulfate, kinetic experiments revealed that apart from alterations in the initial stage of the reaction, the rate remained essentially constant during the period of oxidation, being independent of the amount of cysteine and H_2O_2 , respectively (see Fig. 4).

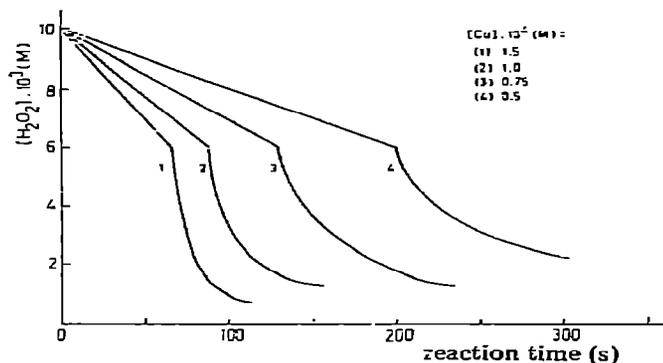


Fig. 4. Decay of the H_2O_2 concentration versus time during the anaerobic oxidation reaction with cysteine in the presence of CuSO_4 . First stage of constant rate (first order in $[\text{Cu}]$) during the period of oxidation, second stage of increasing rate after completion of the oxidation of cysteine to cystine.

The order with respect to copper was found to be close to 1, leading to expression (4):

$$r_{\text{H}_2\text{O}_2(\text{Cu})} = k_{\text{H}_2\text{O}_2(\text{Cu})}[\text{Cu}] \quad (4)$$

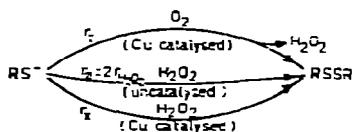
with $r_{\text{H}_2\text{O}_2(\text{Cu})}$ being the copper catalysed reaction rate of H_2O_2 ; $k_{\text{H}_2\text{O}_2(\text{Cu})}$ was found to be 0.4 s^{-1} .

The rate of cysteine consumption in all cases was twice as fast as the rate of disappearance of H_2O_2 in accordance with the stoichiometry of reaction (2). As soon as cysteine has been oxidised completely the solution starts to produce oxygen at a rate that progressively increases with the amount of copper present (see Fig. 4).

Aerobic conditions

In the absence of copper ions the kinetics of the reaction of H_2O_2 with cysteine are not influenced by molecular oxygen, hence eqn. (3) remains valid.

In order to estimate the contribution of a copper catalysed reaction between cysteine and H_2O_2 under aerobic conditions (r_x) one has to realize that in the presence of both copper ions and oxygen, oxidation of cysteine is effected by H_2O_2 as well as by oxygen (see Scheme 1).



Scheme 1.

Moreover, H_2O_2 is not only consumed according to eqn. (2) but also produced during the copper catalysed oxidation of cysteine (see Scheme 1). Hence, the rate r_x cannot be easily found from inspection of the H_2O_2 level in the course of the reaction. However, since the overall rate of cysteine consumption is composed of $r_{\text{tot}} = r_{\text{I}} + r_{\text{II}} + r_x$, an estimate of r_x can be found by measuring separately the value of r_{tot} , r_{I} and r_{II} . It can be seen in Table 2 that r_x (calculated from $r_x = r_{\text{tot}} - r_{\text{I}} - r_{\text{II}}$) is very low even at copper concentrations as high as $2 \times 10^{-4} \text{ mol l}^{-1}$.

In addition we should take into account that under the usual conditions of catalysis the actual concentration of cysteine is much higher (see Table 1) giving rise to a proportional increase of the value of r_{II} . Therefore, we may neglect a catalytic contribution of copper — if there is any — to the reaction between H_2O_2 and cysteine in the presence of oxygen. This is in strong contrast with the results obtained under anaerobic conditions (Table 2).

The relative concentration of the $\text{Cu}^{\text{II}}(\text{RS}^-)_2$ complex under different reaction conditions

The $\text{Cu}^{\text{II}}(\text{RS}^-)_2$ complex has been put forward in the literature to be present during the catalytic oxidation process of cysteine [1, 7]. Cavallini *et al.* [1] reported that the $\text{Cu}^{\text{II}}(\text{RS}^-)_2$ complex exhibits an intensive optical absorption at 330 nm. This absorption at 330 nm is absent after anaerobic

TABLE 2

Rates of cysteine oxidation^a effected by O_2 and H_2O_2

$[\text{Cu}] \times 10^4$ (mol l^{-1})	Rates ^b of cysteine consumption $\times 10^6$ ($\text{mol l}^{-1} \text{ s}^{-1}$)				
	Aerobic				Anaerobic
	r_{tot}	r_{I}	r_{II}	r_x	$r_{\text{Cys(Cu)}}$
1.0	33.1	17.8	13.6	1.7	80
2.0	72.6	56.2	13.6	2.8	160

^a Experimental conditions: $[\text{Cys}]_0 = 4 \times 10^{-3} \text{ mol l}^{-1}$, $[\text{H}_2\text{O}_2]_0 = 1 \times 10^{-2} \text{ mol l}^{-1}$, $[\text{NaOH}] = 0.25 \text{ mol l}^{-1}$.

^b r_{tot} is the initial rate of Cu-catalysed CyS oxidation by O_2 and initially added H_2O_2 . r_{I} is the initial rate of Cu-catalysed CyS oxidation by O_2 only. r_{II} is the rate of *uncatalysed* CyS oxidation by H_2O_2 calculated from eqn. (3). r_x is the contribution of Cu-catalysed oxidation by H_2O_2 in the presence of O_2 calculated from $r_{\text{tot}} - (r_{\text{I}} + r_{\text{II}})$. $r_{\text{Cys(Cu)}}$ is the rate of anaerobic Cu-catalysed CyS oxidation by H_2O_2 .

bleaching of the reaction liquid, resulting in the formation of a Cu(I) complex. Hence, optical spectroscopy gives us a tool to estimate the relative amount of Cu(II) at different conditions. The results of three alternative measurements are depicted in Fig. 5. Curve I shows the intensity of the 330 nm signal during the catalytic oxidation of cysteine by molecular oxygen. The intensity remains constant until the moment of colour change (*i.e.* complete conversion of cysteine). Addition of H_2O_2 did not affect the intensity of the 330 nm signal. Curves II and III are both recorded under anaerobic conditions, namely oxidation of cysteine by H_2O_2 only. Curve II was recorded during an experiment started with a solution of Cu(II) ions, whereas in the case of curve III anaerobic bleaching preceded the experiment. Addition of a small amount of oxygen causes a momentary increase of the optical absorption.

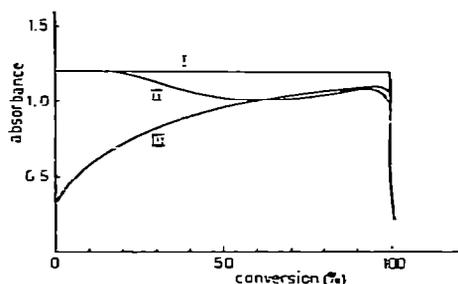


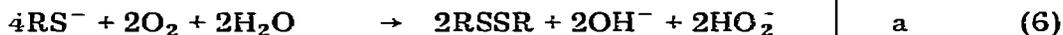
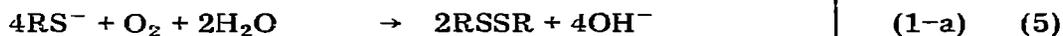
Fig. 5. Absorbance of the Cu(II)-cysteine complex at 330 nm *vs.* relative conversion (%) of cysteine to cystine at different reaction conditions. Curve I, oxidation of cysteine by O_2 and H_2O_2 ; curve II, oxidation by H_2O_2 only, experiment started with Cu(II) solution; curve III, oxidation by H_2O_2 only, experiment started with Cu(I) solution.

Discussion

Selectivity of the oxidation reaction

It was found that the amount of oxygen actually consumed during the oxidation process exceeds the stoichiometric value corresponding to reaction (1). Two effects may account for this excess of oxygen consumption: (1) the observed accumulation of H_2O_2 during the oxidation process; and (2) the formation of higher oxidation products than the disulfide cystine. The data reported in this work are used in the following to find a more quantitative description of the origin of the excess of oxygen consumption.

Presuming that cysteine (RSH) is selectively converted to cystine (RSSR), the stoichiometry of the reaction will then be represented by reaction (7), being a linear combination of reaction (5) (formation of RSSR and H_2O) and reaction (6) (formation of RSSR and H_2O_2):



with RS^- representing the cysteinate ion and HO_2^- being the basic form H_2O_2 (pH \sim 13). It is obvious that the formation of acidic products (RSO_xH) would give rise to a still higher level of oxygen consumption than the value indicated by reaction (7).

It should be noted that the stoichiometric relation according to eqn. (7) represents a mass balance that is of course independent of the way oxygen is reduced. Hence, reactions (5) and (6) are arbitrarily chosen alternatives leading to overall reaction (7). It is easily seen that eqn. (8) is consistent with reaction (7):

$$n_{\text{O}_2} = \frac{1}{4} \Delta [\text{PS}^-] + \frac{1}{2} [\text{H}_2\text{O}_2] \quad (3)$$

where n_{O_2} = oxygen consumed (mol l^{-1}), $\Delta [\text{RS}^-]$ = cysteine converted (mol l^{-1}), $[\text{H}_2\text{O}_2]$ = hydrogen peroxide accumulated (mol l^{-1}).

Equation (8) gives us a tool to examine the selectivity of the oxidation reaction. The relevant experimental data at the moment of complete conversion of cysteine for different copper and cysteine concentrations are presented in Table 1. Rearrangement of these data following eqn. (8) (columns E and F) reveals that the stoichiometry of the oxidation process is completely consistent with eqn. (8). Accordingly, it can be concluded that within the limits of accuracy (about 1%) cysteine is converted selectively to cystine as long as cysteine is present in the reaction liquid.

Hence, the excess of oxygen consumption should be ascribed completely to the accumulation of H_2O_2 in the course of the oxidation process of cysteine. Otherwise, after the moment of complete conversion of cysteine to disulfide a relatively slow uptake of oxygen continues to proceed. This observation suggests that in this stage of the reaction oxidation of disulfide might occur.

Rate of production of H_2O_2

The data found for the kinetics of the reaction between H_2O_2 and cysteine together with those of the accumulation of H_2O_2 are useful to obtain knowledge of the rate of production of H_2O_2 ($r_{\text{H}_2\text{O}_2}(\text{prod})$). In this respect two facts are important. Firstly, it has been shown in this work, that no catalytic effect of copper ions on the reaction of H_2O_2 with cysteine could be measured under oxygen atmosphere. Secondly, decomposition of H_2O_2 during the copper catalysed autoxidation of cysteine can be excluded based upon the following arguments. During the experiment under anaerobic conditions as described by curve III in Fig. 4 both Cu(I) and Cu(II) ions are present. Since the presence of a small amount of dioxygen produced by

decomposition of H_2O_2 would give rise to a fast reoxidation of Cu(I) the conclusion can be drawn that no decomposition of H_2O_2 occurs under these circumstances and that neither Cu(I) nor Cu(II) ions are catalytically active for the decomposition of H_2O_2 as long as cysteine is present in the reaction liquid. Moreover, the constant ratio $[\text{RS}^-]/[\text{H}_2\text{O}_2] = 2/1$, which is observed for the reaction of H_2O_2 with cysteine to be independent of the copper concentration, is in agreement with this conclusion.

Taking into account that the rate of consumption of H_2O_2 obeys eqn. (3), the following expression for the rate of accumulation can now be derived:

$$r_{\text{H}_2\text{O}_2}(\text{acc}) = r_{\text{H}_2\text{O}_2}(\text{prod}) - k_{\text{H}_2\text{O}_2}[\text{RS}^-]_t[\text{H}_2\text{O}_2]_t \quad (9)$$

Rearrangement of eqn. (8) and taking $n_{\text{O}_2} = r_{\text{O}_2}t$ gives

$$\Delta[\text{RS}^-]_t = 4r_{\text{O}_2}t - 2[\text{H}_2\text{O}_2]_t \quad (10)$$

Assuming that the rate of production of H_2O_2 is proportional to the rate of oxygen consumption:

$$r_{\text{H}_2\text{O}_2}(\text{prod}) = pr_{\text{O}_2} \quad (11)$$

with $0 < p \leq 1$.

Substitution of (10) and (11) in (9) gives

$$r_{\text{H}_2\text{O}_2}(\text{acc}) = pr_{\text{O}_2} - k_{\text{H}_2\text{O}_2}[\text{H}_2\text{O}_2]_t\{[\text{RS}^-]_0 - 4r_{\text{O}_2}t + 2[\text{H}_2\text{O}_2]_t\} \quad (12)$$

A computer analysis with numerical integration of eqn. (12) has been carried out. Values of p for various copper and cysteine concentrations are obtained from the best fits to the sets of experimental data. As shown in Table 3, the values of p in all cases are lower than 1 indicating that complete reduction of oxygen to H_2O at the catalytic site has to be taken into account. Moreover, this reaction path seems to be more important at higher concentrations of cysteine, a feature reflected by the decrease of the values of p at higher cysteine concentrations. This observation suggests that during

TABLE 3

Values of the fractional part (p) of dioxygen converted into H_2O_2

$[\text{CyS}]_{1/2} \times 10^2$ (mol l^{-1}) ^a	$[\text{Cu}^{2+}] \times 10^4$ (mol l^{-1})	p^b	$p^c = 1/(1 + 30.2[\text{CyS}]_{1/2})$
1.09	0.5 - 1.0	0.78	0.75
2.175	0.5 - 1.0 - 2.0	0.55	0.60
4.35	1.0 - 2.0 - 4.0	0.50	0.43

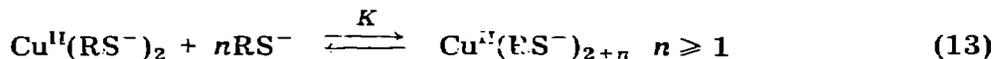
^aSince p values were calculated according to experimental data for the H_2O_2 concentration determined throughout the oxidation experiments mean values of cysteine concentration are used.

^b p calculated from eqn. (12).

^c p calculated from eqns. (12) and (14).

the catalytic oxidation at least two different copper complexes are operative, *i.e.* one leading to a two-electron reduction and another one, being progressively produced at higher concentrations of cysteine, leading to a four-electron reduction of dioxygen. In accordance with the ideas of Cavallini *et al.* [1], the first of these complexes might be the copper(II) dicysteinate complex $[\text{Cu}^{\text{II}}(\text{RS}^-)_2]$.

Based on this reasoning it is plausible to suppose a reaction between $\text{Cu}^{\text{II}}(\text{RS}^-)_2$ and cysteine resulting in a complex with at least three cysteinate ligands:



If only $\text{Cu}^{\text{II}}(\text{RS}^-)_{2+n}$ mediates four-electron transfer to dioxygen, the parameter p will be proportional to the fraction of copper present as $\text{Cu}^{\text{II}}(\text{RS}^-)_{2+n}$, leading to eqn. (14):

$$p = \frac{1}{1 + fK[\text{RS}^-]} \quad (14)$$

where f is a constant of proportionality representing the ratio of turnover numbers of $\text{Cu}^{\text{II}}(\text{RS}^-)_{2+n}$ and $\text{Cu}^{\text{II}}(\text{RS}^-)_2$. In fact eqn. (14) was developed assuming first order kinetics with respect to cysteine for the production of $\text{Cu}^{\text{II}}(\text{RS}^-)_{2+n}$. Alternative kinetic models were investigated but failed to give a satisfying fit to the parameter p .

Computer analysis of eqn. (12) after substitution of eqn. (14) for the value of p led to a best fit value of 30.2 for the constant fK . Calculated plots of the mode of accumulation of H_2O_2 for different copper and cysteine concentrations using $fK = 30.2$ shown in Fig. 2 reveal that a quite reasonable fit to the experimental data is obtained. The variation of the parameter p is described quite satisfactorily by eqn. (14) for $fK = 30.2$ (see Table 3). It should be noted that the value of f will be close to unity since the rate of oxygen consumption at the same copper concentration only slightly increases with increasing concentration of cysteine (Table 1), indicating that turnover numbers of both copper complexes are almost equal.

The role of copper ions in the reaction between H_2O_2 and cysteine

The kinetic results for the uncatalysed reaction between H_2O_2 and cysteine reported in this work are fully consistent with data reported in the literature on the reaction of H_2O_2 and aminothiols in a large range of pH values (8 - 13) [9, 10].

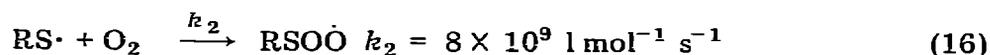
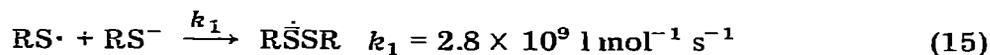
Of particular interest are the findings that the copper complex present under anaerobic conditions is a remarkably effective catalyst for the reaction of H_2O_2 with cysteine, whereas under oxygen atmosphere this catalytic activity is completely absent. The plots of Fig. 5 may shed light on this phenomenon. It is shown that in the presence of oxygen the intensity of the 330 nm absorption due to the amount of Cu(II) dicysteine complex is

essentially constant in the course of the reaction (curve I). However, in the absence of oxygen a loss of intensity is observed despite the presence of H_2O_2 indicating that under these conditions part of the copper is in the reduced state (Fig. 5, curves II and III). The catalytic activity observed for the reaction between H_2O_2 and cysteine should be ascribed therefore to the action of a Cu(I) complex being generated under anaerobic conditions. The lack of this catalytic activity observed when experiments are performed under oxygen atmosphere suggest that during the copper catalysed autoxidation of cysteine no significant amount of a Cu(I) complex will be present.

Hanaki [11] studied the influence of copper on the reaction of cysteine with H_2O_2 at pH 4 - 8 under anaerobis and derived conclusions from these results for the catalytic autoxidation reaction of cysteine. It is demonstrated here that results obtained under anaerobic conditions are not *a priori* valid for the actual catalytic experiments.

The occurrence of free thiy radicals

Free thiy radicals have been suggested in the literature [5, 6] to be operative during the copper catalysed autoxidation reaction of cysteine. An estimate of the selectivity of a free radical reaction might be obtained from kinetic data concerning thiy radicals [12, 13] :



$$\text{pH} \sim 8 - 9$$

It is reasonable to assume that only the first reaction might lead to disulfide as the main product. The fraction of disulfide produced via $RS\cdot$ intermediates in that case is given by

$$\frac{k_1 [RS^-]}{k_1 [RS^-] + k_2 [O_2]} \quad (17)$$

Using formula (17) with $[O_2] = 1.25 \times 10^{-3} \text{ mol l}^{-1}$ ($P_{O_2} = 1 \text{ atm}$) and the highest concentration of cysteine presented in Table 1 with $[RS^-]_{\text{average}} = 8.7 \times 10^{-2} \text{ mol l}^{-1}$ leads to a selectivity of about 96%. However, if the lowest value of the cysteine concentration of Table 1 with $[RS^-]_{\text{average}} = 1.1 \times 10^{-2} \text{ mol l}^{-1}$ is taken, a selectivity of only 75% can be calculated. A comparison of data in columns E and F from Table 1 shows that in all cases the selectivity is close to 100%. Therefore the feasibility of a free radical mechanism as proposed in the literature deserves a critical reconsideration.

References

- 1 D. Cavallini, C. de Marco and S. Dupre, *Arch. Biochem. Biophys.*, **124** (1968) 18.
- 2 A. Hanaki and H. Kamide, *Chem. Pharm. Bull.*, **19** (1971) 1006.
- 3 C. S. Swan and D. L. Trimm, *J. Appl. Chem.*, **18** (1968) 349.

- 4 C. F. Cullis and D. L. Trimm, *Discuss. Faraday Soc.*, 46 (1968) 144 and 184.
- 5 T. J. Wallace, A. Schriesheim, H. Hurwitz and M. B. Glaser, *Ind. and Eng. Chem. (Process Design)*, 3 (1964) 237.
- 6 A. Hanaki and H. Kamide, *Chem. Pharm. Bull.*, 23 (1975) 1671.
- 7 D. Cavallini, C. de Marco, S. Dupre and G. Rotilio, *Arch. Biochem. Biophys.*, 130 (1969) 354.
- 8 A. C. Egerton, A. J. Everett, G. J. Minkoff, S. Rudrakanchana and K. C. Salooja, *Anal. Chim. Acta*, 10 (1954) 422.
- 9 J. P. Barton, J. E. Packer and R. J. Sims, *J. Chem. Soc. Perkin II*, (1973) 1547.
- 10 G. A. Bagiyani, S. A. Grachev, I. K. Koroleva and N. V. Soroka, *Bull. Acad. Sci. Chem. (USSR)*, 5 (1976) 990.
- 11 A. Hanaki and H. Kamide, *Chem. Pharm. Bull.*, 21 (1973) 1421.
- 12 M. Z. Iloffman and E. Hayon, *J. Am. Chem. Soc.*, 94 (1972) 7950.
- 13 J. P. Barton and J. E. Packer, *Int. J. Radiat. Phys. Chem.*, 2 (1970) 159.