Kinetics of the Reaction of Hydrogen Peroxide with Cysteine and Cysteamine

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The rate of the reaction between hydrogen peroxide and cysteine or cysteamine is proportional to $[H_2O_2]$ and $[NH_3^+CHXCH_2S^-]$ (X = H or CO_2^-) consistent with nucleophilic attack by the thiolate ions on peroxide oxygen. The rate decreases at higher pH where loss of the NH_3^+ proton occurs, and it is suggested that hydrogen bonding between this group and hydrogen peroxide facilitates the reaction.

As hydrogen peroxide is a product of the radiolysis of water, its thermal reaction with thiols must be allowed for in determining product yields in the radiolysis of aqueous thiol solutions at higher pH where the rate of this reaction becomes significant. Surprisingly little work has been reported. Tarbell[1] found the reaction of H_2O_2 with 2-mercaptophenylacetic acid to be catalysed by metal ions, and in the presence of ethylenediaminetetra-acetic acid to eliminate this effect he found the rate proportional to $[RSH][H_2O_2][H^+]^{-\frac{1}{2}}$. The conclusions of four studies done concomitantly with radiolysis experiments[2] were that the rate was first order in H_2O_2, fractional order in thiol, and increased with increasing pH.

We have examined the reaction of H_2O_2 with cysteamine and cysteine in the pH range 6—11 and find simple kinetics consistent with the rate-determining step being a nucleophilic attack of the zwitterion $^+NH_3CHXCH_2S^-$ (X = H or CO_2^-) on a neutral hydrogen peroxide molecule. As this work was also primarily done to allow us to correct radiolytic yields for the thermal reaction, experiments were carried out at room temperature, and ionic strength effects were not studied.

EXPERIMENTAL

Reagents.—Cysteamine hydrochloride (Fluka AG), cysteine hydrochloride monohydrate (Riedel-de-Haen), hydrogen peroxide (Riedel-de-Haen, 30%), sodium hydroxide (B.D.H. AnalaR), and citric acid (B.D.H. AnalaR) were used as supplied. Water was triply distilled, the second and third distillations being from alkaline permanganate and acidified dichromate respectively.

pH Measurements.—The pH was altered by addition of NaOH and was measured with a pH meter. Cysteamine solutions were 2×10^{-2}M in citric acid, and cysteine solutions were unbuffered.

Analyses.—Hydrogen peroxide was determined using the titanium method of Eisenburg,[3] and thiol was determined using Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)].[4]

Kinetics.—Reactions were studied at room temperature under an atmosphere of oxygen-free nitrogen. Kinetics of cysteamine, and of cysteine in one experiment, were followed by measuring hydrogen peroxide concentration as a function of time. For all other experiments on cysteine loss of thiol was measured.

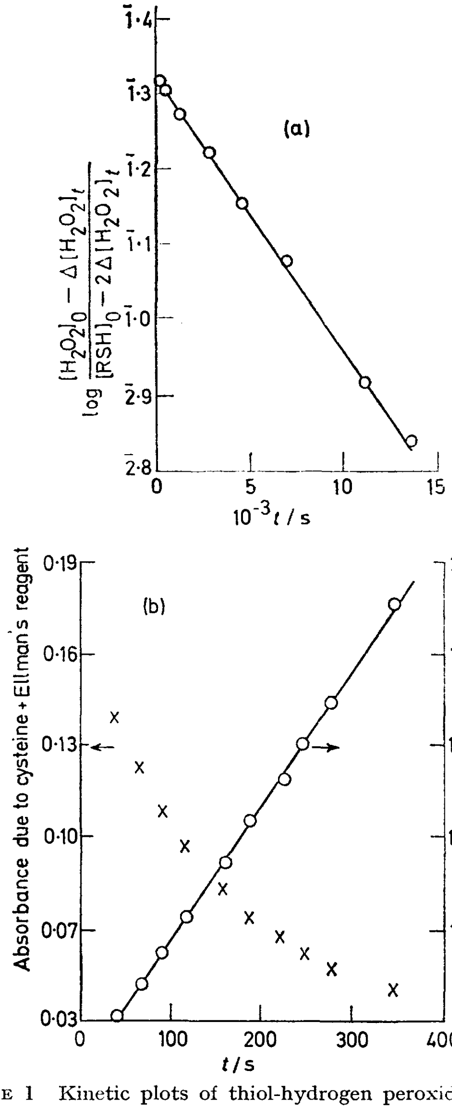

FIGURE 1 Kinetic plots of thiol-hydrogen peroxide reactions: (a) 10^{-3}M-cysteamine and $2·1 \times 10^{-4}$M-hydrogen peroxide pH 6·5; (b) $1·07 \times 10^{-3}$M-cysteine and $5·4 \times 10^{-4}$M-hydrogen peroxide pH 9·55

RESULTS

The stoicheiometry of both reactions was checked by analysis for thiol and peroxide from the same sample, and at all times it was found that $\Delta[RSH] = 2\Delta[H_2O_2]$ consistent with the reaction (1).

$$2RSH + H_2O_2 \longrightarrow RSSR + 2H_2O \qquad (1)$$

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[1] D. S. Tarbell in 'The Chemistry of Organic Sulphur Compounds' ed. N. Kharasch, Pergamon, Oxford, 1961, vol. 1, p. 98.

[2] (*a*) A. El Samahy, Ph.D. Thesis, University of Delaware, 1964; (*b*) A. A-M. Al-Thannon, Ph.D. Thesis, University of Delaware, 1967; (*c*) V. G. Wilkening, M. Lal, M. Arends, and D. A. Armstrong, *J. Phys. Chem.*, 1968, **72**, 185; (*d*) R. V. Winchester, Ph.D. Thesis, University of Auckland, 1968.

[3] G. M. Eisenburg, *Ind. and Eng. Chem.* (*Analyt.*), 1943, **15**, 327.

[4] G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70.

In all but two experiments the initial thiol concentration was close to 1×10^{-3}M, the initial hydrogen peroxide concentrations being 2×10^{-4}M with cysteamine and *ca.* 5×10^{-4}M with cysteine. The exceptions were $9{\cdot}24 \times 10^{-4}$M-cysteamine with $4{\cdot}62 \times 10^{-4}$M-$H_2O_2$ and $1{\cdot}36 \times 10^{-3}$M-cysteine with 5×10^{-4}M-H_2O_2. Plots of $\log\{([H_2O_2]_0 - \Delta[H_2O_2]_t/[RSH]_0 - 2\Delta[H_2O_2]_t)\}$ against time (where $\Delta[H_2O_2]_t$ is peroxide consumed at time t) when $[RSH]_0 \neq 2[H_2O_2]_0$ and plots of $[H_2O_2]_t^{-1}$ against time when $[RSH]_0 = 2[H_2O_2]_0$ were linear for over 70% reaction (Figure 1).

The variation of the second-order rate constants, k, calculated from the slopes of these plots (see Discussion section) varied with pH, and plots of k against $K_a/([H^+] + K_a)$ where K_a is the acid dissociation constant for reaction (2) are shown in Figure 2. The values of pK_a were taken as 8·35 and 8·53 for cysteamine and cysteine respectively.[5]

$$NH_3^+CHXCH_2SH \longrightarrow NH_3^+CHXCH_2S^- + H^+ \quad (2)$$

DISCUSSION

If the rate equation is of the form $-d[H_2O_2]/dt = k[RSH][H_2O_2]$ then the integrated form, assuming the stoicheiometry of reaction (1), is $\log\{([H_2O_2] - \Delta[H_2O_2]_t)/([RSH]_0 - 2\Delta[H_2O_2]_t)\} = (2[H_2O_2]_0 - [RSH]_0)kt/2{\cdot}3 + \log([H_2O_2]_0/[RSH]_0)$. When $[RSH]_0 = 2[H_2O_2]_0$ it takes the form $[H_2O_2]_t^{-1} = 2kt + [H_2O_2]_0^{-1}$ All our kinetic data are consistent with this rate expression and the values of k were determined from the slopes of these plots. The plots of k against pH suggest that the thiolate ion RS^-, rather than RSH reacts in the rate controlling step and that the fundamental rate equation is $-d[H_2O_2]/dt = k'[RS^-][H_2O_2]$. As $[RS^-]/[RSH]_T = K_a/([H^+] + K_a)$ where $[RSH]_T$ is the total thiol concentration, $([RSH] + [RS^-])$, $k = k'K_a/([H^+] + K_a) = k'[RS^-]/[RSH]_T$. Figure 2 shows k to be proportional to $K_a/([H^+] + K_a)$ up to pH = pK_a, but that it deviates markedly from a straight line at higher pH. Values of k' found from plots are 10 and 12·4 l mol^{-1} s^{-1} for cysteamine and cysteine respectively. The deviation can be explained by the fact that both thiols can act as diprotic acids and at higher pH are ionised according to reaction (3). Figure 2 also shows k as a function of $[NH^+_3CHXCH_2S^-]/[RSH]_T$ at higher pH where the extent of reaction (3) becomes appreciable. pK_a Values for reaction (3) were measured at 10·85 for cysteamine and taken as 10·03 for cysteine.[5] The results imply that the rate of attack of $NH_2CHXCH_2S^-$ on H_2O_2 is very much less than that of $NH_3^+CHXCH_2S^-$.

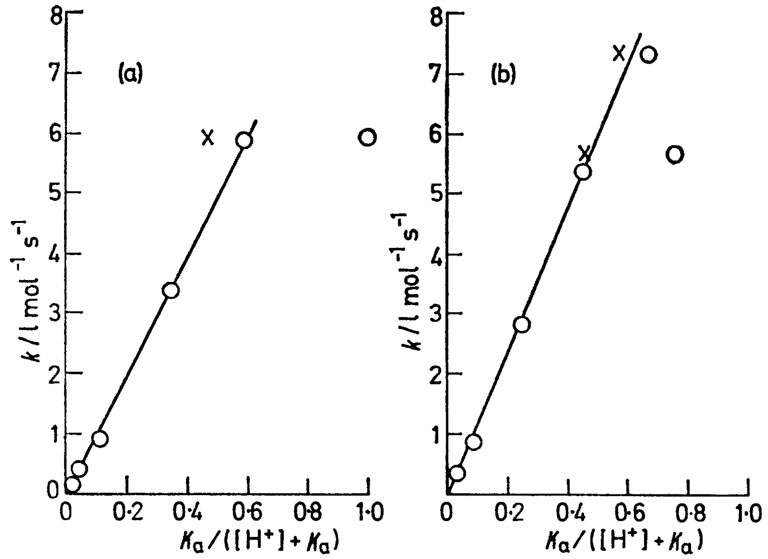
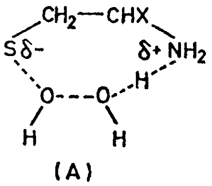


FIGURE 2 Rate constants as a function of thiolate ion fraction: (a) cysteamine; (b) cysteine. Abscissae: ○, $K_a/([H^+] + K_a) = [RS^-]/[RSH]_T$; ×, $[NH_3^+CHXCH_2S^-]/[RSH]$ where $NH_2CHXCH_2S^-$ is present in significant amounts.

$$NH_3^+CHXCH_2S^- \longrightarrow NH_2CHXCH_2S^- + H^+ \quad (3)$$

It is well established that acid catalyses some reactions of hydrogen peroxide, as it protonates one of the hydroxy-groups, facilitating heterolytic O–O bond fission. We suggest our rate-determining steps involve a nucleophilic attack of the thiolate ion on the oxygen of the peroxide and that hydrogen bonding occurs between the displaced hydroxy-group and the NH_3^+ group of the thiol, lowering the activation energy compared with the $NH_2CHXCH_2S^-$ case. Thus we envisage a transition state of the form (A). The pK_a of hydrogen peroxide is too high to account for the decrease in rate being attributed to conversion of H_2O_2 to HO_2^-.



(A)

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[5] R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 1955, **77**, 5877.

The sulphenic acid, RSOH, formed would presumably react fast with RSH or RS^- to give disulphide and water [equation (4)].

$$RSOH + RSH \longrightarrow RSSR + H_2O \qquad (4)$$

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