COMMENTARY

On the Kinetics and Mechanism of the Reaction of Cysteine and Hydrogen Peroxide in Aqueous Solution

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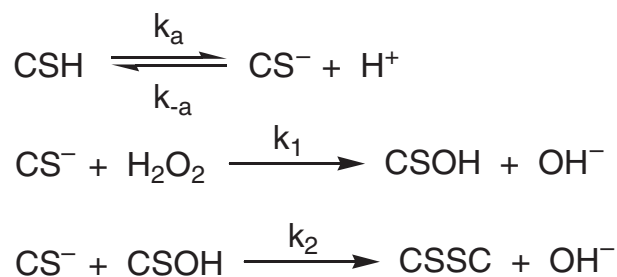Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

The kinetics and mechanism of oxidation of cysteine by hydrogen peroxide have been recently investigated by Luo, Smith, and Anderson.[1] The disappearance of cysteine (CSH) and hydrogen peroxide (H_2O_2) and the appearance of cystine (CSSC) were monitored by HPLC and analysis of that data lead the authors to propose the mechanism of Scheme I: rapid equilibrium to form cysteinate (CS^-), followed by irreversible oxidation of CS^- by H_2O_2 to give cysteine sulfenic acid (CSOH), and nucleophilic attack on CSOH by CS^- to yield CSSC. Nonlinear least-squares fitting of the observed concentration-time profiles yielded values of $k_1 = 15.2 \pm 0.1/M \cdot s$ and $k_2 = 720 \pm 70/M \cdot s$ for the second-order rate constants at $25°C$ and pH 6.0.[1] Regrettably, we do not agree with the analysis of Luo, Smith, and Anderson. We suggest herein that their data afford no insight into the mechanism that follows the rate-determining step, oxidation of CS^- by H_2O_2. Furthermore, as cited in the original study, the kinetics and mechanism of that step have been previously established.[2-4]

The kinetics for the mechanism of Scheme I can be defined by the following differential equations that only differ from those presented in the original study[1] in that the acid-base equilibrium is included:

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$$CSH \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} CS^- + H^+$$

$$CS^- + H_2O_2 \xrightarrow{k_1} CSOH + OH^-$$

$$CS^- + CSOH \xrightarrow{k_2} CSSC + OH^-$$

Scheme I. Mechanism for the reaction of cysteine and hydrogen peroxide as proposed by Luo, Smith, and Anderson.

$$\frac{d[CSH]}{dt} = -k_a[CSH] + k_{-a}[CS^-][H^+] \qquad (1)$$

$$\frac{d[CS^-]}{dt} = k_a[CSH] - k_{-a}[CS^-][H^+] - k_1[CS^-][H_2O_2] - k_2[CS^-][CSOH] \qquad (2)$$

$$\frac{d[H_2O_2]}{dt} = -k_1[CS^-][H_2O_2] \qquad (3)$$

$$\frac{d[CSOH]}{dt} = k_1[CS^-][H_2O_2] - k_2[CS^-][CSOH] \qquad (4)$$

$$\frac{d[CSSC]}{dt} = k_2[CS^-][CSOH] \qquad (5)$$

Since protonation of CS^- is expected to be diffusion-controlled, much faster than the subsequent reactions of CS^-, we can define the

partitioning of CSH/CS$^-$ according to the equilibrium constant k_a and the mass balance equation $[CSH]^T = [CSH] + [CS^-]$ Thus, Eqs. 1 and 2 can be defined in terms of the total concentration of cysteine ($[CSH]^T$):

$$\frac{d[CSH]^T}{dt} = -k_1[CS^-][H_2O_2] - k_2[CS^-][CSOH]$$

(6)

where

$$[CS^-] = \frac{[CSH]^T}{1 + \frac{[H^+]}{k_a}}$$

(7)

Eqs. 3–5 remain unchanged. Although these ordinary differential equations (Eqs. 3–6) do not have an analytical solution, they can be solved numerically using the Euler–Cauchy method.[5] This approach permits a direct comparison between the kinetic model that was originally proposed (Model I) and a simpler model (Model II) for which $k_2[CS^-][CSOH] >> k_1[CS^-][H_2O_2]$. Model II is defined by the following set of differential equations:

$$\frac{d[CSH]^T}{dt} = -2 \times k_1[CS^-][H_2O_2]$$

(8)

$$\frac{d[H_2O_2]}{dt} = -k_1[CS^-][H_2O_2]$$

(9)

$$\frac{d[CSSC]}{dt} = k_1[CS^-][H_2O_2]$$

(10)

Figure 1 illustrates the concentration-time profiles for the two models at pH 6.0 for $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 2$ mM (the same conditions that were employed in Figure 3A of the original study).[1] Figure 1 demonstrates that the two models are practically indistinguishable for these conditions. However, this is not necessarily the case for higher $[H_2O_2]/[CSH]$ ratios. Figure 2 illustrates $d[CSSC]/dt$ for the two models for $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 2$, 4, and 9.2 mM (the three conditions employed in the original Fig. 3).[1] As expected, the rate of the reaction increases with increasing $[H_2O_2]$. Figure 2 also illustrates the differences between the two models, which become more pronounced at higher $[H_2O_2]$. The largest differences between the slopes (an hence the rates) for the two models occur at the beginning of the reactions, before a steady-state $[CSOH]$ is achieved (*vide infra*). We note that only one data point is apparent in the
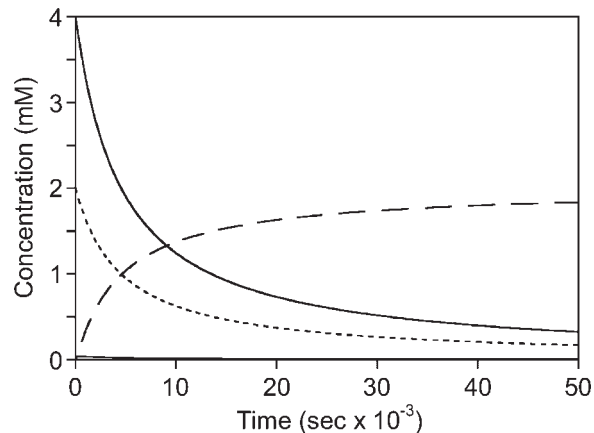

Figure 1. Simulation of the concentration versus time plots for Models I and II of $[CSH]^T$ (solid line), $[H_2O_2]$ (short dashed-line), $[CSOH]$ (in the baseline), and $[CSSC]$ (long dashed-line) for the reaction of $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 2$ mM at pH 6.0. Except for the presence of CSOH (scarcely visible in the baseline early in the reaction), the corresponding traces for the two models are practically superimposable.

presteady-state regime in the original Figure 3C ($[H_2O_2]_0 = 9.2$ mM).[1]

The validity of Model II depends on whether or not the steady-state approximation[6] is appropriate for the mechanism of Scheme I. To apply the steady-state approximation, it is necessary for CSOH to be sufficiently reactive such that it does not accumulate with respect to CSH, CS$^-$,
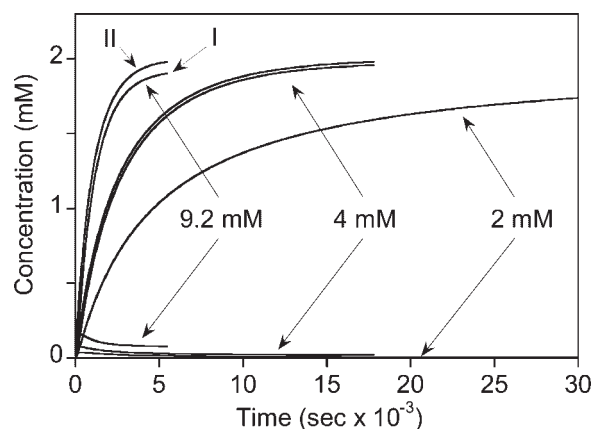

Figure 2. Simulation of the [CSSC] versus time plots for Models I and II for $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 2$, 4, and 9.2 mM at pH 6.0. The differences between the two models (bottom of the graph) illustrate the greatest significance between the slopes (and hence the rates) occur at the beginning of the reactions.