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Research Article

Kinetics and Mechanism of Captopril Oxidation in Aqueous Solution Under Controlled Oxygen Partial Pressure

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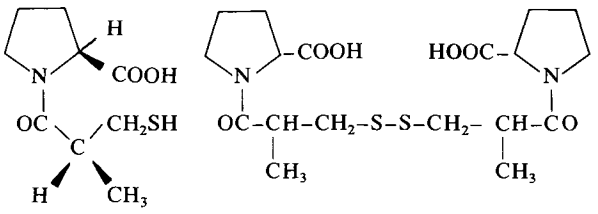
The stability of captopril in aqueous solution at 32°C was studied in the pH range 6.6 to 8.0 under controlled oxygen partial pressure (90–760 mm Hg) with and without the addition of cupric ion. The oxidation product, captopril disulfide, was found to be the sole degradation product. A change in reaction rate from first order to zero order occurs as the captopril concentration decreases. The concentration at which this transition takes place is a function of the pH, oxygen partial pressure, and cupric ion concentration. The apparent first-order rate constants show a first-order dependency on both the oxygen partial pressure and the cupric ion concentration. However, the apparent zero-order rate constants show a first-order dependency on oxygen partial pressure and a second-order dependency on cupric ion concentration. As the pH increases from 6.6 to 8.0, the first-order process becomes more predominant. A mechanism which consists of cupric ion- and molecular oxygen-catalyzed oxidation is proposed to explain those observations.

KEY WORDS: captopril oxidation; captopril stability; thiol; captopril disulfide; oxidation kinetics.

INTRODUCTION

Captopril (1-[(2S)-3-mercapto-2-methyl-1-oxopropyl]-L-proline; 1) is a potent and specific inhibitor of the enzyme which catalyzes the conversion of angiotensin I to angiotensin II (1,2). As a result of this angiotensin-converting enzyme (ACE) inhibitory activity, captopril is an effective antihypertensive agent. In addition, captopril is the first nonpeptidic ACE inhibitor and it can be administered both orally and parenterally.



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Characteristic of thiols, captopril reportedly undergoes oxidation to form the dimer, captopril disulfide (2) (3). In addition, amide hydrolysis has also been reported in aqueous solution (3). Timmins *et al.* (3) showed that oxidation predominates in the pH range 2 to 5.6 and becomes increasingly important as the pH increases.

Previous studies have not controlled the oxygen partial pressure above the reaction (3–5). The current study investigates the oxidative mechanism in aqueous solution under controlled oxygen partial pressure. Further, no evidence was found for captopril stabilization by cyclodextrins, which have been reported to form inclusion complexes which stabilize some compounds against oxidation.

MATERIALS AND METHODS

Apparatus. Requirements of the apparatus were (1) to allow the selection of oxygen partial pressure, (2) to maintain constant oxygen partial pressure throughout an experiment, (3) to permit rapid equilibrium between the oxygen gas phase and the reaction solution, and (4) to provide convenient introduction and sampling of captopril. An apparatus similar to that used by Sokoloski and Higuchi (6) was employed.

The rate of captopril oxidation in 0.1 *M* phosphate buffer at pH 6.62 (μ = 0.18), under 1-atm oxygen partial pressure at 32°C was used to evaluate two stirring methods. The continuous diffusion of oxygen bubbles through the reaction was used as a control. Mechanical stirring was chosen since it provided rates which were similar to the control while those using magnetic stirring were lower.

Analysis. An isocratic high-performance liquid chromatographic (HPLC) assay (Model 332, Beckman Instruments Inc.) detected captopril (1) and its degradation product (2) at 210 nm using an ultraviolet detector and integrator (Models 1040A and 3090A, Hewlett Packard, Inc.), a reversed-phase C-18 column (μ-Bondapak C18, Waters Associates), and a 20-μl injection loop. A mobile phase containing 25% acetonitrile and 75% filtered, degassed aqueous

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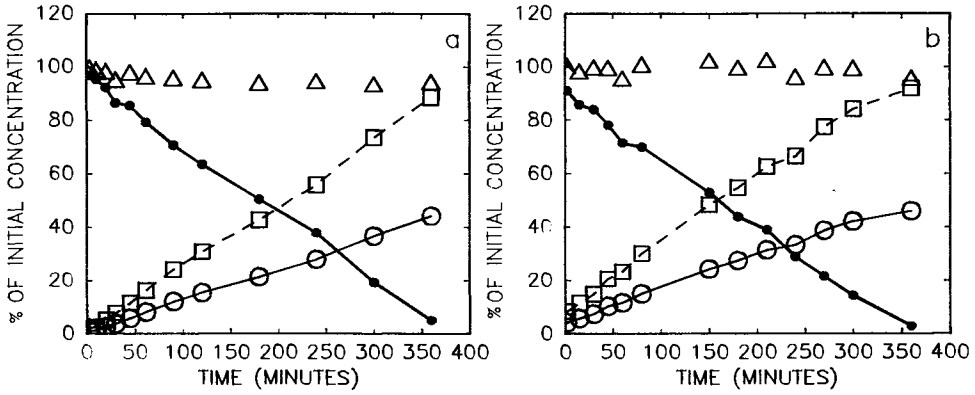

Fig. 1. Mass balance during captopril oxidation at 32°C, pH 6.62 (0.1 *M* phosphate buffer): captopril (●); captopril disulfide (○); two times the disulfide (□); captopril plus twice the disulfide (△). Conditions: (a) $[Cu^{2+}] = 1.35 \times 10^{-5}$ *M*, $pO_2 = 322$ mm Hg; (b) $[Cu^{2+}] = 0$, $pO_2 = 733$ mm Hg.

phosphoric acid (0.05%) was used at a 1-ml/min flow rate. Captopril and its disulfide had retention times of 5.6 and 10.9 min with capacity factors of 1.24 and 3.32 in the detection ranges (10^4 *M*) of 0.1–1.0 and 0.05–0.5 having coefficients of variation of 1.8%.

Reaction samples taken at various times indicated quantitative conversion of 1 to 2. The UV spectra during chromatography, taken at the upslope, apex, and downslope for each peak (1040A detector), were consistent with those for the reference standards. As illustrated in Fig. 1, the mean percentage recovery during a typical reaction was 99.0% (±SD = 2.1%).

Kinetic Studies. The 5-gal oxygen source carboy was filled with water and the desired oxygen partial pressure was calculated from the measured volumes displaced by high-purity oxygen and nitrogen and the ambient atmospheric pressure (6).

The distilled water used in preparing all solutions was filtered (Milli-Q_2 System, Millipore Co., Bedford, Mass.) to minimize contamination by trace metals. Successive recrystallization of buffer salts from hot water was used to remove trace metals (7).

Twenty-five milliliters of buffer was placed in the 50-ml reaction flask. After evacuating the head space for 5 min at 32 ± 0.2°C, the vessel was connected to the oxygen source and equilibrated with stirring for 1 hr. One milliliter of an aqueous captopril stock solution was introduced and the reaction was protected from light. The initial captopril concentration was approximately 1.9×10^{-4} *M* unless otherwise specified. The pH before and after the reaction was constant. Approximately 0.8-ml samples of reaction mixture were taken as a function of time. After cooling, 0.5-ml aliquots were quenched to pH 2 to 3 by dilution with 1 or 2% phosphoric acid solution. All reactions were adjusted to constant ionic strength ($\mu = 0.18$) using NaCl.

Various volumes (10 to 50 μl) of cupric acetate stock solution were used to provide Cu^{2+} concentrations ranging from 2.7×10^{-6} to 1.35×10^{-5} *M*. The influence of pH was examined from pH 6.6 to pH 8.0 under pure oxygen with a Cu^{2+} concentration of 1.35×10^{-5} *M* at 32°C. Ethylenediaminetetraacetate and 8-hydroxyquinoline at concentrations of 2.80×10^{-4} *M* were used to examine the influence of chelating agents on the oxidation of captopril at pH 6.62.

Various ratios of oxygen and nitrogen provided oxygen partial pressures ranging from 90 mm Hg to pure oxygen at 32°C, pH 6.62, with a Cu^{2+} concentration of 1.35×10^{-5} *M*. Initial rate studies were employed under low oxygen partial pressures (90–125 mm Hg) owing to the slow rate of oxidation. Four different initial concentrations of captopril ranging from 1.0×10^{-4} to 5.0×10^{-4} *M* were used for each estimate. Loss of captopril and formation of captopril disulfide were both measured during the initial 10% of the reaction.

Influence of Cyclodextrins. Three cyclodextrins (α, β, and γ, P. L. Biochemicals Inc., Milwaukee, Wis.) were examined as possible stabilizers against captopril oxidation by the formation of inclusion complexes. Three conditions were employed.

(i) A pH 6.62, buffer containing approximately 1.0×10^{-3} *M* cyclodextrin (α, β, or γ) and 1.35×10^{-5} *M* Cu^{2+} ion was prepared. Reactions were carried out by the addition of captopril in the usual manner.

(ii) Captopril (5.0×10^{-4} *M*) and cyclodextrin (2.5×10^{-3} *M*) were dissolved in 25 ml of 0.001 *N* HCl and stirred under nitrogen at 60°C for approximately 18 hr. A 10-ml sample of this captopril–cyclodextrin mixture was mixed with 15 ml of 0.167 *M* phosphate buffer solution at pH 6.60 to make the final Cu^{2+} concentration of 1.35×10^{-5} *M*.

(iii) The latter procedure was duplicated using 32°C in place of 60°C during the 18-hr equilibration step.

RESULTS

Identification of Degradation Products. The captopril disulfide peak was validated by comparison of its retention time and its UV spectra at various positions on the HPLC peak to those of the reference standard. Enriching the reaction mixture with captopril disulfide showed only one peak at the retention time of the product which yielded the expected total area. The concentration of captopril plus twice the concentration of captopril disulfide equals the initial concentration throughout the reaction, indicating that captopril disulfide is the only degradation produce under all conditions in this study (Fig. 1).

Table I. Effect of the Initial Captopril Concentration on Observed Rates[a]

Captopril concentration (10^4 M)	k_0 (10^6 M/min)	k_1 (min^{-1})
5.26 ± 0.01	1.14 ± 0.02	6.01
3.55 ± 0.13	1.05 ± 0.01	6.35 ± 0.02
1.93 ± 0.02	1.02 ± 0.01	—
1.31	0.936	—

[a] 0.1 M phosphate buffer, pH 6.62, μ = 0.18, $[Cu^{2+}] = 1.35 \times 10^{-5}$ M, pure O_2 at 32°C.

Factors Influencing Captopril Oxidation Rate. The influence of the initial captopril concentration was examined at pH 6.62 with pure oxygen in the presence of Cu^{2+} (Table I). Apparent first-order loss is evident at high captopril concentrations, while zero-order behavior predominates when the captopril concentration is less than $\sim 1.8 \times 10^{-4}$ M (Fig. 2). The corresponding plots using disulfide data provide similar results. However, initial rates under low oxygen partial pressures (90–125 mm Hg) were directly proportional to the captopril concentration over the same concentration range, thus suggesting apparent first order. These results indicate that first-order and zero-order behaviors depend upon the experimental conditions.

The addition of Cu^{2+} to the reaction was found to change the reaction rate from first-order to zero-order. The captopril concentration at which the order changed from first to zero order decreased as the Cu^{2+} concentration decreased.

A change from first to zero order was also observed during studies using oxygen partial pressures above ~180 mm Hg. The captopril concentration at which the order changed decreased as the oxygen partial pressure decreased. At oxygen partial pressures of 90–125 mm Hg, only first-order behavior was studied since initial rates were determined owing to slow oxidation.

Influence of pH. The effect of pH was examined under pure oxygen in the presence of 1.35×10^{-5} M Cu^{2+} at 32°C in the pH range 6.6 to 8.0. Both first-order and zero-order rates were observed. Figure 3 shows three pairs of apparent first-order and zero-order rate constants as a function of pH. Doubling the buffer concentration from 0.05 to 0.1 M at constant ionic strength produced only a 3% increase in the rate of captopril oxidation (Fig. 3 inset). No significance is attached to this difference since the standard deviation of the rate constants determined throughout this investigation varies from 1 to 4%. The small apparent increase could be due to trace metals and/or minor buffer catalysis.

Influence of Chelating Agents and Cyclodextrins. Loss of captopril was examined as a function of time with and without the chelating agents EDTA and 8-hydroxyquinoline. While the control was completely oxidized within 10 hr, no reaction was detected in 50 hr in the presence of either agent.

The effect of cyclodextrins (α, β, and γ) were examined under conditions where the predominant reaction pathway is zero order. The resultant zero-order rate constants were similar to those in the control studies ($k_0 = 1.0 \times 10^{-7}$ M/min), indicating that cyclodextrin–captopril inclusion complexes were not formed or the inclusion process does not protect the captopril against oxidation.

DISCUSSION

Reaction Order with Respect to Captopril. The oxidation of captopril to its disulfide is the predominant degradation pathway in the pH range of 6.6 to 8.0 at 32°C with or without the addition of cupric ion. Depending upon the reaction conditions, the rate is first order or zero order with respect to captopril. First-order reactions change to zero order when the captopril concentration decreases below a minimum value. The concentration at which this change occurs is a function of the pH, cupric ion concentration, and oxygen partial pressure.

Effects of Trace Metals. Literature methods for removal of trace metals from buffer salts (7) effected no change in the oxidation rate, indicating that the salts did not contain trace metals or the process did not totally remove them. To verify further the catalysis by trace metals, EDTA and 8-hydroxyquinoline were shown to stabilize captopril. Since these are two different chemical types of chelating agents, this rate reduction is most likely due to inactivation of trace metals rather than direct participation by the agents.

Cupric Ion Dependency. The linear dependency of observed first-order rate constants on the cupric ion concen-

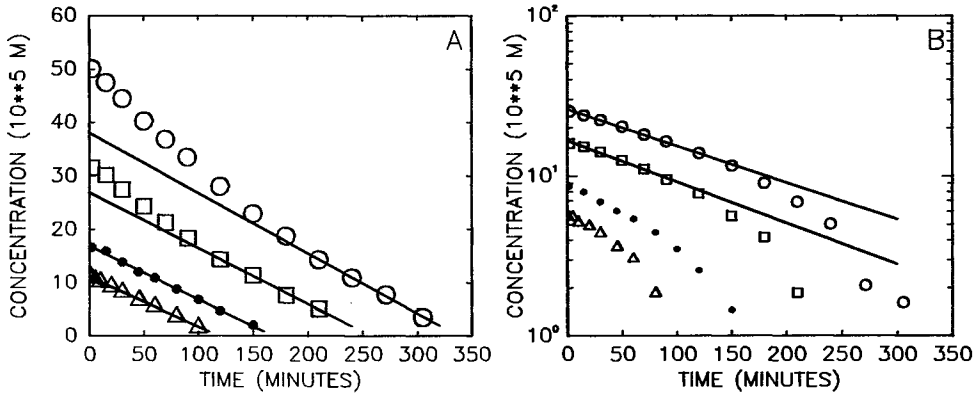

Fig. 2. (A) Captopril concentrations as a function of time and (B) first-order plots using captopril data at pH 6.62, 0.1 M phosphate buffer (μ = 0.18), pure O_2, $[Cu^{2+}] = 1.35 \times 10^{-5}$ M at 32°C. Initial captopril concentrations: 52.4×10^{-5} (○), 34.2×10^{-5} (□), 19.5×10^{-5} (●), and 13.2×10^{-5} (△) M.

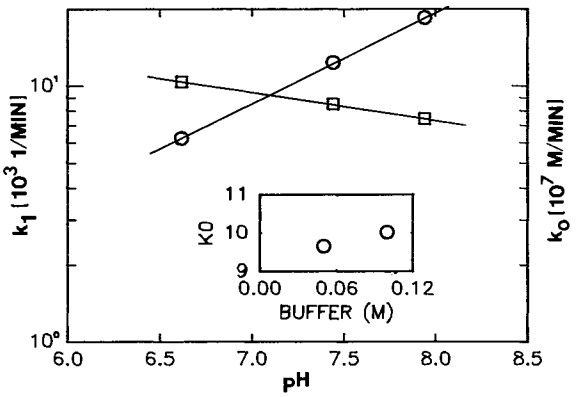

Fig. 3. Apparent zero-order (□; 10^7 k_0 as M/min) and first-order (○; 10^3 k_1 as min^{-1}) rate constants as a function of pH in the presence of pure oxygen and $[Cu^{2+}] = 1.35 \times 10^{-5}$ M at 32°C. Inset shows apparent zero-order rate constants for two buffer concentrations at pH 6.6.

tration is shown in Fig. 4A. The intercept agrees with the first-order rate constant determined for captopril oxidation when no Cu^{2+} is added. In contrast, the zero-order rate constants are described by a second-order dependency since k_0/Cu^{2+} versus Cu^{2+} is linear (Fig. 4B). In addition to these observed cupric ion dependencies, the captopril concentration at which the reaction changed from first to zero order decreased as the Cu^{2+} concentration decreased.

Oxygen Dependency. The apparent first- and zero-order rate constants are linearly related to the oxygen partial pressure (Fig. 5). Thus the rate of loss of captopril is either first or zero order with respect to captopril, but it is first order with respect to $[O_2]$ in both cases. This indicates that oxygen is involved in both the first-order and the zero-order rate-limiting step.

Mechanism. Previously proposed mechanisms for thiol oxidation in the presence and absence of heavy metal ions (Schemes I and II) do not adequately describe the present results. A proposed mechanism combining simultaneous heavy metal ion-catalyzed and direct molecular oxygen-catalyzed oxidation (Scheme III) is consistent with the observed change in rate as a function of reaction conditions.

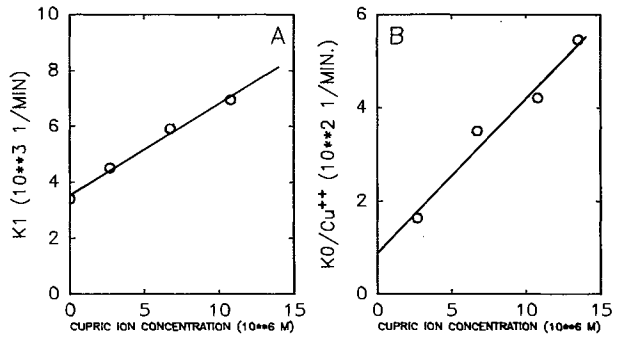

Fig. 4. Rate constants as a function of added cupric ion concentration at pH 6.62, 0.1 M phosphate buffer (μ = 0.18) in the presence of pure oxygen at 32°C. (A) Apparent first-order rate constants; (B) observed zero-order rate constants (corrected for the observed k_0 value of 2.83×10^{-7} M/min in the absence of added copper) divided by the added cupric ion concentration.

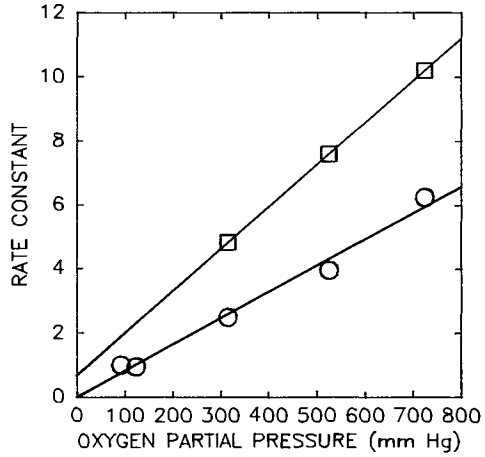

Fig. 5. Apparent zero-order (□; 10^7 k_0 as M/min) and first-order (○; 10^3 k_1 as min^{-1}) rate constants as a function of oxygen partial pressure at pH 6.62, 0.1 M phosphate buffer (μ = 0.18), $[Cu^{2+}] = 1.35 \times 10^5$ M, 32°C.

The following mechanism was proposed for the autooxidation of thiols by molecular oxygen (8–10).

$$RSH + B^- \underset{}{\overset{K}{\rightleftharpoons}} RS^- + BH \quad (1)$$

$$RS^- + O_2 \xrightarrow{k_2} RS\cdot + O_2^- \quad (2)$$

$$RS^- + O_2^- \xrightarrow{k_3} RS\cdot + O_2^{-2} \quad (3)$$

$$2RS\cdot \xrightarrow{k_4} RSSR \quad (4)$$

$$2O_2^{-2} + 2BH \xrightarrow{k_5} O_2 + 2B^- + 2OH^- \quad (5)$$

Scheme I

Using this scheme and applying steady-state assumptions for RS · and O_2^-, the rate law can be described by Eq. (6), which predicts a first-order dependency on thiol concentration after the initial lag time,

$$d[RSH]_T/dt = -2k_2\{K[B^-]/(K[B^-] + [BH])\}[O_2][RSH]_T \quad (6)$$

where $[RSH]_T$ represents the total thiol concentration, K is the equilibrium constant for step 1, and $[O_2]$ is the dissolved oxygen concentration. In the present study, first-order behavior was not in effect under all conditions. In addition, this scheme cannot explain the rate change from first to zero order. This phenomenon of order change has also been observed by Cullis *et al.* (11), while a zero-order process was reported by Rippie and Higuchi for the oxidation of 2,3-dimercapto-1-propanol (BAL) (12,13). Scheme I cannot accommodate these observations.

A mechanism proposed for the case where heavy metal ions are present is shown in Scheme II (14).

$$RS^- + M^{(n+1)+} \longrightarrow RS\cdot + M^{n+} \quad (7)$$

$$2RS\cdot \longrightarrow RSSR \quad (8)$$

$$2M^{n+} + O_2 \longrightarrow 2M^{(n+1)+} + O_2^{-2} \quad (9)$$

$$O_2^{-2} + 2H_2O \longrightarrow H_2O_2 + 2OH^- \quad (10)$$

Scheme II

Cullis *et al.* (15) and Swan and Trimm (16) observed that the reaction order with respect to thiol concentration is dependent upon the type of heavy metal ions present. A zero-order reaction was observed when copper and cobalt were added, while the addition of nickel resulted in a first-order reaction. According to Scheme II, when step 9 becomes rate limiting the reaction rate will become independent of the thiol concentration and the reaction order becomes zero order with respect to thiol. Conversely, when step 7 becomes rate limiting, the rate is first order. In the current study, reactions were first order when the captopril concentration was high and zero order when the concentration was low. According to Scheme II, a high captopril concentration would make step 9 rate limiting and a zero-order reaction would result.

Although heavy metal ion-catalyzed thiol oxidation is usually faster than that catalyzed by molecular oxygen, both reactions exist when metal ions are present. Competition between these two pathways can explain the current observations (Scheme III).

$$RS^- + Cu^{2+} \xrightarrow{k_1} RS\cdot + Cu^+ \quad (11)$$

$$2Cu^+ + O_2 \xrightarrow{k_2} 2Cu^{2+} + O_2^{-2} \quad (12)$$

$$RS^- + O_2 \xrightarrow{k_3} RS\cdot + O_2^- \quad (13)$$

$$RS^- + O_2^- \xrightarrow{k_4} RS\cdot + O_2^{-2} \quad (14)$$

$$2RS\cdot \xrightarrow{k_5} RSSR \quad (15)$$

$$O_2^{-2} + 2H_2O \xrightarrow{k_6} H_2O_2 + 2OH^- \quad (16)$$

Scheme III

Employing steady-state assumptions for O_2^-, the rate of loss of captopril can be described by

$$d[RS^-]/dt = -k_1[RS^-][Cu^{2+}] - 2k_3[RS^-][O_2] \quad (17)$$

Since the oxidation of cuprous (Cu^+) to cupric (Cu^{2+}) ion is known to be slow when the pH of the reaction is increased (17), reaction (12) may become rate-limiting relative to reaction (11). Applying steady-state assumptions for Cu^{2+}, Eq. (17) can be rewritten as

$$d[RS^-]/dt = -2k_2[Cu^+]^2[O_2] - 2k_3[RS^-][O_2] \quad (18)$$

When the captopril concentration is small, the contribution of the second term would become insignificant and the reaction would approach apparent zero order with respect to captopril:

$$d[RS^-]/dt \simeq -2k_2[Cu^+]^2[O_2] \quad (19)$$

However, when the captopril concentration is increased, direct oxidation by molecular oxygen would become significant and the rate would appear first order with respect to captopril.

Both the observed first-order and the observed zero-order rate constants are increased by the addition of cupric ion. When the cupric ion concentration is low, the rate-limiting step for cupric ion-catalyzed oxidation would be reaction (11). Consquently, the reaction would appear first order with respect to captopril as shown in Eq. (17). In addition, as the cupric ion concentration decreases, the change in the rate-limiting step from reaction (11) to reaction (12) would occur at lower captopril concentrations. Consequently, the change in order was observed at lower captopril concentrations. When the cupric ion concentration is increased, the role of reaction (12) as a rate-limiting step would increase and cupric ion-catalyzed oxidation would become zero order. The observed zero-order rate law indicated a second-order dependency on cupric ion concentration as expected from Eq. (19).

The saturated oxygen solubility can be described by Henry's law (18) when the oxygen pressure is below 1 atm; $[O_2] = k[pO_2]$. A first-order dependency on dissolved oxygen concentration for the rate of loss captopril shown by Eqs. (18) and (19) can therefore be equated to the observed first-order dependency on oxygen partial pressure.

According to Eqs. (17) and (18), the reaction would be expected to occur in the absence of metal ions through the molecular oxygen pathway. Since no measurable rate was observed in the presence of chelating agents and oxidation occurred without the addition of cupric ions, the rate constants in Eqs. (17)–(19) probably include catalytic effects attributable to trace metal ions. The lack of a measurable rate in the absence of oxygen, together with intercept values which approach zero in plots of the observed rate constants as a function of oxygen partial pressure, are consistent with the rate expression in Eq. (18).

When the pH was increased from 6.62 to 7.94, the captopril concentration at which the rate changed from first order to zero order was also decreased. As the pH is increased, the concentration of thiol anion (RS^-) would increase. Consequently, direct oxidation of captopril by molecular oxygen would become increasingly significant, thus competing more favorably with the cupric ion-catalyzed pathway. The apparent first-order rate process would therefore become more dominant. In summary, Scheme III presents a mechanism which is consistent with current experimental observations.

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