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Oxyhalogen–Sulfur Chemistry: Kinetics and Mechanism of Oxidation of Captopril by Acidified Bromate and Aqueous Bromine

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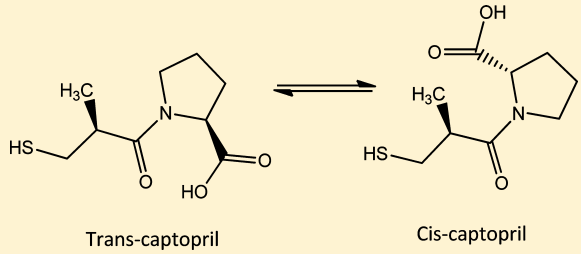
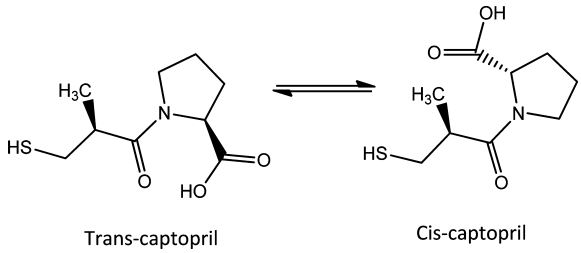
ABSTRACT: By nature of their nucleophilicity, all thiol-based drugs are oxidatively metabolized in the physiological environment. The key to understanding the physiological role of a hypertension drug, (2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid, medically known as captopril is through studying its oxidation pathway: its reactive intermediates and oxidation products. The oxidation of captopril by aqueous bromine and acidified bromate has been studied by spectrophotometric and electrospray ionization techniques. The stoichiometry for the reaction of acidic bromate with captopril is 1:1, BrO_3^- + $(C_4H_6N)(COOH)(COCHCH_3CH_2)-SH$ → $(C_4H_6N)(COOH)(COCHCH_3CH_2)-SO_3H$ + Br^-, with reaction occurring only at the thiol center. For the direct reaction of bromine with captopril, the ratio is 3:1; $3Br_2$ + $(C_4H_6N)(COOH)(COCHCH_3CH_2)-SH$ + $3H_2O$ → $(C_4H_6N)(COOH)-(COCHCH_3CH_2)-SO_3H$ + 6HBr. In excess acidic bromate conditions the reaction displays an initial induction period followed by a sharp rise in absorbance at 390 nm due to rapid formation of bromine. The direct reaction of aqueous bromine with captopril was much faster than oxidation of the thiol by acidified bromate, with a bimolecular rate constant of (1.046 (±0.08) × 10^5 M^{-1} s^{-1}. The detection of thiyl radicals confirms the involvement of radicals as intermediates in the oxidation of Captopril by acidified BrO_3^-. The involvement of thiyl radicals in oxidation of captopril competes with a nonradical pathway involving 2-electron oxidations of the sulfur center. The oxidation product of captopril under these strong oxidizing conditions is a sulfonic acid as confirmed by electrospray ionization mass spectrometry (ESI-MS), iodometric titrations, and proton nuclear magnetic resonance (^{1}H NMR) results. There was no evidence from ESI-MS for the formation of the sulfenic and sulfinic acids in the oxidation pathway as the thiol group is rapidly oxidized to the sulfonic acid. A computer simulation analysis of this mechanism gave a reasonably good fit to the experimental data.



INTRODUCTION

High blood pressure is one of the major risk factors for cardiovascular diseases including coronary heart disease, peripheral artery disease, and stroke.[1−6] Angiotensin-I converting enzyme (ACE) plays a critical physiological role in regulation of blood pressure by converting angiotensin-I to angiotensin-II, a potent vasoconstrictor.[7,8] The critical role of ACE in cardiovascular and renal diseases makes it an attractive target for drug design in the prevention of hypertension. Extensive structure−activity studies showed that the simple structure of the Ala−Pro analog (D-2-methylsuccinyl-L-proline) was optimal for binding to ACE in all but one respect: its zinc-binding carboxyl group.[9] Replacement of this carboxyl group by a sulfhydryl (SH) group resulted in a 1000-fold increase in the inhibitory potency, with the resulting compound, captopril (Scheme 1). Captopril became the most potent competitive inhibitor and the first useful antihypertensive drug designed to bind to the active site of ACE.[9]

Scheme 1



Captopril (1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline) was the first nonpeptidic orally active ACE inhibitor to be introduced for the treatment of hypertension and congestive

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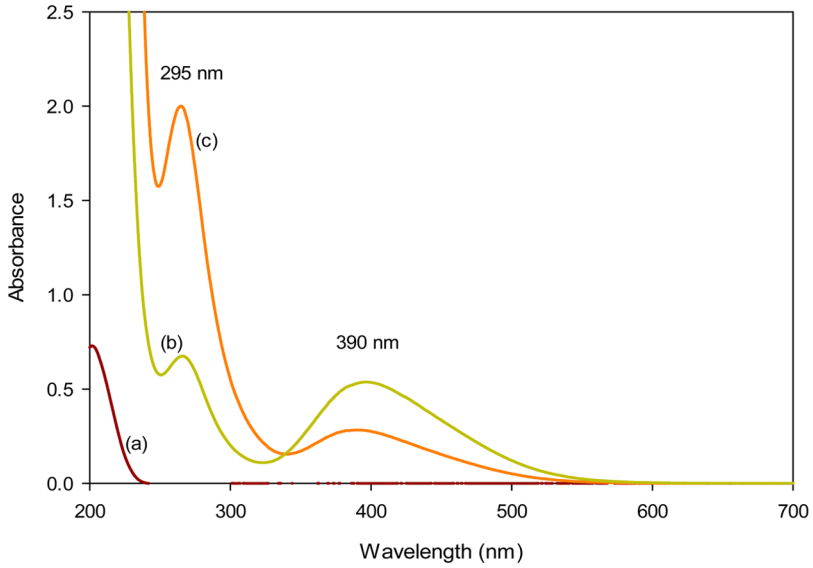

Figure 1. UV/vis spectral scans of captopril, bromine, and the product of captopril and acidified bromate. Bromine has two wavelengths, 266 and 390 nm. Captopril does not absorb in the UV/vis region, and there was no intereference on the bromine peak at 390 nm, which was used to quantify bromine concentrations.

heart failure.[10] Captopril is the prototypic human somatic Angiotensin-I converting enzyme (ACE) inhibitor which inactivates ACE by binding via its sulphydryl group to the zinc center.[11,12] ACE is known to cleave and inactivate the vasodilator bradykinin, and these actions result in raised blood pressure.[12] For that reason ACE is a primary target for drugs used to control hypertension.[13]

Orally, captopril is an effective ACE inhibitor and is widely used to treat high blood pressure, congestive heart failure, and cardiovascular disease[14] as such or in combination with other drugs.[15−18] After single oral dosing of captopril, the antihypertensive action is only effective for 6−8 h. As a result, clinical use requires a daily dose of 37.5−75 mg to be taken three times in equally divided doses.[19,20] Unfortunately, the usefulness of captopril has been associated with the development of side effects which include dry coughing, rash, and taste disturbances[21,22] which are attributed to the thiol group.[11] Because captopril binds strongly to metal cations, its use can lead to translocation and/or elimination of metals such as Cu, Mn, Ca, and Zn from various body tissues, which can cause deficiency of such metals.[23] Captopril is also used in the in vitro studies for the inhibition of proliferation of a variety of cell lines.[24]

Captopril and various other reduced sulfhydryl (SH) group containing converting enzyme inhibitor compounds have antioxidant properties; for example, they are potent scavengers of hydroxyl radicals (OH.) as well as hypohalite radicals (OCl.).[25−27] Some in vitro studies indicate that captopril functions as an antioxidant by increasing the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase.[28] Captopril is easily converted to its disulfide dimer and forms conjugates with other thiols.[29] Under anaerobic and in aerobic conditions, captopril has been reported to give different products, with disulfide being the major degradation product under aerobic conditions (which is a significant pharmaceutical problem[30]), and proline is obtained after anaerobic hydrolysis.[31]

Sulfur compounds in the physiological environment undergo a variety of metabolic reactions, namely oxidations, reductions, hydrolysis and conjugations. Sulfur in most organic configurations is nucleophilic, and nucleophilic atoms are usually susceptible to metabolic oxidation.[32−34] The oxidation of sulfur-containing compounds represents an important aspect of sulfur metabolism. These oxidations appear to be involved in many cellular functions, including the reductive degradation of polypeptide hormones and proteins, regulation of protein synthesis, maintenance of intracellular redox potential, and protection of the cell from oxidative damage.[35] The study of mechanism of the oxidation the sulfhydryl (SH) group of captopril is thus important because it predicts possible bioactivation metabolites and controls the inhibitory potency of the drug.[36] Some work done previously in our group has shown that in a strongly oxidizing in vitro environment, the oxidation of sulfhydryl-containing molecules such as cysteamine and *N*-acetylcysteine yields the sulfonic acid as the final product and no sulfate is observed; meaning that C−S bond remained intact.[37,38] Many of the toxic organosulfur compounds are oxidatively metabolized to sulfate, with a concomitant cleavage of the C−S bonds and subsequent release of damaging genotoxic reactive oxygen species.[39−41] Our previous work had established that thiols with an amine group on the β-carbon are not oxidatively metabolized to sulfate; specific examples including cysteamine,[42] hypotaurine[43] and cysteine.[44] The aim of this study is to report on the oxidation of captopril by strong oxidants, acidified bromate and bromine, and to determine the mechanism and possible metabolites, stable and unstable, as well as the products. This study will go a long way in determining its dose-limiting toxicological effects.

EXPERIMENTAL SECTION

Materials. The following reagent grade chemicals were used without further purification: sodium bromate, perchloric acid (70−72%), sodium bromide, bromine, sodium perchlorate, soluble starch, sodium thiosulfate (Fisher), captopril (Sigma), and DCl and D_2O (Aldrich). Bromine solutions, being volatile, were kept capped and standardized spectrophotometrically before each set of experiments. Stock solutions of captopril were prepared just before use.

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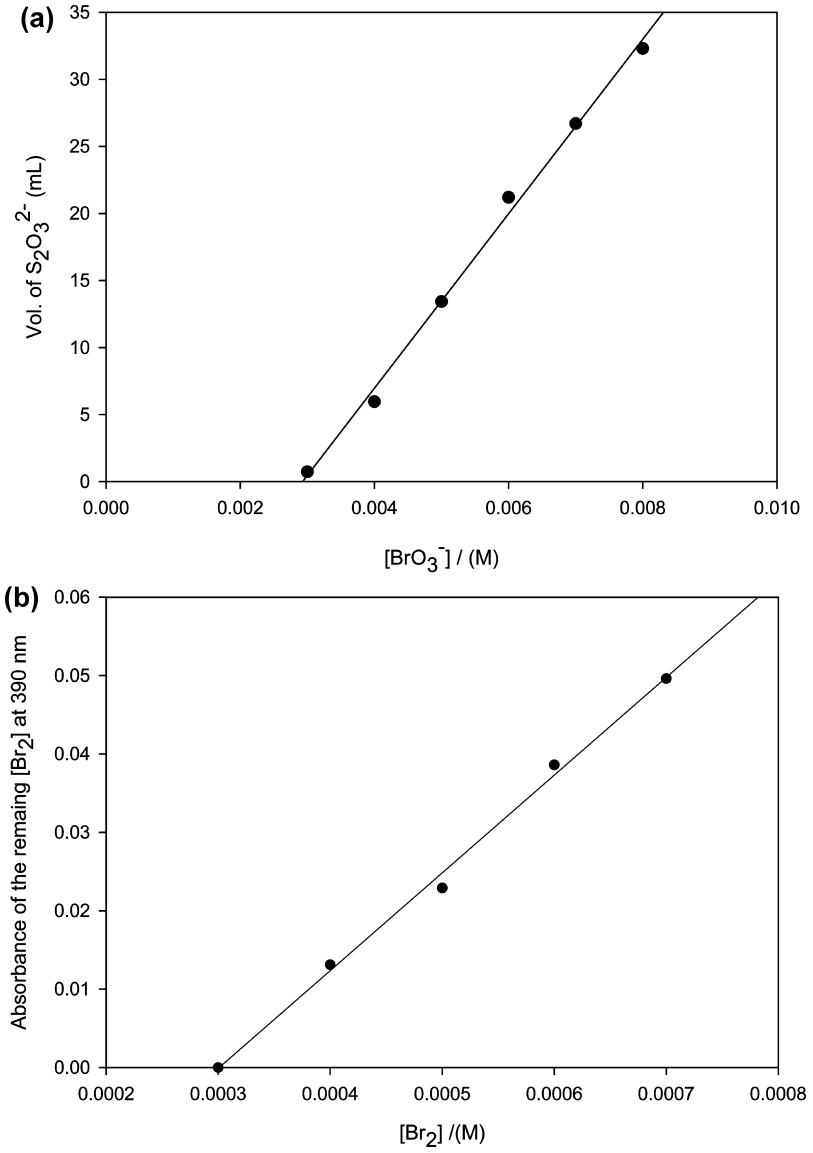

Figure 2. (a) Stoichiometric plot generated from iodometric titration of sodium thiosulfate against liberated I_2 following complete oxidation of captopril in acidic bromate. For a fixed captopril concentration of 0.003 M, the intercept on the $[BrO_3^-]$ axis is 0.0029 M. The ratio of $[BrO_3^-/CAP]$ is 1:1. (b) Stoichiometric determination of the bromine−CAP reaction by bromine titration. Fixed $[CAP]_0 = 1 \times 10^{-4}$ M and varied $[Br_2]$ and the absorbance of the remaining bromine was plotted against initial bromine concentration. The intercept on the $[Br_2]$ axis is 0.0003 M giving a 1:3 ratio for $[Br_2]/[CAP]$.

Methods. The rapid reactions of captopril with bromine were followed on a Hi-Tech Scientific SF61-DX2 double-mixing stopped-flow spectrophotometer. These reactions were monitored by following consumption of bromine at 390 nm (ε = 142 M^{-1} cm^{-1}). Captopril has no absorbance in the visible region, whereas aqueous bromine has an isolated peak at 390 nm (Figure 1). Thus absorbance at this peak could be used for analytical determination of aqueous bromine. Slower reactions involving sulfonic acid formation following oxidation of captopril by acidified bromate were monitored on a conventional Perkin-Elmer Lambda 25 UV−vis spectrophotometer. All kinetics experiments were performed at 25.0 ± 0.1 °C and at an ionic strength of 1 M ($NaClO_4$). All solutions were prepared using doubly distilled deionized water from a Barnstead Sybron Corp. water purification unit capable of producing both distilled and deionized water (Nanopure). Mass spectra of product solutions were taken on a Thermo Scientific LTQ-Orbitrap Discovery mass spectrometer (San Jose, CA) equipped with an electrospray ionization source operated in the negative mode. All EPR spectra were recorded on a Bruker Biospin e-scan spectrometer designed to perform EPR measurements in the X-band-range at room temperature. ^1H NMR spectra of captopril and its oxidation products were recorded with a Bruker AMX-400 MHz spectrometer using D_2O as the solvent and internal standard.

Stoichiometric Determinations. Stoichiometric determinations were performed by an iodometric titration method in which varying amounts of bromate were reacted with fixed concentrations of captopril in highly acidic conditions. The excess bromate was evaluated by addition of acidified excess iodide with the liberated iodine next titrated against standard sodium thiosulfate with freshly prepared starch as indicator. Bromine−captopril stoichiometric determinations were per-

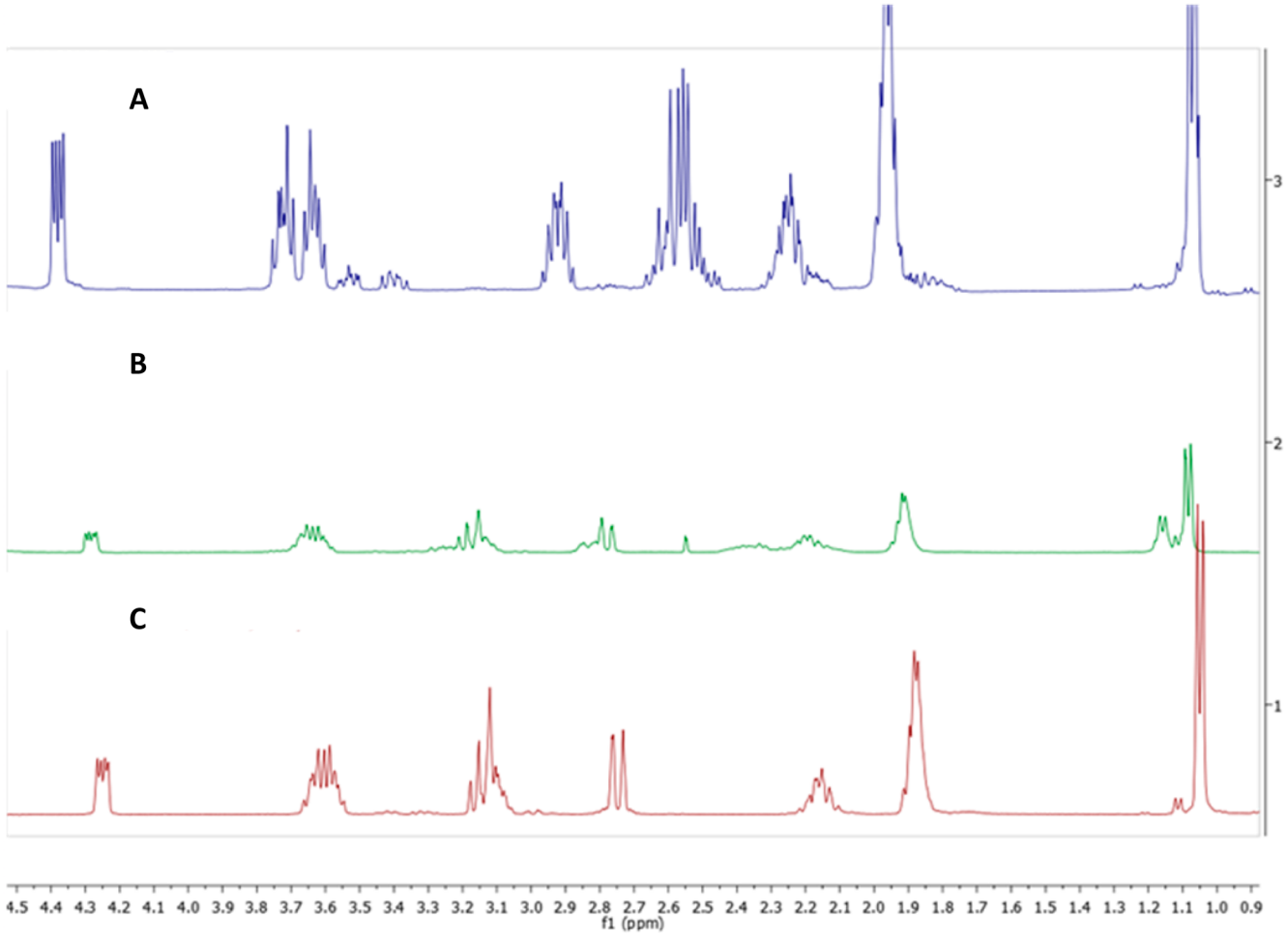

Figure 3. (A) ^{1}H NMR spectrum for captopril showing the *S*-methyl protons peak at 2.55 ppm and the proton next to the *S*-methyl s peak at 2.95 ppm. (B) and (C) show spectra of oxidation products of CAP by acidified bromate and CAP by bromine, respectively. Both show a shift in *S*-methyl protons, from 2.55 to 2.75 ppm, and the proton next to the *S*-methyl s peak, from 2.95 to 3.15 ppm, due to oxidation of the sulfur center.

formed in excess bromine with excess bromine determined spectrophotometrically as well as iodometrically.

RESULTS AND DISCUSSION

Stoichiometry. A series of experiments were performed with constant captopril concentration of 3 mM while varying excess acidified bromate. The same standard thiosulfate solution was used in a titrimetric analysis of excess oxidizing power left after complete consumption of the substrate, captopril. The exact and precise thiosulfate concentration was not important, for as long as the same solution was used throughout the whole series of titrimetric analyses. A plot of volume of thiosulfate needed vs initial bromate concentrations was linear (Figure 2a), as expected, and the zero thiosulfate volume intercept represented the concentration of bromate needed to just consume the 3 mM captopril with no excess oxidizing bromate left to effect an iodometric titration. Figure 2a gives an intercept of 2.96 mM bromate as the stoichiometric equivalent to 3 mM captopril. This indicated a 1:1 stoichiometry of bromate to captopril and also indicated that reactivity only occurred at the thiol group. By assuming that captopril can be represented as RSH (a thiol), we could represent the stoichiometry as

$$RSH + BrO_3^- \rightarrow RSO_3H + Br^- \quad (R1)$$

This represented a 6-electron oxidation of the sulfur center from −2 to +4. The stoichiometry of the oxidation of captopril by aqueous bromine was spectrophotometrically determined (Figure 2b). Excess bromine concentrations were used for a fixed amount of captopril and residual bromine absorbance noted. Plot of bromine absorbance should give an intercept value (zero absorbance) of the bromine concentration needed to just deplete the fixed amount of captopril. 01 mM captopril was used in Figure 2b with a concomitant intercept of 0.3 mM bromine, indicating a 1:3 stoichiometry:

$$RSH + 3Br_2 + 3H_2O \rightarrow RSO_3H + 6Br^- + 6H^+ \quad (R2)$$

All our studies were carried out in excess bromate so that formation of bromine could be used to study the reaction. Excess bromate conditions would bring into play the BrO_3^-/Br^- reaction after complete consumption of the substrate, captopril:

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2(aq) + 3H_2O \quad (R3)$$

The final stoichiometry in excess acidic bromate conditions is a linear combination of reactions R1 and R3, which consumes all the bromide formed in reaction R1. This stoichiometry is achieved by 5R1 + R3:

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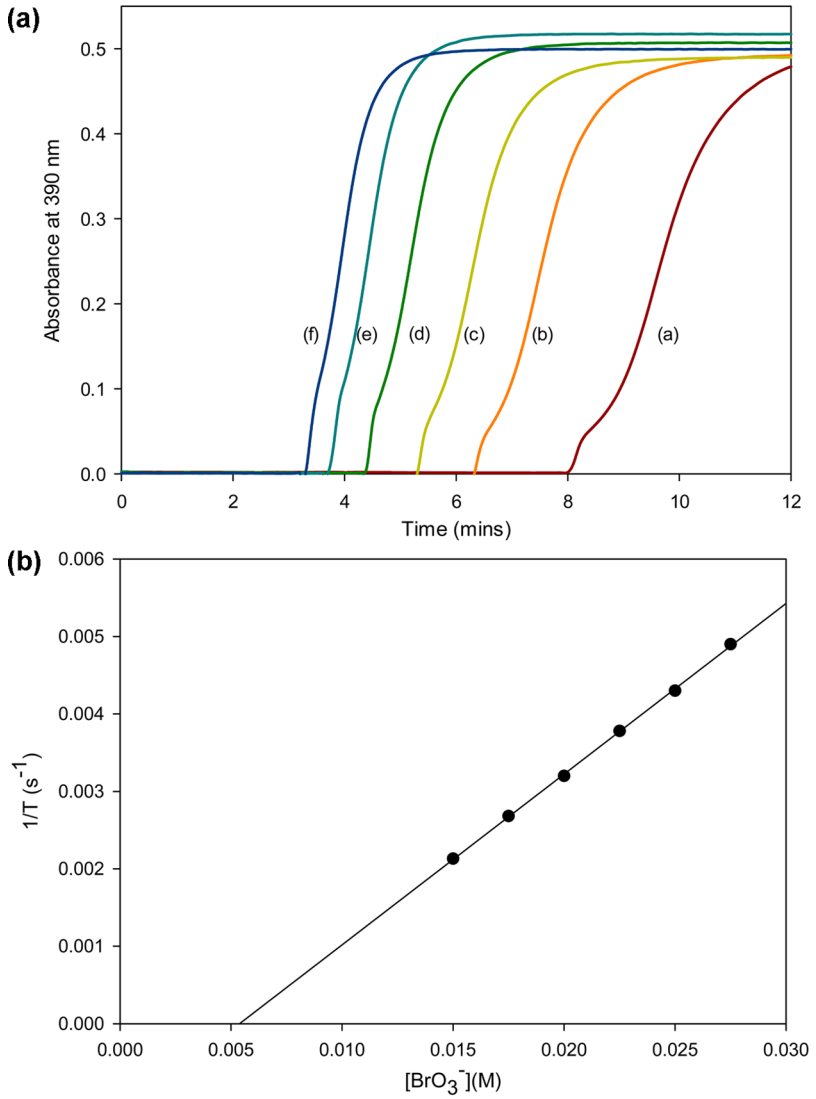

Figure 4. (a) Absorbance traces showing the effect of varying bromate concentrations on the rate of reaction. $[CAP]_0$ = 0.005 M; $[H^+]_0$ = 0.4 M; $[BrO_3^-]_0$ = (a) 0.015 M, (b) 0.0175 M, (c) 0.020 M, (d) 0.0225 M, (e) 0.0250 M, and (f) 0.0275 M. (b) Direct inverse relationship between induction time against initial bromate concentration for conditions shown in Figure 6a.

$$6BrO_3^- + 5RSH + 6H^+ \rightarrow 5RSO_3H + 3Br_2(aq) + 3H_2O \quad (R4)$$

At these conditions, concentration of captopril is limiting, with respect to bromine formation and in all reactions performed in excess bromate, amount of bromine obtained was exactly 60% of the initial captopril concentrations as expected from stoichiometry R4 (vide infra, Figure 7).

Proton NMR experiments were also utilized to show that the carbon backbone of captopril is not altered during its oxidation by acidic bromate and aqueous bromine. Figure 3, spectrum A shows the normal spectrum of captopril. It shows the expected *S*-methyl protons peak at 2.55 ppm and the proton at the adjacent asymmetric carbon center showing up at 2.95 ppm. Figure 3 spectrum B shows the spectrum of the product of captopril oxidation by excess acidified bromate. It shows essentially the same carbon skeleton features of the parent compound except for the expected downfield shift of both peaks to 2.75 and 3.15 ppm, respectively, due to the stronger electron withdrawing sulfonic acid group. Spectrum C shows a stoichiometric equivalent bromine concentration (1:3 ratio). It shows the same spectrum as the one derived from excess acidified bromate solution. The poor resolution in excess bromate is derived from the low pH, highly acidic conditions that are necessary for bromate oxidations. Spectra B and C show that the same product is obtained with bromate and in aqueous bromine oxidations and that the sole reactivity occurs on the thiol group.

Kinetics. All kinetics experiments were performed in excess acidic bromate conditions so that the reaction could be followed spectrophotometrically by bromine production. The acid was a catalyst that was not involved in the reaction stoichiometry (reaction R1). In this mode, the reaction was characterized by an initial quiescent period, followed by a rapid formation of bromine. Reactions R1−R3 occur concurrently in the reaction mixture at any time.

Formation of bromine occurs when the volume of reaction R3 exceeds that of reaction R2. The relative rates of these three reactions determine the length of the quiescent period (induction time) and the rate of formation of bromine after the quiescent period. At constant acid and captopril concentrations, bromate concentrations shorten the induction

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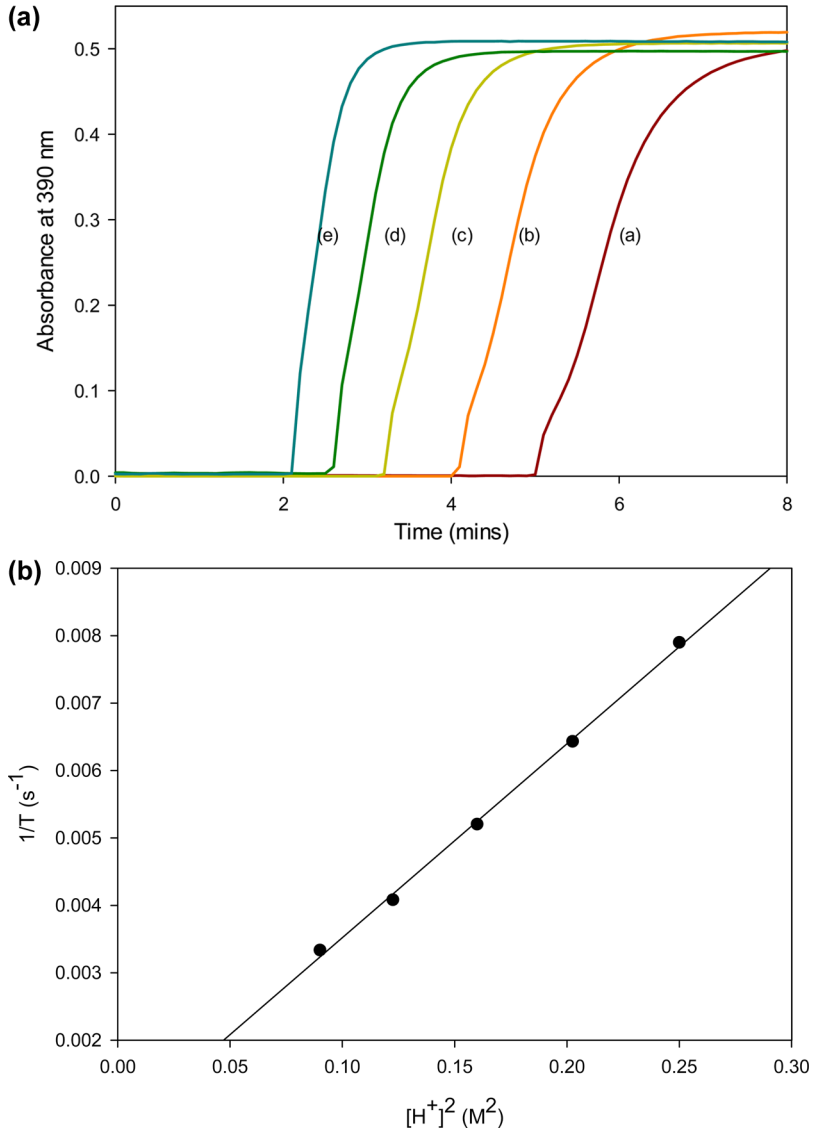

Figure 5. (a) Absorbance traces showing the effect of varying acid concentrations. $[CAP]_0$ = 0.005 M; $[BrO_3^-]_0$ = 0.02 M; $[H^+]_0$ = (a) 0.3 M, (b) 0.35 M, (c) 0.4 M, (d) 0.45 M, and (e) 0.5 M. The final bromine formed is independent of $[H^+]_0$ as the final bromine remained the same. The induction time became shorter with increasing $[H^+]_0$. (b) Inverse square linear relationship between induction time against acid concentrations for the experimental conditions in (a).

period and also increase rate of formation of bromine (Figure 4a). At excess bromate concentrations, final bromine formed in Figure 4a is invariant because captopril is the limiting reagent (reaction R4). A plot of inverse of induction time versus initial bromate concentrations is linear (Figure 4b). This is kinetically significant. Bromine production indicates total consumption of captopril, and thus the rate of reaction is related to the time taken for consumption of captopril. For a first-order dependence, there should be an inverse relationship between induction time and concentration of the variable substrate. Thus one can predict that the rate of reaction of captopril and bromate is first-order in bromate. One can also predict stoichiometry R1 from the plot shown in Figure 4b. The intercept bromate concentration indicates the bromate concentration needed to satisfy stoichiometry R1, where the induction time approaches infinity and the inverse of the induction time is zero. For the concentrations used for Figure 4a, captopril was fixed at 5 mM. The bromate intercept concentration is 5.1 mM, indicating a 1:1 stoichiometry.

Acid also shortened the induction period (Figure 5a). It did not alter the amount of final bromine formed for a fixed bromate to captopril ratio because its action is merely catalytic and not involved in the final stoichiometry (reaction R1). Acid dependence, for a range of mildly acidic concentrations, showed an inverse square dependence on the induction period (Figure 5b). This dependence became inverse first-order at high acid concentrations.

If the $[BrO_3^-]_0/[CAP]_0$ ratio, R, is maintained high, much greater than the stoichiometric ratio of 1.2 needed in reaction R4, any changes in initial captopril concentrations produce an invariant induction period. Figure 6 shows a series of kinetics traces with $8.3 > R > 4.6$ with an invariant induction period and a final bromine concentration conforming to stoichiometry R4. A plot of captopril concentration (independent axis) versus final bromine concentration gave a straight line through the origin and with a slope of 0.6 (data not shown). Such plots as shown in Figure 6 have been observed in several other reaction

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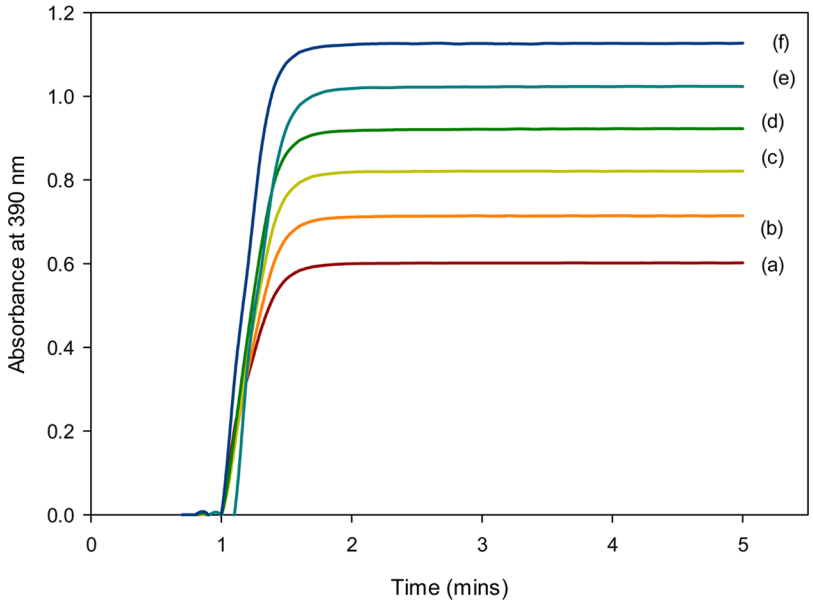

Figure 6. Absorbance traces showing the effect of varying CAP concentrations on the amount of bromine formed. $[H^+]_0$ = 0.5 M; $[BrO_3^-]_0$ = 0.05 M; $[CAP]_0$ = (a) 0.006 M, (b) 0.007 M, (c) 0.008 M, (d) 0.009 M, (e) 0.010 M, and (f) 0.011 M. The traces show an increase in bromine formed as CAP concentration increases from (a) to (f).

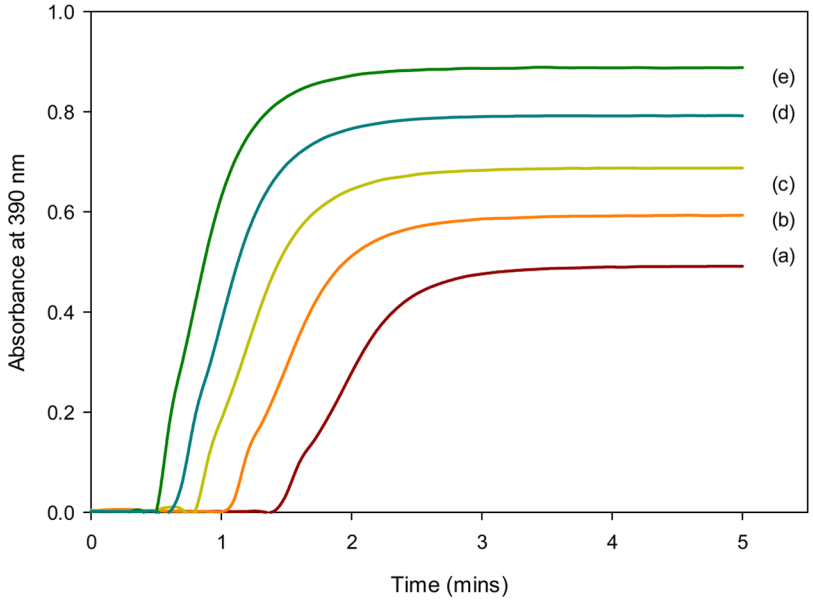

Figure 7. Absorbance traces showing the effect of varying bromide concentration. $[CAP]_0$ = 0.004 M, $[H^+]_0$ = 0.3 M, $[BrO_3^-]_0$ = 0.04 M, $[Br^-]_0$ = (a) 0.001 M, (b) 0.002 M, (c) 0.003 M, (d) 0.004 M, and (e) 0.005 M. Addition of bromide (from trace (a) to (e)) reduced the induction time and increased the amount of bromine formed.

systems involving an induction period dependent on total consumption of the reductant.

Bromide is a product of the reaction (reaction R1) but is also heavily involved in the global dynamics of the reaction through reaction R3. Its initial addition to the reaction mixture will alter three specific aspects of the reaction: induction period, final amount of bromine formed, and rate of formation of bromine at the end of the induction period (Figure 7). In Figure 7, bromate, acid, and captopril concentrations were fixed. A plot of initial bromide concentration (independent axis) versus final bromine concentration was a straight line, with the intercept on the bromine concentration axis (representing zero added bromide) giving the bromine concentrations expected from stoichiometry R4 (plot not shown). Though catalytic, bromide is not an autocatalyst. Autocatalysis involves a rapid increase in reaction rate, which is incommensurate with its involvement in the form of standard mass-action kinetics. Figure 7, however, shows simple linear kinetics dependence of the reaction on bromide and not the sigmoidal increase in rate on addition of bromide, a hallmark of autocatalysis.

The direct reaction of captopril with bromine was exceedingly fast, almost diffusion controlled, and approaching the limits of detection of our stopped-flow instrument (vide supra). Figure 8 shows a series of kinetic traces performed in excess bromine concentrations over captopril. This figure shows that the reaction is complete within 10 ms. Excess bromine

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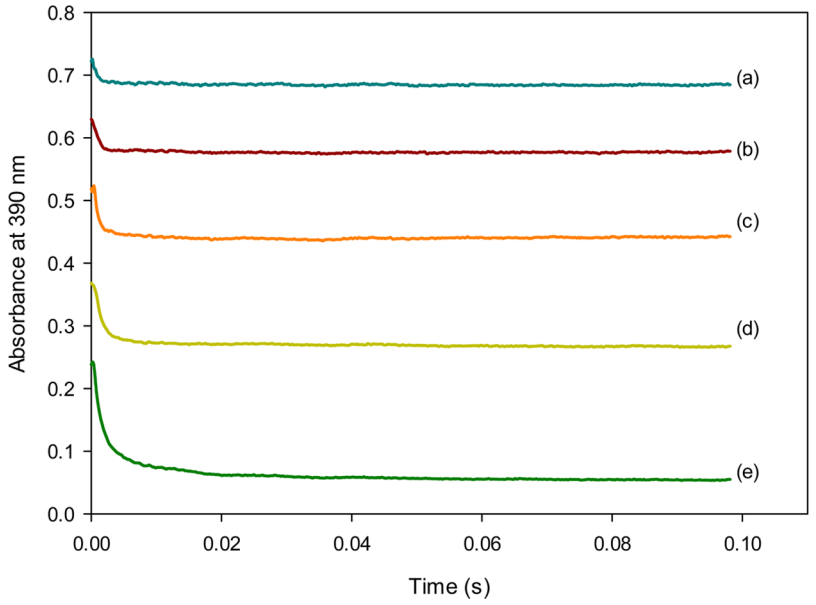

Figure 8. Absorbance traces showing the effect of varying captopril concentration on bromine depletion. $[Br_2]_0$ = 0.0025 M, $[Br^-]$ = 1.0 M and varied $[CAP]_0$ = (a) 0.0002 M, (b) 0.0003 M, (c) 0.0004 M, (d) 0.0005 M, and (e) 0.0006 M.

concentrations obtained at the end of the reaction conformed to stoichiometry R2. This rapid reaction rate, at least 10^4 times faster than the main reaction (Figures 5a, 6, and 7), indicates that, at the moment of observation of bromine production, all the substrate (captopril) would have been completely consumed. This simplifies the kinetics analysis of the observed global dynamics of this reaction.

Mechanism. The overall reaction mechanism involves the well-known oxyhalogen/oxybromine kinetics and sulfur oxidation kinetics, which are not as well-known. Rate of depletion of captopril has a first-order dependence on bromate and a second-order dependence on acid, as expected from acidic bromate oxidations. These bromate oxidations were extensively studied and experimentally determined during the study of the Belousov–Zhabotinskyi reaction, which involves a metal ion catalyzed oxidation of an organic substrate with an ionizable proton by acidified bromate. Generally, the rate of reaction can be summed by a fourth-order rate law:

$$\text{Rate} = k_0[BrO_3^-][H^+]^2[\text{Red.}] \quad (1)$$

In eq 1, Red. can be any 2-electron reductant. Involvement of acid is through protonation of bromate to bromic acid, followed by the acidification of bromic acid to produce the active oxidizing species:

$$H^+ + BrO_3^- \leftrightharpoons HBrO_3 \quad (R5)$$

$$HBrO_3 + H^+ \leftrightharpoons H_2BrO_3^+ \quad (R6)$$

$$H_2BrO_3^+ + 2e^- \rightarrow HBrO_2 + OH^- \quad (R7)$$

With reaction R7 as the rate-determining step, then the overall rate law eq 1 can be justified. Standard oxybromine kinetics involve Br^- as the 2-electron reductant which is oxidized to HOBr:

$$H_2BrO_3^+ + Br^- \leftrightharpoons HBrO_2 + HOBr \quad (R8)$$

Composite reaction R8 is written as

$$BrO_3^- + 2H^+ + Br^- \leftrightharpoons HBrO_2 + HOBr \quad (R9)$$

If sequence R5–R9 is correct, according the standard oxybromine kinetics, then oxidation of the sulfur center should proceed through 2-electron oxidations via sulfenic (S(I)), sulfinic (S(II)), and sulfonic (S(IV)) acids. This is a sequence that has been suggested in several oxidations of thiols and thiocarbamides.[45]

$$HBrO_2 + RSH \rightarrow HOBr + RSOH \quad (R10)$$

RSOH would represent the unstable sulfenic acid which can rapidly be oxidized to the sulfinic acid:

$$HOBr + RSOH \rightarrow Br^- + H^+ + RSO_2H \quad (R11)$$

Or react with a captopril molecule to form the dimeric species in a condensation-type reaction:

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (R12)$$

Due to its instability, it can also disproportionate to the sulfinic acid and thiol:

$$2RSOH \rightarrow RSO_2H + RSH \quad (R13)$$

The final distribution of the oxo-acids is based on amount of oxidant relative to reductant and the relative stability of the oxo-acids.

Figure 9, however, shows the formation of radicals during the oxidation of captopril. Radicals were trapped by 5,5-dimethyl-1-pyrroline *N*-oxide, DMPO, on an X-band EPR spectrometer. None of the standard DMPO adduct radical spectra such as those with hydroxyl radical and superoxide radical are evident in the spectra shown in Figure 9. The spectra appear to result from more than one radical species. The Belousov–Zhabotinskyi reaction mechanism in highly acidic media has established the existence of the bromine dioxide radical as the backbone in the generation of autocatalysis which is the essential nonlinearity for oscillatory behavior:[46−48]

$$BrO_3^- + HBrO_2 + H^+ \rightarrow 2BrO_2^{\bullet} + H_2O \quad (R14)$$

Oxidation by bromine dioxide radical is via a one-electron transfer:

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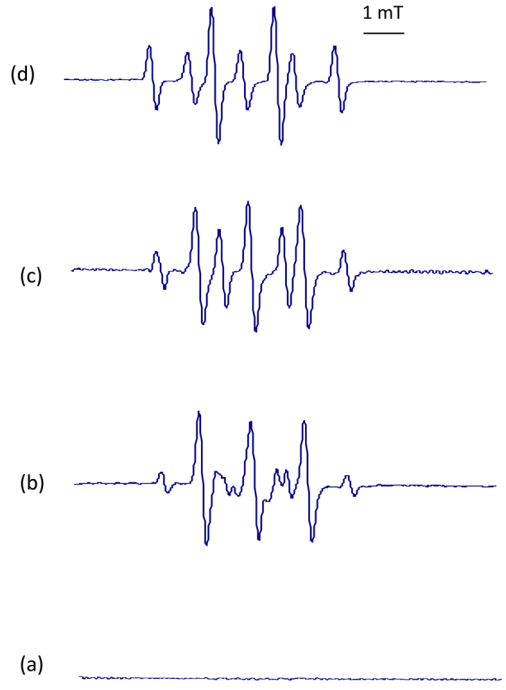

Figure 9. EPR spectra of thiyl radical generated during the oxidation of CAP using spin trap [5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)]$_0$ = 0.06 M, [H$^+$]$_0$ = 0.01 M, [BrO$_3^-$]$_0$ = 0.015 M, and varied [CAP]$_0$ = (a) 0 M, (b) 0.01 M, (c) 0.02 M, and (d) 0.04 M.

$$BrO_2^{\bullet} + H^+ + Red. \rightarrow HBrO_2 + Red.^+ \quad (R15)$$

Combining reaction R14 with two reaction R15s shows that $HBrO_2$ is produced autocatalytically via quadratic autocatalysis in which 1 mol of bromous acid, $HBrO_2$ results in 2 mol of bromous acid, resulting in an ever-increasing reaction rate. Bromine dioxide formation would encourage formation of the thiyl radical:

$$BrO_2^{\bullet} + RSH \rightarrow HBrO_2 + RS^{\bullet} \quad (R16)$$

The abstraction of the hydrogen atom should be facile to form the more stable bromous acid.

Termination of these radicals would be through the formation of the captopril dimer:

$$2RS^{\bullet} \rightarrow RSSR \quad (R17)$$

Oxidation Pathway of Captopril. The oxidation pathway of captopril could be mapped by a series of experiments in which varying ratios of oxidant are used with captopril initially in excess up to stoichiometric amount of oxidant and eventually to where oxidant is in excess. At each ratio an ESI spectrum is taken to deduce the stable metabolites before formation of final product as expected in stoichiometry R1. This could be achieved by the use of aqueous bromine (Figure 10a–c). In the spectrum shown in Figure 10a, a 1:3 ratio of bromine to captopril is utilized in which 1 equiv of oxidizing power is used. This represents a 2-electron oxidation of the expected 6 electrons for a full oxidation to the sulfonic acid. The final spectrum is surprisingly simple, with the expected dominant peak for the substrate, captopril, at m/z = 131.52, the single negatively charged substrate at m/z = 216.07, and a relatively strong peak for the dimer, RSSR, at m/z = 215.06. A small peak for the thiosulfinate, RSS(=O)R, at 231.06 is also observed as well as the product, sulfonic acid, at 264.05. This simple experiment shows that none of the oxo-acids of captopril, sulfenic acid, and sulfinic acid are stable, and any oxo-acid formed would disproportionate to the original substrate and the sulfonic acid. The dimeric species could be formed from either reaction R12 (2-electron oxidation) or R17 (1-electron oxidation). However, the thiosulfinate at m/z = 231.05 can only be formed from a 2-electron oxidation:

$$2RSOH \rightarrow RSS(=O)R + H_2O \quad (R18)$$

The 1:2 reductant to oxidant spectrum is more telling (Figure 10b). In this spectrum, the thiosulfinate peak increases to nearly the same level of abundance as the dimer at the expense of the product peak at m/z = 264.05. This clearly shows that, in excess and/or increasing oxidant ratios, the 2-electron pathway dominates. Had the one-electron pathway been dominant, then the autocatalytic pathway would display its hallmark of sigmoidal kinetics. These were not observed in this system. It is possible for the thiosulfinate to be formed by oxidation of the dimeric species, but its relative stability militates against its ease of oxidation to the thiosulfinate. Figure 10c shows the full stoichiometric equivalent of oxidant, a 1:3 ratio of captopril to bromine that shows only peaks for the product at m/z = 264.05 and 131.53. Further increases in oxidant concentration ratios showed no other products.

Computer Simulations. The full mechanism is compiled in Table 1 and used to simulate the reaction. This simple 21-reaction scheme was able to satisfactorily model the kinetics of the reaction, both the acid and bromate dependencies. The model, when compared to experimental data, is shown in Figure 11. Experimental traces are derived from data in Figure 5. Kinetics data for reactions M4 to M7 were derived from literature values. Reactions M8 and M9 were unimportant with respect to the simulations for as long as they were faster than reaction M7. Though pathway M7–M9 was viable, it was not dominant, as observed from the reaction kinetics. We assumed all bromine dioxide radicals formed reacted via reaction M8 and neglected the possible formation of Br_2O_4. Parameters for reactions M12 to M14 were estimated from this study. Reactions M15 and M16 were merely rearrangement reactions that were considered fast and not rate-determining because there was no evidence of the formation of stable sulfenic or sulfinic acids. The simulations shown in Figure 11 considered excess oxidant conditions with product spectra as in Figure 10c with no formation of the thiolsufinate. The three additional reactions, M19–M21 were not important for the purposes of the model, and only relevant in conditions of excess reductant. These are not the conditions utilized in the simulated Figure 5 data. Thus they could be removed from the model with very little effect on the overall simulations when the bromine formation was being observed.

In the absence of added bromide, the most important parameter was the forward rate constant of reaction M1 (this was assumed to be irreversible). The stability of the dimeric species meant that kinetics parameters for reactions M17 and M18 were also important. A relevant assumption in our simulations was that reaction M5 was rapid enough for us to ignore all oxidations of the substrate by bromous acid, though, clearly, bromous acid is a relevant oxidant. Clearly, reactions M10, M11, M13, and M14 were not that important because none of the metabolites involved in these reactions were detected on ESI spectra.

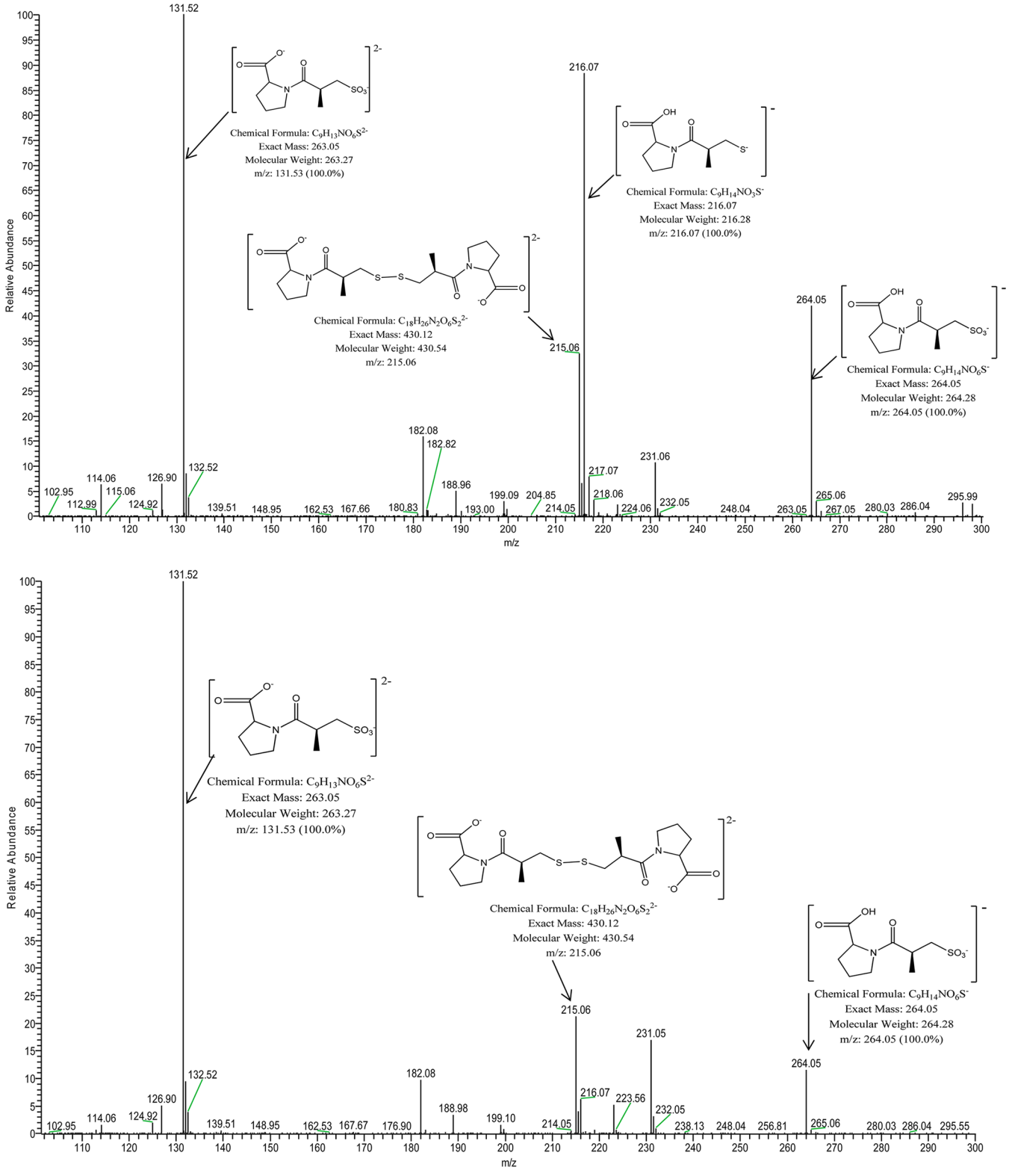

Figure 10. continued

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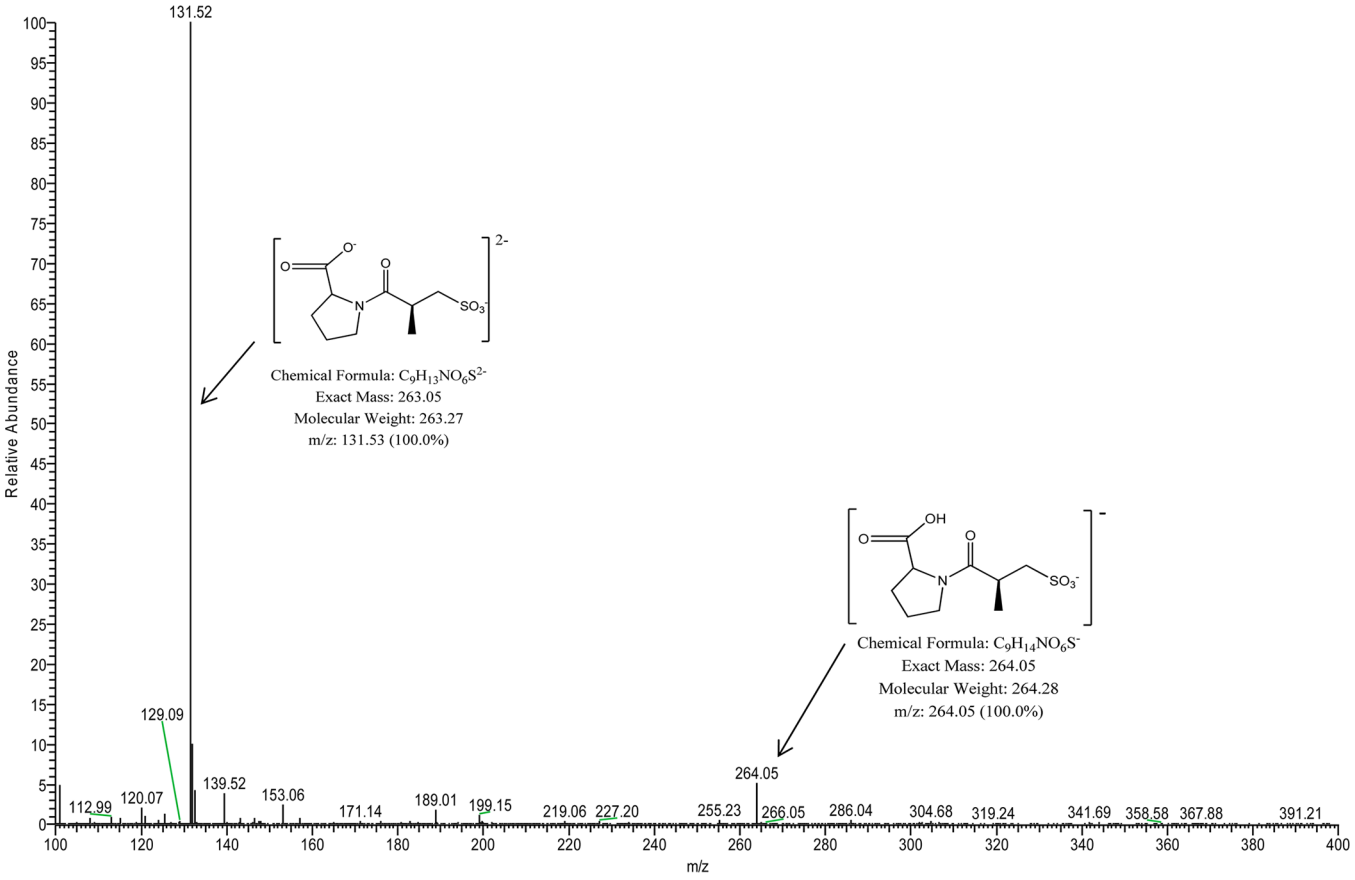

Figure 10. (a) Negative mode ESI-MS spectrum of stoichiometric 1:1 ratio [CAP−Br_2] solution using 10:90 methanol−water solvent after three solvent extractions with (95%) *n*-hexane. This spectrum was taken 5 min into the reaction. (b) Negative ESI-MS spectrum of stoichiometric 1:2 ratio [CAP−Br_2] solution taken after 1 h. (c) Spectrum taken of the expected full stoichiometric ratio of 1:3. Only one product, the sulfonic acid, is obtained at these conditions.

Table 1. Mechanism Used for Simulating the S-Oxidation of Captopril (RSH = Captopril)

reaction	reaction	k_f, k_r[a]
M1	$BrO_3^- + RSH + H^+ \rightarrow HBrO_2 + RSOH$	8.0×10^{-1}; 0
M2	$HBrO_2 + RSH \rightarrow HOBr + RSOH$	1.0×10^{8}; 0
M3	$HOBr + RSH \rightarrow Br^- + H^+ + RSOH$	1.0×10^{10}; 0
M4	$BrO_3^- + 2H^+ + Br^- \rightarrow HBrO_2 + HOBr$	2.1; 1.0×10^{-4}
M5	$HBrO_2 + Br^- + H^+ \rightarrow 2HOBr$	2.0×10^{6}; 2.0×10^{-5}
M6	$HOBr + Br^- + H^+ \rightarrow Br_2 + H_2O$	8.9×10^{9}; 1.1×10^{2}
M7	$BrO_3^- + HBrO_2 + H^+ \rightarrow 2BrO_2^\bullet + H_2O$	1.1×10^{-4}; approx 0
M8	$BrO_2^\bullet + RSH \rightarrow RS^\bullet + HBrO_2$	5.0; 0
M9	$2RS^\bullet \rightarrow RSSR$	100
M10	$HOBr + RSOH \rightarrow RSO_2H + Br^- + H^+$	1.0×10^{9}
M11	$HOBr + RSO_2H \rightarrow RSO_3H + Br^- + H^+$	1.0×10^{9}
M12	$Br_2(aq) + RSH + H_2O \rightarrow RSOH + 2Br^- + 2H^+$	1.05×10^{6}
M13	$Br_2(aq) + RSOH + H_2O \rightarrow RSO_2H + 2Br^- + 2H^+$	1.0×10^{9}
M14	$Br_2(aq) + RSO_2H + H_2O \rightarrow RSO_3H + 2Br^- + 2H^+$	2.5×10^{10}
M15	$RSO_2H + RSO_2H \rightarrow RSOH + RSO_3H$	1.0×10^{-6}
M16	$RSOH + RSH \rightarrow RSSR + H_2O$	1.0×10^{-4}
M17	$RSSR + HOBr + H_2O \rightarrow 2RSOH + Br^- + H^+$	1.0×10^{3}
M18	$RSSR + Br_2 + 2H_2O \rightarrow 2RSOH + 2Br^- + 2H^+$	1.0×10^{4}
M19	$2RSOH \rightarrow RSS(=O)R + H_2O$	5×10^{-1}; 2×10^{-2}
M20	$RSS(=O)R + Br_2 + H_2O \rightarrow RSO_2H + RSH$	1.0×10^{6}
M21	$RSSR + HOBr \rightarrow RSS(=O)R + H^+ + Br^-$	1.0×10^{5}

[a]Forward and reverse rate constants, separated by a semi-colon. Except for reactions involving water, the units of kinetics rate constants derived from the reactions' molecularity.

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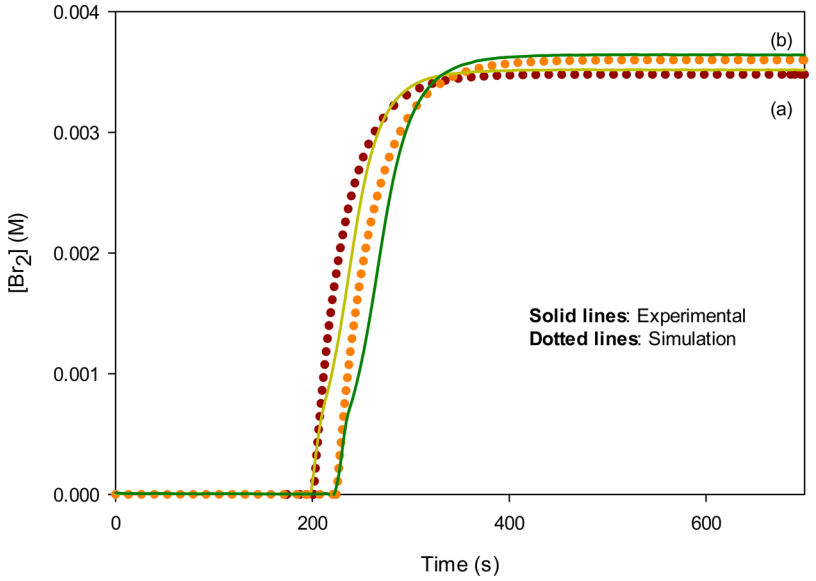

Figure 11. Simulation traces for reaction of acidified bromate with CAP for experimental conditions in Figure 6a traces (f) and (g).

CONCLUSION

This simple mechanistic study shows that captopril can be bioactivated oxidatively, to the dimeric species, and in highly oxidative environments it can only be oxidized to the sulfonic acid. This has been a characteristic of many biologically active thiols such as cysteine, glutathione, and cysteamine.[49−51] Most thiol/thiocarbamide-based toxicities have been attributed to the early cleavage of the C−S bond before oxidative saturation of the sulfur center. These highly reactive sulfur leaving groups then generated a cascade of reactive oxygen species which are genotoxic. Because the C−S bond in captopril is not cleaved, then the above argument cannot be used here. The acknowledged toxicity of captopril is thus not asserted from its oxidative bioactivation. Rather, the unreacted captopril and its dimer should take the active toxic form. Work in our laboratories determined that dimeric forms of small biologically active thiols do react with sulfhydryl groups of protein and/or peptides to form mixed disulfides, which are recognized as foreign by the immune system, thereby eliciting an immune response from the biological system.[52−54] Its binding to relevant biological metal ions should be the genesis of its toxicity because its oxidative bioactivation does not result in any toxic metabolites.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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