Antioxidant Chemistry: Hypotaurine−Taurine Oxidation by Chlorite[1]

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Extensive experimental data have been collected on the oxidation of hypotaurine, $H_2NCH_2CH_2SO_2H$, by chlorite and chlorine dioxide. Hypotaurine is stable and reacts slowly with chlorite to give taurine, hypotaurine sulfonic acid, and monochloro- and dichlorotaurine. However, it reacts rapidly with chlorine dioxide with a second-order rate constant of 801 M^{-1} s^{-1} to give taurine. Oxidation occurs simultaneously at the sulfur center (to give the sulfonic acid) and at the nitrogen center (to give the chloramines). The stoichiometry of the reaction was experimentally determined to be $ClO_2^- + H_2NCH_2CH_2SO_2H + H^+ \rightarrow ClHNCH_2CH_2SO_3H + H_2O$. The formation of dichlorotaurine is favored only in high acid environments.

Introduction

Although the physiological roles antioxidants play in the human body have been studied[2] extensively, in vitro antioxidant chemistry studies are still in their infancy. There is a need to study mechanistic details of how antioxidants protect tissue from harmful oxidants. A good start would be to identify common antioxidants and study their reactivity patterns in vitro.

The biochemistry and clinical biology communities have different definitions of an antioxidant.[3] In this paper, we will assume that an antioxidant is a substance that, when present in low concentrations, can significantly delay oxidation of an oxidizable substrate.[4] Previous work suggests that hypotaurine and taurine fall into this category. A number of reactive oxygen species ($^{\bullet}OH$ and $O_2^{\bullet -}$) are produced in vivo, and they oxidize and damage normal tissue.[5] $^{\bullet}OH$ can easily be produced in biological systems by the metal-ion-dependent decomposition of hydrogen peroxide.[6] Antioxidants should be able to scavenge all reactive oxygen species (ROS) and thus protect tissue from damage and also to reduce inflammation.[7]

The major antioxidants most commonly mentioned (cysteine, glutathione, taurine, hypotaurine, vitamin E, ascorbic acid, superoxide dismutase, and caeruloplasmin)[8] all contain an amino group. The formation of *N*-chloro- and *N*-bromomines has been suggested as possible mechanisms by which the most common harmful oxidants, HOCl and HOBr, can be scavenged.[9] With the high concentrations of Cl^- (0.10 M) and Br^- (0.001 M) in the human body, HOCl and HOBr can be easily produced from the myeloperoxidase-catalyzed peroxidation of chloride and bromide ions, respectively.[10] These neutrophil-derived prooxidants, HOCl and HOBr, are important in bacterial killing but also damage tissue at inflammation sites.[11]

A recent study showed that cephalosporins are scavengers of HOCl.[12] Even some cephalosporins without a primary amino group (such as cefamandale) have been shown to have powerful antioxidant activity with respect to HOCl, leading to a reduced antibiotic activity of the cephalosporin.[13] Cephalosporins contain thioether groups: speculation is that these groups may be responsible for mopping up HOCl.[12] Specific studies are thus needed for each suspected antioxidant compound before its mechanism for antioxidant activity can be deduced.

Apart from its antioxidant activity, taurine has been implicated in several other physiological roles.[14] However, there is no adequate mechanistic data on how it functions in these roles. Most reports on mechanistic studies are speculative. For example, it has been reported that in physiological concentrations taurine can, in vitro, kill schistosoma mansoni.[15] Also, taurine has been shown to inhibit calcium-activated respiration in small mammalian liver mitochondria,[16] to inhibit nerve impulses in nerve tissues,[17] and to reduce cholesterol in vertebrates.[18]

Taurine and hypotaurine are closely linked. Taurine, with its special stability, is considered a metabolic end product of methionine and cysteine.[19] Most metabolic pathways give hypotaurine as a precursor to taurine.[19] Hypotaurine differs from taurine only in the oxidation state of the sulfur center and the resulting change in the acidity of the molecule.

In general, hypotaurine is much more reactive than taurine. Recent experimental data have shown that hypotaurine reacts rapidly and efficiently with the hydroxyl radical, superoxide radical, and hydrogen peroxide.[20] The ability to scavenge these reactive oxygen species rapidly is a prerequisite for a molecule to act as an antioxidant in vivo.[21] The other metabolic precursors of taurine, cysteic acid and cysteamine, are also poor scavengers of ROS as is taurine. Taurine also appears to be inefficient in moderating HOCl toxicity because the *N*-chloramine produced can still deactivate α_1-antiproteinase.[22]

Some of our recent work examined the reactivity of taurine.[23] We found taurine to be extremely inert and surprisingly inactive to oxidation by the reactive radical oxyhalogen species ClO_2 and by acidified bromate.[23] It did appear that, whatever physiological roles taurine might play, it does not seem to be a strong and effective antioxidant.

In this paper we report an extensive kinetics and mechanistic study of the reaction of hypotaurine with chlorite and chlorine

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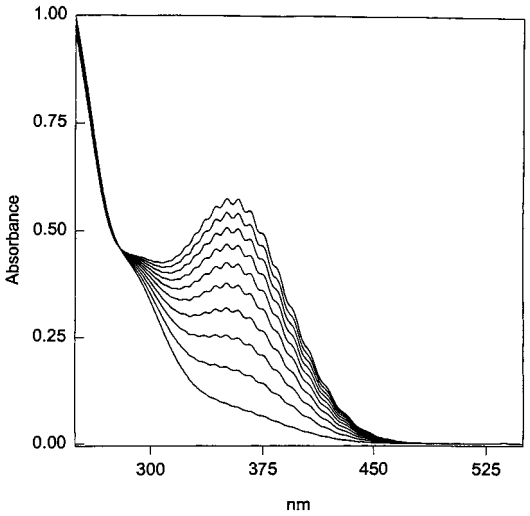

Figure 1. UV−vis spectral scans (60 s intervals) of the ClO_2^-−hypotaurine reaction. Absorbance readings are observed only at λ = 360 nm, the absorption maximum wavelength for chlorine dioxide. $[ClO_2^-]_0 = 1.01 \times 10^{-2}$ M; $[H_2NCH_2CH_2SO_2H]_0 = 9.99 \times 10^{-4}$ M.

dioxide. A full mechanistic study of hypotaurine's reactivity can help in inferring to its reactivity in vivo.

Experimental Section

Materials. The following reagent-grade chemicals were used without further purification: hypotaurine (Aldrich), potassium iodide, sodium thiosulfate, and perchloric acid (69−72%) (Fisher). Sodium perchlorate solution was first filtered and then recrystallized twice from deionized water before use. Sodium chlorite was first recrystallized to approximately 96% assay before being used. Fresh sodium chlorite solutions were prepared before each set of experimental data was acquired. Chlorine dioxide was prepared by a method previously described.[24] It was standardized by its absorptivity coefficient of 1265 M^{-1} cm^{-1} at 360 nm.

Methods. Product identification was by UV, IR, and ^1H NMR spectroscopy. The proton NMR spectra were obtained on a JEOL GX 270 spectrometer using D_2O as the solvent and internal standard. Kinetics experiments were carried out at 25 ± 0.5 °C and an ionic strength of 0.50 M ($NaClO_4$). The reactions were followed spectrophotometrically using the absorbance of ClO_2 at 360 nm. The slower reactions of chlorite and hypotaurine were followed on a Perkin-Elmer Lambda 2S UV/vis spectrophotometer, while the faster reactions of ClO_2 and hypotaurine were followed on a Hi-Tech Scientific SF-61AF stopped-flow spectrophotometer.

Results

Product Identification. Taurine and hypotaurine do not absorb in the visible region of the spectrum. Chlorotaurine has a weak absorption at 250 nm. Figure 1 shows that ClO_2^-−hypotaurine mixtures give a featureless spectrum between 200 and 500 nm. The only signal in the visible region is the absorbance of chlorine dioxide at 360 nm. ^1H NMR spectral data were sufficient to conclude that hypotaurine is oxidized to taurine, which is in turn further oxidized to *N*-chlorotaurine. Figure 2 shows NMR spectra of the substrate as well as its progressive transformation to chlorotaurine. Hypotaurine itself has a proton NMR spectrum shown in Figure 2a with two triplets that are displaced to low field with respect to taurine. These triplets integrate as two protons each. The positions of the triplets in hypotaurine and taurine are also heavily dependent on the pH of the solution, and so all the spectra shown in Figure 2 were run in $DClO_4$. Upon mixing a 1:2 mole ratio of ClO_2^- to hypotaurine, the spectrum in Figure 2b is obtained. Two new sets of triplets appear, with one set centered at positions δ = 3.24 and the other at 3.05 (indicative of taurine and/or chlorotaurine). The NMR spectra of taurine and chlorotaurine are nearly identical. The other set of triplets are centered at δ = 4.2 and 3.55. Further addition of chlorite shows the progressive decrease in the intensity of this set of protons. This is shown in the series of spectra b, c, and d. In Figure 2d, excess chlorite effectively wipes out these triplet sets of protons. The only product left in the reaction mixture is chlorotaurine. The spectrum shown in Figure 2e is acidified taurine ($DClO_4$) which has been added for comparison with the reaction product spectrum. The transient peaks in spectra b and c are due to the N-chlorination of the hypotaurine before it has been oxidized to taurine:

$$HOCl + H_2NCH_2CH_2SO_2H \rightarrow Cl(H)NCH_2CH_2SO_2H + H_2O \quad (R1)$$

As the oxidation proceeds, the *N*-chlorohypotaurine, $Cl(H)NCH_2CH_2SO_2H$, is oxidized to *N*-chlorotaurine, $Cl(H)NCH_2CH_2SO_3H$, and the peaks gradually disappear:

$$Cl(H)NCH_2CH_2SO_2H + [O] \rightarrow Cl(H)NCH_2CH_2SO_3H \quad (R2)$$

In conditions of excess ClO_2^-, the remaining chlorite could be quantitatively determined by using excess acidified iodide and titrating the liberated iodine with thiousulfate after correcting for the contribution from *N*-chloramine.[25] In excess hypotaurine the released *N*-chloramine could also be quantitatively determined by adding acidified iodide (as well) and analyzing for iodine:[26]

$$Cl(H)NCH_2CH_2SO_3H + 2I^- + H^+ \rightarrow I_2(aq) + H_2NCH_2CH_2SO_3H + Cl^- \quad (R3)$$

The reaction presents very little in terms of possible side reactions, and we confirmed only *N*-chlorotaurine (^1H NMR spectra), ClO_2 (spectrophotometry, λ = 360 nm), and Cl^- (gravimetric analysis and after prolonged standing) as the products of the reaction in excess ClO_2^-.

Stoichiometry. The stoichiometry of the chlorite−hypotaurine reaction was established as

$$ClO_2^- + H_2NCH_2CH_2SO_2H + H^+ \rightarrow Cl(H)NCH_2CH_2SO_3H + H_2O \quad (R4)$$

The stoichiometry was obtained by running a series of experiments with excess ClO_2^- and measuring the ClO_2 obtained after 12 days of incubation. Any excess ClO_2^- will be converted to ClO_2 after all hypotaurine has been consumed with the stoichiometry:[27]

$$5ClO_2^- + 4H^+ \rightarrow 4ClO_2 + Cl^- + 2H_2O \quad (R5)$$

Required stoichiometry is when no production of ClO_2 is observed at the highest possible ratio of the oxidant. Any further addition of chlorite above that required for stoichiometry R4 will result in excess chlorite, which will show up as chlorine dioxide according to stoichiometry R5. A plot of final absorbance of ClO_2 (after an incubation period of 12 days) against the initial ClO_2^- concentrations gives a straight line with an

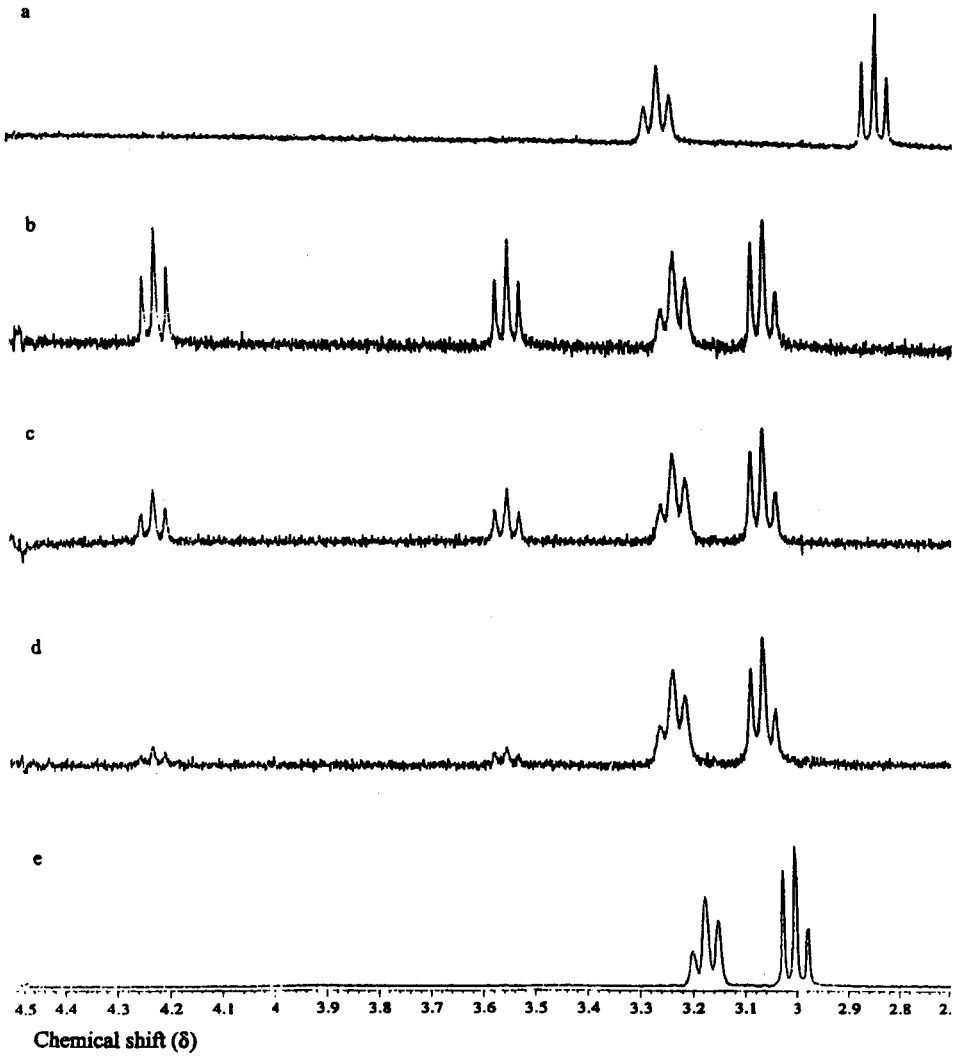

Figure 2. ^{1}H NMR spectra of the reactants, intermediates, and products. (a) Standard spectrum of hypotaurine in $DClO_4$ (pH < 3). (b) Appearance of spectrum after mixing a 1:2 ratio of ClO_2^-:$H_2NCH_2CH_2SO_2H$. The triplets observed at $\delta = 3.55$ and 4.22 are due to an intermediate species, $ClHNCH_2CH_2SO_2H$, *N*-chlorohypotaurine. (c) Addition of more ClO_2^- to the mixture in (b) sees the progressive disappearance of the triplets at $\delta = 3.55$ and 4.22. (d) In excess ClO_2^- the peaks at $\delta = 3.55$ and 4.22 disappear completely, leaving peaks characteristic of chlorotaurine. The same spectrum for chlorotaurine was observed in ref 23. (e) Spectrum of taurine in $DClO_4$ inserted for comparison with the product spectrum in (d). NMR spectra of taurine and chlorotaurine are very similar (see ref 23).

intercept on the ClO_2^-−concentration axis. The intercept on this axis is concentration one needs to satisfy stoichiometry R4 without any excess ClO_2^- left to produce ClO_2 as in reaction R5. Reaction R5 is a slow decomposition reaction whose end point is reached after several days and thus unreliable for the determination of this reaction's stoichiometry. The value of the intercept from these data clearly supports stoichiometry R4.

The stoichiometry of the ClO_2−hypotaurine reaction was determined by titration. A fixed amount of hypotaurine was titrated against standardized aqueous ClO_2, and the end point was determined as the point at which the yellow chlorine dioxide color no longer vanishes. The detection of the end point could be enhanced by the addition of freshly prepared starch solution with mercuric iodide as a preservative. Starch without mercuric iodide did not give a complex with ClO_2.[28] The titration exercise gave a ClO_2:hypotaurine stoichiometric ratio of 4:5. The products were more difficult to identify. NMR spectra confirmed the formation of *N*-chlorotaurine only. We thus inferred the following stoichiometry:

$$4ClO_2 + 5H_2NCH_2CH_2SO_2H \rightarrow 4Cl(H)NCH_2CH_2SO_3H + H(OH)NCH_2CH_2SO_3H + 2H_2O \quad (R6)$$

The oxime formed in (R6) can condense with another taurine molecule to form a tauryl dimer. NMR spectra could not give a distinctly different spectrum for the products of the ClO_2− hypotaurine reaction compared to the original spectrum of hypotaurine. The two triplet peaks did not shift from their original positions.

Reaction Dynamics. *The Induction Period.* The reaction of chlorite with hypotaurine was much faster than the corresponding reaction with taurine.[1] Reaction solutions displayed a finite induction period of between 10 and 1000 s (depending on initial conditions) before the formation of ClO_2 in excess ClO_2^-: $[ClO_2^-]_0 > [H_2NCH_2CH_2SO_2H]_0$. No ClO_2 formation is observed in excess hypotaurine. Figure 3a shows the effect of varying chlorite at constant acid and hypotaurine concentrations. Figure 3b shows that there is a linear dependence between the induction time and the inverse of the initial chlorite concentrations, $[ClO_2^-]_0$. At fixed hypotaurine concentrations, however, this linearity is lost at low chlorite concentrations (see Figure 3b). This is to be expected as we shall see from the data in Figure 5 and suggests that the precursor reaction to formation of ClO_2 is dependent on the first power in the concentrations of ClO_2^-. In the range of acid strength, 2.0×10^{-2} M > $[H^+]_0$

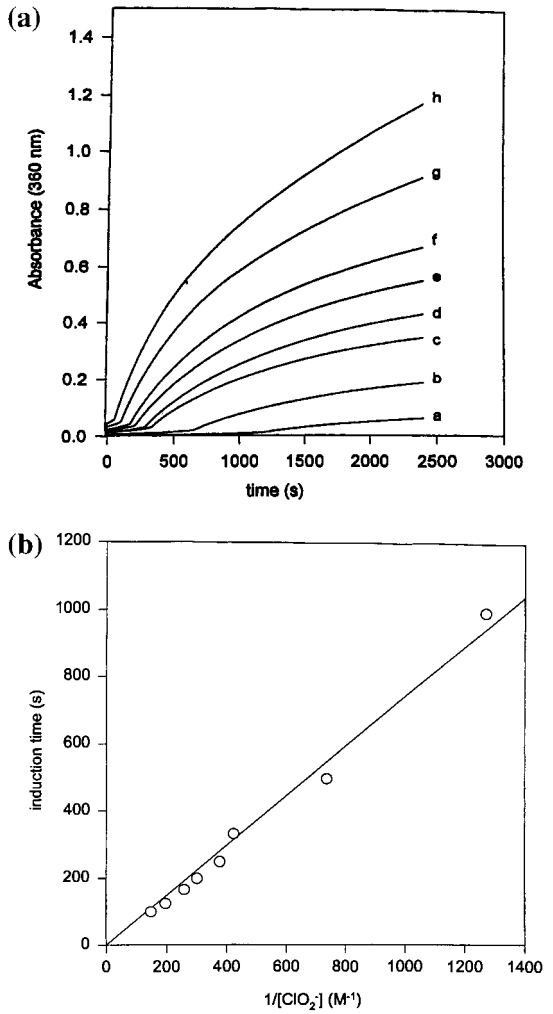

Figure 3. (a) Reaction dynamics in excess ClO_2^-. There is a progressive increase in the $[ClO_2^-]_0$ in going from (a) to (h). As $[ClO_2^-]_0$ increases, there is a shortening of the induction period and an increase in the rate of formation of the ClO_2. $[H_2NCH_2CH_2SO_2H]_0 = 1.0 \times 10^{-3}$ M, $[H^+]_0 = 0.050$ M. $[ClO_2^-]_0 =$ (a) 1.0×10^{-3} M with increments of 1.0×10^{-3} M in (b) to (f); (g) 8.0×10^{-3} M, (h) 1.0×10^{-2} M. (b) Plot of the induction time vs the inverse of the chlorite concentration. The straight line indicates that the hypotaurine consumption reaction has first-order dependence with respect to the chlorite concentrations.

$> 1.0 \times 10^{-3}$ M, there is also a linear dependence of the induction period on the inverse of the acid concentration. Linearity is lost in highly acidic environments, as the experimental data become irreproducible with what appears to be a limiting effect. Figure 4 shows this effect within the linear region. As the acid concentrations are increased past 0.02 M, the induction period stays constant at about 310 s (from Figure 4), and as the acid concentrations are increased further, past 0.1 M, the induction period starts to increase, showing acid retardation at high acid concentrations.

The effect of varying hypotaurine concentrations also was complex. In high excess of ClO_2^-, such that ratio $R = [ClO_2^-]_0/[H_2NCH_2CH_2SO_2H]_0 > 4.0$, variation of hypotaurine concentrations did not change the induction period of the reaction (see Figure 5), although it did affect the rate of formation of chlorine dioxide at the end of the induction period. Increasing the hypotaurine concentrations so that $R < 4$ had the effect of increasing the induction period, as it now took much longer to oxidize the hypotaurine completely and allow the ClO_2 to accumulate.

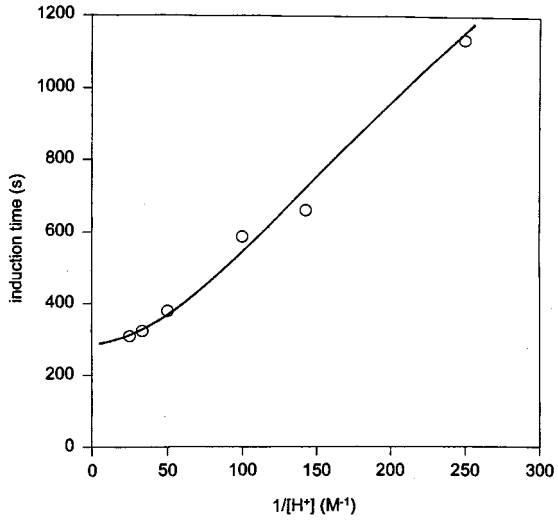

Figure 4. Dependence of the induction period on the acid concentrations. Acid is merely acting as a catalyst in this reaction. This plot is only valid within the narrow acid concentration range plotted.

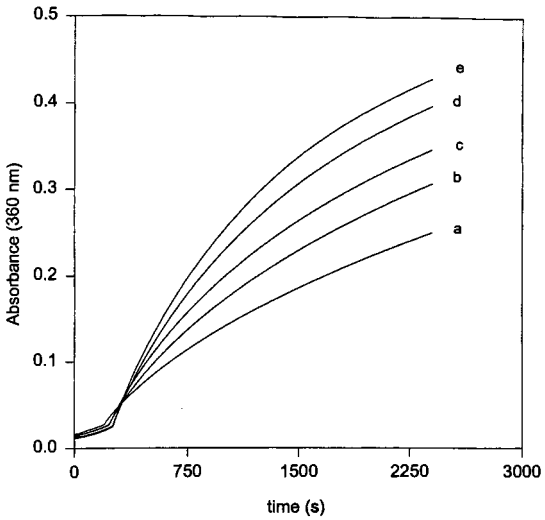

Figure 5. Reaction dynamics showing the "peacock-type" absorbance traces under conditions of $[ClO_2^-]_0 \gg [H_2NCH_2CH_2SO_2H]_0$. $[ClO_2^-]_0 = 4.02 \times 10^{-3}$ M. $[H_2NCH_2CH_2SO_2H]_0 =$ (a) 2.0×10^{-4} M, (b) 4.0×10^{-4} M, (c) 6.0×10^{-4} M, (d) 8.0×10^{-4} M, and (e) 1.0×10^{-3} M. The induction period gets longer as the hypotaurine concentrations are increased further. Though the induction period does not increase, the rate of formation of ClO_2 increases with $[H_2NCH_2CH_2SO_2H]_0$ (see plot shown in Figure 7b).

Formation of Chlorine Dioxide. The relationship between the initial reactant concentrations and the rate of formation of ClO_2 at the end of the induction period can lead to information on the rate of formation of the reactive intermediates that control the formation of ClO_2. The log−log plot shown in Figure 6a was derived from the data shown in Figure 3a. Initial rates were estimated as the tangents of the chlorine dioxide formation curves. Only about 2% of the ClO_2 formation data was used for each curve. The log−log plot gave a slope of 0.94 ± 0.07; thus, the rate of formation of ClO_2 has a first-order dependence on the initial concentration of the chlorite. At the end of the induction period the reagent concentrations, especially when $[ClO_2^-]_0 \approx [H_2NCH_2CH_2SO_2H]_0$, will be vastly different from the concentrations at the beginning of the reaction. The existence of a relationship between initial concentrations and the reaction rate at the beginning of the induction period indicates that the two processes (consumption of hypotaurine and formation of chlorine dioxide) are connected. The data in

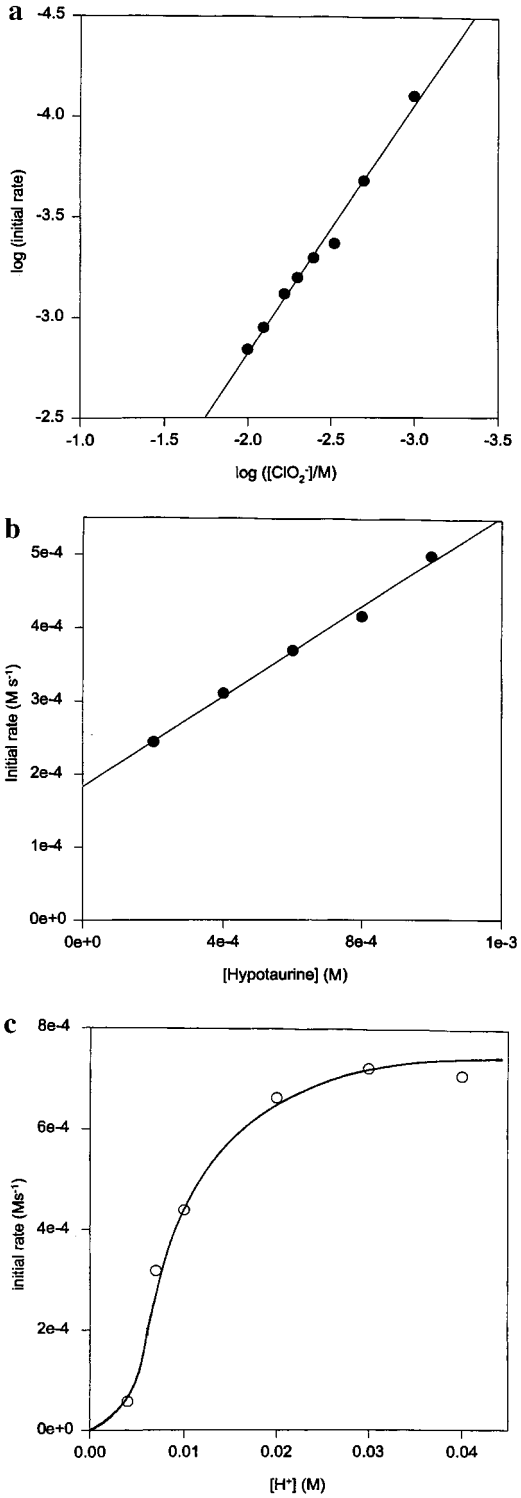

Figure 6. Plots showing the dependence of the ClO_2^-−formation on the initial reagent concentrations. (a) A log−log plot of chlorite concentrations against initial rate of formation of ClO_2. The slope of 1.0 confirms unit order with respect to $[ClO_2^-]_0$. Data used for this plot derived from data in Figure 4a. (b) The effect of the hypotaurine concentrations on the initial rate of formation of ClO_2. This is from data shown in Figure 6. (c) Complex dependence of the rate of formation of ClO_2 with respect to acid. Plot shows a saturation as well as a retardation as the acid is increased further.

Figure 5 were also used to evaluate the plot shown in Figure 6b. The initial rate of formation of chlorine dioxide is also dependent to the first power on the initial hypotaurine concentrations. The positive intercept observed in Figure 6b is expected since even without the hypotaurine, formation of ClO_2 is expected from the standard decomposition of chlorite as shown in stoichiometry R5.[27] Figure 6c shows the complex dependence of the initial rate of formation of ClO_2 on the acid concentrations. At low acid concentrations, there is a reasonably linear relationship between the rate and the acid concentrations, but by pH 2 there is a rapid saturation followed by a retardation of the reaction as the acid concentrations are further increased. This is the same type of effect observed in the data shown in Figure 4.

The ClO_2−$H_2NCH_2CH_2SO_2H$ Reaction. The interactions between hypotaurine and chlorine dioxide are crucial in determining the global reaction mechanism. How rapidly does ClO_2 oxidize hypotaurine? If this process is slow (or nonexistent), then both ClO_2 and hypotaurine can coexist in the reaction solution, and the induction period data cannot be effectively associated with any of the kinetics parameters we seek for the chlorite−hypotaurine reaction. A rapid ClO_2−$H_2NCH_2CH_2SO_2H$ reaction would indicate that any ClO_2 formed during the induction period will be quickly mopped up by hypotaurine, and formation of ClO_2 would then indicate complete consumption of the hypotaurine substrate.

The ClO_2−$H_2NCH_2CH_2SO_2H$ reaction was found to be fast (Figure 7a,b), complete in typically less than 10 s. This can be compared with data shown in Figures 3a and 5 in which typical induction times of the chlorite−hypotaurine reaction are of the order of 100 s, and complete reaction may require over 3000 s. Thus, the rapid nature of the ClO_2−$H_2NCH_2CH_2SO_2H$ reaction simplifies deduction of a plausible mechanism from the reaction data. Figure 7a shows the effect of varying $[ClO_2]_0$ at constant $[H^+]_0$ and $[H_2NCH_2CH_2SO_2H]_0$; the insert is a log−log plot of initial rates vs $[ClO_2]_0$, with a slope of 1.06 ± 0.11. Figure 7b shows the effect of varying hypotaurine; the insert shows the log−log plot, which gives a slope of 0.98 ± 0.05. The reactions involving oxidation by ClO_2 were reproducible, and no special experimental precautions were needed for these sets of experiments.

Mechanism

Hypotaurine has a pK_b of 9.56.[29] At the pH conditions utilized in these experiments, we expect the amino group to be fully protonated:

$$H_2NCH_2CH_2SO_2H + H^+ \rightleftharpoons [H_3NCH_2CH_2SO_2H]^+ \quad \text{(R7)}$$

The first step in the mechanism is the formation of the active oxidizing species, HOCl. HOCl is formed by the two-equivalent reduction of ClO_2^-:

$$ClO_2^- + 3H^+ + 2e^- \rightleftharpoons HOCl + H_2O \quad \text{(R8)}$$

Initially, the only reductant is hypotaurine, and thus its oxidation is the initiation reaction:

$$ClO_2^- + H_2NCH_2CH_2SO_2H + H^+ \rightarrow H_2NCH_2CH_2SO_3H + HOCl \quad \text{(R9)}$$

The formation of HOCl in reaction R9 triggers several rapid reactions. HOCl can rapidly oxidize the remaining hypotaurine to taurine

$$HOCl + H_2NCH_2CH_2SO_2H \rightarrow H_2NCH_2CH_2SO_3H + Cl^- + H^+ \quad \text{(R10)}$$

or oxidize hypotaurine to *N*-chlorohypotaurine

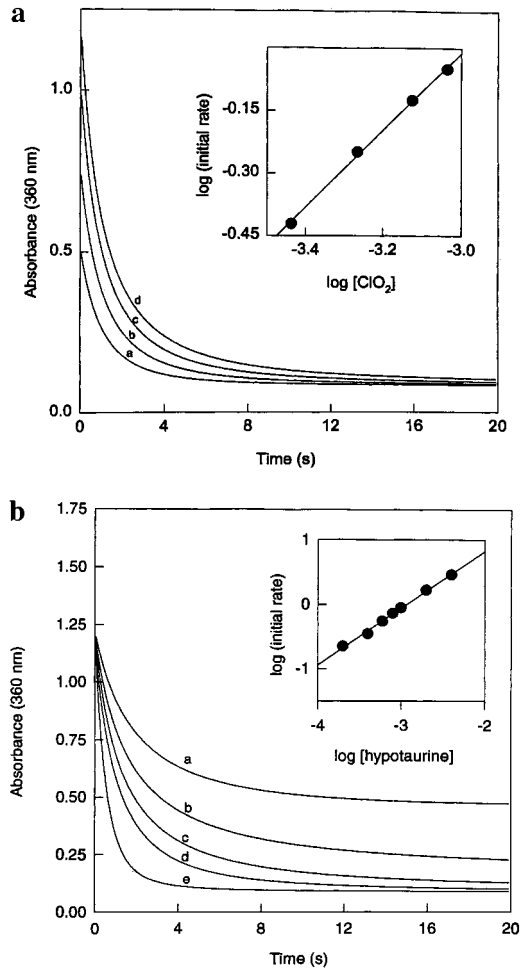

Figure 7. (a) Direct reaction of ClO_2 with $H_2NCH_2CH_2SO_2H$ in unbuffered solutions. The reaction is very fast. The insert shows a log−log plot of the $[ClO_2]_0$ and initial rate of the reaction which gives a value of unit dependence of the reaction on $[ClO_2]_0$. $[H_2NCH_2CH_2SO_2H]_0 = 1.0 \times 10^{-3}$ M. $[ClO_2]_0$ = (a) 3.7×10^{-4} M, (b) 5.4×10^{-4} M, (c) 7.5×10^{-4} M, (d) 9.2×10^{-4} M, and (e) 1.8×10^{-3} M. (b) Effect of hypotaurine concentrations on the $ClO_2-H_2NCH_2CH_2SO_2H$. The insert shows a log−log plot of the data which also gives an order of 1.0 in $[H_2NCH_2CH_2SO_2H]_0$. $[ClO_2]_0 = 9.2 \times 10^{-4}$ M. Ratio = $[ClO_2]_0/[H_2NCH_2CH_2SO_2H]_0$ = (a) 4.61, (b) 2.30, (c) 1.54, (d) 1.15, and (e) 0.92.

$$HOCl + H_2NCH_2CH_2SO_2H \rightarrow ClHNCH_2CH_2SO_2H + H_2O \quad (R11)$$

Oxyhalogen reactions will also be activated by formation of HOCl. The most important reaction is the formation of ClO_2 from ClO_2^-:[30]

$$HOCl + 2ClO_2^- + H^+ \rightarrow 2ClO_2 + Cl^- + H_2O \quad (R12)$$

Unless there is a reaction that consumes ClO_2 as rapidly as it is produced by reaction R12, we expect an almost instant accumulation of yellow ClO_2 as soon as the reaction commences as has been observed in the oxidation of aldehydes by ClO_2^-.[31] Our kinetics data, e.g., Figures 3a and 5, show that the reaction exhibits a finite induction period in the formation of ClO_2. These induction periods can be up to thousands of seconds depending on initial conditions.

Apart from some of the oxyhalogen species, there are only two possible reductants in the reaction medium: hypotaurine and *N*-chlorohypotaurine. The reaction of taurine and ClO_2 can be ignored since in a previous study, reaction mixtures of ClO_2 and taurine showed no tendency to react over long periods of time.[23] However, ClO_2 can be consumed by reaction with hypotaurine:

$$2ClO_2 + H_2NCH_2CH_2SO_2H + H_2O \rightarrow 2ClO_2^- + H_2NCH_2CH_2SO_3H + 2H^+ \quad (R13)$$

Reaction R13 is fast enough to mop up all the ClO_2 molecules as soon as they are formed from reaction R12. The data for reaction R13 are shown in Figure 7a,b where the reaction is 90% complete within 1.00 s. Reaction R13 is not an elementary step. The kinetics data clearly implicate bimolecular kinetics with orders of unity in the concentrations of chlorine dioxide and hypotaurine. The initial step of reaction R13 is a one-electron oxidation of hypotaurine to form an unstable radical organic species which rapidly reacts with more ClO_2 to form taurine. The rate of the reaction was deduced to be

$$-d[ClO_2]/dt = k_0[ClO_2][H_2NCH_2CH_2SO_2H] \quad (1)$$

with $k_0 = 801$ M^{-1} s^{-1}. In excess chlorite, it has been established that the formation of HOCl from ClO_2^- is autocatalytic such that

$$ClO_2^- + HOCl + 3H^+ + 2e^- \rightleftharpoons 2HOCl + H_2O \quad (R14)$$

The mechanism for the autocatalytic sequence described by the composite reaction R14 is well-known.[32] The expected rapidly increasing ClO_2 concentrations (due to quadratic autocatalysis) are not observed due to limited concentrations of ClO_2^- and the fact that nonautocatalytic pathways for the consumption of HOCl, as in reactions R10 and R11, also contribute to the overall reaction dynamics.

Product Formation. The ^1H NMR spectra shown in Figure 2 confirm that the reaction is quite complex, with a set of reactions that can be derived by permuting the set of oxidants in the reaction medium (ClO_2^-, HOCl, and ClO_2) with the set of reductants ($H_2NCH_2CH_2SO_2H$, $H_2NCH_2CH_2SO_3H$, and $ClHNCH_2CH_2SO_2H$). The NMR data could not give evidence of further oxidation of *N*-chlorotaurine to dichlorotaurine because the NMR spectra of both the mono- and dichloro compounds are very similar:

$$HOCl + ClHNCH_2CH_2SO_3H \rightarrow Cl_2NCH_2CH_2SO_3H + H_2O \quad (R15)$$

The stoichiometry of the reaction, however, clearly indicates monochlorotaurine as the overwhelmingly favored product. Dichlorotaurine is favored in highly acidic environments.[33] Our experiments were carried out under mildly acidic conditions, hence the dominance of the monochlorotaurine product.

Explanation of Acid Dependence. Linear acid dependence with respect to the induction period and rate of formation of chlorine dioxide is obtained within a narrow range of acid concentrations, $0.005 M < [H^+] < 0.02$ M. Protonation can occur at the nitrogen group of the hypotaurine as in reaction R7. Protonation of ClO_2^- can also occur, with Cl(III) existing as either the protonated $HClO_2$ form or unprotonated ClO_2^- anion:

$$ClO_2^- + H^+ \rightleftharpoons HClO_2 \quad (R16)$$

Since the pK_a of chlorous acid is about 2.5,[34] Cl(III) species below pH = 2.5 exist predominantly as $HClO_2$.

TABLE 1: Reactions Used in the Simulation of the $ClO_2^- - H_2NCH_2CH_2SO_2H$ Reaction

no.	reaction
M1	$ClO_2^- + H^+ \rightleftharpoons HClO_2$
M2	$H_2NCH_2CH_2SO_2H + H^+ \rightarrow [H_3NCH_2CH_2SO_2H]^+$
M3	$ClO_2^- + H_2NCH_2CH_2SO_2H + H^+ \rightarrow H_2NCH_2CH_2SO_3H + HOCl$
M4	$ClO_2^- + H_2NCH_2CH_2SO_3H + H^+ \rightarrow H(OH)NCH_2CH_2SO_3H + HOCl$
M5	$HOCl + H(OH)NCH_2CH_2SO_2H + H_2O \rightarrow H(OH)NCH_2CH_2SO_3H + H^+ + Cl^-$
M6	$H(OH)NCH_2CH_2SO_3H + H^+ + Cl^- \rightarrow Cl(H)NCH_2CH_2SO_3H + H_2O$
M7	$HOCl + H_2NCH_2CH_2SO_2H \rightarrow H_2NCH_2CH_2SO_3H + Cl^- + H^+$
M8	$HOCl + 2ClO_2^- + H^+ \rightarrow 2ClO_2(aq) + Cl^- + H_2O$
M9	$2ClO_2(aq) + H_2NCH_2CH_2SO_2H + H_2O \rightarrow 2ClO_2^- + H_2NCH_2CH_2SO_3H + 2H^+$
M10	$HOCl + H_2NCH_2CH_2SO_3H + H_2O \rightarrow H(OH)NCH_2CH_2SO_3H + Cl^- + H^+$
M11	$ClO_2^- + Cl(H)NCH_2CH_2SO_2H + H^+ \rightarrow Cl(H)NCH_2CH_2SO_3H + HOCl$
M12	$HOCl + Cl(H)NCH_2CH_2SO_2H \rightarrow Cl(H)NCH_2CH_2SO_3H + Cl^- + H^+$
M13	$2Cl(H)NCH_2CH_2SO_3H + H^+ \rightleftharpoons Cl_2NCH_2CH_2SO_3H + [H_3NCH_2CH_2SO_2H]^+$

TABLE 2: Rate Laws and Rate Constants Used in Computer Simulations

reaction	forward reaction	reverse reaction
M1	$1.0 \times 10^{10}[H^+][ClO_2^-]$	$3.16 \times 10^7[HClO_2]$
M2	$5.0 \times 10^8[H_2NCH_2CH_2SO_2H][H^+]$	$1.0 \times 10^7[[H_3NCH_2CH_2SO_2H]^+]$
M3	$5.0 \times 10^2[ClO_2^-][H_2NCH_2CH_2SO_2H][H^+]$	
M4	$55[ClO_2^-][H_2NCH_2CH_2SO_3H][H^+]$	
M5	$1.0 \times 10^3[HOCl][H(OH)NCH_2CH_2SO_2H]$	
M6	$1.0 \times 10^6[H(OH)NCH_2CH_2SO_2H][Cl^-][H^+]$	
M7	$1.5 \times 10^3[HOCl][H_2NCH_2CH_2SO_3H]$	
M8	$1.06 \times 10^6[HOCl][ClO_2^-][H^+]$	$1.0 \times 10^2[ClO_2]^2[Cl^-]$
M9	$8.01 \times 10^2[ClO_2][H_2NCH_2CH_2SO_2H]$	
M10	$1.1 \times 10^2[HOCl][H_2NCH_2CH_2SO_3H]$	
M11	$1.5 \times 10^2[ClO_2^-][Cl(H)NCH_2CH_2SO_2][H^+]$	
M12	$1.0 \times 10^3[HOCl][Cl(H)NCH_2CH_2SO_2][H^+]$	
M13	$5.0 \times 10^6[Cl(H)NCH_2CH_2SO_3]^2[H^+]$	$1.0 \times 10^4[Cl(H)NCH_2CH_2SO_3H][[H_3NCH_2CH_2SO_3H]^+]$

In this reaction system, the formation of HOCl is the rate-determining step giving the rate of reaction as

$$-\mathrm{d}[H_2NCH_2CH_2SO_2H]/\mathrm{d}t = k_1[ClO_2^-][H_2NCH_2CH_2SO_2H][H^+] \quad (2)$$

ClO_2^- is a better nucleophile than $HClO_2$ and would react much faster with hypotaurine and/or protonated hypotaurine. $HClO_2$ is not completely unreactive, but it reacts slowly with hypotaurine. Thus, no simple approximations could be made to evaluate k_1. Taking into account the equilibrium shown in reaction R16, the rate of reaction R9 will be given by

$$-\mathrm{d}[H_2NCH_2CH_2SO_2H]/\mathrm{d}t = k_1[Cl(III)]_T[H_2NCH_2CH_2SO_2H][H^+]/(1 + K_a[H^+]) \quad (3)$$

where $[Cl(III)]_T = [ClO_2^-] + [HClO_2]$ and K_a is the acid dissociation constant for chlorous acid. Thus, simple first-order kinetics in acid will be expected at low acid concentrations. As acidity increases, one will notice a saturation followed by a retardation. This is illustrated in the data shown in Figure 6c.

Computer Simulations. The reaction network used to simulate the reaction system is shown in Table 1. The rate laws and rate constants used in the simulations are shown in Table 2. Only one oxyhalogen reaction (reaction M8) and two protolytic reactions (reactions M1 and M2) were used. The remaining 10 reactions were interactions between an oxyhalogen species and one of the four possible amino acid species in solution. Reaction M1 was needed to explain the acid retardation that is observed in pH conditions lower than the pK_a of chlorous acid (see Figure 6c). The kinetics parameters used for this reaction were taken from the work of Epstein et al.[35] Lower rate constants could be used for (M1) as long as the pK_a was maintained at 2.5. Higher rate constants for (M1) rendered the simulations stiff with the simulations not giving any better fit to the data. If, as is assumed in the proposed mechanism, $HClO_2$ is slower reacting (cf. reactions M3 and M4), then the acid retardation can be justified from the increase in the slower-reacting form of Cl(III) species as acid concentrations are increased. Reaction M2 was not important in influencing the rate of reaction since the protonated species was dominant at the pH conditions used for these experiments. High rate constants for both the forward and reverse reactions of reaction M2 made it unimportant in determining the overall global dynamics of the reaction. Reaction M3 was important since it is the reaction initiator which produces the reactive intermediate, HOCl. Reaction M3 could be split into two reactions in which the initial products are taurine and OCl^-. OCl^- is then quickly protonated to form HOCl. The addition of another rapid protolytic reaction, $H^+ + OCl^-$, however, made the integration extremely stiff, also without any improvement in the results obtained. Reaction M4 is also an initiator reaction in which the oxidation occurs at the nitrogen center. Reactions M6 + M10 can be combined to give reaction R11 in which the chlorotaurine is formed directly without formation of Cl^-. The use of (R12) instead of (M6) + (M10) did not give a better fit to the data. We assumed that all chlorine dioxide is formed in reaction M8 and is consumed only in (M9). The other route for the consumption of chlorine dioxide is the reverse reaction of (M8). This route could have been important in high Cl^- concentrations, but in these simulations, Cl^- concentrations never rose above 10^{-6} M since reaction M6 instantly consumed all the chloride formed in the reaction.

Estimation of Some of the Rate Constants. *Reaction M3.* The final value used was estimated from the best fit to the induction period. This estimation could be made by modeling a network of reactions M3 + M8 + M9. The rough value obtained could then be refined to fit the overall reaction data.

Reaction M4. This was estimated from Chinake et al. (ref 1, part 22). In this reference, taurine was reacted with chlorite. The chlorine dioxide formed was unreactive toward taurine, and so the rate of formation of ClO_2 in this reaction gave a good

estimate of the ClO_2−taurine reaction after accounting for the stoichiometric factors.

Reaction M5. This reaction's rate constant was initially estimated from the data in Figure 2. The rate at which the two spurious triplets ($\delta = 4.22$ and 3.55) disappear gives a good estimate of the rate of consumption of $H(OH)NCH_2CH_2SO_2H$ and $Cl(H)NCH_2CH_2SO_2H$.

Reaction M6. This reaction was assumed to be fast and not rate determining. The chosen value of 1.0×10^6 M^{-2} s^{-1} was sufficient to allow it to consume the Cl^- ions as soon as they are formed.

Reaction M7. The initial guess value for the kinetics parameters for this reaction was made by running a crude Br_2−hypotaurine stopped-flow study ($\lambda = 390$ nm). In general, the Cl_2 and HOCl oxidations are slightly faster than the corresponding bromine analogues.

Reaction M8. Rate constants were taken directly from the work of Peintler et al.[35]

Reaction M9. Rate constants were estimated directly from this study.

Reaction M10. Rate constants were estimated from our recent work on the bromine−taurine reaction.[36]

Reactions M11 and M12. These were purely guessed and were not important in determining the overall global dynamics of the reaction system.

Reaction M13. This reaction was only important for stoichiometric consistency and did not affect the reaction dynamics.[33] Our stoichiometric determinations showed that very little dichlorotaurine is formed, and so the equilibrium of reaction M13 was pushed to the left.

Results of the Simulations. The results of the simulations are shown in Figure 8a,b. The data shown in Figure 7b on the direct reaction between ClO_2 and hypotaurine are modeled in Figure 8a. The mechanism in Table 1 could be used to generate the simulations shown in Figure 8a, but a much faster and more accurate simulations set could be obtained from the use of reactions M1, M2, M3, M5, M9, and M13 only. For these sets of simulations, the experimentally determined rate constant for reaction M9 was the most important. The kinetics parameters for reactions M1 and M2 were not important as long as they represented rapid equilibria in both directions. Data in Figure 5 were simulated in Figure 8b. These simulations could be generated by considering only three important reactions in the mechanism: (a) consumption of hypotaurine, reaction R4; (b) formation of chlorine dioxide, reaction M8; and (c) consumption of chlorine dioxide, reaction M9. Reaction M3 was then added to enhance reaction M8. Such a small set of reactions could easily predict the induction period and the rate of formation of chlorine dioxide. Acid dependence could then be simulated by addition of reactions M1 and M2. ClO_2^- oxidations are characterized by autocatalysis in HOCl[37] mediated via the intermediate Cl_2O_2. Reaction M8 can be split to show that ClO_2 is produced via quadratic autocatalysis in HOCl. The rapid ClO_2−hypotaurine reaction made it unnecessary to include this autocatalytic step in the overall reaction mechanism. For the purposes of what we needed to prove, the mechanism in Table 1 appears to be adequate in describing the reaction of hypotaurine with chlorite.

Conclusion

These experiments have shown that hypotaurine is much more active than taurine and can better be classified as an antioxidant. Taurine does not seem to be effective as an antioxidant for many of the common chemicals that attack physiological tissue, except for HOCl and HOBr. For effective antioxidant activity, the compound must quickly detoxify the common oxidants to protect tissue. Taurine does not seem to be able to do this, but hypotaurine does. Any reactions of hypotaurine will take it to the more stable and relatively unreactive taurine. It is not clear how or whether hypotaurine can be regenerated after being converted to taurine. The fact that it is in the metabolic pathway may be sufficient to maintain its concentrations in physiological environments.

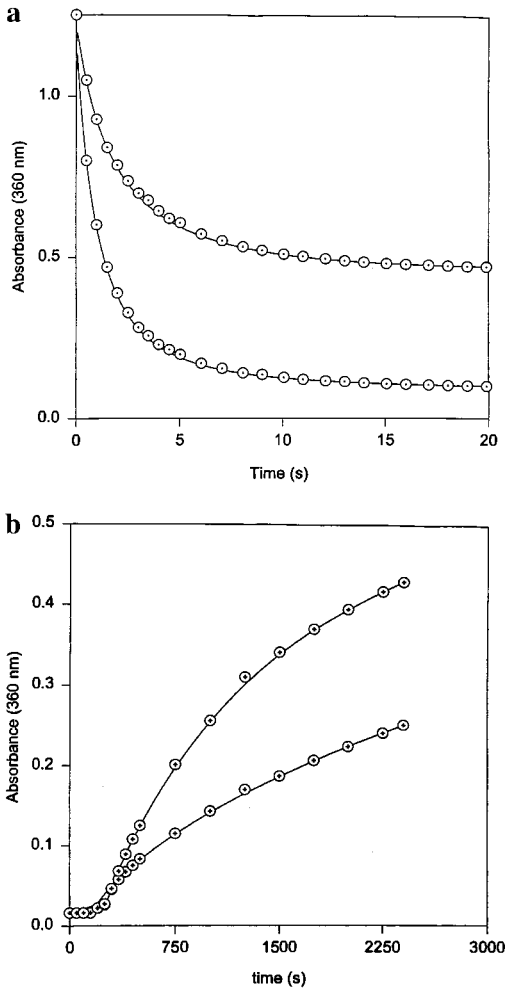

Figure 8. (a) Computer simulations of the ClO_2−hypotaurine reaction using the mechanism shown in Table 1. For these simulations, initial chlorite concentrations were set to zero. $[ClO_2]_0 = 9.2 \times 10^{-4}$ M. Ratio $= [ClO_2]_0/[H_2NCH_2CH_2SO_2H]_0 =$ (a) 4.61 and (b) 0.92. Modeling that "peacock-type" absorbance traces shown in Figure 5. There is good agreement between the model and the experimental data especially with respect to the induction period and the rate of formation of chlorine dioxide after the induction period.

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