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Complex Kinetics in the Reaction of Taurine with Aqueous Bromine and Acidic Bromate: A Possible Cytoprotective Role against Hypobromous Acid

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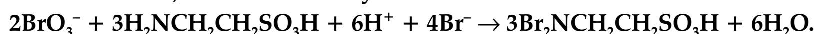
ABSTRACT

The most abundant aminoacid in the human body, 2-aminoethanesulphonic acid ($H_2NCH_2CH_2SO_3H$), is surprisingly stable and reacts exceedingly slowly even with the most powerful oxidizing agents like acidic bromate. Oxidation occurs only on the nitrogen centre to give the corresponding N-derivatives. No activity is observed at the sulphonic acid group and no cleavage of the C–S bond is observed. The stoichiometry of the oxidation of 2-aminoethanesulphonic acid by bromate is complex, yielding a mixture of monobromo- and dibromotaurines, oximes as well as the corresponding dimeric azo-compounds. In the presence of added bromide, the stoichiometry of the reaction is:



$$2BrO_3^- + 3H_2NCH_2CH_2SO_3H + 6H^+ + 4Br^- \rightarrow 3Br_2NCH_2CH_2SO_3H + 6H_2O.$$

Monobromotaurine is formed as an intermediate product before formation of the dibromotaurine. Aqueous bromine reacts quantitatively with 2-aminomethanesulphonic acid according to the stoichiometry:

$$H_2NCH_2CH_2SO_3H + 2Br_2 \rightarrow Br_2NCH_2CH_2SO_3H + 2Br^- + 2H^+.$$

This reaction is strongly inhibited by acid due to the deactivation of the amino group to electrophilic attack by protonation. The formation of N-bromotaurines is suggested as a possible mechanism by which taurine can moderate the oxidative toxicity of bromine and hypobromous acid in the slightly basic physiological environments.

KEYWORDS

Taurine, oxyhalogen, hypotaurine, toxicity, oxidation.

1. Introduction

The reactions of small organic sulphur compounds with oxyhalogens have presented to us a wide range of nonlinear behaviour.[2] In a recently established series we have exclusively examined the kinetics and mechanisms of the oxidation of sulphur compounds with oxyhalogens and halogens.[3] Any reasonable explanation of the origin of nonlinear behaviour involving sulphur compounds has to include the kinetics and mechanism of the main driving reactions. The reaction of chlorite and thiourea,[4] for example, had shown complex patterning[5] and symmetry-breaking bifurcations,[6] clock reaction characteristics,[7] and even chemical chaos.[8] We postulated a mechanism which included a steady and progressive oxidation of the sulphur centre in thiourea from the sulphenic acid to the sulphinic acid, the sulphonic acid and finally to the sulphate after cleavage of the C–S bond.[7] Other kinetic studies which involved the oxidation of formamidinesulphinic acid by chlorite gave results that supported this postulated mechanism.[9]

The oxidations of sulphonic acids are difficult to deduce mechanistically especially if they have an amino group at the α- or β-positions. The reaction of chlorite with aminomethanesulphonic acid is very slow and does not give quantitative formation of sulphate, as expected from the cleavage of the C–S bond under the influence of a strong oxidizing agent such as chlorite.[10] Sulphamic acid and methylsulphamic acid do not give sulphate either upon oxidation as the sulphonic acid group cannot and is not further oxidized. This behaviour seems prevalent in all aminoacids based on the sulphonic acid group.[11]

The most important β-aminosulphonic acid in physiological mechanisms is 2-aminoethanesulphonic acid, taurine.[13] Taurine has been implicated in several physiological roles, but there has been no firm and conclusive mechanistic study of how it functions. For example, what is the mechanism by which taurine and isethionic acid inhibit calcium-activated respiration in liver mitochondria?[14] *In vivo* taurine studies that have been done so far have only helped to establish that there is a special stability associated with taurine, and that the amino group of the molecule is the only one that can engage in reactions.[10] The range of taurine implication in several roles in the human body made us want to take a closer look at its reactivity.

Taurine is a major constituent of the free aminoacid pool in most animal tissues, and often occurs in concentrations higher than those of all the other aminoacids.[15] It is also highly concentrated in neurologically excitable tissues like the brain and in membranes such as the retina.[16] Taurine can be acquired through dietary processes, but also, due to its stability, has been considered the biochemically inert end-product of methionine and cysteine metabolism.[17] Among the several roles of taurine is osmoregulation, in which it regulates the osmotic pressure of the cells.[18] It also attaches to cells and stimulates processes like glycolysis.[19] In conjunction with eosinophils, taurine is known to kill the schistosomula of *Schistosoma mansoni* and *S. haematobium in vitro*.[20]

The most controversial role taurine is supposed to play in the

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human body, however, is as an antioxidant.[21] It has been implied that taurine, hypotaurine and their metabolic precursors act as antioxidants *in vivo*.[22] The mechanism of such a process is still very sketchy, but must involve the scavenging of the standard reactive oxygen species: superoxide radical,[23] $O_2^{\bullet-}$, hydroxyl radical,[24] $^{\bullet}OH$, and peroxide,[25] H_2O_2, and perhaps other damaging oxyhalogen species such as HOBr and HOCl.

The link between taurine and possible physiological mechanisms is difficult to assess. It is known, however, that myeloperoxidase (MPO) and eosinophil peroxidase (EPO) catalyse the oxidation of bromide and chloride ions by H_2O_2 to produce HOBr or HOCl,[26] which rapidly react with amines to give long-lived bromo- and chloramines.[27] Could the bromamines formed regulate the oxidative toxicity of the peroxides?

MPO and EPO have a role in killing microorganisms and inactivation of viruses by leukocytes.[28] Apart from Cl^- and Br^-, these enzymes also catalyse the oxidation of SCN^- by H_2O_2 to produce the (pseudo)halogen, hypohalous acid, HOX or the hypohalite anion, OX^-. HOX/OX^- are in rapid equilibrium and they possess antimicrobial and antiviral activity.[29] However, they also damage host tissues, contribute to DNA damage, and cause inflammatory tissue injury.[30] Blood concentrations of chloride ions are about 0.1 M, bromide ions 0.001 M, iodide 10^{-6} M, and thiocyanate between 0.02 and 0.12 mM.[31] The abundance of chloride suggests that it is the physiological substrate for MPO. However, even though EPO has antimicrobial activity in the presence of H_2O_2 and Cl^-, some studies have shown that EPO is less active than MPO with Cl^- as substrate.[32] There is evidence to suggest that Br^- may be the physiological substrate for EPO:[33]

$$H_2O_2 + Br^- \rightarrow HOBr + H_2O \text{ (catalysed by EPO/H}^+\text{).} \qquad \text{R1}$$

Toxicity of HOBr can then be moderated by amines by producing milder oxidizing bromamines:

$$HOBr + RNH_2 \rightarrow RNHBr + H_2O. \qquad \text{R2}$$

We had conjectured that the amine group of taurine might play such a role as in reaction R2, above, in cytoprotectivity. Bromide oxidation, however, has always been difficult to measure in physiological conditions. The formation, by the more abundant chloride ions, of monochloramines can lead to the direct formation of monobromamines:

$$RNHCl + Br^- \rightarrow RNHBr + Cl^-. \qquad \text{R3}$$

Thus formation of bromamines does not indicate that HOBr toxicity had been moderated by the amine.[34] An experimental study that isolated the bromide ion could shed light on the possible cytoprotectivity of amines to possible tissue damage by HOBr.

Recent studies have demonstrated that human neutrophils stimulated with phorbol myristate acetate, PMA, or *opsonized zymosan* particles destroyed cell targets by a myeloperoxidase-dependent procedure that could be inhibited by compounds known to scavenge HOCl.[35] Attempts to measure the direct chloride peroxidation products by intact cells were quantitatively performed by the use of taurine to form chlorotaurines. Our own previous studies have also clearly shown the formation of chloramines from the oxidation of taurine by chlorite under neutral to slightly acidic pH conditions.[10] The monochloramine initially formed disproportionated in acidic conditions to give back taurine and dichlorotaurine.

The present work focuses on the bromide ion and the possible moderation of bromine and hypobromous acid toxicity by taurine. In its oxidized form, we introduce bromine in the form of Br(V), BrO_3^- and Br(0), Br_2(aq). These conditions are extremely oxidizing, and taurine should be able to protect tissue from oxidative damage from Br_2 and HOBr by the formation of relatively stable bromotaurines. There are two important factors that need to be considered before such an assertion can be made, and we intend to investigate both factors in this manuscript: (a) how rapidly can taurine mop up the halogen and oxyhalogen at physiological pH's; and (b) can taurine be regenerated such that it can continue moderating the toxicity of HOBr? The rate of reaction of taurine with the oxidant should be faster than the rate of damage of tissue by the oxidant for taurine to qualify as an antioxidant.

The primary aim of the work reported here is to examine the oxidation of taurine to form bromotaurines and to evaluate the mechanism of the reaction. Mechanistic data on this reaction will assist in evaluating some of the physiological roles of taurine.

2. Experimental

2.1. Materials

The following reagent grade chemicals were used without further purification: sodium bromate, sodium perchlorate, potassium iodide, perchloric acid (72%), sodium thiosulphate (Fisher), bromine, taurine and D_2O (Aldrich). Aqueous bromine solutions were standardized by adding excess potassium iodide and titrating the liberated iodine against standard thiosulphate with freshly prepared starch as indicator.[36] The concentration was also determined by measuring the absorbance at 390 nm where the extinction coefficient of bromine[37] had been deduced as 142 l mol^{-1} cm^{-1}. Bromine solutions, being volatile, were kept capped and were standardized before each set of experimental runs.

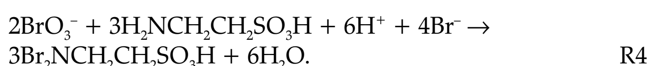
2.2. Methods

The reactions of bromate and taurine were slow enough to be followed on a Perkin Elmer Lambda 2S UV-visible spectrophotometer. The absorbance of bromine at 390 nm was monitored. The faster reactions of bromine and taurine were followed on a Hi-Tech Scientific SF61AF stopped-flow spectrophotometer. ^{1}H NMR spectra of taurine and the bromotaurines were obtained on a JEOL GX 270 spectrometer using D_2O as the solvent and internal standard. All kinetic experiments were performed at 25.0 ± 0.5°C and an ionic strength of 1.0 M ($NaClO_4$). The stoichiometric determinations were performed by varying the amount of bromate while keeping the taurine concentration constant. The required stoichiometry was determined as the point just before the reaction solution produced bromine.

3. Results

3.1. Stoichiometry

The stoichiometry of the BrO_3^-–taurine reaction is very complex in highly acidic media. The notable result was the lack of sulphate in the product solution. Thus the C–S bond in taurine could not be cleaved as had been observed in other small organic sulphur molecules when subjected to a strong oxidizing agent. The reaction gave a mixture of products: $Br(H)NCH_2CH_2SO_3H$, $Br_2NCH_2CH_2SO_3H$ and $HO(H)CH_2CH_2SO_3H$, all of which give nearly identical ^{1}H NMR spectra. Reproducible stoichiometry could be obtained from solutions in which Br^- was deliberately added to the reaction mixture to produce a mixture of the monobromo- and dibromotaurines:



$$2BrO_3^- + 3H_2NCH_2CH_2SO_3H + 6H^+ + 4Br^- \rightarrow 3Br_2NCH_2CH_2SO_3H + 6H_2O. \qquad \text{R4}$$

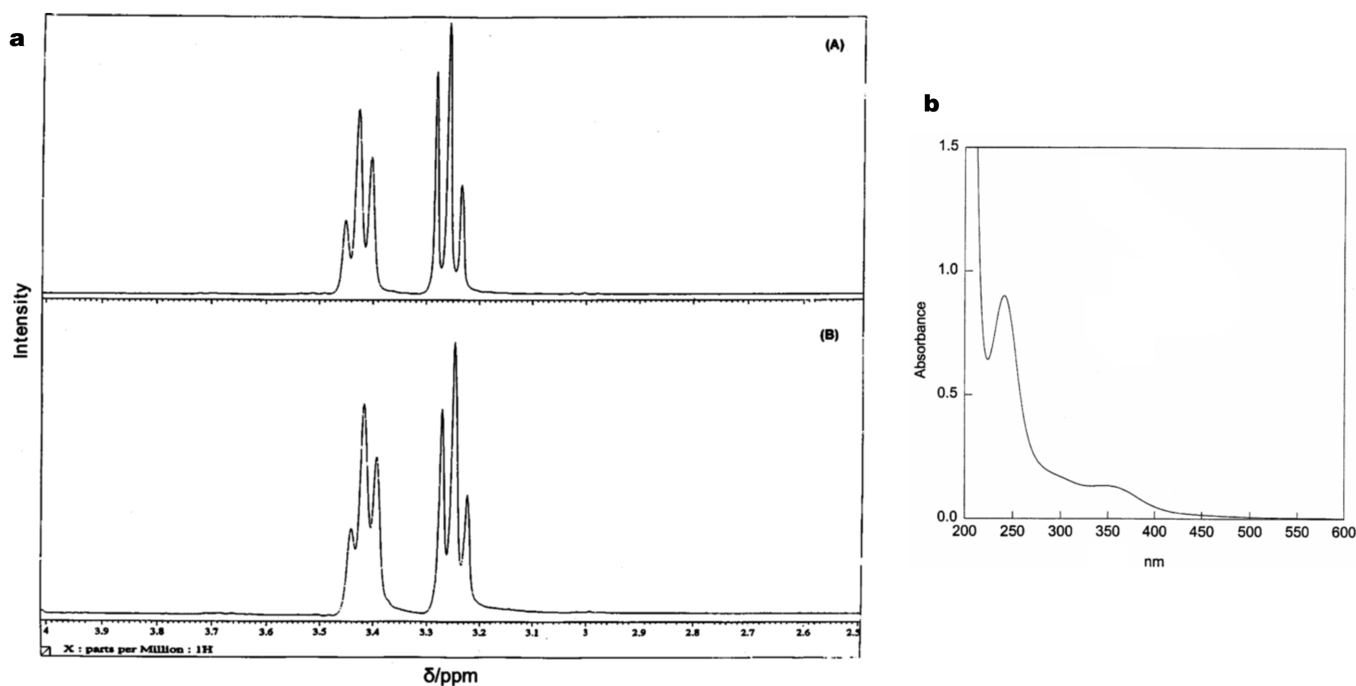
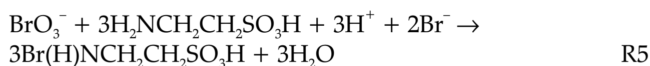


Figure 1. **a**, ^{1}H NMR spectral traces of pure taurine at pH 5 (spectrum A) and spectrum of the bromate–taurine reaction at pH 0 (spectrum B). The basic CH_2-CH_2 backbone is maintained throughout. **b**, UV spectrum of the product solution of the bromine–taurine reaction showing the strong peak at 240 nm which signifies dibromotaurine. $[Br_2]_0$ = 0.006 M, $[Taurine]_0$ = 0.003 M

Stoichiometry R5 is first obtained before R4:

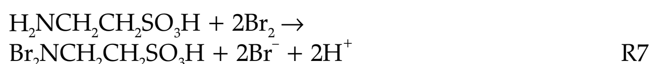


$$BrO_3^- + 3H_2NCH_2CH_2SO_3H + 3H^+ + 2Br^- \rightarrow 3Br(H)NCH_2CH_2SO_3H + 3H_2O \quad \text{R5}$$

and in the absence of further bromide, the monobromotaurine formed in stoichiometry R5 disproportionated in high acid environments to give dibromotaurine and taurine:

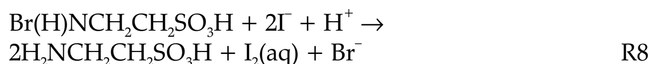
$$2Br(H)NCH_2CH_2SO_3H \rightarrow Br_2NCH_2CH_2SO_3H + H_2NCH_2CH_2SO_3H \quad \text{R6}$$

The protonation of the amine group at high acid concentration tends to pull the equilibrium of reaction R6 to the right. The stoichiometry of the Br_2–taurine reaction, however, was very clean and gave a 1:2 stoichiometric ratio:



$$H_2NCH_2CH_2SO_3H + 2Br_2 \rightarrow Br_2NCH_2CH_2SO_3H + 2Br^- + 2H^+ \quad \text{R7}$$

Monobromotaurine and dibromotaurine could easily be quantified in the reaction product by their rapid reaction with excess iodide to produce iodine which could then be spectrophotometrically determined:

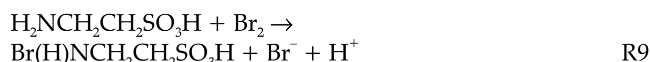


$$Br(H)NCH_2CH_2SO_3H + 2I^- + H^+ \rightarrow 2H_2NCH_2CH_2SO_3H + I_2(aq) + Br^- \quad \text{R8}$$

Excess iodide was essential in reaction R8 to give the relatively inert I_3^- which discouraged any electrophilic iodination of the amino group. The oxidizing equivalents with respect to thiosulfate did not change after addition of excess iodide. Monobromotaurine retained 2 mol of oxidizing equivalent, and its concentration could also be determined by its ability to oxidize 5-thio-2-nitrobenzoic acid to its dimer.[38]

3.2. Product Identification

The products were identified by the use of ^{1}H NMR and UV spectroscopy. Figure 1a shows a series of NMR spectra of pure taurine (A), taurine in high acidic environments (pH 0.00, spectrum B) and the product solution of the bromate–taurine reaction (C). In all three spectra the two characteristic triplets from the CH_2-CH_2 backbone of taurine are evident. The slight downfield shift of the two triplets is expected from the protonation of the amino group in taurine (spectrum B). The free C–N bond rotation (after protonation) also removes the slight splitting of the triplet peaks observed in spectrum A. The product solution spectrum shows a further slight shift downfield as expected when the hydrogens on the amino group are replaced by bromine. These NMR spectra show that the basic structure of taurine is unaltered during this oxidation. No sulphate was detected in the product solution, confirming that no reactivity occurs on the sulphonic acid moiety. The UV spectrum of the bromine–taurine reaction product is shown in Fig. 1b. The dibromotaurine product was easily identified by its UV spectrum and its ^{1}H NMR spectrum. The UV spectrum of dibromotaurine shows a strong absorption peak at 240 nm with an absorptivity of 2713 l mol^{-1} cm^{-1} and another peak at 336 nm with ε = 371 l mol^{-1} cm^{-1}. Monobromotaurine, on the other hand, has a single, relatively weak absorption peak at 288 nm (ε = 430 l mol^{-1} cm^{-1}). The product spectrum obtained in Fig. 1b is exactly the one given in the literature for dibromotaurine. The spectrum was taken from reaction solutions derived from stoichiometric amounts of bromine and taurine to remove the expected bromine peak at 390 nm. The dibromotaurine solution gave the same NMR spectrum as the one in Fig. 1a (spectrum C). Equimolar solutions of bromine and taurine gave monobromotaurine as the predominant product:



$$H_2NCH_2CH_2SO_3H + Br_2 \rightarrow Br(H)NCH_2CH_2SO_3H + Br^- + H^+ \quad \text{R9}$$

At high acid concentrations, however, the monobromotaurine slowly decayed to be replaced by a mixture of taurine, monobromo- and dibromotaurine as in reaction R6.

3.3. Reaction Dynamics

The bromate–taurine reaction was extremely slow and sometimes needed days (a week or two) for the reaction to proceed to

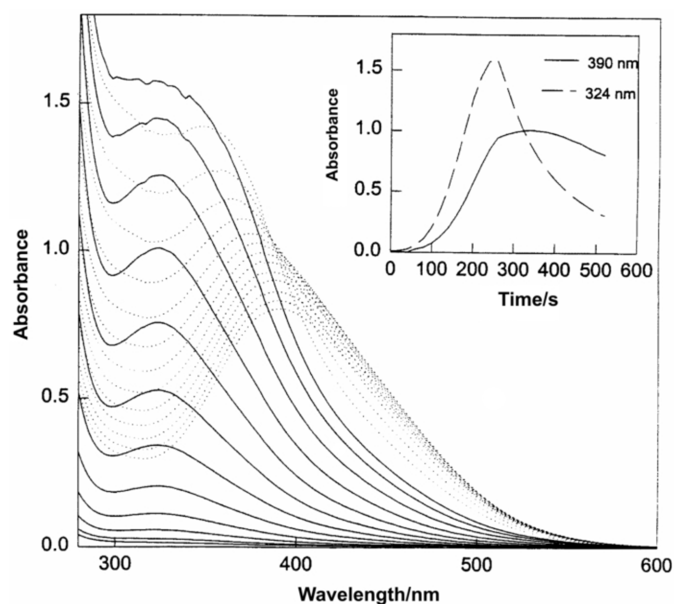


Figure 2 Spectral scans of the bromate–taurine reaction taken every 30 s. The insert shows the specific activities at 390 nm and 324 nm. The absorbance at 324 nm is of the monobromotaurine intermediate species, which attains a maximum concentration before falling to accommodate the formation of the product species dibromotaurine. Bromine concentrations (λ = 390 nm), are depleted due to their reaction with monobromotaurine to form dibromotaurine. $[BrO_3^-]_0$ = 0.100 M, $[H^+]_0$ = 2.00 M, $[Taurine]_0$ = 0.010 M. To avoid confusion, the dotted spectra were taken after the peak in bromine concentrations had been reached. Solid lines represent rising absorbance at 390 nm

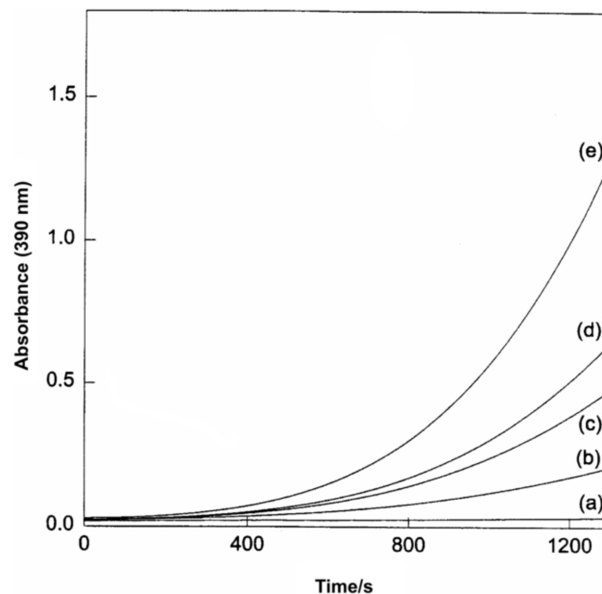
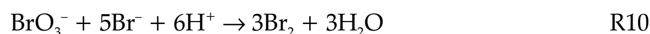


Figure 3 Absorbance traces showing the effect of acid on the bromate–taurine reaction. $[Taurine]_0$ = 0.10 M, $[BrO_3^-]_0$ = 0.40 M, $[H^+]_0$ = (a) 1.0 M, (b) 1.2 M, (c) 1.5 M, (c) 1.8 M, (e) 2.0 M

completion. This is an indication of how stable and unreactive taurine is, even when subjected to an excess of a strong oxidizing agent. In the UV and visible regions of the spectrum the reaction mixture showed absorption at 240 nm, 324 nm and 390 nm. The peak at 240 nm is by far the strongest. Figure 2 shows UV spectral scans taken every 30 s. The solid lines represent the first 360 s, where the peak at 324 nm increases, and the dotted lines represent the region in which the peak at 324 nm rapidly decreases with a concomitant decrease in the peak at 390 nm. No discernible peak is evident in the spectra at 390 nm during the first 360 s of the reaction. The observed absorbance readings obtained at 390 nm are a summation of contributions from bromine, monobromo- and dibromamines. Taurine itself shows no strong absorption at this wavelength. The insert shows specific activities at the two particular wavelengths of 324 nm and 390 nm. Absorption at 324 nm is attributed mainly to monobromotaurine and that at 390 nm to Br_2. Although literature claims that monobromotaurine has an absorption peak at 288 nm, this peak is not observed due to interference from other species also absorbing at 288 nm, to the extent that there is no real correlation between the peak at 288 nm and the concentration of monobromotaurine. Applying the Beer-Lambert law for monobromotaurine at 288 nm gives an anomalously high reading. Initially there is an accumulation of monobromotaurine as well as Br_2. The monobromotaurine concentration reaches a peak at the same time that bromine concentrations attain a peak and then decays. Although 324 nm does not represent an absorption peak for monobromotaurine, it is displaced far enough away from the regions where the dibromotaurine absorbs too strongly.

From an analysis of the combination of the slow reaction R4 and the very rapid reaction R7, the bromate–taurine reaction appeared to be one of those typical clock reactions with variable stoichiometry based on initial concentrations with an induction period before formation of bromine. The observed reaction dynamics, however, did not give a clear induction period in conditions of $[BrO_3^-]_0 \gg [H_2NCH_2CH_2SO_3H]_0$. Figure 3 shows that the reaction gives a slow bromine formation even at the initial part of the reaction. This rate of bromine formation keeps increasing in what appears to be an autocatalytic process. The initial rate of formation of bromine gave an $[H^+]_0^2$ dependence. The effect of $[BrO_3^-]_0$ was nearly the same as that for acid except for the dependence of the initial rate of formation of bromine which was unit order with respect to $[BrO_3^-]_0$.

The effect of taurine on the reaction is shown in Fig.s 4a and 4b. Figure 4a shows the UV spectra of products at t = ∞. For as long as $[BrO_3^-]_0 \gg [H_2NCH_2CH_2SO_3H]_0$, the amount of bromine produced is proportional to $[H_2NCH_2CH_2SO_3H]_0$. This is due to the extension of stoichiometry R4. After full consumption of taurine and monobromotaurine, any further bromide formed in reactions R7 and R9 will be converted to bromine by the excess bromate:



$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O \qquad R10$$

Figure 4b shows the rapid autocatalytic increase in bromine as the initial taurine concentrations are increased.

The direct reaction of taurine and bromine was extremely fast, especially in neutral and slightly basic environments. Figure 5 shows a series of experiments run at varying initial taurine concentrations. All the experiments shown were run in excess bromine, thus ensuring complete oxidation of the taurine to dibromotaurine. The observed initial rapid increase in absorbance is due to the formation of the tribromide species, Br_3^-. The production of Br^- in the initial stages of reaction R3 triggers the rapid equilibrium:[39]

$$Br_2 + Br^- \rightarrow Br_3^- \quad K_{eq} = 17 \qquad R11$$

Bromine has an absorptivity of 142 l mol^{-1} cm^{-1} at λ = 390 nm while the tribromide complex has an absorptivity of 1006 l mol^{-1} cm^{-1}.[39] The isosbestic point for the Br_2/Br_3^- species is at 442 nm. The absorptivity for both species at this wavelength is too low to be of any analytical use. Br_2, being a stronger electrophile, reacts much faster than Br_3^-, hence the initial accumulation of Br_3^-.

The effect of acid on the bromine–taurine reaction was both

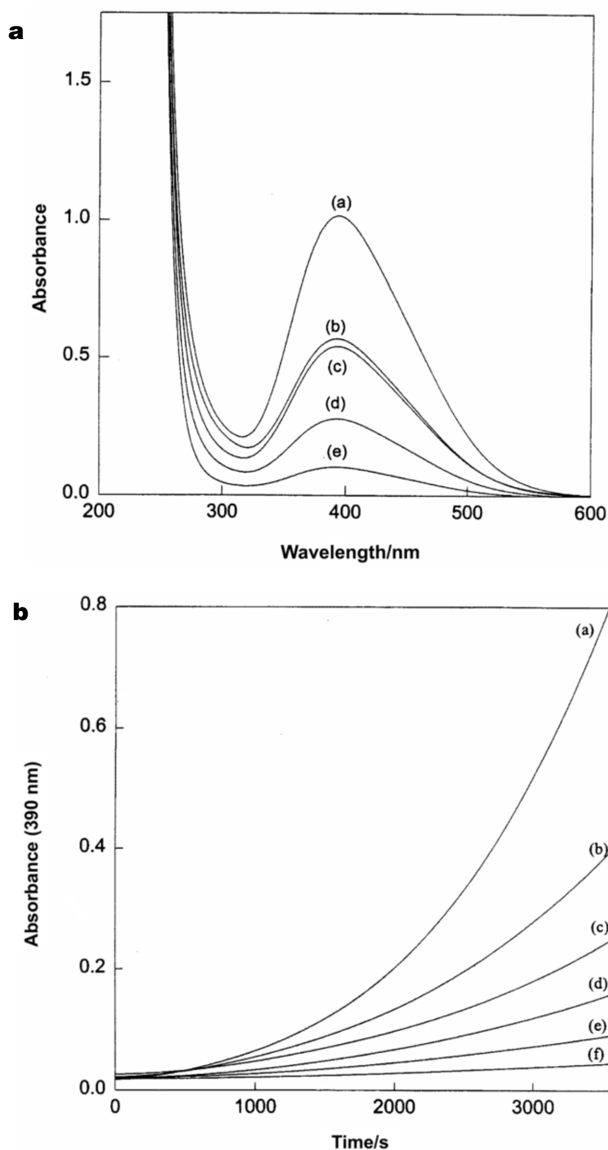


Figure 4. **a**, Effect of taurine in conditions of excess bromate concentrations. These spectral scans were taken after the reaction had been incubated for 14 days. $[BrO_3^-]_0 = 0.10$ M, $[H^+]_0 =$ (a) 2.0 M, $[\text{Taurine}]_0 =$ (a) 0.02 M, (b) 0.01 M, (c) 0.008 M, (d) 0.005 M, (e) 0.0025 M, (f) 0.001 M. **b**, The absorbance–time traces for the reaction solutions with t_∞ shown in Fig. 4a. The traces show a rapidly-increasing rate of production of bromine, but no real discernible induction period

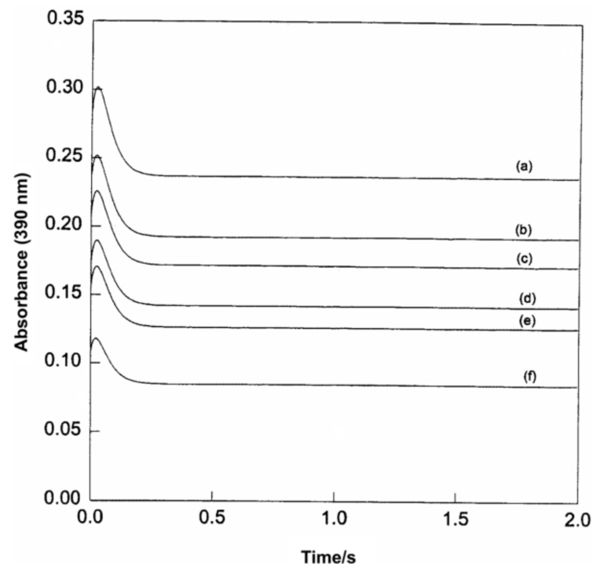


Figure 5 The direct reaction of bromine and taurine. The reaction is very fast and at pH > 4 the reaction is essentially over in 0.5 s. $[\text{Taurine}]_0 = 0.005$ M. $[Br_2]_0 =$ (a) 0.01 M, (b) 0.009 M, (c) 0.008 M, (d) 0.007 M, (e) 0.006 M, (f) 0.005 M

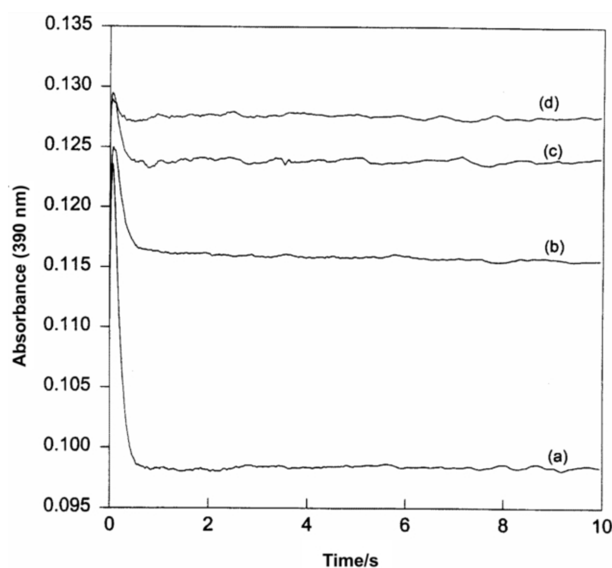
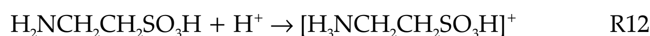


Figure 6 Effect of acid on the direct bromine–taurine reaction shown in Fig. 5. $[Br_2]_0 = 0.012$ M. $[\text{Taurine}]_0 = 0.005$ M. $[H^+]_0 =$ (a) 0.012 M, (b) 0.023 M, (c) 0.058 M, (d) 0.12 M

dramatic and surprising. Figure 6 shows some absorbance traces of experiments run at varying acid concentrations. There is a strong inhibitory effect by acid on the reaction such that the reaction virtually shuts down when the acid concentrations exceed 0.100 M. Within a small range of acid concentrations, $0.0 < [H^+] < 0.10$, there is an inverse dependence of the initial rate on acid concentration. At $[H^+]_0 > 0.10$ M the acid retardation attains a limiting effect. To appreciate the retarding effect of acid better, one should remember that all runs shown in Fig. 6 give, finally, the same value for bromine absorbance.

3.4. Mechanism

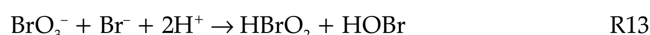
Owing to the inertness of taurine at the C–S bond, the postulated mechanism is very simple and not complicated by possible intermediate species formed from taurine. The only possible taurine species are taurine itself, mono- and dibromotaurine. The mechanism involves mostly oxyhalogen reactions, whose kinetics and kinetic parameters are well known. With a taurine pK_b of 9.46,[40] and pH conditions lower than 1.00, taurine will be nearly 100% protonated at these reaction conditions:



$$H_2NCH_2CH_2SO_3H + H^+ \rightarrow [H_3NCH_2CH_2SO_3H]^+ \qquad \text{R12}$$

All reactivity will be on the protonated nitrogen centre. For all reference to taurine in this manuscript, we assume the protonated form.

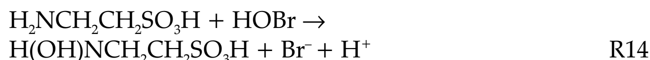
With Br^- initially added to the reaction mixture, the initiation reaction is a pure oxyhalogen reaction in which the reactive intermediates, HOBr and $HBrO_2$, are formed:



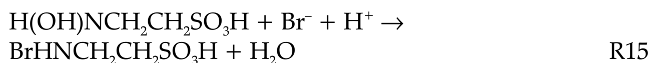
$$BrO_3^- + Br^- + 2H^+ \rightarrow HBrO_2 + HOBr \qquad \text{R13}$$

Reaction R13 is well known in all bromate oxidations and is responsible for the observed second order kinetics in $[H^+]$ for all

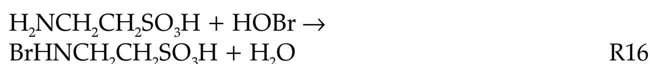
bromate oxidations.[41] Br^- can be replaced by any 2-electron reductant. Hypobromous acid formed in R13 rapidly oxidizes taurine in a 2-electron transfer process:


$$H_2NCH_2CH_2SO_3H + HOBr \rightarrow H(OH)NCH_2CH_2SO_3H + Br^- + H^+ \qquad R14$$

followed by:


$$H(OH)NCH_2CH_2SO_3H + Br^- + H^+ \rightarrow BrHNCH_2CH_2SO_3H + H_2O \qquad R15$$

Reactions R14 and R15 can be represented by a composite reaction R16:

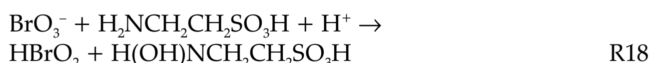

$$H_2NCH_2CH_2SO_3H + HOBr \rightarrow BrHNCH_2CH_2SO_3H + H_2O \qquad R16$$

Br^- produced in R14 can be used to produce more reactive species:

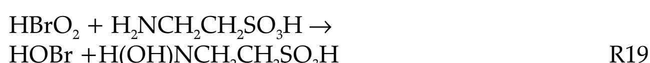
$$HBrO_2 + Br^- + H^+ \rightarrow 2HOBr \qquad R17$$

We thus expect an autocatalytic build-up of the reactive species and hence the rate of reaction should also increase with time. This can be observed in the rate of formation of bromine (see Figs 3 and 4b).

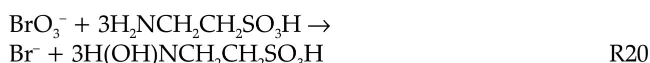
In the absence of initial concentrations of bromide, the reactive species are formed by the direct oxidation of taurine by bromate:


$$BrO_3^- + H_2NCH_2CH_2SO_3H + H^+ \rightarrow HBrO_2 + H(OH)NCH_2CH_2SO_3H \qquad R18$$

followed by successive 2-electron reductions to give HOBr and Br^-:

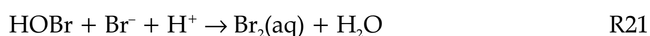

$$HBrO_2 + H_2NCH_2CH_2SO_3H \rightarrow HOBr + H(OH)NCH_2CH_2SO_3H \qquad R19$$

The final stage in the reduction of the bromine centre is reaction R14. The overall stoichiometry of this initiation reaction is:


$$BrO_3^- + 3H_2NCH_2CH_2SO_3H \rightarrow Br^- + 3H(OH)NCH_2CH_2SO_3H \qquad R20$$

The bromide produced in reaction R20 can then activate reaction R13 to form the reactive species. Reaction R18 is not the rate-determining step as the reaction follows second order dependence in $[H^+]$, which indicates that reaction R13 is ultimately the rate-determining step in this reaction. Standard bromate solutions contain at least 10^{-6} M of Br^-, which exists as a contaminant.[42] This is sufficient to initiate reaction R8. The production of Br^- is autocatalytic (from reaction R13), and so very little Br^- is needed to initiate the reaction.

There is only one reaction pathway for the formation of bromine in the reaction:[43]


$$HOBr + Br^- + H^+ \rightarrow Br_2(aq) + H_2O \qquad R21$$

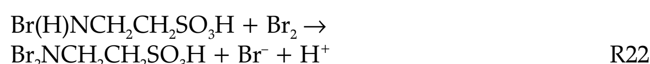
The rate of bromine formation is thus dependent on the rate of formation of HOBr and Br^-. Br^- can be consumed to form bromine as in reaction R21, above, or can react with the oxime, reaction R15, to give the bromotaurines. Reactions R15 and R21 occur simultaneously, hence the absence of a noticeable induction period for this reaction. Figure 2 insert shows that Br_2 concentrations rapidly increase, but later start to fall at the same time as the concentration of monobromotaurine starts to fall. Figure 6 shows that in pH conditions less than 2, the direct reaction between taurine and bromine is very slow. Hence bromine formed from reaction R21 accumulates without any appreciable consumption of bromine from reactions R7 and R9. The protonation of the nitrogen centre strongly retards the electrophilic attack by bromine. If one assumes that the protonated taurine species is inert, then the rate of the bromine–taurine reaction becomes:

$$-d[Br_2]/dt = [H_2NCH_2CH_2SO_3H]_T[Br_2]/(1 + K_{eq}[H^+]) \qquad (1)$$

where $[H_2NCH_2CH_2SO_3H]_T$ is the total taurine concentration which combines the protonated and the unprotonated forms ($[H_2NCH_2CH_2SO_3H] + [H_3NCH_2CH_2SO_3H^+]$). K_{eq} is the equilibrium constant of reaction R12. In the limit of high acid concentrations, we expect the experimentally-observed inverse acid dependence kinetics from this reaction.

3.5. Formation and Consumption of Monobromotaurine

Figure 2 shows that monobromotaurine and bromine initially increase in concentration, and towards the end of the reaction, they both decrease. The accumulation of bromine is simply due to the very slow bromine–taurine reaction in high acid conditions. Another slow reaction occurs between Br_2 and bromotaurine:


$$Br(H)NCH_2CH_2SO_3H + Br_2 \rightarrow Br_2NCH_2CH_2SO_3H + Br^- + H^+ \qquad R22$$

Reaction R22, being slow in high acid environments, cannot mop up all the bromine as it is formed, hence its continued accumulation. Any Br^- formed in reaction R22 will produce more bromotaurine (reaction R15) and more bromine (reaction R21). Bromotaurine will continue to increase until all the taurine has been consumed and converted to bromotaurine. After the total consumption of taurine, there will be no more reactions in solution which can produce bromotaurine. There are two possible routes for the consumption of bromotaurine: reactions R6 and R22. In the presence of excess oxidant, reaction R22 predominates, which means both Br_2 and bromotaurine will be simultaneously consumed (Fig. 2). Reaction R6, should it be significant, will produce taurine which will react with Br_2 as in reaction R9. This sequence will be kinetically indistinguishable from reaction R21.

3.6. Computer Simulations

The reaction was modelled using the abbreviated mechanism shown in Table 1 (with the reactions now re-numbered M1 to M11). The kinetic parameters for the well-known oxyhalogen reactions M1–M3 were available from the literature.[44] The forward rate constant for reaction M7 was evaluated from this study (see Fig. 5). Out of the remaining seven reactions M5 and M10 were fast and not rate-determining in this reaction network. M10, however, was important in determining the observed retardation by acid through the assumption that no electrophilic attack occurred on the protonated nitrogen centre. The forward and reverse constants of M10 are related by the pK_b of taurine. Strict adherence to this ratio, however, gave poor simulation fits to the data. This could be attributed to the presence of the strongly acidic sulphonic acid group. For example, after fixing the forward rate constant at $1.0 \times 10^8 \times 1\ mol^{-1}\ s^{-1}$, the reverse reaction rate will also be fixed by pK_b at $2.9 \times 10^3\ s^{-1}$. Using this value did not reproduce the rate of formation of bromine. Reaction M11 was used for stoichiometric consistency only: it was not rate-determining with respect to consumption of taurine, even though it is one of the slowest reactions in the scheme. The full reaction stoichiometry can be obtained from a linear combination of the following reactions:

2M1 + 3M2 + 2M3 + 3M6 + 3M11.

The most important reactions in the simulations were M1 and M4. When Br^- was initially added to the reaction mixture, reac-

Table 1 Mechanism used for the bromate-taurine simulations.

Reaction no.	Reaction	Rate constants
M1	$BrO_3^- + Br^- + 2H^+ \rightarrow HBrO_2 + HOBr$	2.1; 1.0×10^4
M2	$HOBr + Br^- + H^+ \rightarrow Br_2 + H_2O$	8.9×10^9; 1.10×10^2
M3	$HBrO_2 + Br^- + H^+ \rightarrow 2HOBr$	2.5×10^6; 2.0×10^{-5}
M4	$BrO_3^- + RNH_2 + H^+ \rightarrow RNHOH + HBrO_2$	82
M5	$RNHOH + Br^- + H^+ \rightarrow RNHBr + H_2O$	5.5×10^3
M6	$HOBr + RNH_2 \rightarrow RNHOH + Br^- + H^+$	1.0×10^5
M7	$Br_2 + RNH_2 \rightarrow RNHBr + Br^- + H^+$	3.8×10^5
M8	$2RNHBr + H^+ \rightarrow RNBr_2 + RNH_3^+$	4.2×10^6; 1.0×10^{-1}
M9	$HBrO_2 + RNH_2 \rightarrow RNHOH + HOBr$	1.5×10^3
M10	$RNH_2 + H^+ \rightarrow RNH_3^+$	1.0×10^8; 4.2×10^5
M11	$RNHBr + Br_2 \rightarrow RNBr_2 + H^+ + Br^-$	45

The units for the rate constants are deduced from the molecularity of the reaction except where the solvent, water, is involved. Note that all acid concentrations were not varied, and thus reactions M4 and M5 are effectively bimolecular, and reaction M10 is first order.

tion M1 became the rate-determining step. Without added Br^-, the reaction became most sensitive to the kinetic parameters for reaction M4. The sequence of reactions

M4 + M9 + M6

produced Br^- such that M4 was only important in the initial stages of the reaction, but after enough Br^- had been formed, reaction M1 became the rate-determining step. To facilitate the simulations we assumed the acid concentrations were in such large excess that they were constant. This allowed us to use what appear to be termolecular steps in the reaction mechanism: reactions M4 and M5. In both cases, the third reagent has a constant concentration, which reverts the reactions to pseudo-second order. Reaction M8 is a composite of two consecutive steps. The reaction scheme was simulated using semi-implicit fourth order Runge-Kutta techniques.[45] The comparison between experimental results and our simulations is shown in Fig. 7. There was a reasonable fit between our model and experiments. The simulations were able to reproduce the acid effect which was most important because, while high acid concentration catalysed the oxyhalogen reactions, it inhibited the needed electrophilic attack on the amino group of taurine. The simulations could not reproduce the observed data for absorbance at 324 nm (Fig. 2). This is because the absorbance at 324 nm is due to a resultant of several species and not just monobromotaurine. The simulations, however, do predict the cresting of the concentrations of monobromotaurine before falling to make way for dibromotaurine.

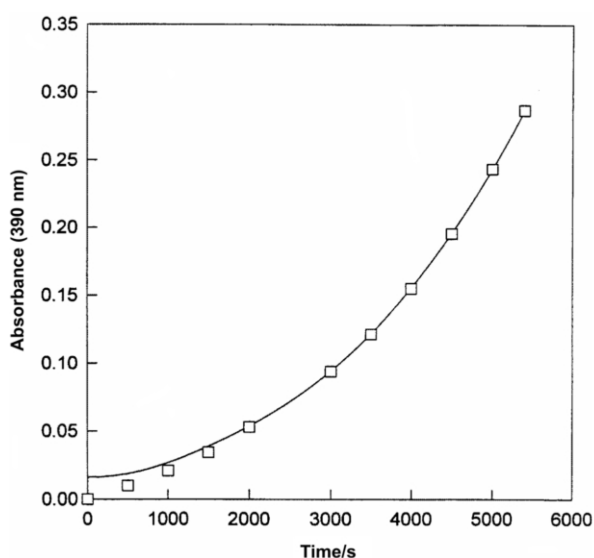


Figure 7 Comparison between experimental and modelling results (squares). The observed disagreement between the simulations and experimental data at the beginning of the reaction is expected since the experimental data will contain background absorbance which cannot be simulated. $[BrO_3^-]_0 = 0.08$ M, $[H^+]_0 = 2.5$ M, $[\text{Taurine}]_0 = 0.008$ M.

4. Conclusion

The reaction of taurine with acidic bromate is exceedingly slow, due to the opposing effects of acid. Production of the electrophilic oxidizing species derived from bromate is greatly enhanced by acid, while electrophilic attack on the nitrogen centre of taurine is strongly retarded by acid. Although the reaction of aqueous bromine with taurine is very fast, it is also retarded by acid. In physiological pH conditions of 7.4, we would expect taurine rapidly to mop up any Br_2 and HOBr, thus moderating any toxicities derived from these species. The ability of taurine to protect tissue from damage from halogens and oxyhalogens is enhanced by its very high concentration. Though it may not be as efficient as other antioxidants, its high concentration will more than make up for its relatively sluggish reactivity. We are not certain of the ultimate fate of the bromotaurines in physiological conditions, but some percentage is expected to be regenerated through disproportionation. Taurine is constantly being formed from cysteine and methionine metabolism and so its concentration can be regarded as constant in physiological environments.

Recent experimental data have shown that the major metabolic precursor to taurine, hypotaurine, reacts rapidly and efficiently with the hydroxyl radical, $^{\bullet}OH$, superoxide radical, $O_2^{\bullet -}$ and hydrogen peroxide, H_2O_2.[46] The abilities to scavenge these reactive oxygen species (ROS) rapidly is a prerequisite for a molecule to act as an antioxidant *in vivo.*[47] The other metabolic precursors of taurine; cysteic acid and cysteamine are also poor scavengers of ROS together with taurine. The cytoprotective role of taurine may be solely restricted to the deactivation of HOBr because taurine appears inefficient in moderating HOCl toxicity since the N-chloramine produced can still deactivate α_1-antiproteinase.[48]

Acknowledgements

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References and Notes

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