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Antioxidant chemistry

Reactivity and oxidation of DL-cysteine by some common oxidants†

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The reactivity of DL-cysteine, a physiologically important aminothiol, was studied by reacting it with several well known oxidants. No activity was observed on the amino and carboxyl groups. The only reactivity of physiological significance was at the sulfur centre. Reactions of cysteine with hydrogen peroxide show that the thiol group is capable of mopping up free radicals by forming thyl radicals, as expected in its role as an antioxidant. A four-electron oxidation of cysteine gave reasonably stable cysteine sulfinic acid. Oxidants in the form of peracids do oxidize cysteine only as far as the sulfinic acid. Stronger oxidizing agents can oxidize cysteine as far as the cysteine sulfonic acid. No further oxidation can be detected as the C—S bond is not cleaved. The inertness of the amino group in cysteine makes it incapable of reversibly mopping up the dangerous oxyhalogens HOCl and HOBr which are produced by myeloperoxidase-catalysed oxidation of halides by hydrogen peroxide, as is the case with taurine. A detailed mechanism, together with a computer simulation study of the oxidation of cysteine by acidified bromate, is proposed.

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There has not been, to date, an exhaustive study of the metabolic functions of small-molecular-weight amino acids. The sulfur-based amino acids, especially, have not been adequately characterized. In some of our recent work, we studied the reactivity of the most common amino acid in the human body, taurine.[2] Taurine has been implicated in several physiological roles, including antioxidant activity,[3] and yet no possible mechanism through which taurine could act as an antioxidant had been established.

We recently began a series of studies on oxyhalogen–sulfur chemistry in which we tried to elucidate systematically the oxidation of a sulfur centre in small organic molecules.[4] Our studies established an oxidation algorithm which involves successive two-electron oxidations of the sulfur centre up to the sulfonic acid stage before cleavage of the C—S bond to form sulfate.[5] For the physiologically active sulfur-based amino acids which we have studied so far: taurine,[2] hypotaurine,[6] methionine[7] and glutathione,[7] this algorithm, however, is not followed. Oxidation of taurine occurs only on the amino group, with no activity on the sulfonic acid moiety. Hypotaurine, when oxidized by oxyhalogens, is oxidized to taurine and then to bromo- and chlorotaurines and no further.[6] Methionine is oxidized to give a mixture of the sulfoxide and the sulfone, while glutathione is oxidized only to the S—S dimeric species. No cleavage of the C—S bond is observed in any of the cases. The reversible nature of the oxidations (even by very strong oxidants like HOCl and HOBr) observed with these aminothiols and aminosulfonic acids[2] tends to lend them well to physiological uses, as there is the possibility of substrate regeneration for further use.

Cysteine is an aminothiol with three reactive centres: the carboxylic acid, the amino group and the sulfur centre. Cysteine is particularly interesting in that it is a non-essential amino acid which can be synthesized by the human body through the metabolism of methionine,[8] a process which produces the very important amino acids homocysteine, hypotaurine and taurine.[9]

Several claims have been made for the physiological relevance of cysteine. Cysteine is said to contain sulfur in a form that can inactivate free radicals,[10,11] especially the reactive oxygen species, ROS, and thus can protect and preserve cells.[12] Thus it should act physiologically as an antioxidant and can extend cell life span and protect against toxic substances.[12] Cysteine is supposed to protect glutathione from powerful oxidants because it is much more easily oxidizable than glutathione.[13] It is also supposed to control protein folding in the endoplasmic reticulum by maintaining the redox state of the extracellular fluids.[14]

Some clinically negative aspects related to cysteine mention that it contributes to kidney stones and is contraindicated for diabetics.[15] Also, cysteine's homologue, homocysteine, has been implicated as playing a critical role in destroying arteries and, subsequently, contributing to heart attacks.[16] Homocysteine, however, is extremely inert and reacts exceedingly slowly (reaction times of the order of days) even with the most powerful oxidants (*e.g.* aqueous bromine). Removing a $-CH_2-$ group from homocysteine gives cysteine, a much faster reacting thiol.

The sulfhydryl group in cysteine seems to play a physiologically significant role.[17] Here, we report on the reaction dynamics, stoichiometries and mechanisms of the oxidation of cysteine by peroxyacetic acid, hydrogen peroxide, acidified bromate and aqueous bromine. It is hoped that this study will help in evaluating the oxidation products of cysteine and lead to a plausible mechanism for its action as an antioxidant. We also hope to determine whether there is any activity on the amino group of the cysteine molecule.

Experimental

Materials

All stock solutions and their subsequent dilutions were prepared from doubly distilled deionized water. The following reagents were used without further purification: sodium bromate, sodium bromide, perchloric acid, 72%, (Fisher); nitrate metal salts, (Aldrich); DL-cysteine, DL-cystine, DL-cysteine sulfinic acid, stabilized hydrogen peroxide (30 wt.% solution in water), (Acros), and peroxyacetic acid (32 wt.%) (Sigma). Bromine solutions were stored in sealed brown winchester bottles as a precautionary measure. All cysteine

† Part 32 in the series: Nonlinear dynamics in chemistry derived from chemistry. Part 31: ref. 1.

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solutions were prepared fresh to ensure reproducibility.[1] All reagents were stored as recommended by the vendor.

Stoichiometry

The stoichiometric studies were performed on a Perkin-Elmer Lambda 2S UV–VIS spectrophotometer. The stoichiometry of the reaction of cysteine with bromate was determined by varying the ratios of bromate to DL-cysteine in an acidic medium. The mixtures were incubated for at least a 24 h period before stoichiometric determinations were performed. The amount of Br_2 produced or consumed was quantified spectrophotometrically by measuring its absorbance at 390 nm and the product was identified by ^1H NMR spectroscopy.

^1H NMR studies

^1H NMR spectra were run at 270 MHz on a JEOL-270 MHz spectrometer at 25 °C. Commercial grade deuterated water (D_2O) and $DClO_4$ (Aldrich) were used as supplied. The solvent peak, D_2O $\delta = 4.67$), was used as the internal standard.

Kinetic determinations

Reactions were slow enough to be followed on a conventional Perkin-Elmer UV–VIS Lambda 2S spectrophotometer interfaced to a 486 DX DEC computer for data acquisition, storage and manipulation. Solutions were first vigorously mixed with a magnetic stirrer prior to introduction to the spectrophotometer. A constant time lag of 25 s was maintained between mixing of reagents and commencement of data acquisition.

Stopped-flow measurements

The kinetics of the cysteine–bromate, metal ion effect and the bromine–cysteine reactions were followed on a Hi-Tech Scientific SF61-DX2 double mixing stopped-flow spectrophotometer interfaced to a pentium 133 MHz computer. Time-dependent absorbance changes corresponding to the formation of bromine were measured at 390 nm ($\varepsilon = 142$ dm^3 mol^{-1} cm^{-1})[18] for a duration of at least 1000 s. All concentrations reported are post mixing and reactions were run at 25.0 ± 0.5 °C with a Neslab RTE-101 thermostat. All reactions were run with at least a two-fold excess of bromate concentrations over cysteine in highly acidic environments (pH < 1.00).

Results

Stoichiometry and product identification

Product identification by ^1H NMR shows that the final sulfur-containing product of the oxidation of cysteine by acidic bromate is cysteine sulfonic acid [$H_3N^+CH(CO_2^-)CH_2SO_3H$] and the stoichiometry of the reaction was experimentally deduced to be:

$$BrO_3^- + H_3N^+CH(COO^-)CH_2SH \rightarrow H_3N^+CH(COO^-)CH_2SO_3H + Br^- \quad (1)$$

Quantitative tests for the presence of sulfate[19] were negative, indicating that the C—S bond was not cleaved under these conditions. The centre of reactivity is the sulfur atom, with the other functionalities merely modifying its reactivity.

It was recognized that the reaction that forms Br_2 is an extraneous pure oxyhalogen reaction that occurs in excess BrO_3^-. Br_2 would only be produced when BrO_3^- was in stoichiometric excess (see Fig. 1). When $[BrO_3^-]_0 \gg [\text{cysteine}]_0$ the amount of Br_2 formed was proportional to the cysteine concentration up to some maximum value. However, when

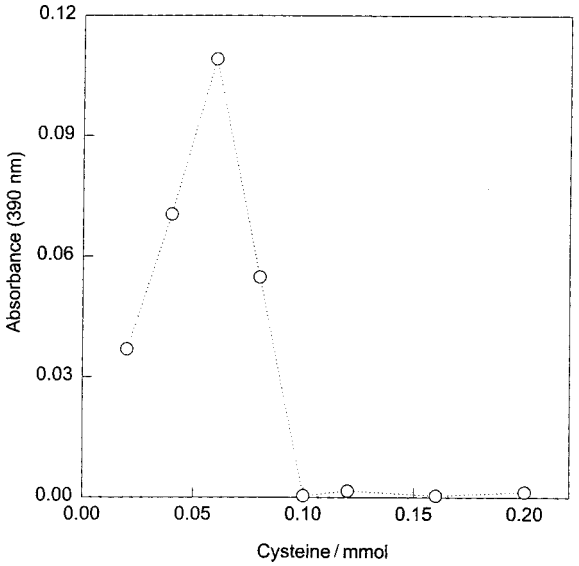

Fig. 1 Determination of the stoichiometry of the bromate–cysteine reaction. The initial amount of cysteine was varied at constant bromate (0.080 mmol). Cell path length is 10 mm, volume 4 cm^3. Wavelength used 390 nm. The reactions were run in a medium with an acid strength, $[H^+]_0$, of 0.065 M. The reaction mixtures of varying ratios of DL-cysteine to bromate were incubated for a period of at least 24 h, after which aliquots from each mixture were analysed by taking absorbance readings at 390 nm.

$[BrO_3^-]_0 \geqslant [\text{cysteine}]_0$, the amount of Br_2 obtained starts to fall with increase in cysteine, to the point where the cysteine concentrations used are sufficient to completely consume the BrO_3^- ions, leaving no more BrO_3^- ions to produce Br_2. This is the stoichiometric point for the bromate–cysteine reaction. The data in Fig. 1 were used to evaluate the stoichiometry (1).

In the highly acidic environments used in this series of experiments (pH < 2) it is expected that both the amino and thiol centres will be highly protonated:

$$H_3N^+CH(CO_2^-)CH_2SH + H^+ \rightleftharpoons H_3N^+CH(CO_2^-)CH_2S^+H_2 \quad (2)$$

The oxidation of cysteine was performed using four oxidants: hydrogen peroxide, peroxyacetic acid, sodium bromate and aqueous bromine. When reactions were performed in an NMR tube, the organic products could be identified by ^1H NMR spectroscopy. The ^1H NMR spectrum of DL-cysteine has two main distinguishing features. First, there is an unresolved doublet of a doublet at a chemical shift of 3.85 ppm, arising from the proton of the —CH— group. The other peak comprises two sets of doublets due to the inequivalent —CH_2— protons[20] coupling with each other and with the —CH— proton. By monitoring each of the oxidation reactions by ^1H NMR it was possible to establish that the oxidation products of the H_2O_2 reaction are different from those of the peroxyacetic acid reaction, which in turn are different from those of the bromate and bromine oxidation reactions (Fig. 2A). It is clear from the ^1H NMR spectra that H_2O_2 oxidation of DL-cysteine leads to a mixture of unidentifiable products. The peroxyacetic acid oxidation cleanly forms cysteine sulfinic acid. This could be established by comparing the ^1H NMR of the oxidation product with commercial cysteine sulfinic acid (Aldrich). On the other hand, BrO_3^- and Br_2 oxidation of DL-cysteine show the ^1H NMR spectrum of cysteine sulfonic acid (Fig. 2B). By reacting an authentic sample of DL-cysteine sulfinic acid with Br_2 and running the ^1H NMR spectrum of the product, it was shown that the product is the sulfonic acid (Fig. 2B). The oxidized DL-cysteine products, *i.e.* the sulfinic and the sulfonic acids, show a downfield shift for

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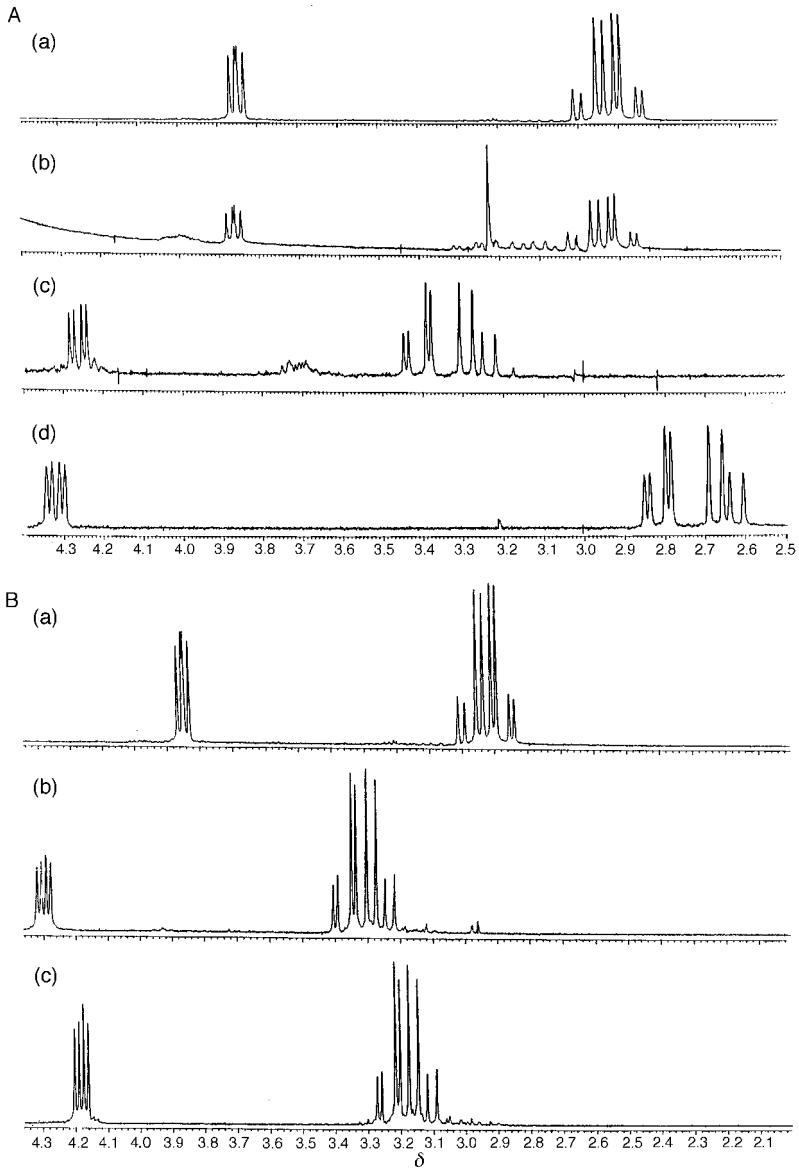

Fig. 2 A ^{1}H NMR traces of oxidation products of cysteine with different oxidants. (a) Spectrum of pure CySH, (b) CySH + H_2O_2, (c) CySH + peroxyacetic acid, (d) spectrum of pure DL-cysteine sulfinic acid. The shift observed in spectrum (c) [from the standard spectrum (d)] is due to the acidic environment. B ^{1}H NMR traces of the products of cysteine oxidation by: (a) pure CySH, (Aldrich), (b) CySH + acidified bromate, (c) cysteine + Br_2(aq). The shift observed again in spectra (b) and (d) is due to the acidic environment of spectrum (b). Bromate oxidations occur only at highly acid conditions.

both the —CH— and the —CH_2— peaks compared to those of cysteine. Unlike the —CH— peaks of cysteine, those of sulfinic and sulfonic acids are well resolved doublets of a doublet.

The clearly distinguishing features of the ^{1}H NMR spectra of the sulfinic acid and sulfonic acid derivatives are the gaps between the high-intensity doublets for the CH_2 group of each acid. The sulfinic acid gap is much larger; $\Delta\delta = 0.07$ ppm compared with $\Delta\delta = 0.02$ ppm for the sulfonic acid.[21] Apart from this, all other spectral features are essentially the same for both acids. Based on the *in situ* NMR studies above, it was established that the strong oxidants, BrO_3^- and Br_2, cleanly oxidise DL-cysteine all the way to the sulfonic acid. The reaction dynamics of these reactions are described below.

Reaction dynamics

In the presence of excess BrO_3^- ($[BrO_3^-]_0/[\text{cysteine}]_0$ ratio $r > 1$), the oxidation of cysteine gives a long quiescent period with no absorbance activity at 390 nm and very little activity in the redox potential of the reaction mixture (calomel reference). After an induction period, whose length was determined by the initial conditions, there is a sudden and rapid production of aqueous Br_2. No bromine production was observed with $r < 1$.

Acid dependence. As expected from most oxyhalogen oxidations, acid strongly catalysed the reaction. Fig. 3A shows a series of experiments at varying acid concentrations. Increase in the acid concentration reduced the induction period and also increased the rate of formation of bromine at the end of the induction period. The acid did not, however, affect the final amount of bromine formed. A plot of induction period *vs.* the square of the inverse of the acid concentrations gave a straight line (Fig. 3B), indicating that the precursor reaction to the formation of bromine is catalysed by acid to the second power.[22]

Bromate dependence. Bromate also decreased the length of the induction period (Fig. 4A). When $1 < r \leqslant 1.2$ the final amount of bromine formed was proportional to the initial bromate concentration. At $r > 1.2$ the ratio of cysteine to the amount of bromine formed was 3:2 exactly. The stoichiometry of the reaction, taking into account the formation of bromine was:

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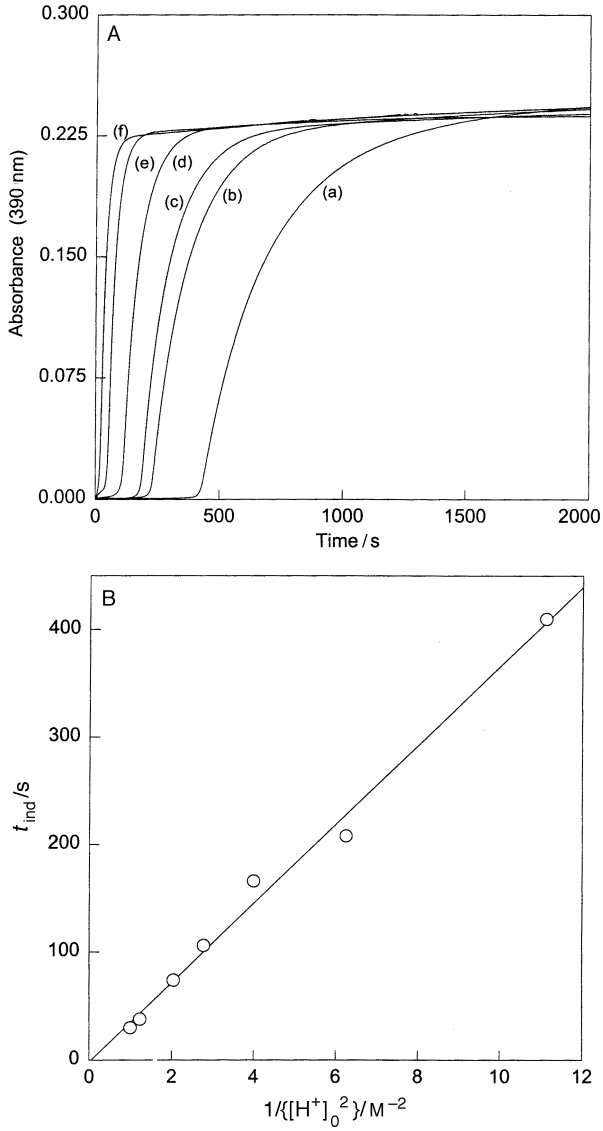

Fig. 3 A, Absorbance traces at $\lambda = 390$ nm in two-fold excess of $[BrO_3^-]_0$ over cysteine at varying initial acid, $HClO_4$, concentration. $[CySH]_0 = 0.0026$ M, $[BrO_3^-]_0 = 0.0050$ M; $[H^+]_0 =$ (a) 0.30, (b) 0.40, (c) 0.50, (d) 0.70, (e) 0.90 and (f) 1.00 M. B, Acid dependence of the induction period for data shown in A.

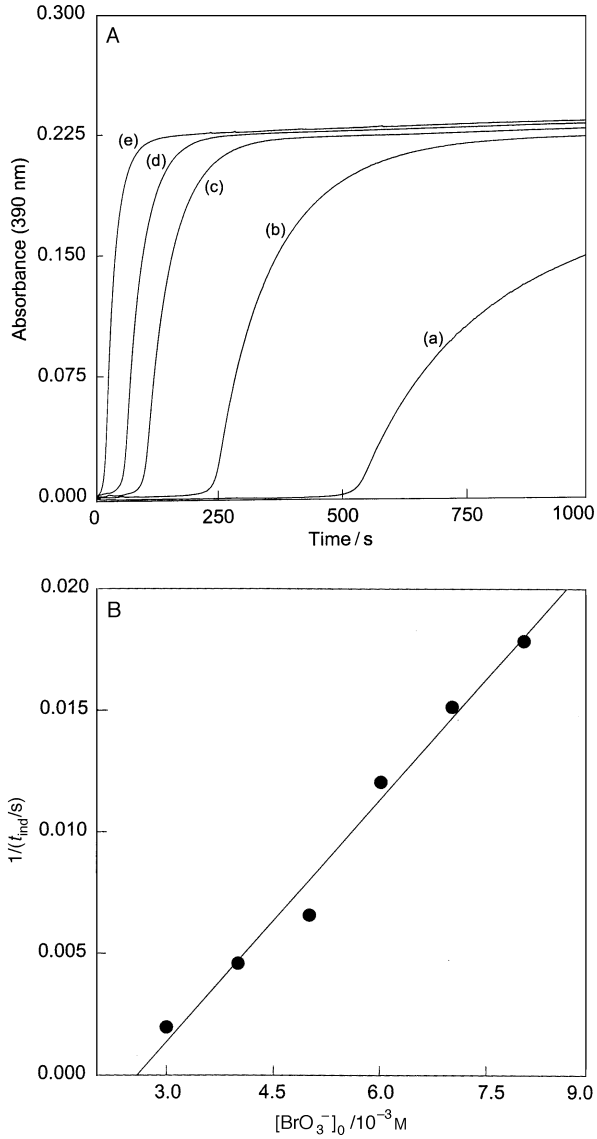

Fig. 4 A, Absorbance traces at $\lambda = 390$ nm in excess $[BrO_3^-]_0$ over $[CySH]_0$ at varying $[BrO_3^-]_0$ and constant acid strength. Final amount of bromine formed is the same for all traces. $[H^+]_0 = 0.70$ M; $[CySH]_0 = 0.0026$ M; $[BrO_3^-]_0 =$ (a) 0.003, (b) 0.0040, (c) 0.0060, (d) 0.0080 and (e) 0.012 M. B, Inverse of induction time *vs.* $[BrO_3^-]_0$ for conditions in A.

$$6BrO_3^- + 5Cys\text{-}SH + 6H^+ \rightarrow$$

$$5Cys\text{-}SO_3H + 3Br_2(aq) + 3H_2O \quad (3)$$

where Cys-SH is cysteine and Cys-SO_3H is cysteine sulfonic acid. This is the stoichiometry obtained at the formation of the maximum amount of Br_2 in Fig. 1.

All Br^- formed in stoichiometric reaction (1) reacts with the excess bromate to give bromine:[23]

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2(aq) + 3H_2O \quad (4)$$

The stoichiometry of reaction (3) is a composite of [5 × reaction(1)] + reaction(4). The induction period is inversely proportional to the initial bromate concentrations (Fig. 4B). The plot shown, with the inverse of the induction period *vs.* $[BrO_3^-]_0$ is valuable in confirming the deduced stoichiometric reaction (1).[24] The $[BrO_3^-]_0$ axis intercept shows that the reaction produces no Br_2 ($t_{ind} = \infty$) when $[BrO_3^-]_0 = 0.0026$ M. This is the limiting bromate concentration before formation of Br_2. Formation of Br_2 denotes an excess of BrO_3^- over that required for stoichiometry (1). The cysteine concentration used for the series of experiments was constant and fixed at 0.0026 M, thus confirming stoichiometry (1) of 1 : 1.

Cysteine dependence. The cysteine dependence of the reaction is more complex than that for acid or bromate. The ratio, r, was the most important parameter in this set of experiments. With very large values of r (>15), variations in the cysteine concentration did not affect the induction period. The cysteine concentration, however, did affect the rate of formation of Br_2. With small r ($1.2 < r < 5.0$) increasing cysteine concentration increased the induction period (see Fig. 5A). This would suggest that the complete consumption of cysteine could be the precursor reaction to the formation of Br_2. Lower cysteine concentrations need very little time for complete consumption (oxidation) by bromate. The amount of Br_2 formed was proportional to the initial cysteine concentration so long as $r > 1.2$ (Fig. 5B). 1 mole of cysteine, according to stoichiometry (1), gives 1 mole of Br^-. This Br^- will determine the final amount of Br_2 formed, according to the stoichiometry of reaction (3). Fig. 5C shows the induction period data

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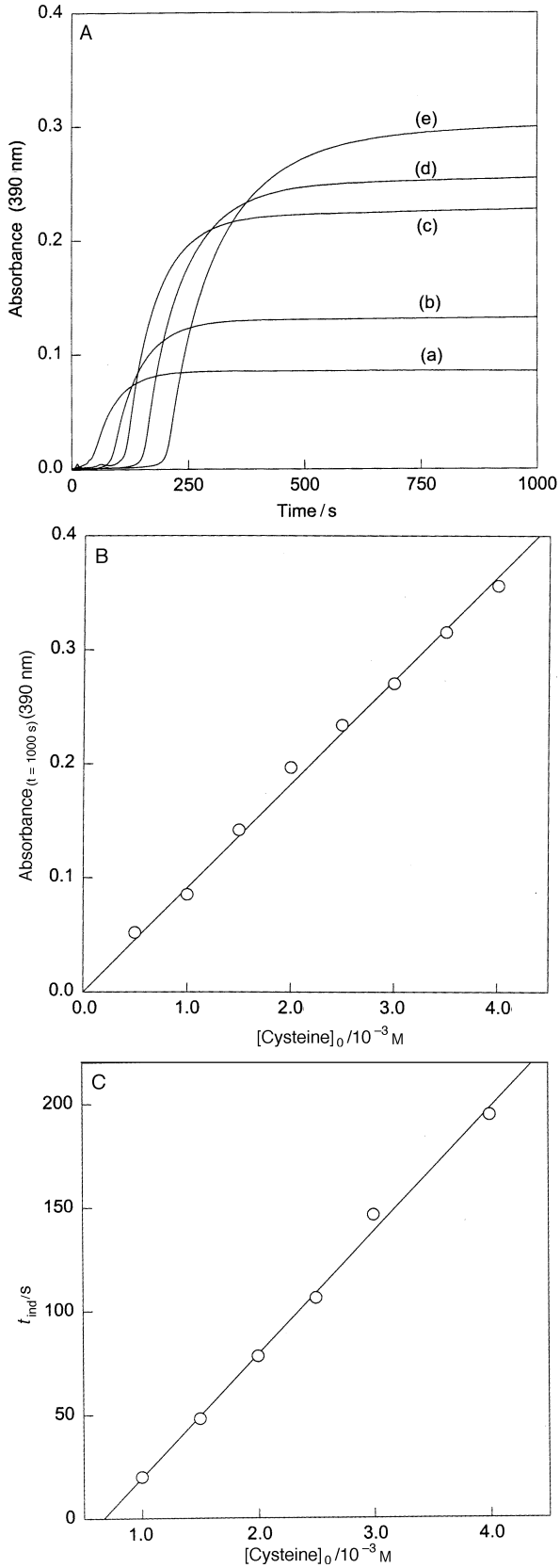

Fig. 5 A, Effect of varying $[CySH]_0$ in excess $[BrO_3^-]_0$ conditions. $[H^+]_0 = 0.70$ M; $[BrO_3^-]_0 = 0.005$ M; $[CySH]_0 =$ (a) 0.001, (b), 0.0015, (c) 0.0025, (d) 0.0030, and (e) 0.0035 M. B, Maximum absorbance at $\lambda = 390$ nm for the data shown in A. The absorbances were taken after 1000 s, the values did not change significantly after 48 h. C, Induction time *vs.* $[CySH]_0$ for the conditions in A.

for small values of r ($1.2 < r < 5.0$). These data show a discontinuity as r goes below unity because the induction period increases to ∞.

Effect of bromide. Bromide appeared to have no effect on the reaction. Most bromate oxidations show bromide catalysis, since the bromate–bromide reaction is responsible for the production of the reactive species which perform the bulk of the oxidation: $HBrO_2$, HOBr, and Br_2.

$$BrO_3^- + Br^- + 2H^+ \rightarrow HBrO_2 + HOBr \qquad (5)$$

Reaction (5) is recognized as the initiation reaction for all bromate oxidations, and it is usually the rate-determining step.[25] With the BrO_3^-–cysteine reaction being insensitive to initial bromide concentrations, then there must be another reaction that can produce Br^- and subsequently $HBrO_2$, HOBr and Br_2, more rapidly than reaction (5) for the reaction's initiation. There is no unambiguous way of testing for the efficiency of the direct bromate–cysteine reaction, but the following experiment was performed. Dry crystals of $NaBrO_3$ and DL-cysteine were mixed together into an intimate powdery mix. Without addition of acid, water was carefully added drop-wise (in a fume hood) to this mixture. As soon as the first drop of water was added, the mixture exploded with rapid formation of bromine (observed as a yellowish-brown gas) due to the violent bromate–cysteine reaction. Thus the reaction is capable of initiating itself to the extent that the addition of bromide delivers no catalytic effect. Fig. 6 shows data for a series of experiments with varying initial bromide concentrations. $[Br^-]_0$ was changed by a factor of 20 with no noticeable effect on the reaction. Even for $[Br^-]_0$ concentrations in excess of the initial cysteine concentrations, there was no effective variation in the induction period. The only (expected) difference was the increase in the final Br_2 concentrations with increased bromide.

Direct Br_2–cysteine reaction. The direct oxidation of cysteine by aqueous bromine was extremely fast (see Fig. 7) and, for approximately equimolar Br_2–cysteine solutions, the reaction was essentially over within 0.1 s. The stoichiometry of the reaction was determined by both spectrophotometric and titrimetric techniques[26] to be:

$$3Br_2 + Cys\text{-}SH + 3H_2O \rightarrow Cys\text{-}SO_3H + 6Br^- + 6H^+ \qquad (6)$$

The reaction occurred in two stages. The first was so rapid (to the point of diffusion control) that it could not be resolved, even on the advanced Hi-Tech Scientific DX2 stopped-flow

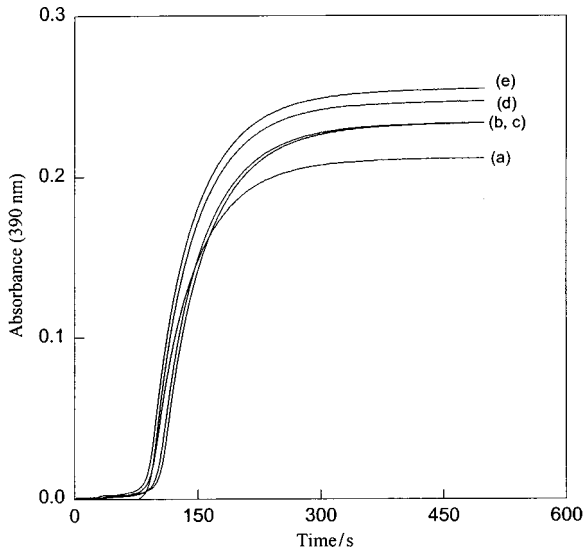

Fig. 6 Effect of Br^- on the bromate–CySH reaction. $[CySH]_0 = 0.0025$ M; $[H^+]_0 = 0.70$ M; $[BrO_3^-]_0 = 0.005$ M; $[Br^-]_0 =$ (a) control, no Br^- added, (b) 0.0005, (c) 0.0010, (d) 0.005 and (e) 0.0100 M

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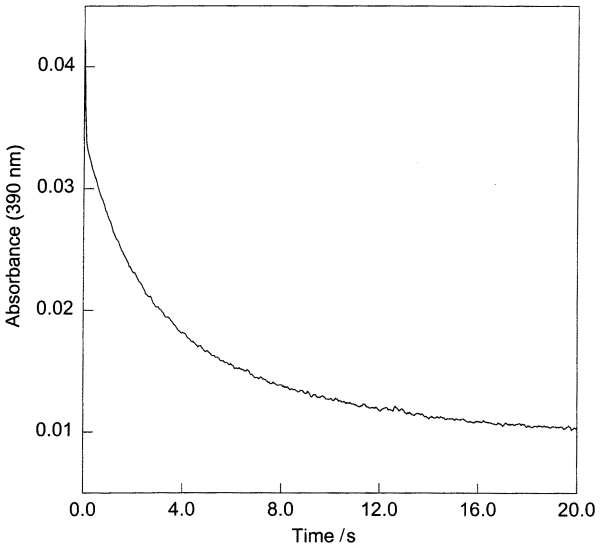

Fig. 7 Direct reaction of bromine with cysteine. $[Br_2]_0 = 0.00392$ M, and $[CySH]_0 = 0.0005$ M.

spectrophotometer used for these studies. The second (and slower) stage of the reaction occurs after 2 moles of bromine have been consumed for each mole of cysteine. Thus the very first part of the reaction involves the oxidation of cysteine to cysteine sulfinic acid:

$$2Br_2 + \text{Cys-SH} + 2H_2O \rightarrow \text{Cys-SO}_2\text{H} + 4Br^- + 4H^+ \quad (7)$$

Fig. 7 shows that by the time the reaction is captured by the stopped-flow spectrophotometer (mixing time, essentially), it is essentially complete. The initial absorbance expected for Fig. 7 was 0.265.

The second stage of the reaction is the further oxidation of the more stable sulfinic acid:

$$Br_2 + \text{Cys-SO}_2\text{H} + H_2O \rightarrow \text{Cys-SO}_3\text{H} + 2Br^- + 2H^+ \quad (8)$$

From the kinetics data we estimate an upper-limit diffusion-controlled rate constant for the first stage of the reaction to be $4.5 \pm 1.3 \times 10^6$ dm^3 mol^{-1} s^{-1}.

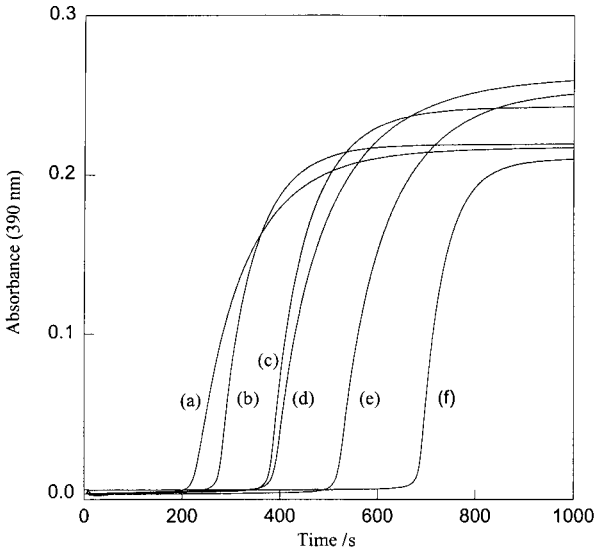

Fig. 8 Effect of Cu^{2+} on the CySH–bromate reaction. $[CySH]_0$ (0.0026 M) was premixed with acid, $[H^+]_0$ (0.70 M) and varying amounts of Cu^{2+} for traces (b)–(e). These mixtures were incubated for a period of 6 h before they were reacted with $[BrO_3^-]_0$ (0.0050 M). (a) control, no Cu^{2+} added, $[Cu^{2+}]_0$ = (b) 0.0025, (c) 0.0050, (d) 0.0075 and (e) 0.025 M. Trace (f) is direct reaction of cystine (0.0012 M) with same $[BrO_3^-]_0$ and $[H^+]_0$ conditions as in (a)–(e).

Effect of metal ions. A number of metal ions were used to examine possible metal-ion catalysis. The only notable set of results was obtained with Cu^{II} ions. The results obtained were unexpected. Cu^{II} ions seemed to have an inhibitory effect on the overall oxidation of cysteine (see Fig. 8). Higher concentrations Cu^{II} ions considerably lengthened the induction period of the reaction. The inhibitory effect was much more pronounced when the cysteine solutions were first incubated with the Cu^{II} ions for between 1–6 h before subjecting them to reaction with acidified bromate. Unincubated solutions did not show any Cu^{II} ion effects.

Mechanism

The bromide effect suggests that the first step of the reaction is production of the reactive species by direct reaction of bromate with DL-cysteine:

$$BrO_3^- + \text{Cys-SH} + H^+ \rightarrow \text{Cys-SOH} + HBrO_2 \quad (9)$$

The sulfenyl acid formed in reaction (9), Cys-SOH, is very unstable and has never been isolated.[27] It can be oxidized further [reaction (10)] or dimerize to form cystine [reaction (11)]:[28]

$$HBrO_2 + \text{Cys-SOH} \rightarrow \text{Cys-SO}_2\text{H} + HOBr \quad (10)$$

$$\text{Cys-SOH} + \text{Cys-SH} \rightarrow \text{Cys-S—S-Cys} + H_2O \quad (11)$$

In the presence of excess oxidant, however, further oxidation of the sulfenyl acid is the predominant pathway.[29] The cystine can also be further oxidized, provided it is not produced in the reaction to the level where it precipitates, as it is sparingly soluble in water.

$$\text{Cys-S—S-Cys} + 2H_2O \rightarrow 2\text{Cys-SOH} + 2H^+ + 2e^- \quad (12)$$

Both products of reactions (10) (cysteine sulfinic acid) and (11) (cystine) are extremely stable and have been isolated as stable products.[30] Further reaction of HOBr releases Br^- which reacts with BrO_3^- as in reaction (4) to release more reactive species:

$$HOBr + \text{Cys-SH} \rightarrow \text{Cys-SOH} + Br^- + H^+ \quad (13)$$

The Br^- formed in reaction (13) can also form Br_2:[31]

$$HOBr + Br^- + H^+ \rightarrow Br_2 + H_2O \quad (14)$$

The Br_2, as soon as it is formed, will immediately react with DL-cysteine or any other cysteine species in solution not yet oxidatively saturated (at the sulfur centre):

$$Br_2 + \text{Cys-SH} + H_2O \rightarrow \text{Cys-SOH} + 2Br^- + 2H^+ \quad (15)$$

$$Br_2 + \text{Cys-SOH} + H_2O \rightarrow \text{Cys-SO}_2\text{H} + 2Br^- + 2H^+ \quad (16)$$

$$Br_2 + \text{Cys-SO}_2\text{H} + H_2O \rightarrow \text{Cys-SO}_3\text{H} + 2Br^- + 2H^+ \quad (17)$$

Fig. 7 data show that reactions (15) and (16) are extremely fast and nearly diffusion-controlled. Reaction (17), though not diffusion-controlled, is still one of the fastest reactions in the reaction mixture. In excess bromate, we expect the cysteine species to mop up all bromine species in solution as soon as they are formed. The only time Br_2 production is observed (end of the induction period) is after all the cysteine species have been oxidized to cysteine sulfonic acid. Stoichiometry (1) should go to completion before Br_2 production can occur. In excess bromate, the rate of formation of Br_2 should be proportional to the initial cysteine concentration, as the composite reaction for formation of Br_2 is given by reaction (4). The data in Fig. 5A confirm this assertion. The formation of Br_2 is a pure oxyhalogen reaction with no interference from the

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cysteine species. This could be proved by running control experiments with the cysteine substituted by an equal concentration of Br^- and observing the rate of formation of Br_2 which was identical to the post-induction period rate.

Other oxidants

While the oxidation of cysteine is extremely facile, the oxidation of cysteine sulfinic acid is not as rapid. Peroxyacetic acid can easily oxidize cysteine to its sulfinic acid but cannot take it all the way to the sulfonic acid. Hydrogen peroxide is a powerful oxidizing agent and should oxidize cysteine to the sulfonic acid. The oxidation by H_2O_2, however, is predominantly *via* free radical mechanisms.[32] Physiologically, cysteine is known as a free radical scavenger because of the thiol group. H_2O_2 can produce a variety of free radical species, including the acidic form of the superoxide radical $HO_2^{\cdot}$.[33] and the hydroxyl radical, $HO^{\cdot}$.[34] Both the superoxide and hydroxyl radicals are one-electron oxidants, which can form thiyl free radicals with cysteine, Cys-$S^{\cdot}$.[35] The predominant pathway for free-radical mediated oxidation of thiols is *via* the dimer:[35]

$$RSH - e^- \rightarrow RS^{\cdot} + H^+ \quad (18)$$

$$RS^{\cdot} + RS^{\cdot} \rightarrow RS - SR \quad (19)$$

As noted before, the dimer, cystine, is extremely insoluble (and only sparingly soluble in highly acid conditions), and is rapidly precipitated. Other possible products include sulfoxides and thiosulfinates.[36] There is lack of product control with free radical mechanisms. This explains the unidentifiable solid products obtained with hydrogen peroxide, as well as the complex ^1H NMR spectrum. Rapid and quantitative formation of cystine yields a precipitate.

Effect of Cu^{II} ions

It has been reported that reactions of sulfur compounds, especially thiols, are strongly catalysed by Cu^+ and Cu^{2+} ions.[37] The decomposition of S-nitrosothiols is one such reaction that is catalysed by Cu^+ ions.[38] Cu^{2+} ions strongly inhibited the reaction (see Fig. 8). The retardation is due to the formation of cystine. The Cu^{II} ions can oxidize the thiol group *via* a one-electron free radical mechanism:[39]

$$2Cu^{2+} + 2RSH \rightarrow 2Cu^+ + RSSR + 2H^+ \quad (20)$$

The cystine dimeric species, RSSR, is very stable and only slightly soluble in water. The conversion of the cysteine to the more stable, slower reacting cystine, significantly retards the reaction. The cystine that precipitates is lost to further reaction, but the dissolved cystine can be oxidized further to give the unstable sulfenyl acid as in reaction (12). In the presence of excess oxidant, the sulfenyl acid is rapidly oxidized to the cysteine sulfinic acid [*e.g.* reaction (16)].

Computer simulation

The proposed mechanism was distilled to yield the set of reactions shown in Table 1. The first three reactions, (M1)–(M3) are pure oxyhalogen reactions whose kinetics parameters were obtained from the literature.[40] In this mechanism, the bulk of the oxidation was assumed to be carried out by HOBr and Br_2. Oxidation of cysteine by HOBr and Br_2 was assumed to be fast and not rate-determining. The rate-determining steps were taken as the rates of formation of $HBrO_2$ and Br_2 as well as the rate of oxidation of cysteine sulfinic acid. The simulations were very sensitive to the kinetics parameters for reaction (M4) as it is the one which initiates the reaction. Owing to the non-linear production of HOBr, [as in reaction (M2)] reaction (M4) is not the rate-determining step. Reaction (M5) was not very important in this reaction scheme. Owing to the rapid reaction (M2), reaction (M5) could be omitted from the mechanism without loss of structure in our simulations. Reactions (M6) and (M7) were rendered fast and non-rate-determining. Reaction (M8) was slower than reactions (M6) and (M7) (see data in Fig. 7) and controlled the rate of formation of Br_2 in the later stages of the reaction. Thus, although the kinetics parameters for reactions (M5)–(M8) were estimated, they had no effect on the simulations so long as they were fast and not rate-determining.

Reactions (M9)–(M11) were treated in the same manner as their HOBr counterparts [reactions (M6)–(M8)]. The simulations were thus controlled only by the kinetics parameters of reactions (M1)–(M4) with (M4) being the only reaction with adjustable kinetics parameters as the other values were set to literature data. The ordinary differential equations generated from Table 1 were numerically integrated by semi-implicit Runge–Kutta techniques.[41] The simulations gave reasonably good fits to the experimental data (see Fig. 9). The simulations were also able to predict quantitatively both the acid dependence (Fig. 3A and B) and bromate-dependence (Fig. 4A and B).

Conclusion

The most important result is that cysteine is extremely capable of acting as an antioxidant with respect to the reactive oxygen species: superoxide and peroxoradicals and hydroxyl radicals. The mechanism of this interaction involves the formation of the cystine dimer *via* the thyl radicals. The dimer can be reduced back to the cystine through an appropriate $2e^-$ reduction, such that cysteine can continue to moderate radical toxicity. For physiological purposes, the regeneration of the antioxidant is essential. Cysteine can also mop up harmful oxidants such as HOCl and HOBr. The efficiency of cysteine as a radical and oxyhalogen scavenger is related to the rate at which it reacts with radicals, oxyhalogens and halogens. Thus, cysteine should be preferentially oxidized over

Table 1 Mechanism for the oxidation of DL-cysteine by bromate in an acidic medium

reaction	reaction	forward and reverse rate constant(s)
(M1)	$BrO_3^- + 2H^+ + Br^- \rightleftharpoons HOBr + HBrO_2$	2.1; 1.0×10^4
(M2)	$HBrO_2 + Br^- + H^+ \rightleftharpoons 2HOBr$	2.0×10^6; 2.0×10^{-5}
(M3)	$HOBr + Br^- + H^+ \rightleftharpoons H_2O + Br_2$	8.9×10^8; 110
(M4)	$BrO_3^- + CySH + H^+ \rightarrow CySOH + HBrO_2$	210
(M5)	$HBrO_2 + CySH \rightarrow CySOH + HOBr$	4.0×10^3
(M6)	$HOBr + CySH \rightarrow CySOH + Br^- + H^+$	1.0×10^5
(M7)	$HOBr + CySOH \rightarrow CySO_2H + Br^- + H^+$	5.0×10^5
(M8)	$HOBr + CySO_2H \rightarrow CySO_3H + Br^- + H^+$	3.2×10^3
(M9)	$Br_2 + CySH + H_2O \rightarrow CySOH + 2Br^- + 2H^+$	4.5×10^6
(M10)	$Br_2 + CySOH + H_2O \rightarrow CySO_2H + 2Br^- + 2H^+$	1.2×10^4
(M11)	$Br_2 + CySO_2H + H_2O \rightarrow CySO_3H + 2Br^- + 2H^+$	1.0×10^3

Rate constants separated by a semi-colon denote forward and reverse rate constants. Rate constant units were deduced from the reaction's molecularity, except for those that involve water, where the water was not considered.

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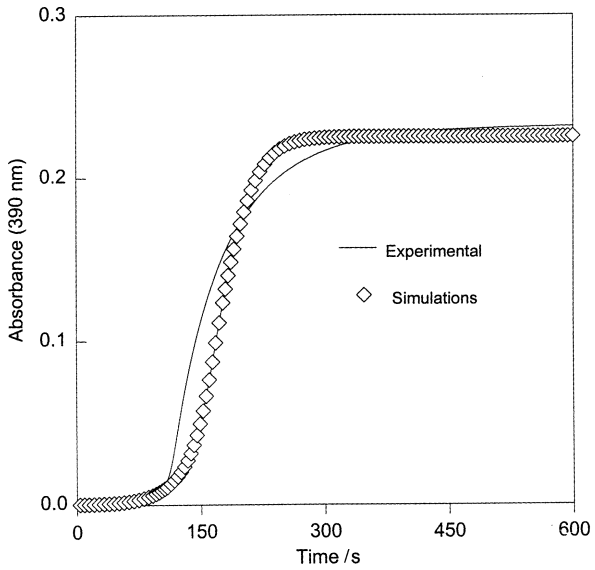

Fig. 9 Computer simulations using the model shown in Table 1. The simulated trace is trace (d) of Fig. 3A with $[CySH]_0 = 0.0026$ M, $[BrO_3^-]_0 = 0.005$ M, $[H^+]_0 = 0.70$ M. This simple model was able to reproduce the acid and bromate effects.

cell tissue for it to act as an antioxidant. Our experimental results which showed that the reaction of bromine and cysteine is close to diffusion-control limits, prove that the oxidation of cysteine is facile and rapid. In contrast to taurine and hypotaurine, the amino group of cysteine is inert and no reactivity has been detected at the nitrogen centre.

We would like to thank the University of the North for giving leave of absence to J.D. This work was supported by the National Science Foundation grant Number CHE-9632592 awarded to R.H.S. We also acknowledge several useful discussions with Prof. Gannett.

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Paper 7/08863I; *Received 9th December*, 1997

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