A Reinvestigation of the Oxidation of Cysteine by $Br_2^{-\cdot}$ and $I_2^{-\cdot}$. Evidence for CyS∴Br⁻ and CyS∴I⁻

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The existence of the species CyS∴Br^- and CyS∴I^-, which may be regarded as complexes between the thiyl radical from cysteine and bromide or iodide ions, is reported, and their properties discussed in relation to other sulphur- and halogen-containing species which also have a three-electron half-order bond. The oxidation of cysteine by $I_2^{-\cdot}$ is shown to be much more complex than hitherto reported, and pH-dependent equilibria involving iodine atoms, iodide ions, cysteine, and cysteinyl radicals are described. The first measured rate constant for the reaction of iodine (as I_3^-) with a thiol is also reported.

Oxidising radicals generated by the radiolysis of aqueous solutions saturated with nitrous oxide and containing chloride, bromide, iodide, or thiocyanate ions have been used as 'selective probes' in enzymatic deactivation studies.[1] Pulse radiolysis studies have shown that these radicals are the dimeric species $X_2^{-\cdot}$ formed in the series of fast reactions (1)—(4), the equilibrium (4) being established in less than a micro-

$$H_2O \rightsquigarrow OH + e_{aq}^- + H\cdot \quad (1)$$

$$e_{aq}^- + N_2O \xrightarrow{H^+} \cdot OH + N_2 \quad (2)$$

$$\cdot OH + X^- \longrightarrow HOX^{-\cdot} \longrightarrow OH^- + X\cdot \quad (3)$$

$$X\cdot + X^- \rightleftharpoons X_2^{-\cdot} \quad (4)$$

second.[2-9] In the paper which pioneered the 'selective probe' technique [10] Adams *et al.* reported a study of reactions of these radicals with a wide range of amino acids, and showed the radicals to be more selective than ·OH in that they reacted with only a few of the amino acids in acidic or neutral solution. They reported rate constants for their reactions with tryptophan (Cl, Br, SCN), cysteine (Cl, Br, I, SCN), tryosine (Cl, Br, SCN), histidine (Cl, Br), and methionine (Cl, Br, SCN), the reactivities decreasing generally from tryptophan to methionine and from Cl to SCN. This and subsequent work showed these radicals were also more regioselective than ·OH in position of attack on the amino acid.[1]

Further work has shown that some of these reactions between $X_2^{-\cdot}$ and amino acids are more complex than first thought. Posener *et al.*[11] have shown that the reaction between $(SCN)_2^{-\cdot}$ and tryptophan does not necessarily go to completion and that equilibria (5)—(8) are involved. Asmus and his co-workers have made a detailed study of reactions of these $X_2^{-\cdot}$ radicals with sulphides including methionine and have shown that equilibria (9)—(11) occur.[12,13]

The equilibrium constants K_9, K_{10}, and K_{11} all decrease in the order X = Cl, Br, I. Asmus also pointed out that the similarity in the spectra of $R_2SX\cdot$ and $X_2^{-\cdot}$ species, and lack of nanosecond time resolution facilities, resulted in Adams *et al.* failing to see a fast reaction between $Cl_2^{-\cdot}$ and $Br_2^{-\cdot}$ with methionine.[13] Spectroscopic studies of the methionine–$(SCN)_2^{-\cdot}$ reaction were made difficult by the fact that $(SCN)_2^{-\cdot}$ and $(R_2S)_2^{+\cdot}$, and therefore presumably $R_2SSCN\cdot$, have

$$TrpH + (SCN)_2^{-\cdot} \rightleftharpoons TrpH^{+\cdot} + 2SCN^- \quad (5)$$

$$TrpH^{+\cdot} + SCN^- \rightleftharpoons TrpH + SCN\cdot \quad (6)$$

$$SCN\cdot + SCN^- \rightleftharpoons (SCN)_2^{-\cdot} \quad (7)$$

$$TrpH^{+\cdot} \rightleftharpoons Trp\cdot + H^+ \quad (8)$$

$$X_2^{-\cdot} + R_2S \rightleftharpoons R_2SX\cdot + X^- \quad (9)$$

$$R_2SX\cdot + R_2S \rightleftharpoons (R_2S)_2^{+\cdot} + X^- \quad (10)$$

$$R_2SX\cdot \rightleftharpoons R_2S^{+\cdot} + X^- \quad (11)$$

almost identical spectra. However it was shown indirectly that $(SCN)_2^{-\cdot}$ does react with methionine.

In very thorough investigations using pulse radiolysis techniques Asmus [14,15] has studied the stabilisation occurring when one-electron oxidised sulphur-centred radicals complex to a sulphide (R_2S) or halide ion X^-. A three-electron $\sigma_2\sigma_1^*$ bond is formed. The same type of bonding exists in the species $X_2^{-\cdot}$ themselves and in equilibria involving dimeric radical anions composed of different halides or pseudohalides. Asmus represents these as $R_2S\therefore^{+}SR_2$, $R_2S\therefore X$, and $X\therefore^{-}X$.

The radiation chemistry of aqueous solutions of thiols has been extensively studied [16] and one of the earlier successes of pulse radiolysis was the discovery that thiyl radicals form complex ions with thiolate anions at pH where the thiol is partially ionised.[17] The species $RSSR^{-\cdot}$ is a further example of

$$RSH \rightleftharpoons RS^- + H^+ \quad (12)$$

$$RS\cdot + RS^- \rightleftharpoons RSSR^{-\cdot} \quad (13)$$

three-electron $\sigma_2\sigma_1^*$ bonding and may be represented as $RS\therefore^{-}SR$. The $RSSR^{-\cdot}$ intermediates have an absorption maximum at *ca.* 420 nm and this property has been much used to follow reactions involving thiols, and involving peptides and enzymes containing cysteine residues. Cysteine is the only amino acid found in pulse radiolysis studies to react with $I_2^{-\cdot}$, and use has been made of this fact to probe the chemistry of the enzyme lactate dehydrogenase.[18]

In this paper we present the first reported evidence for the formation of a complex between a thiyl radical and a halide

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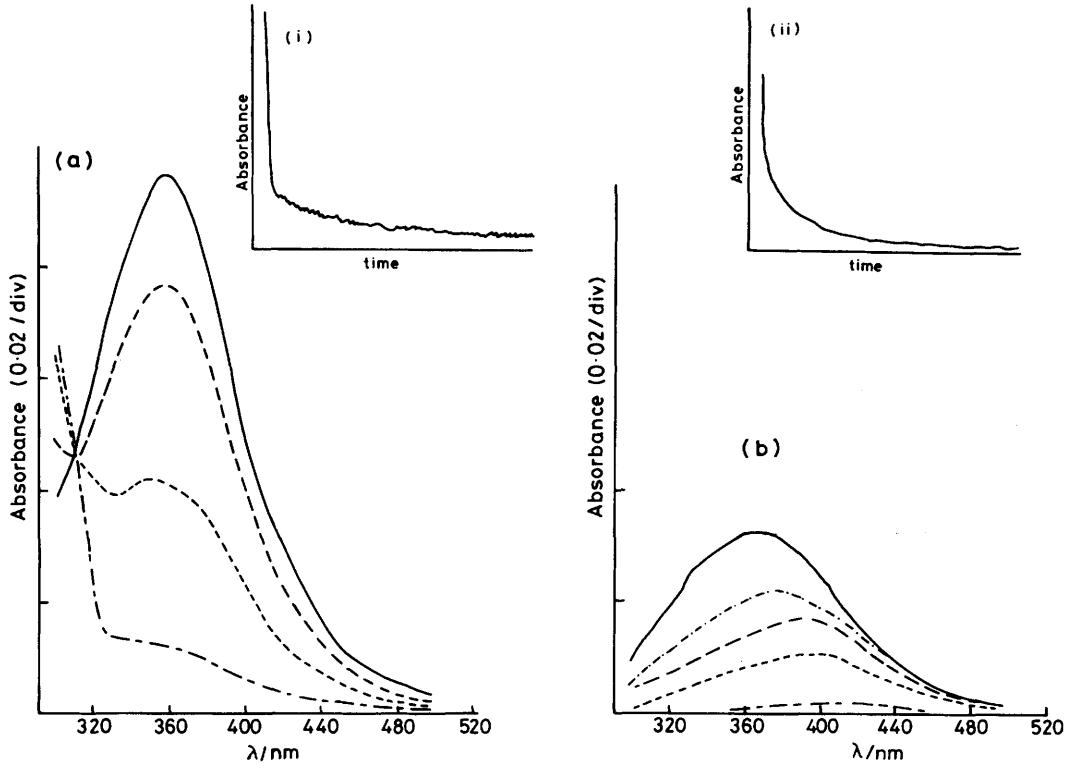

Figure 1. Time-resolved spectra of N_2O-saturated 2.0M-potassium bromide solution at pH 5.8 (a) without cysteine, 10 Gy pulse; (b) plus 5 × 10^{-3}M-cysteine, 9 Gy pulse. All spectra shown in this paper were measured at 10 nm intervals and at seven times after the pulse: —— 2 μs, — · — · — 5 μs, — — — 10 μs, · · · · · 20 μs, - - - - - 50 μs, - · - · - · - 100 μs, — - — - — 400 μs. For clarity spectra at all time intervals are not necessarily shown. Inset: absorbance against time traces over first 400 μs for 5 × 10^{-3}M-cysteine, (i) 0.1M-KBr; (ii) 2.0M-KBr

ion. From pulse radiolysis experiments involving cysteine we present evidence for the formation of the complex ions CyS.·.Br^- and CyS.·.I^-. We could find no evidence for the formation of the corresponding CyS.·.SCN^- ion.

These results could be of significance in interpretation of data obtained in studies involving $X_2^{-\bullet}$ radicals reacting with peptides and enzymes.

Experimental

Pulse radiolysis experiments were done on the Brunel University 4 MeV linear accelerator with associated equipment for kinetic spectroscopy and computer analysis.[19] 0.2 μs pulses producing doses in the range 2—20 J kg^{-1} (Gy) (0.2—2 krad) in a 1.5 cm optical pathlength cell were used, the dose being varied by altering the filament current. Dosimetry was carried out by simultaneously measuring the absorbance at 500 nm due to $(SCN)_2^{-\bullet}$ produced in an aerated 0.01M-KSCN solution taking $G\varepsilon = 2.12 \times 10^{-4}$ m^2 J^{-1} and the charge collected on a plate behind the cell, the charge being proportional to absorbance. In subsequent experiments the plate charge was measured and converted by the computer into a dose reading. When concentrated solutions of KBr were used it was noticed that this apparent dose reading for a fixed gun filament current decreased as the concentration increased. This was investigated and the charge collected on the plate for similar pulses was measured at the different KBr concentrations used. Corrections to the increased dose absorbed by these solutions were made assuming the decrease in charge collected was due to a corresponding increase in absorbed dose by the more dense solution. At the highest KBr concentration of 3.2M the correction factor by which the apparent dose was multiplied was 2. Consequently the percentage error in the measured dose of these solutions may be greater than that for dilute solutions normally used in pulse radiolysis experiments.

All solutions were freshly prepared with doubly distilled or Millipore-filtered water. Solutions were saturated with nitrogen, nitrous oxide, or N_2O–O_2 mixture (British Oxygen Ltd.) using the syringe bubbling technique.[20]

L-Cysteine (free base and hydrochloride), L-cystine (Sigma), potassium iodide, bromide, and thiocyanate, propan-2-ol, acetone, and t-butyl alcohol (B.D.H. AnalaR) were used as supplied. pH was adjusted with analytical grade hydrochloric acid or sodium hydroxide.

Results and Discussion

The rate constants for the oxidation of many substrates by $X_2^{-\bullet}$ ions have been measured by following the exponential decay of the absorbance due to $X_2^{-\bullet}$ as a function of substrate concentration, the observed first-order rate constants increasing linearly with concentration. The rate constants reported in ref. 10 were all determined in this way, and those for reactions of cysteine were found to be pH dependent indicating the thiolate anion was more reactive than its conjugate acid, the thiol. Rate constants were given as 1.8 × 10^8 dm^3 mol^{-1} s^{-1} for $Br_2^{-\bullet}$ at pH 6.6, 5 × 10^7 dm^3 mol^{-1} s^{-1} for $(SCN)_2^{-\bullet}$ at pH 6.6, and 1.1 × 10^8 dm^3 mol^{-1} s^{-1} for $I_2^{-\bullet}$ at pH 6.8. At these pH values 2—3% of the thiol is dissociated, and from the data presented in ref. 10 it is not clear whether $(SCN)_2^{-\bullet}$ and $I_2^{-\bullet}$ do in fact react with the undissociated thiol. A measurement below pH 6 in the case of $Br_2^{-\bullet}$ implied that this radical anion does oxidise undissociated cysteine with k 1.4 × 10^8 dm^3 mol^{-1} s^{-1}.

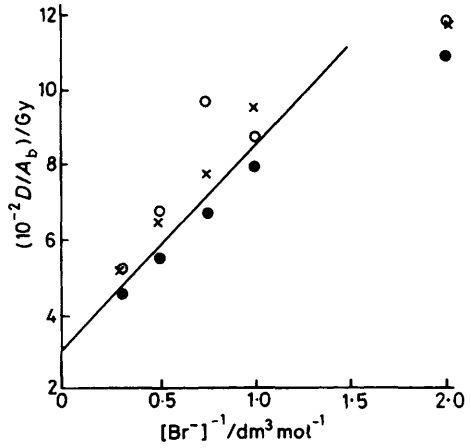

Figure 2. Plot of dose/(absorbance at discontinuity) against (bromide concentration)$^{-1}$: ○, highest gun filament current (*ca.* 30 Gy); ×, middle gun filament current (*ca.* 15 Gy); ●, lowest gun filament current (*ca.* 7 Gy)

Bromide.—Figure 1 shows the time-resolved spectra observed on pulsing an N_2O-saturated 2M-KBr solution with and without 5×10^{-3}M-cysteine (free base form). $Br_2^{-\bullet}$ on its own disproportionates to Br^- and Br_3^- and decays according to second-order kinetics. Cercek [21] has shown that in concentrated bromide solutions the equilibrium (14) becomes

$$Br_2^{-\bullet} + Br^- \rightleftharpoons Br_3^{\bullet 2-} \qquad (14)$$

important, that $K_{14} = 20$ dm^3 mol^{-1}, that $\lambda_{max.}(Br_3^{2-\bullet}) = \lambda_{max.}(Br_2^{-\bullet}) = 360$ nm and that at this wavelength $\varepsilon(Br_3^{2-\bullet}) > \varepsilon(Br_2^{-\bullet})$. Thus, at the high concentration used in this work the initial spectrum recorded after the pulse may be due to both $Br_2^{-\bullet}$ and $Br_3^{2-\bullet}$. In the presence of cysteine the decay at 360 nm is initially much faster but a new product with an absorbance maximum at *ca.* 390 nm is evident [Figure 1(b)]. The inset shows the decay curve at 380 nm for 0.1 and 2.0M-KBr solutions. Clearly a two-step decay process occurs, the fast decay of $Br_2^{-\bullet}$ (or $Br_3^{2-\bullet}$) due to reduction by cysteine followed by the slower decay of the product. The pH of the above solution containing cysteine was 5.6, and time-resolved spectra recorded at pH 1 and 4 also showed the presence of an absorbing product. However at pH 7.5 the absorbance due to $Br_2^{-\bullet}$ ($Br_3^{2-\bullet}$) decayed more rapidly to give as a transient product a species with an absorbance maximum at 420 nm and a spectrum similar to that of $CySSCy^{-\bullet}$. The rapid decay at pH < 6 confirms the fact that $Br_2^{-\bullet}$ ($Br_3^{2-\bullet}$) does oxidise the undissociated thiol group, but to give a product in addition to $CyS^\bullet$ as this radical has negligible absorbance in the spectral region [22] shown in Figure 1(b). The absorbance A_b at the kinetic discontinuity shown in the inset to Figure 1 was found to increase with increasing KBr concentration. A_b was measured as a function of KBr concentration at three gun filament currents and the results are shown in Figure 2, a plot of D/A_b against 1/[KBr] where D is the dose of the pulse. At fixed gun filament current the dose per pulse increased with increasing KBr concentration due to the corresponding increase in density of the solution and the dose was determined by the method outlined in the Experimental section.

We propose that the observed transient product is $CyS.\therefore Br^-$, a further example of the $\sigma^2\sigma^{*1}$ class of radicals described by Asmus.[12–15] The increase in A_b with increasing bromide ion concentration can be accounted for by equilibrium (15).

$$CyS^\bullet + Br^- \rightleftharpoons CyS.\therefore Br^- \qquad (15)$$

It is readily shown that for the above equilibrium equation (i) holds where $[CyS^\bullet]_T = [CyS^\bullet] + [CySBr^{-\bullet}]$ and equals the

$$\frac{1}{[CySBr^{-\bullet}]} = \frac{1}{[CyS^\bullet]_T}\left(1 + \frac{\gamma_{CySBr^{-\bullet}}}{K_{15}[Br^-]\gamma_{Br^-}}\right) \qquad (i)$$

concentration of $Br_2^{-\bullet}$ produced at the end of the pulse. As $A_b = \varepsilon l[CySBr^{-\bullet}]$ where ε is the molar absorptivity of $CySBr^{-\bullet}$ and l is the length of the cell, and $[CyS^\bullet]_T = \Gamma D$ where Γ is the yield (mol J^{-1}) and D the dose per pulse (J dm^{-3}) equation (ii)

$$D/A_b = (\varepsilon l\Gamma)^{-1}\left(1 + \frac{\gamma_{CySBr^{-\bullet}}}{K_{15}[Br^-]\gamma_{Br^-}}\right) \qquad (ii)$$

follows. High concentrations (up to 3.2M) of KBr were used in testing this expression and activity coefficients should be considered. Cysteine exists at the pH of these experiments (*ca.* 5.6) as the zwitterion $^-O_2CCH(NH_3^+)CH_2SH$. Assuming bond formation between S and Br in the complex radical does not greatly alter the pK_a values of the carboxylic acid and ammonium groups it is reasonable to equate the activity coefficients of $CySBr^{-\bullet}$ and Br^- as both carry overall a single negative charge.

There is considerable uncertainty in the experimental points shown in Figure 2. The high concentrations of KBr lead to considerable uncertainty in the dose figures, possibly up to 30% for the most concentrated. As the concentration of KBr increased the discontinuity in the decay curves at 380 nm became more difficult to measure accurately. Further, especially at higher dose per pulse, decay of $CyS^\bullet$ and $CySBr^{-\bullet}$ overlaps with that of $Br_2^{-\bullet}$. In view of these uncertainties we estimate the intercept to be 300 ± 60 J dm^{-3} and the slope 550 ± 70 J dm^{-6} mol. To determine ε from the intercept the value of Γ is required. For dilute aqueous solutions saturated with N_2O this is commonly equated with the sum of the yields of $^\bullet OH$ and e_{aq}^-, 0.56 μmol J^{-1}. However at the high concentration of KBr used there is the possibility of direct action (*i.e.* interaction of Compton electrons directly with Br^-) and of spur scavenging of $^\bullet OH$ by Br^- and this could alter Γ considerably. Allowing a possible 20% error we estimate ε at 380 nm as 4 000 dm^3 mol^{-1} cm^{-1} ± 60% and K_{15} as 0.55 dm^3 mol^{-1} ± 100%. Inspection of several spectra show $\lambda_{max.}$ for $CySBr^{-\bullet}$ to be 390 nm, with ε at this maximum *ca.* 5% greater than at 380 nm.

At higher pH where the CyS^- ion exists there will be competition between CyS^- and Br^- for thiyl radicals. K_{16} has been determined as 6.5×10^3 dm^3 mol^{-1}.[22] Combining this with K_{15} it is possible to calculate the equilibrium constant for reaction (17) as $K_{17} = K_{16}/K_{15} = 1.2 \times 10^4$ ± (100%). That the equilibrium constant is large is verified by the fact that when the solutions containing cysteine and bromide are pulsed at pH 7.5 the spectrum of the product present immediately after the decay of $Br_2^{-\bullet}$ is that of $CySSCy^{-\bullet}$.

A very brief study of the decay of $CySBr^{-\bullet}$ at 390 nm was made at the natural pH of the 5×10^{-3}M cysteine in the free base form (*ca.* 6). The decays were not exponential, and a strong second-order component was indicated by a decrease in half-life with increasing dose per pulse [although a plot of $1/(A_t - A_\infty)$ against time deviated from linearity]. At constant dose the half-life increased with increasing bromide ion concentration. These results suggest that reaction (18) is not important and the probable mode of decay is *via* (19) and (20), similar in principle to the decay of disulphide radical anions of cysteamine [17] and cysteine [23] in the presence of thiolate anion.

When the solutions of cysteine and bromide at pH 5—6

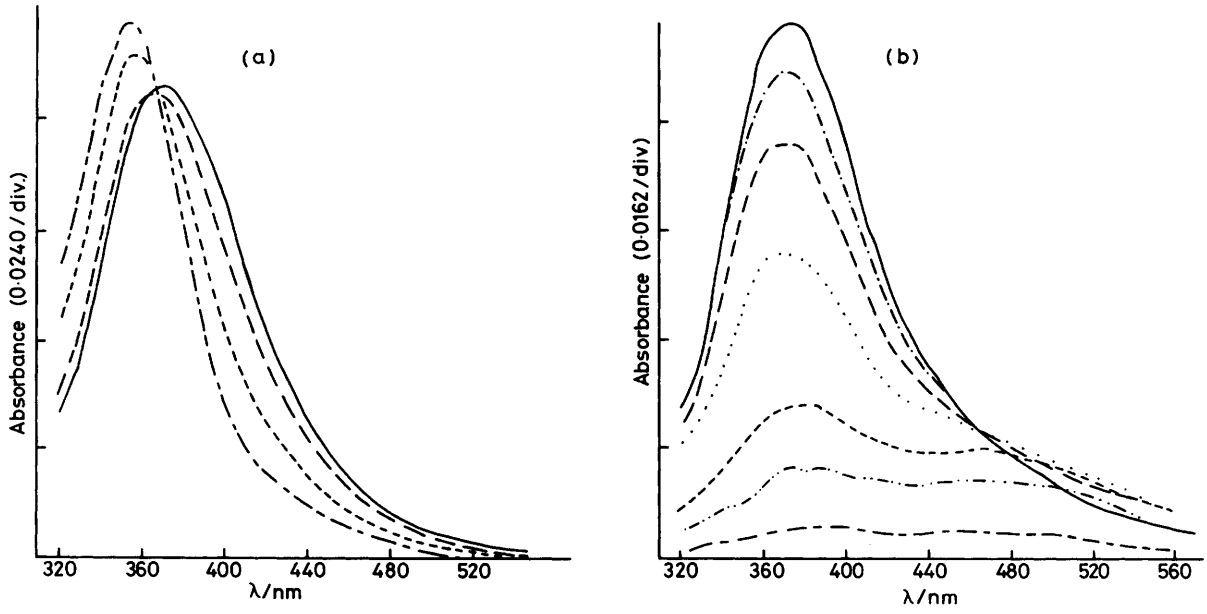

Figure 3. Time-resolved spectra of N_2O-saturated 0.1M-potassium iodide solution at pH 5.8 (a) without cysteine, 10 Gy pulse; (b) plus 5×10^{-3}M cysteine, 9 Gy pulse

$$CyS\bullet + CyS^- \rightleftharpoons CyS\therefore SCy^- \quad (16)$$

$$CyS\therefore Br^- + CyS^- \rightleftharpoons CyS\therefore SCy^- + Br^- \quad (17)$$

$$2\ CyS\therefore Br^- \longrightarrow CySSCy + 2Br^- \quad (18)$$

$$2\ CyS\bullet \longrightarrow CySSCy \quad (19)$$

$$CyS\bullet + CyS\therefore Br^- \longrightarrow CySSCy + Br^- \quad (20)$$

$$CyS\bullet\therefore Br^- + O_2 \longrightarrow CySBr + O_2^{-\bullet} \quad (21)$$

$$CyS\bullet + O_2 \longrightarrow CySOO\bullet \quad (22)$$

were saturated with a 80 : 20 N_2O–O_2 gas mixture instead of pure N_2O, the rate of decay of $CySBr^{-\bullet}$ at 390 nm increased by a factor of *ca.* 10. The decay did not give a good first-order fit, but the half-life was independent of dose per pulse. There are two possible pathways for the enhanced rate of decay, reaction (21), and (−15) followed by (22), the rate of which is close to diffusion controlled.[23,24] Further work is necessary before the occurrence of reaction (21) can be established. This would be of interest as $CySSCy^{-\bullet}$ reacts fast with oxygen (k 4.8×10^8 $dm^3\ mol^{-1}\ s^{-1}$) [23] while $Br_2^{-\bullet}$ does not.

Iodide.—When bromide is replaced by iodide the chemistry appears much more complex. Figure 3 shows the time-resolved spectra for N_2O-saturated 0.1M-potassium iodide solution (a) without and (b) with 5×10^{-3}M-cysteine (free base and natural pH 5.8). In the absence of cysteine $I_2^{-\bullet}$ disproportionates to the stable products I^- and I_3^-. In the presence of cysteine no rapid decay of $I_2^{-\bullet}$ corresponding to that of $Br_2^{-\bullet}$ is seen. The initial absorbance, A_1, at 400 nm decreased when the iodide concentration was decreased at constant cysteine concentration and a plot of $1/A_1$ against $1/[I^-]$ was linear. Analysis of this in terms of competition between I^- and CySH for $^\bullet OH$ gave the rate constant ratio $k_{23}/k_{24} = 1.0$, very close to that obtained from the individual rate constants quoted in the literature.[25]

$$\bullet OH + I^- \xrightarrow{I^-} I_2^{-\bullet} + OH^- \quad (23)$$

$$\bullet OH + CySH \longrightarrow CyS\bullet + H_2O \quad (24)$$

The decay of the absorbance at 400 nm was neither exponential nor second order, and the time-resolved spectra show the presence of new transient products exhibiting a broad band. Lowering the pH to 2.4 resulted in a markedly changed time-resolved spectrum as shown in Figure 4. At the lower pH the intermediate product absorbing at 480 nm has gone. A shift in the absorption maximum from 370 to 350 nm over the first 20 μs and an initial increase in A_1 at 320 nm shows $I_2^{-\bullet}$ disproportionating to I_3^- and I^-, while the later decay at 350 nm shows I_3^- (*i.e.* iodine) is reacting with cysteine on a microsecond time scale.

A survey of the literature showed no rate studies of the reaction between iodine and thiols have been reported, although the stoicheiometry of the reaction has been investigated in some detail [26] as iodometry has been used in analysis of thiols. We therefore decided to measure the rate of decay of I_3^-, this study being somewhat novel in that the technique of pulse radiolysis is usually used to study reactions of free radicals. The decay was followed at 370 nm, the isosbestic point of the $I_2^{-\bullet}$ disproportionation reaction, and a large dose per pulse of 24 Gy was used to increase the rate of this $I_2^{-\bullet}$ disproportionation reaction relative to the rate of the reaction of I_3^- with cysteine. Under our conditions the half-life of the former reaction was only 30 μs compared to the 100—2 000 μs for the latter reaction. At pH 2.4 the decay was exponential and the observed first-order rate constant proportional to cysteine concentration as shown in Figure 5(a), the bimolecular rate constant being 1.5×10^6 $dm^3\ mol^{-1}\ s^{-1}$. Danely and Oestler [26] have postulated that the first step in the oxidation of thiols by iodine is reaction (25) and our data are consistent

$$I_3^- + RSH \longrightarrow RSI + H^+ + 2I^- \qquad (25)$$

with this in that the disappearance of I_3^- is first order in cysteine. Our rate constant with cysteine is therefore probably the first recorded value of k_{25}.

A study of the effect of pH on this rate constant was then made by varying the pH of solutions containing 2×10^{-3}M-cysteine and the results are shown in Figure 5(b). There is a small but steady increase in rate with increasing pH up to *ca.* 4, and then the rate of absorbance decay increases rapidly, and at the same time this rate deviates markedly from exponential. [The values of k_{25} above pH 4 shown in Figure 5(b) were simply calculated from the half-life of decay.] At pH 5.8 the rate of decay was almost independent of cysteine concentration over the measured range of 2×10^{-3}—1×10^{-4}M. Clearly major changes in mechanism are occurring over this pH range which is perhaps surprising in that no major changes in the protonation of cysteine are occurring between pH 3 and 6 [pK_a(COOH) 1.7, pK_a(SH) 8.3].

When the pH of the 0.1M-KI–5×10^{-3}M-cysteine solution is raised to 7.5 a further major change in the time-resolved spectrum occurs as shown in Figure 6(a). Now a fast initial decay leads to transient products showing a broad spectrum with a maximum at *ca.* 430 nm. When the cysteine concentration is lowered to 2×10^{-3}M [Figure 6(b)] and further to 5×10^{-4}M [Figure 6(c)] a decrease in the rate of decay of I_2^- is evident, and the spectrum of the transient products changes, with some evidence for two bands, one on either side of 430 nm. That the nature of the transient products depends on the $[I^-]/[CySH]$ ratio is shown by varying the iodide concentration at constant (5×10^{-3}M) cysteine concentration. At 0.01M-KI [Figure 6(d)] (where 30% of the primary $^{\bullet}OH$ radicals react directly with cysteine) the spectrum of the product is clearly that of $CySSCy^{-\bullet}$ with an absorption maximum at 420 nm, while at 1.0M-KI [Figure 6(e)] two bands either side of 420 nm are now very evident. This series of experiments shows that $I_2^{-\bullet}$ reacts faster with CyS^- than CySH as shown by Adams,[10] and that at higher CyS^- concentrations, $CySSCy^{-\bullet}$ is the major transient product, but at higher $[I^-]/[CyS^-]$ other transient products are produced.

To investigate these other products further methods of producing $CyS^{\bullet}$ radicals directly in the presence of iodide were sought. Two systems were used. The first was to pulse a nitrogen-saturated solution containing 20% by volume of t-butyl alcohol and 66% of saturated cystine (CySSCy). Here the t-butyl alcohol scavenges $^{\bullet}OH$ radicals to give the relatively unreactive β-alcohol radical [reaction (26)] while the aquated electron adds fast to cystine [reaction (27)]. Under these

$$^{\bullet}OH + (CH_3)_3COH \longrightarrow H_2O + {}^{\bullet}CH_2C(CH_3)_2OH \qquad (26)$$

$$e_{aq}^- + CySSCy \longrightarrow CyS^{\bullet}\!\!:\!\!^{\bullet}SCy^- \qquad (27)$$

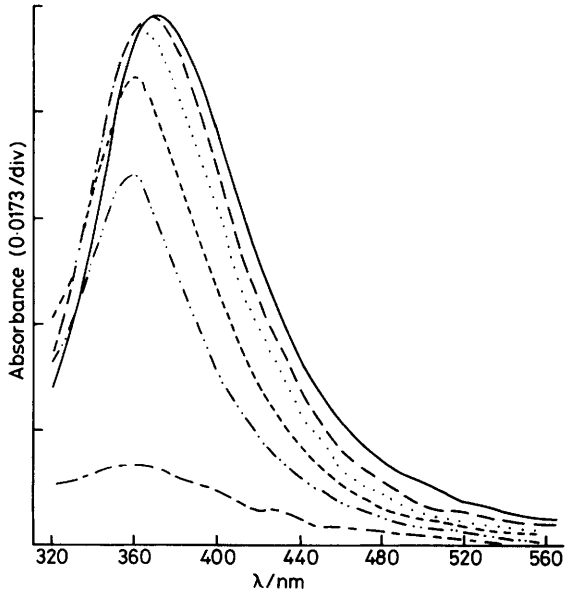

Figure 4. Time-resolved spectra of N_2O-saturated 0.1M-potassium iodide and 5×10^{-3}M cysteine hydrochloride at pH 2.35, 9 Gy pulse

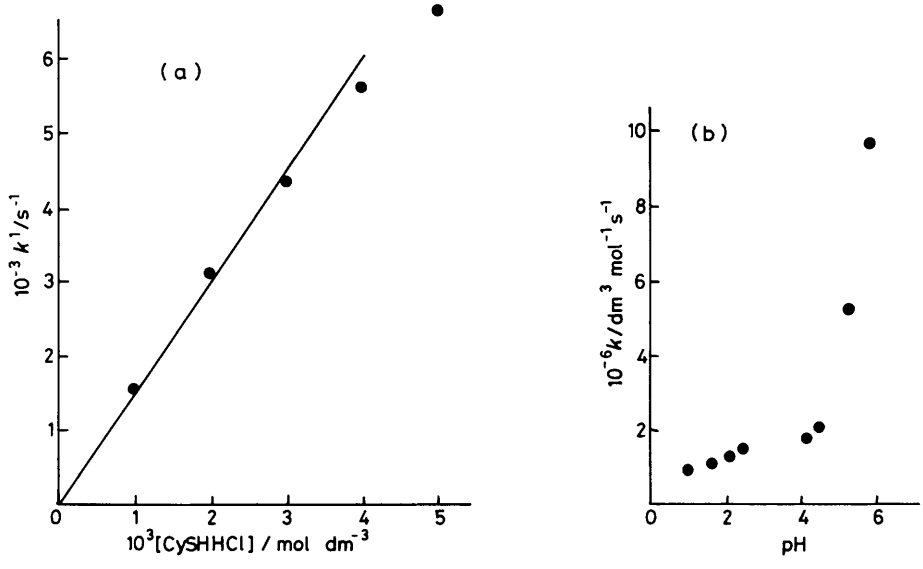

Figure 5. Kinetics of decay of I_3^- at 370 nm following 24 Gy pulse. (a) Pseudo-first-order rate constant against cysteine hydrochloride concentration at pH 2.4. (b) Second-order rate constant against pH 0.1M-potassium iodide, 2×10^{-3}M-cysteine (see Discussion section)

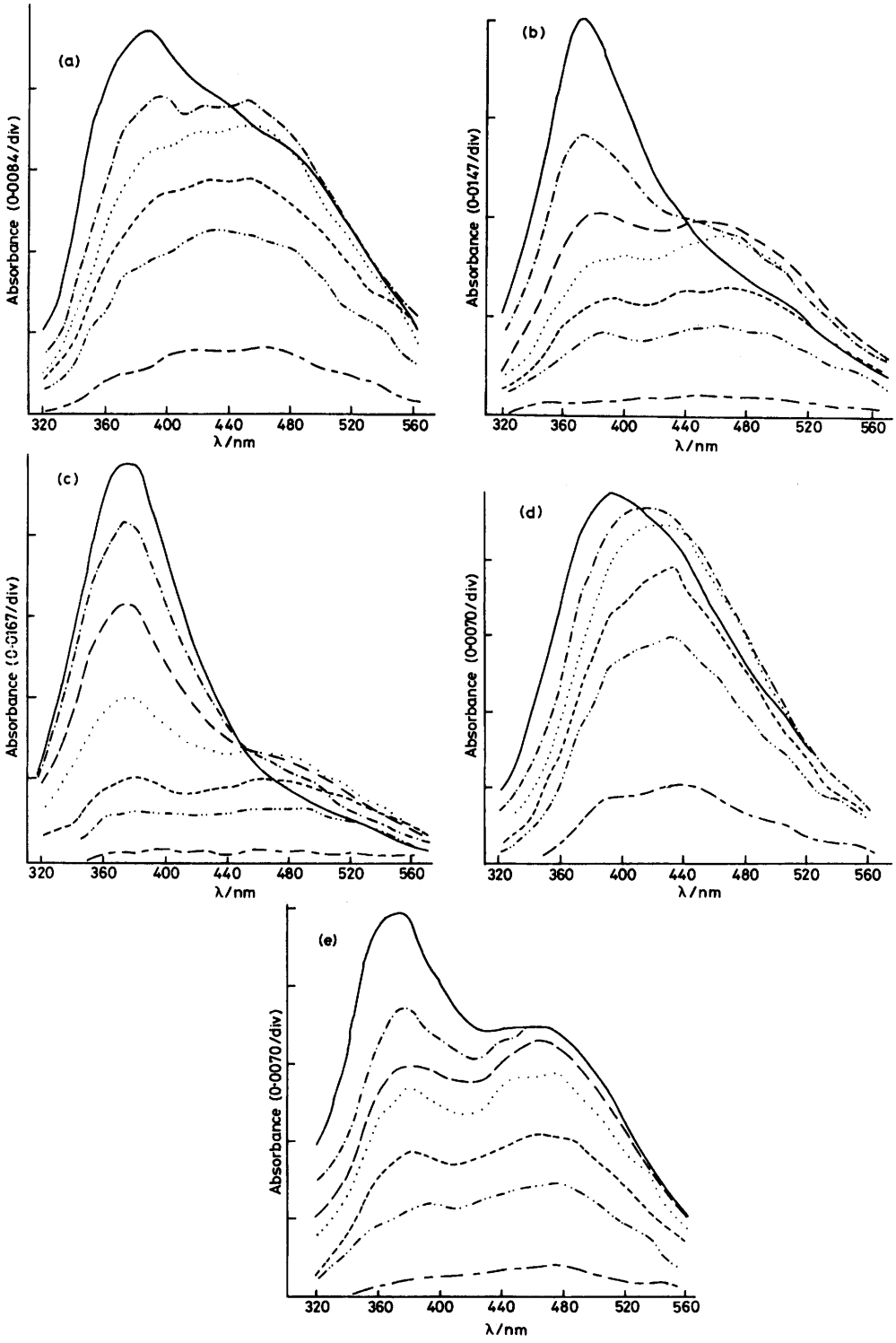

Figure 6. Time-resolved spectra of N_2O-saturated solutions of potassium iodide and cysteine at pH 7.5: (a) 0.1M-KI, 5×10^{-3}M-cysteine, 8 Gy pulse; (b) 0.1M-KI, 2×10^{-3}M-cysteine, 9 Gy pulse; (c) 0.1M-KI, 5×10^{-4}M-cysteine, 9 Gy pulse; (d) 0.01M-KI, 5×10^{-3}M-cysteine, 8 Gy pulse; (e) 1.0M-KI, 5×10^{-3}M-cysteine, 8 Gy pulse

conditions where there is no CyS^-, $CySSCy^{-\bullet}$ decays exponentially with a half-life of 2.4 μs [23] to $CyS^\bullet$ and CyS^- [reaction (−16)]. Figure 7 shows the time-resolved spectrum for a solution of this composition at pH 6.3 also containing 0.02M-KI. Superimposed on part of this spectrum is that of the same solution without I^-. Instead of the rapid decay of $CySSCy^{-\bullet}$ the spectrum quickly changes to one with two bands similar to that of Figure 6(e), and the decay of this is much slower than that of $CySSCy^{-\bullet}$ in the absence of CyS^- or I^-.

This result suggests that $CyS^\bullet$ is rapidly reacting with iodide to give $CyS.\therefore I^-$ [reaction (28)], or possibly this is partially being formed by reaction (29). The question arises as to

$$CyS^{\bullet} + I^{-} \rightleftharpoons CyS.^{\bullet}.I^{-} \quad (28)$$

$$I^{-} + CyS.^{\bullet}.SCy^{-} \rightleftharpoons CyS.^{\bullet}.I^{-} + CyS^{-} \quad (29)$$

$$^{\bullet}OH + (CH_3)_2CHOH \longrightarrow H_2O + (CH_3)_2\dot{C}OH \quad (30)$$

$$e_{aq}^{-} + (CH_3)_2CO \longrightarrow (CH_3)_2\dot{C}O^{-} \xrightarrow{H^+} (CH_3)_2\dot{C}OH \quad (31)$$

$$(CH_3)_2\dot{C}OH + CySH \longrightarrow (CH_3)_2CHOH + CyS^{\bullet} \quad (32)$$

whether the two bands are both due to $CySI^{-\bullet}$, or whether only the 480 nm band is due to this with the 370 nm band being due to $I_2^{-\bullet}$. If the latter is the case it appears the ratio $[I_2^{-\bullet}]/[CySI^{-\bullet}]$ remains constant during the subsequent decay of the radicals.

The above CySSCy–I^-–t-butyl alcohol system has four disadvantages: (i) the pH cannot be lowered below *ca.* 4 as e_{aq}^- will react with H^+ (to give $H^{\bullet}$) rather than with CySSCy; (ii) the precursor of $CyS^{\bullet}$ ($CySSCy^{-\bullet}$) has an absorption spectrum; (iii) the yield of $CyS^{\bullet}$ is only half the primary radical yield from water; and (iv) there is an upper limit to the iodide concentration which can be used because of competition between I^- and t-butyl alcohol for $^{\bullet}OH$. The first three of these can be overcome by using a nitrogen-saturated solution containing 20% propan-2-ol, 10% acetone, and 0.01M-cysteine. Under neutral and acid conditions reactions (30)—(32) occur,[27,28] with k_{32} *ca.* 3×10^8 dm^3 mol^{-1} s^{-1}. Thus the thiyl radical can be produced rapidly from both primary oxidising and reducing species in the presence of H^+ and without an absorbing transient precursor. Any observable transient species formed in this system in the presence of a low concentration of iodide must be due to reaction between $CyS^{\bullet}$ and I^-.

Figure 8(a) shows the time-resolved spectrum for this system in the presence of 0.02M-KI at pH 4.8. Products showing two bands are clearly visible, and the decay at 480 nm is faster than at 380 nm showing these absorbances are due to different species. Figure 8(b) shows the situation at pH 2.45. Over the first 20 μs there is an isosbestic point at 430 nm while the 380 nm band grows in. At longer times the 380 band shifts to a shorter wavelength and decays. This is clear evidence that the 380 nm band is due to $I_2^{-\bullet}$ and therefore under these conditions overall $CyS^{\bullet}$ radicals are oxidising iodide.

We suggest that the 480 nm band is due to $CySI^{-\bullet}$ and that the reaction occurring cleanly over the first 20 μs is effectively (33). Support for this comes from studying the kinetics at 480 and 380 nm. Both the decay and grow-in are exponential and

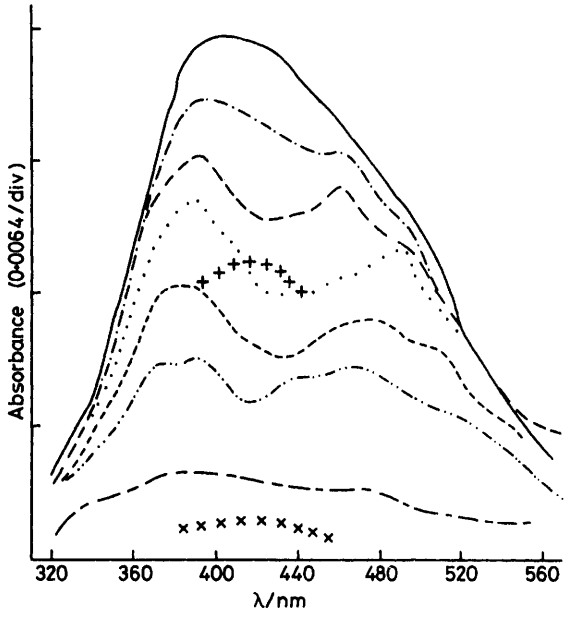

Figure 7. Time-resolved spectra of an N_2-saturated solution containing by volume 20% t-butyl alcohol, 66% saturated cysteine, and 0.02M-potassium iodide at pH 6.3, 10 Gy pulse. Also shown is part of the spectra for the above solution without KI at 2 μs, + + + + and 20 μs, × × × × (see text)

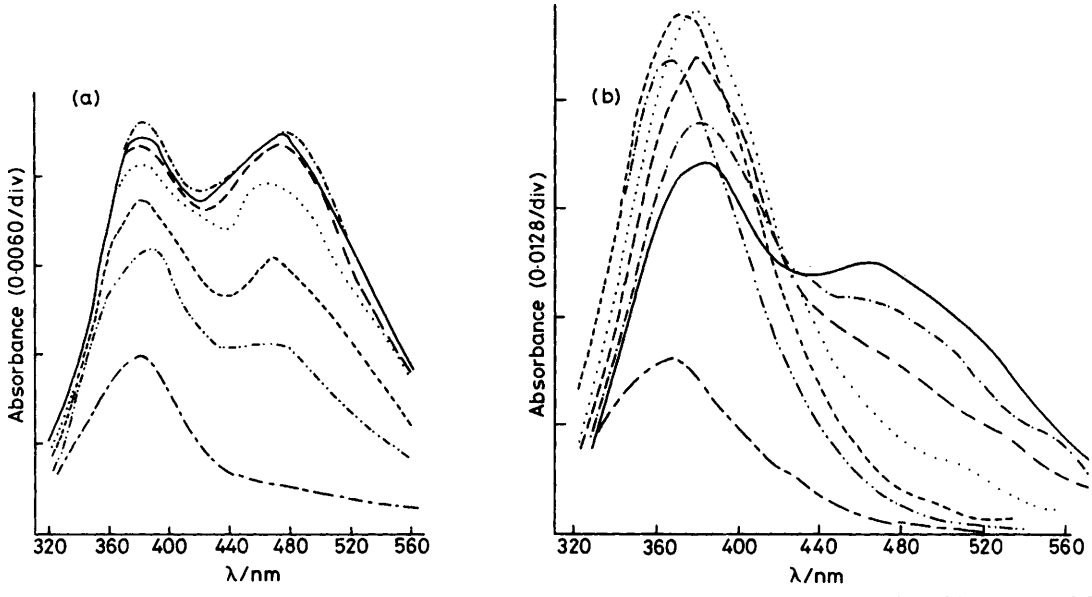

Figure 8. Time-resolved spectra of an N_2-saturated solution containing by volume 20% propan-2-ol, 10% acetone, 0.01M-cysteine, 0.02M-potassium iodide, 9 Gy pulse: (a) pH 4.8; (b) pH 2.4

Table. Effect of dose and iodide concentration on kinetics of transient species in N_2-saturated 0.01M-cysteine hydrochloride solutions (pH 2.45) containing 20% propan-2-ol and 10% acetone. Decay at 480 nm, grow-in at 380 nm [see Figure 8(b)]

λ/nm	Dose/Gy	$[I^-]$/M	$t_{\frac{1}{2}}$/μs
480	10	0.02	6.9
480	6	0.02	7.7
480	11	0.08	6.0
380	11	0.02	4.8
380	6	0.02	5.8
380	10	0.08	3.8
380	5	0.08	4.5

$$CyS^{\bullet}.I^- + I^- + H^+ \longrightarrow CySH + I_2^{\bullet-} \qquad (33)$$

the effect of dose per pulse and iodide concentration at pH 2.45 is shown in the Table. At 380 nm the absorbance rises to a maximum after the pulse and then decays due to the disproportionation of $I_2^{\bullet-}$. The half-lives are measured with respect to the maximum which will be considerably less than it would be if disproportionation of $I_2^{\bullet-}$ were not occurring simultaneously with its formation. Thus the true half-life for the formation of $I_2^{\bullet-}$ by reaction (33) will be longer than that measured, and the error in $t_{\frac{1}{2}}$ will be larger at higher dose where the relative rate of disproportionation of I_2^- will be greater. We therefore believe the half-life figures in the Table are consistent with the decay and grow-in being the result of reaction (33). The rate of the reaction is only marginally changed by a four-fold increase in iodide concentration, implying that the rate-controlling step is not a bimolecular nucleophilic substitution by iodide on the complex radical.

The decay at 480 nm and the grow-in at 380 nm however increase in rate with hydrogen ion concentration. The half-life for the decay at 480 nm in the presence of 0.02M-KI with a 10 Gy dose fell to 4.6 μs at pH 2.0 and *ca.* 2.5 μs at pH 1.1. At pH 3.1 $t_{\frac{1}{2}}$ was 20.7 μs but here, as at pH 4.8, the reaction was clearly not going to completion before appreciable radical–radical decay had occurred.

It is possible to get an estimate of the molar absorptivity of $CySI^{\bullet-}$. From Figure 8(b) it is seen that the magnitude of the decrease in absorbance at 480 nm over 20 μs is almost the same as the increase at 370 nm. As $\varepsilon(I_2^-)$ has values of 11 000 and 1 000 $dm^3\ mol^{-1}\ cm^{-1}$ at 370 and 480 nm respectively [Figure 3(a)] it follows that $\varepsilon_{CySI^{-\bullet}}^{460\ nm} + \varepsilon_{CySI^{-\bullet}}^{370\ nm} = 12\,000\ dm^3\ mol^{-1}\ cm^{-1}$. Assuming the spectrum of $CySI^{\bullet-}$ has a single peak and a band width similar to $I_2^{\bullet-}$ and $CySSCy^-$, $\varepsilon_{CySI^{-\bullet}}^{480\ nm}$ would have a value 8 000—10 000 $dm^3\ mol^{-1}\ cm^{-1}$.

From Figure 7 and taking k_{-16} into account, it can be seen that the yield of $I_2^{\bullet-}$ plus $CySI^{\bullet-}$ formed from $CySSCy^{\bullet-}$ in the CySSCy–I^-–t-butyl alcohol system is comparable with the yield of $CySSCy^{\bullet-}$ initially formed. This implies that the fraction of $CySSCy^{\bullet-}$ yielding free $CyS^{\bullet}$ is small. Thus an estimate of a lower limit for K_{28} can be made. At an iodide concentration of 0.02M we can deduce $[CyS.^{\bullet}.I^-]/[CyS^{\bullet}] > 1$ and $K_{28}[I^-] > 1$ or $K_{28} > 50\ dm^3\ mol^{-1}$.

Figure 6(e) shows that after pulsing 5×10^{-3}M-cysteine and 1.0M-KI at pH 7.5, $CyS.^{\bullet}.I^-$ and $I_2^{\bullet-}$ are formed in approximately equal amounts. From this it is possible to obtain an estimate of K_{-33}. When $[CyS.^{\bullet}.I^-]/[I_2^{\bullet-}] = 1$, $K_{-33} = [H^+][I^-]/[CySH] \simeq 8 \times 10^{-6}\ mol\ dm^{-3}$.

Thiocyanate.—When N_2O-saturated solutions containing 0.1M-KSCN and 5×10^{-3}M-cysteine at pH 7.5 were pulsed the decay of $(SCN)_2^{\bullet-}$ at 480 nm, where $\varepsilon_{(SCN)_2^{-\bullet}}$ is at its maximum, was exponential, and the time-resolved spectrum showed clearly that $CySSCy^{\bullet-}$ was formed as a transient product and therefore $(SCN)_2^{\bullet-}$ is oxidising CyS^-. At pH 5.6 the time-resolved spectra of 0.1M-KSCN was almost unaltered by the presence of 5×10^{-3}M-cysteine. When the thiocyanate concentration was decreased at this CySH concentration the initial yield of the transient decreased and a plot of $1/A_1$ at 480 nm against $1/[SCN^-]$ was linear. Recently Asmus has shown that $Br_2^{\bullet-}$ and $(SCN)_2^{\bullet-}$ do oxidise methionine in neutral solution [13] in contrast to a previous report by Adams,[10] the similarity in the spectra of the products $>S.^{\bullet}.Br$ and $Br_2^{\bullet-}$ in the former case and $>S.^{\bullet}.\overset{+}{S}<$, $>S.^{\bullet}.SCN$, and $(SCN)_2^{\bullet-}$ in the latter being the cause of the initial misinterpretation. As the strength of $(SCN)_2^{\bullet-}$ as an oxidising agent is between that of $Br_2^{\bullet-}$ and $I_2^{\bullet-}$,[13] it seemed possible that $(SCN)_2^{\bullet-}$ could oxidise CySH, and that $CyS.^{\bullet}.SCN^-$ with a spectrum very similar to $(SCN)_2^{\bullet-}$ might be formed in equilibrium with $CyS^{\bullet}$ according to equation (34).

$$CyS.^{\bullet}.SCN^- \rightleftharpoons CyS^{\bullet} + SCN^- \qquad (34)$$

However three experimental approaches showed this not to be the case. When an N_2-saturated solution containing CySSCy, 0.02M-SCN^-, and t-butyl alcohol at pH 5.2 was pulsed, unlike the iodide case, the formation and rapid decay of $CySSCy^{\bullet-}$ was identical to that in the absence of KSCN. When an N_2-saturated solution of 20% propan-2-ol and 10% acetone containing 0.01M-CySH and 0.02M-KSCN at pH 3.5 was pulsed no appreciable transient absorbances were detected again in contrast to the corresponding iodide system. In the N_2O–SCN^-–CySH system at pH 5.6, increasing the cysteine concentration at constant lower $[SCN^-]$ resulted in a decrease in initial absorbance, with a plot of $1/A_1$ against [CySH] being linear. If $(SCN)_2^{\bullet-}$ were oxidising cysteine to produce $CyS^{\bullet}$ in equilibrium with $CySSCN^{\bullet-}$, A_1 would be independent of [CySH]. {Hoffman and Hayon determined the rate constant for reaction (24) by this standard competition method [29] but did not state whether they varied both [CySH] and $[SCN^-]$ and in light of the above discussion it was thought necessary to do the above experiment.} These results show conclusively that $CyS^{\bullet}$ neither forms a complex with SCN^- nor oxidises it to $^{\bullet}SCN$.

Comparison with Other Three-electron Bond Species.—Asmus has shown that the species $X_2^{\bullet-}$, $R_2SSR_2^{\bullet-}$, $R_2SX^{\bullet}$, and $RSSR^{\bullet-}$ may all be described as species with $\sigma_2\sigma_1^*$ three-electron bonds. $CySBr^{\bullet-}$ and $CySI^{\bullet-}$ clearly fit into this class and it is of interest to compare their properties with the related species above. First, the values of $\lambda_{max.}$ and $\varepsilon_{max.}$ are of similar magnitude, and $\lambda_{max.}$ for $CySI^{\bullet-}$ is greater than for $CySBr^{\bullet-}$, consistent with the corresponding $X_2^{\bullet-}$ and $R_2SX^{\bullet}$ species. Secondly K_{28} is greater than K_{15}, *i.e.* the equilibrium constant for reaction (35) is greater for iodide than bromide. The same is found for K_{-11}, the corresponding reaction for sulphides. K_{-11} is 333 and 2 500 $dm^3\ mol^{-1}$ for the dimethyl and diethyl bromide species respectively and $\gg 10^4\ dm^3\ mol^{-1}$ for the corresponding iodide species.[12] However the actual values of K_{15} and probably K_{28} are much smaller than the corresponding values of K_{-11}. This is probably because reaction (−11) involves association of oppositely charged ions, whereas in (15) and (23) the radical is neutral. Indeed it may be argued that the above comparisons support the contention that the transient species observed in this work are indeed $CyS.^{\bullet}.X^-$.

In addition to reactions (9)—(11) Asmus has shown

$$CyS\cdot + X^- \rightleftharpoons CyS\therefore X^- \quad (35)$$

$$R_2S^{+\cdot} + X^- \rightleftharpoons R_2S\therefore X \quad (-11)$$

$$R_2S\therefore SR_2^+ \rightleftharpoons R_2S^{+\cdot} + R_2S \quad (36)$$

$$R_2S\therefore X \rightleftharpoons R_2S + X\cdot \quad (37)$$

$$X_2^{-\cdot} + CySH \rightleftharpoons \begin{matrix} Cy \\ S\therefore X \\ H \end{matrix} + X^- \quad (38)$$

$$\begin{matrix} Cy \\ S\therefore X \\ H \end{matrix} \rightleftharpoons CyS\therefore X^- + H^+ \quad (39)$$

$$\begin{matrix} Cy \\ S\therefore I \\ H \end{matrix} \rightleftharpoons CySH + I\cdot \quad (40)$$

$$I\cdot + I^- \rightleftharpoons I_2^{-\cdot} \quad (41)$$

equilibria (36) and (37) to be significant and he has reported values for the corresponding equilibrium constants.[12] A thiol may be regarded as a sulphide in which one R group is replaced by H, and therefore corresponding equilibria reactions might be expected. Indeed Asmus has recently shown that the species $H_2S^{+}\therefore SH_2$ can be made.[30] Thus for cysteine reaction (9) becomes (38). At low pH $Br_2^{-\cdot}$ does react with cysteine to give an absorbing transient product whereas $I_2^{-\cdot}$ does not. This is consistent with the sulphide case in that K_9 for Br is $>10^4$ time greater than for I.[12]

The difference between a sulphide and a thiol is that the complex in the latter case can lose a proton in a further equilibrium reaction, which for cysteine would be (39). Our iodide system provides evidence for reaction (39) and the cysteine equivalent of reaction (37) [reaction (40)]. In the presence of acid $CyS\therefore I^-$ reacts with iodide to give I_2^- at a rate depending on hydrogen ion concentration but not on iodide concentration. This suggests the mechanism for this reaction is (−39) followed by (40) and (41). As the reaction occurs in the pH range 1—5 it suggests pK_{39} for X = I is in the range 2—4. Reaction (40) is analogous to the rapid breakdown of $RS\therefore SR^-$ to RSH and RS· on protonation.[22] We have not yet made a detailed study of the effect of lowering pH on the corresponding bromide system to provide evidence for equilibria (39) where X is Br. However the time-resolved spectra show conclusively that $Br_2^{-\cdot}$ does oxidise CySH at low pH whereas $I_2^{-\cdot}$ does not and that an absorbing transient species is formed. This suggests that the bromide equivalent of reaction (40) does not occur. The equilibrium constants for the corresponding sulphide reactions show the same halide effect, *i.e.* $K_{37}(Br) < K_{37}(I)$. Equilibrium (39) also accounts for the fact that while $I_2^{-\cdot}$ does not oxidise cysteine at low pH it does so at pH values too low for CyS^- to be the actual reductant, *i.e.* $\begin{matrix} Cy \\ H \end{matrix}\!\!>\!S\therefore I$ must deprotonate for this oxidation to occur.

When CyS· radicals are produced initially at pH *ca.* 6 both $CyS\therefore I^-$ and $I_2^{-\cdot}$ are rapidly produced. From a redox point of view this means CyS· radicals are oxidising iodide. In $CyS\therefore I^-$ the oxidation process is in a sense at a half-way stage. It seems that the direction of oxidation is pH dependent and if $I_2^{-\cdot}$ is to fully oxidise cysteine, a sufficient concentration of CyS^- must be present to shift equilibrium (29) over to $CyS\therefore SCy^-$, *i.e.* to stabilise CyS·.

Conclusions.—The experiments discussed above show the existence of $CyS\therefore Br^-$ and $CyS\therefore I^-$, as further examples of species containing three-electron $\sigma_2\sigma_1^*$ bonds. It is shown that $CyS\therefore I^-$ is an important intermediate in the oxidation of cysteine by $I_2^{-\cdot}$ and in the oxidation of iodide by CyS·. While the formation of $CyS\therefore Br^-$ may be of minor importance in the oxidation of thiols by the so-called selective free radical $Br_2^{-\cdot}$, where relatively low bromide ion concentrations are used, the same cannot be said about the corresponding iodide case. Consequently some reinterpretation of data from selective probe experiments involving enzymes and iodide may be necessary.

The first kinetic data on the rate of oxidation of a thiol by iodine are also reported.

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