Reactions of OH, CH_2OH, Br_2^-, and $(CNS)_2^-$ Free Radicals with Cysteine at p*H* 5.3

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The products of radiolysis of cysteine in nitrous oxide saturated solutions at p*H* 5.3 have been investigated both in the absence of other solutes and in the presence of 1 *M* methanol, 0.5 *M* KBr, and 0.2 *M* NaCNS. The results can be explained by a mechanism in which the free radicals •OH, $•CH_2OH$, $•Br_2^-$, and $•(CNS)_2^-$ all react with the cysteine (CysSH) sulfhydryl group to form CysS• as a major intermediate product. Minor yields (0.2 molecules per 100 eV) of ammonia observed in the case of attack by $•Br_2^-$ may result from attack on the NH_3^+ group or α-hydrogen by this species. Cysteine trisulfide was observed in all systems with a yield 0.12 ± 0.03 molecules per 100 eV and is probably formed from minor secondary reactions of CysS• radicals. In agreement with previous conclusions based on the analysis of products from deaerated solutions the majority of CysS• radicals form CysSSCys.

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On a examiné les produits de radiolyse de la cystéine dans des solutions saturées en oxyde nitreux à un p*H* de 5.3 en l'absence d'autres solutés de même qu'en présence de méthanol 1 *M*, de KBr 0.5 *M* et de NaCNS 0.2 *M*. Les résultats peuvent être expliqués par un mécanisme dans lequel les radicaux libres •OH, $•CH_2OH$, $•Br_2^-$ et $•(CNS)_2^-$ réagissent tous avec le groupe sulfhydryle de la cystéine pour former le radical CysS• comme un produit intermédiaire majeur. Les rendements mineurs (0.2 molécules par 100 eV) d'ammoniac observés dans le cas de l'attaque par $•Br_2^-$ peuvent provenir d'une attaque, par cette espèce, soit sur le groupe NH_3^+ soit sur l'hydrogène en α. Dans tous les cas, on a observé la formation de trisulfure de cystéine et le rendement est de 0.12 ± 0.03 molécules par 100 eV; ce composé se forme probablement à partir de réactions secondaires mineures des radicaux CysS. La majorité des radicaux CysS• forment CysSSCys; cette conclusion est en accord avec d'autres énoncés précédemment et qui étaient basés sur l'analyse des produits provenant de solutions desquelles l'air avait été enlevée.
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Introduction

Previous studies of the γ (1, 2) and pulse radiolysis (3, 4) of aqueous oxygen free solutions of cysteine ($HSCH_2CH(NH_3^+)CO_2^-$) and the related molecule cysteamine ($HSCH_2CH_2NH_3^+$) (5) have shown that the major products can be explained by the following mechanism, in which the abbreviation CysSH is used for the cysteine structure:

[1] $H_2O \rightsquigarrow •OH\ •H\ e_{aq}^-\ H^+\ H_2O_2\ H_2$

[2] $•OH + CysSH \rightarrow CysS• + H_2O$

[3] $•H + CysSH \rightarrow CysS• + H_2$

[4] $•H + CysSH \rightarrow Cys• + H_2S$

[5] $e_{aq}^- + H^+ \rightarrow •H$

[6] $e_{aq}^- + CysSH \rightarrow Cys• + SH^- \ (\xrightarrow{H^+} H_2S)$

[7] $Cys• + CysSH \rightarrow CysH + CysS•$

[8] $CysSH \rightleftharpoons CysS^- + H^+$

[9] $CysS• + CysS^- \rightleftharpoons CysS\overset{\bullet}{S}^-Cys$

[10] $CysS• + CysS• \rightarrow CysSSCys$

[11] $CysS• + CysS\overset{\bullet}{S}^-Cys \rightarrow CysS^- + CysSSCys$

[12] $H_2O_2 + 2CysSH \rightarrow CysSSCys + 2H_2O$

More recently Purdie *et al.* (6) have investigated the radiolysis of penicillamine ($HSC(CH_3)_2CH(NH_3^+)CO_2^-$ = HSPen) and have found clear evidence for the production of a significant yield of penicillamine trisulfide (PenSSSPen). Since this behavior is quite different from previous observations of cysteine radiolysis and may have implications for an understanding of differences in their abilities as protectors (6), we have reinvestigated the cysteine system to determine whether any trisulfide is in fact formed. We have also broadened the scope of our study to determine the products resulting from the reactions of $•(Br_2)^-$, $•(CNS)_2^-$, and $•CH_2OH$ with cysteine. Pulse

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TABLE 1. Solute systems and radical balances at pH 5.3

Solute system	$k_{OH + Solute}$ ($M^{-1}s^{-1} \times 10^{-9}$)	Major reacting radical species	$k_{CysSH + Radical}$ ($M^{-1}s^{-1} \times 10^{-9}$)	Primary radical yields from the literature				$2\{G(CysS)_2 + G(CysSSSCys)\}$ observed experimentally
				G_H	G_{OH}	$G_{e_{aq}^-}$	Total	
N_2O	—	•OH	19.0[a]	0.60	2.90	3.05	6.6[b]	6.8
				0.60	3.15	3.15	6.9[c]	
$N_2O + CH_3OH$	0.9[d]	$•CH_2OH$	0.10[e]	0.60	2.90	3.05	6.6[b]	6.3
$N_2O + Br^-$	1.2[d]	$•Br_2^-$	~0.1[f]	0.50	3.40	3.05	7.0[g]	7.2
$N_2O + CNS^-$	11[d]	$•(CNS)_2^-$	~0.05[f]	0.50	3.40	3.05	7.0[g]	7.2

[a]Taken from ref. 4.
[b]Yields taken from ref. 13.
[c]Yields calculated from yield-reactivity curves in ref. 14 on the basis $\Sigma k_{OH+S}[S] = 1.9 \times 10^8\ s^{-1}$ and $\Sigma k_{e_{aq}^- + S}[S] = 3.5 \times 10^8\ s^{-1}$.
[d]From refs. 16 and 17.
[e]From ref. 9, assuming the same value as for cysteamine.
[f]From ref. 7.
[g]Yields taken from ref. 13 with G_H modified as in ref. 15 and G_{OH} increased by 0.5 per 100 eV on the basis of yield–reactivity curves (ref. 14) to take account of the higher solute concentrations ($\Sigma k_{OH+S}[S] = 0.8–2.2 \times 10^9\ s^{-1}$).

radiolysis experiments (7) show that the rate constants for reaction with CysSH by the former two radicals increase markedly in the pH region where —SH ionizes (pK CysSH 8.3 (8)) and above pH 8 they are assumed to react preferentially with $CysS^-$ (7), presumably to cause oxidation to CysS. Our present interest lay in determining the mechanism of reaction at pH 5.3, where the rate constants are still appreciable (see Table 1) even though CysSH is un-ionized.

In the case of the $\cdot CH_2OH$ radical two reactions are conceivable, since alcohol radicals may

[13] $CysSH + \cdot CH_2OH \rightarrow CysS\cdot + CH_3OH$

[14] $CysSH + \cdot CH_2OH \rightarrow Cys\cdot + CH_2O + H_2S$ (or $SH^- + H^+$)

behave as hydrogen atom acceptors (9) or as reducing agents (10, 11). The prior observation (9) of a pseudo first-order build up of —S$\dot{S}^-$— anions in the pulse radiolysis of N_2O-saturated solutions containing 1.13 mM cysteamine and higher concentrations of methanol was taken as evidence for the cysteamine analogs of reactions 13 and 9, the former being rate controlling. While vitiating the possibility of reaction 14 for the cysteamine system, this evidence does not completely exclude it since minor contributions of —S$\dot{S}^-$— from the sequence of reactions 14, 7, and 9 may not alter the rate of production significantly from that expected for reactions 13 and 9 alone. In the present system the yield of reaction 14 can be determined from the alanine yield.

Experimental

Potassium bromide and sodium thiocyanate from the Baker Chemical Company and L-cysteine from Mann Research Laboratories were used without further purification. The methanol was A.C.S. certified reagent grade. High purity nitrous oxide from the Matheson Co. was freed from traces of NO and other noncondensible gases by several cycles of sublimation, condensation, and pumping at −196 and −132 °C. After this it was stored in a bulb on the vacuum line. Cysteine solutions of pH 5.3 were prepared in fresh, triply distilled water containing the solutes needed for the production of the radicals being investigated. Following this 5 ml aliquots were transferred to the carefully cleaned pyrex cells and attached to the vacuum line. After thorough degassing (2) the samples were equilibrated with N_2O at 760 mm and sealed. Prior to irradiation they were stored in a refrigerator. Immediately after irradiation the analysis for amino-containing products was performed on a Technicon amino acid analyzer. Norleucine was used as an internal standard and elution buffers of pH 3.10, 3.30, and 4.50 were employed in succession for periods of 50, 60, and 50 min, respectively. A final buffer of pH 5.0 was used to elute ammonia. Standardization procedures and other details of the amino acid analyses may be found in ref. 2.

All irradiations were performed at 23 °C in a "Gamma Cell 220" purchased from Atomic Energy of Canada. The dose rate, which was determined by ferrous sulfate dosimetry assuming $G(Fe^{3+}) = 15.6$ (p. 217 of ref. 12), was 4.5×10^{16} eV ml^{-1} min^{-1}.

Results

Chromatograms of the irradiated solutions exhibited clearly resolved peaks due to residual cysteine and to alanine, cystine, cysteine trisulfide, norleucine (internal standard), and ammonia in the order given. The cysteine trisulfide was a minor product in all solutions, while ammonia was only detectable in the KBr solutions. In solutions containing methanol a small unidentified peak appeared before the residual cysteine. It corresponded to a G of about 0.1, assuming a ninhydrin sensitivity equal to that of alanine. No other peaks of any significance were observed.

Experiments with each solute system were performed at several doses in the range 1.0 to 6.0×10^{18} eV ml^{-1}. Yield–dose plots calculated

TABLE 2. G values of amino-containing products and nitrogen balance from N_2O-saturated 10^{-2} M cysteine solutions with added solutes at pH 5.3

	No added solute		1 M CH_3OH		0.5 M KBr		0.2 M NaCNS	
Product	G value	G(N)	G value	G(N)	G value	G(N)	G value	G(N)
CySSCy	3.25±0.25	6.50	3.05±0.2	6.10	3.43±0.3	6.86	3.48±0.2	6.96
CyH	0.88±0.05	0.88	0.89±0.06	0.89	0.83±0.05	0.83	0.77±0.06	0.77
CySSSCy	~0.14	0.28	~0.12	0.24	~0.15	0.30	~0.10	0.20
NH_3					0.20±0.04	0.20		
Total N		7.65		7.25		8.2		7.95
−CySH[a]	7.9±0.5		7.3±0.5		8.5±0.5		8.1±0.5	

[a]Determined from the decrease in the cysSH peak.

from the peak areas were linear, both for the products and for residual cysteine. The G values calculated from their slopes are presented in Table 2. In each case there is an excellent nitrogen balance, showing that the overall stoichiometry:

$$[15]\quad G(-\mathrm{CysSH}) = 2G(\mathrm{CysS})_2 + 2G(\mathrm{CysSSSCys}) + G(\mathrm{CysH})$$

holds. Thus one can conclude that all significant products, other than H_2S which was previously found to follow the alanine yield closely (2), have been determined.

Discussion

The amount of cystine formed from hydrogen peroxide would be negligible here since reaction 12 has a half life of ~10 h (2*b*) and our solutions were analyzed immediately after the radiolyses, which were themselves of less than 2 h duration. Hence only reactions initiated by ·H, ·OH, and e_{aq}^- or by the solute radicals formed from them need be considered. Table 1 gives the yields of primary radicals estimated for our conditions from data in the literature. From the concentration of N_2O (2.8×10^{-2} *M*) and the rate constants for reactions 6 ($9 \times 10^9\ M^{-1}\ s^{-1}$ (1, 2)) and 16 ($9 \times 10^9\ M^{-1}\ s^{-1}$, average of two values on p. 55 of ref. 12):

$$[16]\quad e_{aq}^- + N_2O + (H_2O) \rightarrow N_2 + OH + OH^-$$

we calculate that 0.78 e_{aq}^- per 100 eV would undergo reaction 6. Since $G_H = 0.6$ (ref. 12, p. 140) in the absence of solutes and $k_3/k_4 = 3.5$ (2*a*), a further yield of 0.13 alanyl radicals per 100 eV would be formed in reaction 4, giving a total yield of alanine molecules from reaction 7 of ~0.9 per 100 eV. This is in excellent agreement with the yield reported in Table 2 for solutions with only N_2O and cysteine. The alanine yield in the 1 *M* CH_3OH solutions is the same, showing that reaction 13 is in fact the only important mode of $\cdot CH_2OH$ attack on CysSH at p*H* 5.3. From Table 1 the yields of G_H in 0.5 *M* KBr and 0.2 *M* NaCNS are expected to be lower and this also appears to be reflected in the smaller alanine yields for these solutions in Table 2.

From the rate constants in column two of Table 1 and k_2 in column four it can be shown that 8, 24, and 17% of the OH radicals react directly with CysSH in the CNS^-, Br^-, and CH_3OH solutions, respectively. The remainder are converted to $\cdot(CNS)_2^-$, $\cdot Br_2^-$, and $\cdot CH_2OH$ by reactions 17–18, 19–20, and 21, respectively:

$$[17]\quad OH + CNS^- \rightarrow OH^- + \cdot CNS$$

$$[18]\quad \cdot CNS + CNS^- \rightleftharpoons \cdot(CNS)_2^-$$

$$[19]\quad \cdot OH + Br^- \rightarrow OH^- + \cdot Br$$

$$[20]\quad \cdot Br + Br^- \rightleftharpoons \cdot Br_2^-$$

$$[21]\quad \cdot OH + CH_3OH \rightarrow H_2O + \cdot CH_2OH$$

The ubiquitous occurrence of trisulfide in a yield of 0.1–0.15 molecules per 100 indicates that it is not a product of specific reactions of any one radical, for example:

$$[22]\quad \mathrm{CysSH} + OH \rightarrow \mathrm{Cys}\cdot + \mathrm{HSOH} \rightarrow S + H_2O$$

$$[23]\quad S + \mathrm{CysSSCys} \rightarrow \mathrm{CysSSSCys}$$

In fact the relative constancy of the disulfide and trisulfide yields in Table 2 is best explained if all four radical species attack CysSH to produce CysS· through reactions 2, 13, 24, and 25:

$$[24]\quad \mathrm{CysSH} + \cdot Br_2^- \rightarrow \mathrm{CysS}\cdot + 2Br^- + H^+$$

$$[25]\quad \mathrm{CysSH} + \cdot(CNS)_2^- \rightarrow \mathrm{CysS}\cdot + 2CNS^- + H^+$$

and CysSSSCys arises from secondary reactions of CysS·. One possibility is the cleavage of sulfenium radicals formed in reaction 26, *viz*:

$$[26]\quad \mathrm{CysS}\cdot + \mathrm{CysSH} \rightleftharpoons \mathrm{CysS}\overset{\overset{H}{|}}{\underset{\cdot}{S}}\mathrm{Cys}$$

$$[27]\quad \mathrm{CysS}\overset{\overset{H}{|}}{\underset{\cdot}{S}}\mathrm{Cys} \rightarrow (\mathrm{CysSSH} + \mathrm{Cys}\cdot)_{\text{solvent cage}} \rightarrow (\mathrm{CysSS}\cdot + \mathrm{CysH})_{\text{solvent cage}} \rightarrow \mathrm{CysSS}\cdot + \mathrm{CysH}$$

followed by:

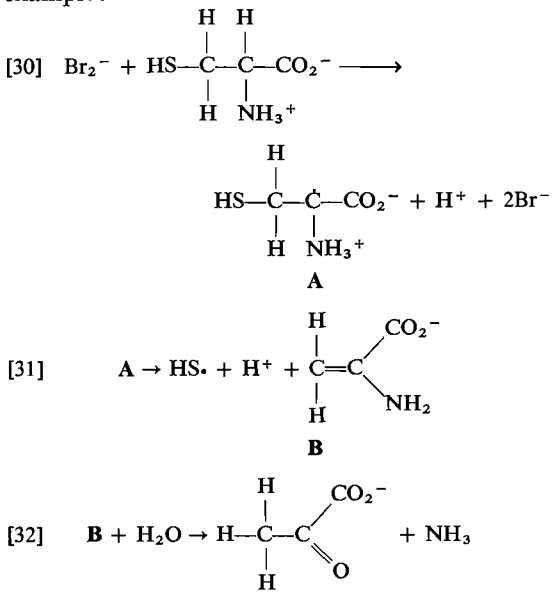
$$[28]\quad \mathrm{CysSS}\cdot + \cdot\mathrm{SCys} \rightarrow \mathrm{CysSSSCys}$$

Tung and Stone (18) have proposed reaction 29

$$[29]\quad \mathrm{CysS}\overset{\cdot}{S}^-\mathrm{Cys} + H_2O \rightarrow \mathrm{CysSS}\cdot + \mathrm{CysH} + OH^-$$

above p*H* 7 and an analogous reaction has been postulated in penicillamine (6). However, further work will be required to confirm the occurrence of reaction 27 for the protonated form of the anion. The important point here is that, in accord with previous conclusions (1–5), cysteine trisulfide is a relatively minor product. Thus the significant difference between the radiation chemistry of penicillamine and cysteine in this respect is confirmed.

The relatively minor pathway responsible for the small yield of ammonia in the $\cdot Br_2^-$ system is not at present understood. However, one may note that the attack of $\cdot Cl_2^-$ on cystine produces ammonia in an even larger yield (19). Possibly the negative charge and strong oxidizing power of these radicals facilitates their attack at the NH_3^+ group (*cf.* ref. 19) or the adjacent α-hydrogen, causing hydrolysis and deamination, for example:



From the major reactions proposed the following radical balance equation should hold:

$$[33]\quad 2[G(\mathrm{CysS})_2 + G(\mathrm{CysSSSCys})] = G_{\mathrm{H}} + G_{\mathrm{OH}} + G_{e_{aq}^-}$$

Reference to Table 1 substantiates its validity within the experimental uncertainty of ±0.5 per 100 eV for all of the solute systems.

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