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RADIATION CHEMISTRY OF THE α-AMINO ACIDS: γ-RADIOLYSIS OF SOLID CYSTEINE[1]

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Abstract

The γ-radiolysis of solid cysteine is shown to yield hydrogen, hydrogen sulfide, ammonia and cystine as major products. Radiolysis data are also given for a series of related compounds: cystamine, N-acetyl cysteine, S-methyl cysteine, glycine and alanine. The proposed reaction schemes provide a basis for correlating the radiation chemistry of these solid-state systems.

Introduction

Radiolysis of the simpler α-amino acids such as glycine and alanine leads to deamination as a major chemical consequence both in aqueous solution[3] and in the solid state.[4] And, there is accumulating evidence that the intermediate processes of deamination in the solid state are closely analogous to those that occur in aqueous solution. For example, in aqueous solution the ionization step[5]

$$H_2O \rightsquigarrow H^+, OH, e^-_{aq} \qquad (1)$$

is followed by[6]

$$e^-_{aq} + NH_3^+CH(R)COO^- \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (2)$$

$$OH + NH_3^+CH(R)COO^- \longrightarrow H_2O + NH_3^+\dot{C}(R)COO^- \qquad (3)$$

where e^-_{aq} represents the hydrated electron. The experimental evidence is that e^-_{aq} adds initially to the C=O linkage of the carboxyl group and that dissociation of the reduced intermediate then ensues.[6b,6c] Subsequent interactions of $\dot{C}H(R)COO^-$ and $NH_3^+\dot{C}(R)COO^-$ yield keto acid and fatty acid as the major organic products,[3c,3d] e.g.,

$$NH_3^+CH(R)COO^- + \dot{C}H(R)COO^- \longrightarrow NH_3^+\dot{C}(R)COO^- + CH_2(R)COO^- \qquad (4)$$

$$2NH_3^+\dot{C}(R)COO^- \longrightarrow NH_2^+{=}C(R)COO^- + NH_3^+CH(R)COO^- \qquad (5)$$

$$H_2O + NH_2^+{=}C(R)COO^- \longrightarrow NH_4^+ + RCOCOO^- \qquad (6)$$

where the hydrolysis of the labile imino-acid intermediate occurs essentially instantaneously.

The identification of reaction 2 prompted the suggestion[6a] that dissociative electron-capture is also involved in the formation of ammonia as a major product in the radiolysis of the α-amino acids in the solid state. Ionic processes in these irradiated polar solids would then be represented by

$$NH_3^+CH(R)COO^- \longrightarrow NH_3^{+\cdot}C(R)COO^- + H^+ + e^- \qquad (7)$$

$$e^- + NH_3^+CH(R)COO^- \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (8)$$

where reactions 7,8 are the stoichiometric equivalents of reactions 1 to 3. Reactions 4,5 which may occur in part in the solid are completed on dissolution of the irradiated solid in water.

It has since been established elsewhere[7] that glyoxylic and acetic acid are indeed formed as major products in the γ-radiolysis of solid glycine with $G(NH_3)$ = G(glyoxylic acid) + G(acetic acid) = 5.

The NH_3^+ group in these solid-state systems does not appear to act as a proton donor

$$e^- + NH_3^+CH(R)COO^- \longrightarrow H + NH_2CH(R)COO^- \qquad (9)$$

since hydrogen yields in the γ-radiolysis of both glycine and alanine (measured after dissolution) are quite low with $G(H_2) \leq 0.2$.[4c,4d]

The recent results obtained with paramagnetic-resonances methods provide convincing physical evidence of the importance of reaction 8 in the radiolysis of the simpler α-amino acids in the solid state:[8] on irradiation at 77°K the initially observed radical has the electron located at the carboxyl group and

on warming to intermediate temperatures the initial radical dissociates to yield $\dot{C}H(R)COO^-$.

Turning now to a consideration of the effects of SH substitution on the radiation chemistry of the α-amino acids we note that in the radiolysis of cysteine, $NH_3^+CH_2(CH_2SH)COO^-$ in oxygen-free aqueous solution the processes of deamination are wholly absent; the reactions of e^-_{aq} and OH occur exclusively at the sulfur moiety[9]

$$e^-_{aq} + RSH \longrightarrow HS^- + NH_3^+CH(\dot{C}H_2)COO^- \qquad (10)$$

$$OH + RSH \longrightarrow H_2O + NH_3^+CH(CH_2\dot{S})COO^- \qquad (11)$$

In the present work we examine the role of the SH function as a competing locus of reaction in the γ-radiolysis of cysteine in the solid state. Data on a number of related (sulfur containing) compounds are also included. Although there have been numerous studies of the paramagnetic-resonance properties of irradiated cysteine,[10] the detailed radiation chemistry of this system has not been elucidated.

Experimental

Sample preparation and irradiation.—All of the organic materials were of reagent grade or of the highest purity available from Nutritional Biochem. Corp., Cyclo Chem. Corp., and Mann Res. Lab. Inc. In some instances several recrystallizations from water were required to obtain acceptable "blank" readings in the various analytical procedures described below.

Pyrex ampoules containing one-gram samples of the polycrystalline solids were degassed on the vacuum line for about 24 hours, then sealed off with the

torch and irradiated in a 10 kc Co^{60} "Gamma-Cell" at a dose rate of 1.3×10^{18} eV/gm/min.

Analyses.—Gaseous products were pumped off following complete dissolution of the irradiated solid in degassed water which was introduced through a break-seal connection to the vacuum line. Gaseous products were identified and assayed by mass spectrometry (Consolidated 120) and by gas-chromatography (Aerograph A90-P3).

Ammonia was determined by a modification of the Conway diffusion method[11] in which a slurry of MgO was used to release ammonia from a solution of the irradiated sample in the outer compartment of the diffusion cell. Conventionally, a saturated K_2CO_3 solution is so employed, but, we found that hydrolytic degradation of cysteine in the K_2CO_3 solution leads to excessively high blanks. Diffusion of ammonia from the MgO solution to the acid compartment (0.1N H_2SO_4) is complete in 8 hours. Diffusates were assayed by means of Nessler reagent.

The "NH_2-free" fraction consists of the totality of organic products that contain an SH group but no NH_2 group. This fraction was isolated by passing the dissolved sample through ion-exchange resin (AG-50W-X8, Bio Rad. Lab.) in the acid form. Cysteine and amino derivatives are retained by the column. The "NH_2-free" fraction was collected and assayed for total SH function after the method of Ellman.[12] β-mercaptopropionic acid was identified by filter-paper chromatography.[13]

Pyruvic acid and total carbonyl were determined after the methods of Johnson and Scholes[14] and Lappin and Clark,[15] respectively.

Hydrogen sulfide was also determined by the Conway technique.[11] Irradiated samples were dissolved in dilute air-free 0.1 M NaOH and transferred to the outer chamber of the diffusion cell. The solution was then acidified with 10 percent H_2SO_4 to liberate H_2S which was collected in 0.1 M NaOH in the inner compartment after a diffusion period of ~5 hours. The diffusates were assayed nephelometrically (Bi_2S_3).

Cystine is sufficiently insoluble in water (~2.5×10^{-4} M at 25°C) to permit a gravimetric determination. Irradiated samples were dissolved in air-free water and allowed to stand for several hours to allow for equilibration. Cystine was removed by filtration through a tared sintered-glass filter and dried at 100°C. A series of blank and "spiked" runs established the solubility corrections.

Results and Discussion

G values for the various products formed in the γ-radiolysis of solid polycrystalline L-cysteine at 30°C are summarized in Table I. Dose-yield plots for all products are linear up to doses in the range 10^{21} eV/gm; typical plots are shown in Fig. 1. Yields of hydrogen, hydrogen sulfide and ammonia from a number of related sulfur-containing compounds are given in Table II.

The fact that carbonyl products are not liberated in any appreciable yield, G(>CO) ~ 0.1, on dissolution of the irradiated cysteine in water indicates that little of the observed ammonia, $G(NH_3) = 1.8$, arises through hydrolysis of labile imino derivatives of the type $NH_2^+{=}C(CH_2SH)COO^-$.

Apparently reactions akin to steps 4-6 of the "glycine" mechanism do not contribute to the radiation chemistry of solid cysteine.

The low carbonyl yields also show that the over-all stoichiometries

$$RSH \rightsquigarrow H_2 + NH_2^+{=}C(CH_2SH)COO^- \qquad (12)^{16}$$

$$RSH \rightsquigarrow H_2S + NH_3^+C({=}CH_2)COO^- \qquad (13)$$

are unimportant since it is clear that the organic products of both reactions 12,13 would yield carbonyl compounds on dissolution, (Amino acrylic acid $NH_3^+C({=}CH_2)COO^-$ is tautomeric with imino propionic acid $NH_2^+{=}C(CH_3)COO^-$).

We suggest that the radiation chemistry of solid cysteine may be interpreted in terms of the reaction scheme:

$$RSH \rightsquigarrow R\dot{S} + H^+ + e^- \qquad (14)$$

$$RSH \rightsquigarrow R\dot{S} + H \qquad (15)$$

followed by

$$H + RSH \rightarrow H_2 + R\dot{S} \qquad (16)$$

$$H + RSH \rightarrow H_2S + NH_3^+CH(\dot{C}H_2)COO^- \qquad (16a)^{17}$$

$$e^- + RSH \rightarrow NH_3 + \dot{C}H(CH_2SH)COO^- \qquad (17)$$

$$e^- + RSH \rightarrow H_2 + NH_2CH(CH_2\dot{S})COO^- \qquad (18)$$

$$e^- + RSH \rightarrow H_2S + NH_2CH(\dot{C}H_2)COO^- \qquad (18a)$$

and by the radical removal steps

$$RSH + \dot{C}H(CH_2SH)COO^- \longrightarrow R\dot{S} + CH_2(CH_2SH)COO^- \quad (19)$$

$$RSH + NH_2CH(\dot{C}H_2)COO^- \longrightarrow R\dot{S} + NH_2CH(CH_3)COO^- \quad (20)$$

$$2R\dot{S} \longrightarrow RSSR \text{ (Cystine)} \quad (21)$$

where reaction 21 occurs mainly on dissolution, although we cannot rule out the possibility that some dimerization occurs in spur regions[18] along the track of the Compton electron.

The ionization act, reaction 14, as formulated here, incorporates the concept that a parent positive ion in a polar medium is converted instantaneously to the corresponding radical species through reaction of the type $RH^+ + RH \longrightarrow R + RH_2^{+\cdot}$.[19] Localization of the radical site at sulfur position would be expected on energetic grounds;[20] EPR studies show that $R\dot{S}$ represents the long-lived radical in γ-irradiated cysteine at room temperature.[10] We assume that any contribution of excited states will arise through homolytic cleavage of the labile S-H bond as shown in reaction 15.[21]

The evidence is that ammonia liberation from cysteine occurs almost exclusively through the dissociative capture of e^- as formulated in reaction 17. This step is, of course, the analogue of reaction 8 of the glycine mechanism. The formation of ammonia via reaction 17 liberates the $\dot{C}H(CH_2SH)COO^-$ radical which we assume abstracts H from the labile S-H bond of cysteine (step 19) to yield β-mercaptopropionic acid. A "NH_2-free" fraction of organic products is produced with $G \geq 1.2$ (Table I) and β-mercaptopropionic acid has been identified as a constituent of this fraction. The fact that the observed yield of "NH_2-free" products is somewhat less than the ammonia yield, $G(NH_3) = 1.8$, is not too surprising

since one might expect that a fraction of the $\dot{C}H_2(CH_2SH)COO^-$ radicals formed in reaction 17 would undergo the rearrangement

$$\dot{C}H_2(CH_2SH)COO^- \longrightarrow CH_3(CH_2\dot{S})COO^- \qquad (22)$$

in competition with the H abstraction step 19. On dissolution, the radicals $CH_3(CH_2\dot{S})COO^-$ are then removed through combination with cysteine radicals $R\dot{S}$.

That e^- adds initially to the carboxyl C=O linkage of cysteine (as in the reaction of e^- with solid glycine) is supported by the results obtained with cystamine $NH_3^+CH_2CH_2SH$ (Table II). We find that cystamine which does not contain the carboxyl group as a trapping center for e^- does not yield ammonia in any appreciable yield $G(NH_3) \leq 0.1$.

The SH group of cysteine represents a competing trapping center for e^-.[22] In fact, as we have noted, e^-_{aq} in aqueous solutions of cysteine reacts exclusively through dissociative attachment to yield $H\bar{S}(H_2S)$. On the other hand, recent studies[23,24] of the radiation chemistry of thiophenol and ethyl mercaptan in the pure liquid state indicate that dissociative capture reactions of the type

$$RSH + e^- \longrightarrow R + H\bar{S} \qquad (23)$$

$$RSH + e^- \longrightarrow R\bar{S} + H \qquad (24)$$

do not occur; these liquid systems are considerably less polar than water and the evidence is that reactions 23,24 become exothermic only if solvation energies in a polar medium can be utilized in the over-all energetics.[23,25] Whether or not such factors are involved in the reactions of e^- in a polar solid such as cysteine we cannot say.

However, simple dissociative attachment of e^- need not necessarily be involved in steps 18,18a. An alternate explanation is that e^- is captured by the sulfhydryl group to give RSH^- and that chemistry then ensues as a consequence of proton transfer from an adjacent NH_3^+ group.[26] Some evidence for such concerted action is to be found in the fact that $G(H_2) + G(H_2S)$ from N-acetylcysteine, $CH_3CONHCH(CH_2SH)COOH$, is markedly lower than the corresponding value for cysteine as shown in Table II. However, as formulated here, reactions 18,18a represent only the over-all stoichiometries.

The reaction scheme given in equations 14-21 leads to the product relationship $G(H_2) + 1/2\ G(H_2S) + 1/2\ G(NH_3) = G(RSSR)$. We find experimentally that $G(H_2) + 1/2\ G(H_2S) + 1/2\ G(NH_3) = 4.8$ and that $G(RSSR) = 5$. The combined yields of the radiation-induced steps 14,15 is given by $G_{14} + G_{15} = G(H_2) + G(H_2S) + G(NH_3) = 6.4$. We cannot separately evaluate the contributions of ionization and excitation via reactions 14 and 15 respectively since we have no knowledge of the relative yields of the branching reactions 16, 16a, and 18, 18a.

Footnotes and References

(1) This work has been supported by the U. S. Atomic Energy Commission.

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(21) Reaction 15 represents a major process in the photolysis of cysteine in the solid state as recently shown by H. C. Box, H. G. Freund and E. E. Budzinzki, J. Phys. Chem., 45, 809 (1966).

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(26) As noted in our discussion of reaction 9, the evidence is that the NH_3^+ group itself does not react with e^- to yield hydrogen. See also ref. 6b.

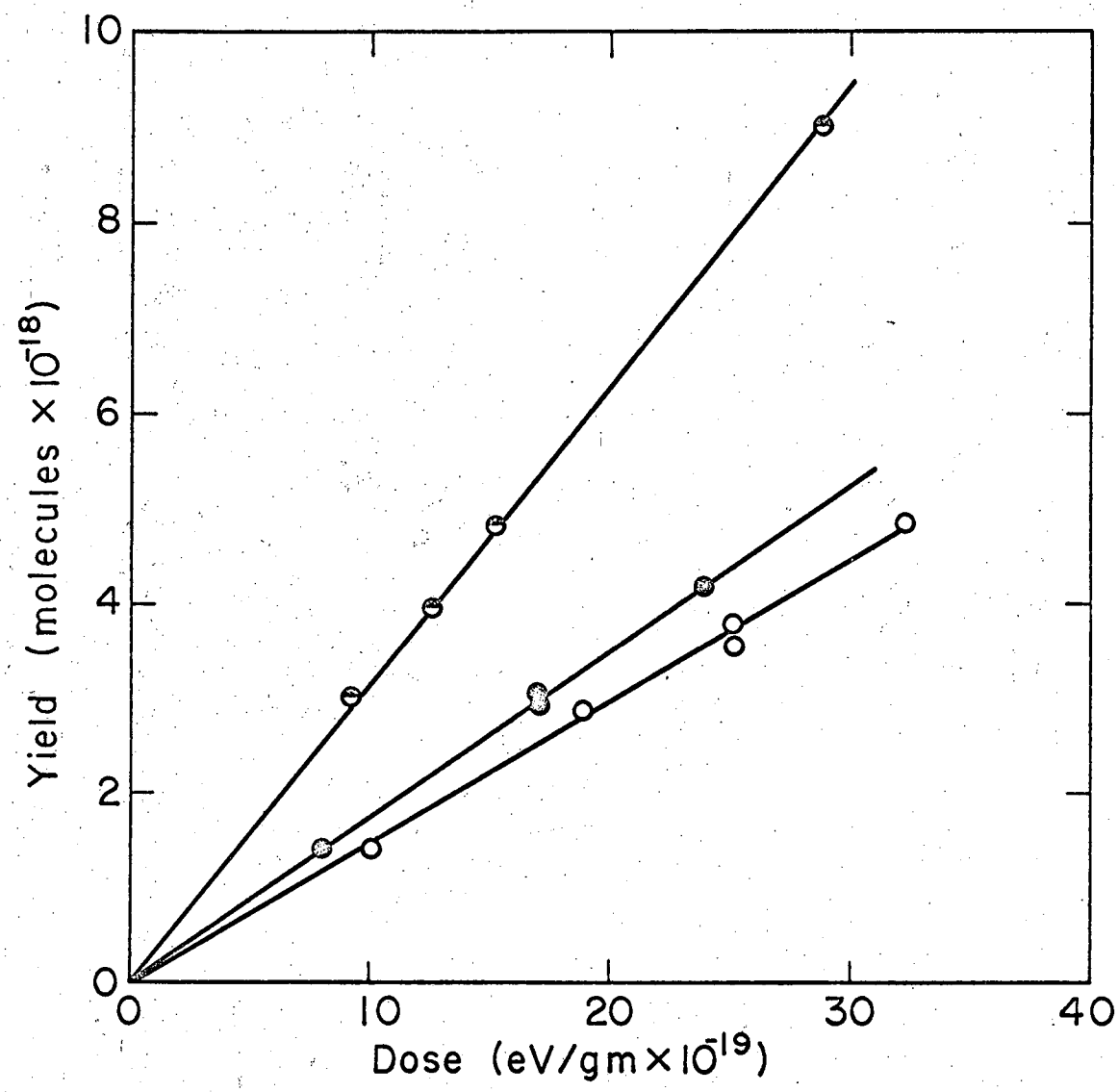

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Fig. 1 Production of hydrogen (⊖), ammonia (●), and hydrogen sulfide (O), from solid cysteine as a function of γ-ray dosage.

Table I. Product yields in the γ-radiolysis of solid L-cysteine.

Product	G
Hydrogen	3.1 ± 0.1
Hydrogen sulfide	1.5 ± 0.1
Ammonia	1.8 ± 0.1
Cystine	5.0 ± 0.5
"NH_2-free" fraction	1.0 ± 0.1
Pyruvic acid	$\leq$ 0.1
Total carbonyl	$\leq$ 0.1

Table II. Comparative product-yields in the γ-radiolysis of cysteine and related compounds in the solid state.

	G		
	H_2	H_2S	NH_3
Cysteine			
$NH_3^+CH(CH_2SH)COO^-$	3.1	1.5	1.8
Cystamine			
$(NH_3^+CHCH_2SH)Cl^-$	5.6	1.2	< 0.1
N-acetyl cysteine			
$CH_3CONHCH(CH_2SH)COOH$	0.5	0.9	< 0.1
S-methyl cysteine			
$NH_3^+CH(CH_2SCH_3)COO^-$	~ 0.2	—[a]	5.1
Glycine			
$NH_3^+CH_2COO^-$	~ 0.2		5.2
Alanine			
$NH_3^+CH(CH_3)COO^-$	~ 0.2		5.4

a) H_2S was not anticipated nor was it observed as a product from S-methyl cysteine; other measurements with S-methylcysteine gave, $G(CH_4) \sim 0.5$; $G(CH_3SH) \leq 0.1$. These results indicate that e^- is not captured via dissociative attachment at the sulfur locus; the observed H_2 and NH_3 yields suggest that the "glycine" mechanism is of major importance in the radiolysis S-methyl cysteine.

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