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The Roles of Thiyl Radicals in the Radiolysis of a Mixed Aqueous Solution of Cysteine and Formate—

Hydrogen Abstraction from Formate and the Formation of Carbon Dioxide

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Attempts were made to elucidate the role of thiyl radicals in the γ-radiolysis of aqueous solutions containing L-cysteine and formate, and the evidence was obtained for the abstraction of hydrogen and the acceleration of the CO_2 formation from formate by cysteine thiyl radicals.

Experimental

Triply-distilled water (pH 5.0) containing formic acid and cysteine was degassed in Pyrex ampoules and irradiated by a ^{60}CO γ-ray source (dose rate: 9×10^4 rads·h^{-1}, dose: 9×10^4 rads).[1] Amino acids were analyzed on an amino-acid analyzer. The gaseous products were analyzed as has been described.[2] The tritium liberated from ^{3}H-formate was measured as has been described.[3] The oxalic acid was estimated by measuring the permanganate reduction in acidic media (2 N sulfuric acid) at room temperature. The permanganate was colorimetrically determined at 525 nm. Before the analysis, the cysteine was removed with cation exchange resin.

Results and Discussion

In the radiolysis of aqueous solutions under deaerated and neutral conditions, formate reacts with hydroxyl radicals (OH) to yield carboxyl radicals ($\cdot CO_2^-$) (1), and $\cdot CO_2^-$ dimerized to oxalic acid[4,5] (2):

$$OH + HCO_2^- \xrightarrow{k_1} \cdot CO_2^- \qquad (1)$$

$$2\cdot CO_2^- \longrightarrow HCO_2CO_2^- \qquad (2)$$

Cystein (CySH) reacts with OH to yield cysteine thiyl radicals (CyS·) (3) and then reacts with e^-_{aq} to yield hydrogen sulfide and alanyl radicals (Cy·)(4). Cy· reacts with another molecule of cysteine to yield alanine (CyH) and CyS· (5). The CyS· produced through both the reactions dimerizes to cystine (CySSCy) (6).[6-8]

$$OH + CySH \xrightarrow{k_3} CyS\cdot \qquad (3)$$

$$e_{aq}^- + CySH \longrightarrow Cy\cdot + H_2S \qquad (4)$$

$$Cy\cdot + CySH \longrightarrow CyH + CyS\cdot \qquad (5)$$

$$2CyS\cdot \longrightarrow CySSCy \qquad (6)$$

The other reactions are considered to be less important than the reactions described above.[4-8]

In this investigation we have studied the radiolysis of a mixed solution of formate and cysteine. As is shown in Fig. 1, the yields of hydrogen sulfide and alanine were not affected by the formate. This is because, at pH 5.0, only 0.6—7% of formate exists in its acid form, and this form reacts efficiently with e_{aq}^-.

Hydroxyl radicals (OH) are competed for by the formate ion and cysteine ($k_1=2.9\times10^9$ M^{-1} sec,$^{-1}$[9] $k_3=3\times10^9$ M^{-1} sec^{-1}[10]). However, oxalic acid was not produced at all in the presence of 10^{-3} M cysteine at three concentrations of formate (10^{-3}, 10^{-2}, and 10^{-1} M), as is shown in Fig. 1. This indicates that, at 10^{-3} M, cysteine completely scavenges the $\cdot CO_2^-$ (7):

$$\cdot CO_2^- + CySH \xrightarrow{k_7} HCO_2^- + CyS\cdot \qquad (7)$$

If the $\cdot CO_2^-$ is completely converted to CyS·, the OH radicals which have reacted with formate by reaction (1) will also produce CyS· and an unchanged yield of cystine may be anticipated, as is observed in the solution containing 10^{-3} and 10^{-2} M of formate (Fig. 1).

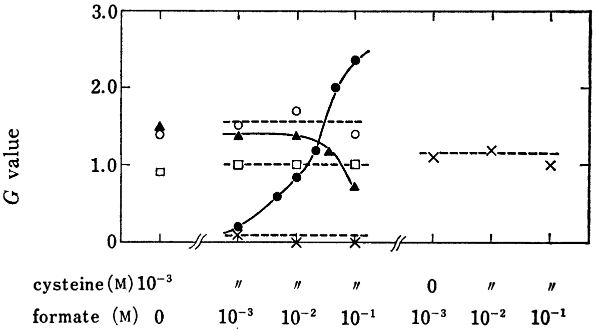

Fig. 1. Yields of the products from crysteine—formate solutions of various compositions.
×···×: oxalic acid, □···□: H_2S, ○···○: alanine, ●···●: CO_2, ▲···▲: cystine.
pH 5, dose: 9×10^4 rads.

It is well known that thiyl radicals abstract hydrogen from aldehydes.[11] In order to test whether or not

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Table 1. ^{3}H-liberation into water from ^{3}H-formate in the γ-radiolysis of its deaerated aqueous solution and addition effect of cysteine on it

Concn. of formate	cpm in 0.5 ml water distilled	
	Without cysteine	With 10^{-3} M cysteine
10^{-3} M	2210	1749
10^{-2} M	2372	13820
10^{-1} M	2699	136562

^{3}H-formate (50 μCi (nominal)/mmol) was used.
dose: 9×10^4 rads.

CyS· abstracts hydrogen of the formate, a tracer experiment using ^{3}H-formic acid was made. As is shown in Table 1, the liberation of tritium from formate to water was almost constant for the three concentrations of formate in the absence of cysteine, but it markedly increased with the concentration of formate in the presence of 10^{-3} M cysteine, the rate of the increase being approximately proportional to the formate concentration. This can be explained by the hydrogen abstraction by CyS· from the formate (8):

$$\mathrm{CyS\cdot} + \mathrm{HCO_2^-} \xrightarrow{k_8} \mathrm{CySH} + \cdot\mathrm{CO_2^-} \qquad (8)$$

The reaction is the reverse of (7), and CyS· and $\cdot\mathrm{CO_2^-}$ will be mutually converted by (7) and (8). If k_8 is much smaller than k_7, $[\cdot\mathrm{CO_2^-}]$ will be much smaller than [CyS·], yielding no oxalic acid. However, the rate of (8) and the rate of the liberation of hydrogen from formate will increase proportionally with the formate concentration, as is observed. As is shown in Fig. 1, the yield of cystine in the presence of 10^{-1} M formate was lowered, while that of carbon dioxide reciprocally increased. This result may be explained as follows: at high formate concentrations, reaction (6) may compete with a reaction of CyS· which produces $\mathrm{CO_2}$. The species which reacts with CyS· to yield carbon dioxide may be the $\cdot\mathrm{CO_2^-}$ or the $\cdot\mathrm{CO_2^-}$-formate complex.$^{12)}$

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