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Flame-induced Reactions of Sulfur-containing Amino Acids in an Aqueous Solution

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Abstract: Hydrogen-oxygen flames, when blown against an aqueous solution of methionine, induced conversion reactions to homoserine, 2-aminobutyric acid and glutamic acid. Besides the already-known reactions by a hydroxyl radical, a contribution of a hydrogen atom from hydrogen-rich flames to the reaction was recognized. We successfully controlled the vigorous oxidation of the system using a radical scavenger. © 1998 Elsevier Science Ltd. All rights reserved.

In our previous study,[1] flames have been shown to cause an oxidation reaction in an aqueous solution when blown against the solution surface. This reaction was caused by a hydrogen, a town gas and an ethylene flame, but not by an acetylene flame. The aqueous-phase reaction is a new type of plasma-induced reaction, which utilizes an active molecular species produced in a gas phase, and has been proved to proceed by a stepwise oxidation mechanism due to a hydroxyl radical generated in the flame. This method can be utilized as a substitute for the conventional hydroxyl radical generating systems, that is, the Fenton reagent, a hydrogen peroxide-ultraviolet system, γ-rays, and so on. Our reaction system can introduce a hydroxyl radical continuously into a water phase and does not need any chemical oxidizing reagents, so that the reaction mixture is kept always clean and can be performed with very simple apparatus. In the present study, first we investigated in further detail the reactions of amino acids containing a sulfur atom, which is labile to radicals, in order to examine reactions caused by flames. Second, it was attempted to control the strong oxidizing ability of the reaction system by employing hydrogen-oxygen flames with several flow rates as well as by the use of a radical scavenger.

The apparatus is shown in Fig. 1. The operating conditions are summarized in the Table. The flame from a burner was blown down to the surface of an aqueous solution (*ca.* 250 ml) containing amino acid (*ca.* 0.5 mmol/l). The initial pH of the solution was adjusted to 3 with 2N hydrochloric acid. The temperature of the solution was kept at 40-50 °C throughout the reaction by circulating cold water through the jacket of the reaction vessel. The solvent was supplied continuously from the solvent reservoir to restore the loss of the sample solution. At appropriate time intervals,

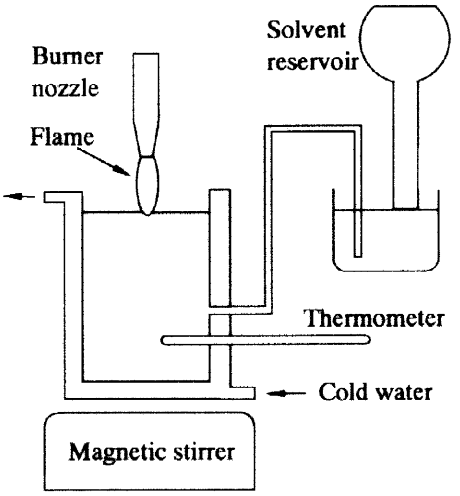

Fig. 1. Apparatus for flame-induced reaction.

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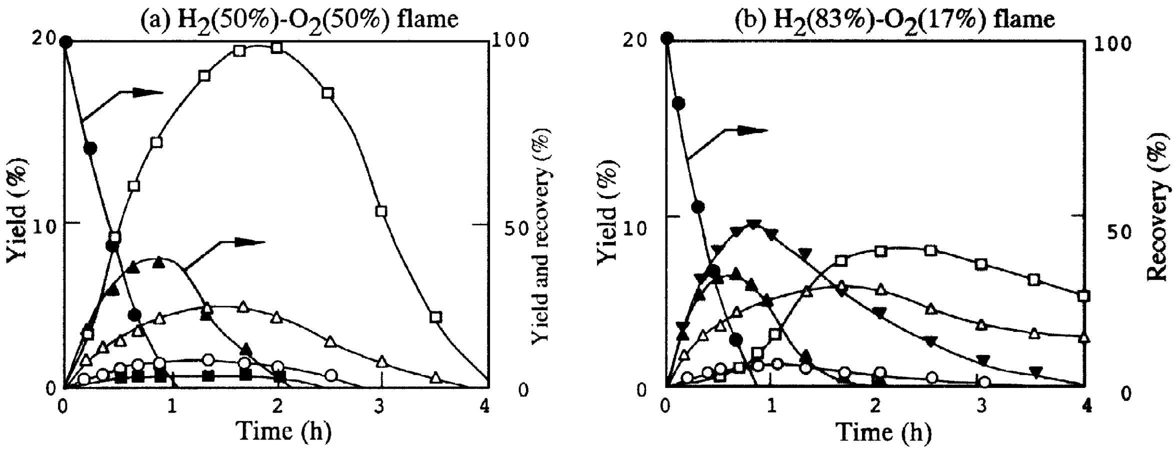
0.5-ml aliquots of the solution were withdrawn and analyzed with an amino acid analyzer.

Figure 2 shows the reaction curves for the conversion of methionine in an aqueous solution using (a) a hydrogen(50%)-oxygen(50%), (b) a hydrogen(83%)-oxygen(17%), and (c) a hydrogen diffusion flame. Methionine was smoothly oxidized and disappeared within 2 hours under all the reaction conditions. The primary product of the oxidation was methionine sulfoxide and the sulfoxide was further oxidized to afford homocysteic acid. In the next step of the oxidation, the flame desulfurized homocysteic acid to produce aspartic acid. The yield of aspartic acid decreased in the prolonged reaction and further oxidized products were detected, which are omitted in the figure.

Table . Flame Conditions Employed for Flame-induced Reaction.

Flame	Flow rate of gas (l/min)		Burner-solution
	Hydrogen	Oxygen	distance* (cm)
H_2(50%)-O_2(50%)	1.0	1.0	3
H_2(83%)-O_2(17%)	4.0	0.8	5
H_2 diffusion	3.0	-	4

* Distance from a burner nozzle tip to the solution surface.



(c) H_2 diffusion flame

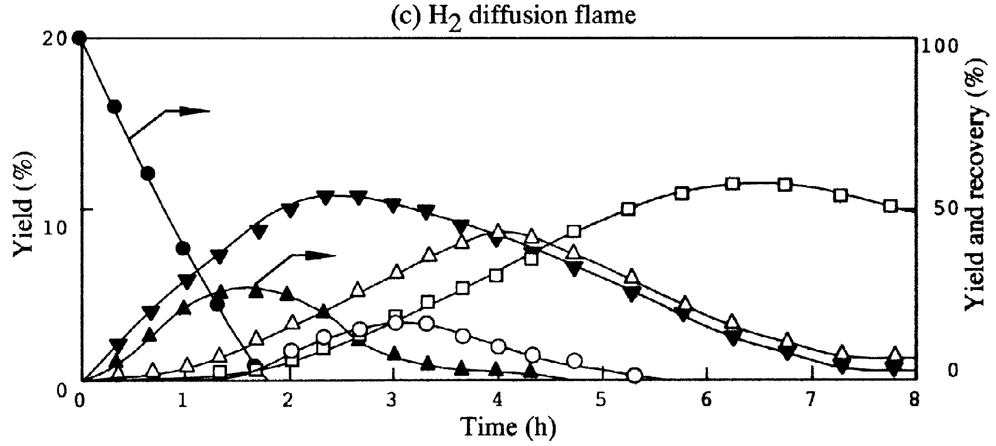
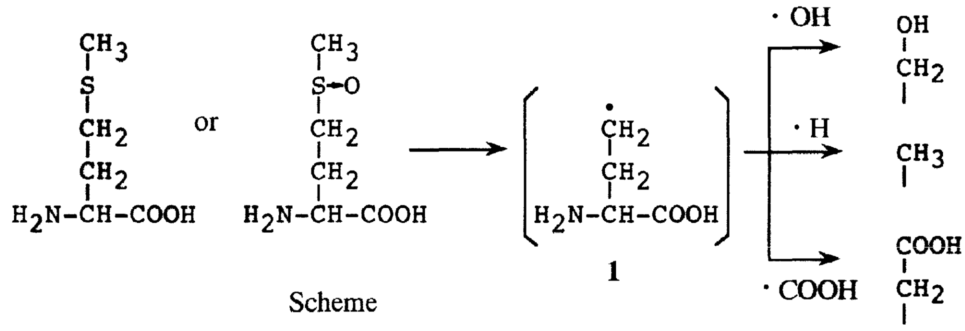


Fig. 2. Reaction curves for the flame-induced reactions of methionine in water.
Symbols: ● , methionine; ▲, methionine sulfoxide; ○ , homoserine; ■ , methionine sulfone; △ , homocysteic acid; □ , aspartic acid; ▼ ,2-aminobutyric acid.

In addition to the above-mentioned oxidation products, homoserine was formed in the reaction mixtures and by the latter two flames, 2-aminobutyric acid was formed. These two amino acids were formed after methionine sulfoxide and before homocysteic acid. This fact indicates that the carbon-sulfur bond was cleaved between the reaction time of hydroxylation or hydrogenation and that of oxidation of sulfur. Thus, in separate experiments, homoserine and 2-aminobutyric acid were not produced from the starting materials with the sulfur atom fully oxidized, namely, methionine sulfone and homocysteic acid by using all the three flames, but was produced from methionine sulfoxide. It has been confirmed by ESR experiments that the 3-carboxy-3-aminopropyl radical (**1**, in the Scheme) is formed as an intermediate from methionine and its sulfoxide by the action of a hydroxyl radical generated by γ-rays or the Fenton reagent.[2-6] The flame-induced reaction of methionine probably forms the same intermediate, and the coupling of radical **1** and a hydroxyl radical might produce homoserine. 2-Aminobutyric acid could be also generated through the intermediate (**1**). Abstraction of the hydrogen atom by this radical from other organic compounds present in the reaction mixture was precluded by the fact that 2-aminobutyric acid was absent throughout the reactions employing the hydrogen(50%)-oxygen(50%) flame. Therefore, the coupling reaction of radical **1** with the hydrogen atom was considered to produce 2-aminobutyric acid. This product would offer the first experimental evidence of introduction of a hydrogen atom from the flames into the water phase. This was observed in the flames from hydrogen-rich mixtures ($H_2/O_2 \geqq 5$).



Scheme

Figure 3 shows the reaction curves for the flame-induced oxidation of methionine in 30% aqueous formic acid solution with the (a) and (b) flames. It was observed that glutamic acid was generated in the presence of formic acid. Formic acid is a kind of radical scavenger, which reacts with a hydroxyl radical to produce a hydroxycarbonyl radical as follows.[7] The radical is expected to react with the above-stated intermediate (**1**), resulting in formation of glutamic acid (Scheme).

$$\cdot OH + HCOOH \longrightarrow H_2O + \cdot COOH$$

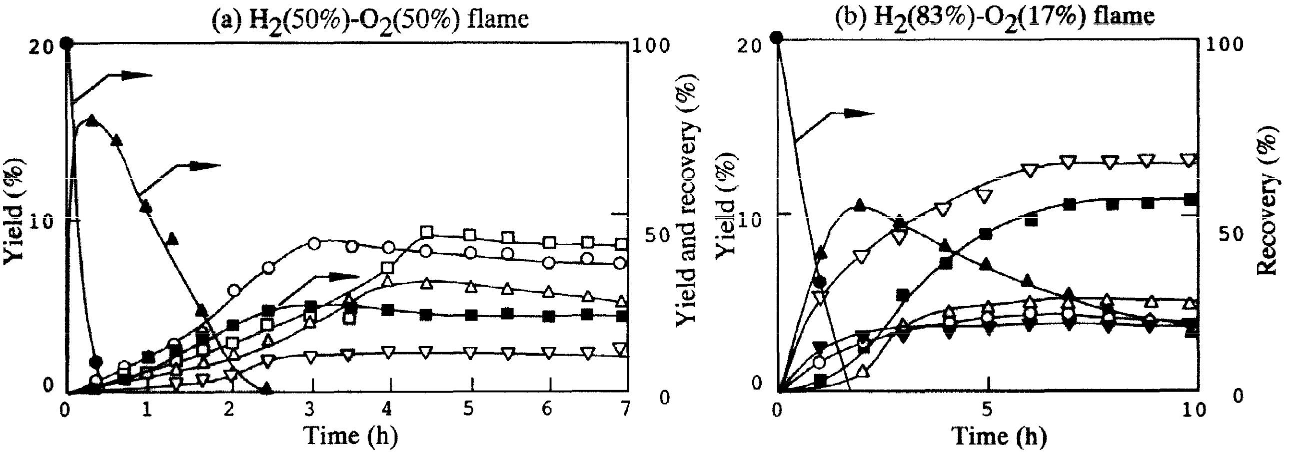


Fig. 3. Reaction curves for the flame-induced reactions of methionine in 30% aqueous formic acid. Symbols: ▽ , glutamic acid; others are as shown in the legend of Fig. 2.

In our previous study[1], we estimated that oxidation was only caused by a hydroxyl radical. The use of methionine which is labile to radical reactions due to the sulfur atom and undergoes homolysis of a carbon-sulfur bond enabled us to discover the contribution of a hydrogen atom and the new conversion reactions shown in the Scheme. We confirmed that lanthionine and S-methylcysteine underwent the same reactions to produce serine, alanine, and aspartic acid. These conversions are regarded as a new type of modification reaction of amino acids.

Alteration of the flame composition influenced the reaction rate, and the reaction rate with the flame (c) was notably slower than that with the flame (b). The reaction was also slowed in the presence of formic acid. The slow reaction rate would be due to the decrease of hydroxyl radicals which react in the water phase, since a reduction of the oxygen content of a hydrogen-oxygen mixture leads to a decrease of hydroxyl radicals in a flame; moreover, formic acid also decreases hydroxyl radicals in a reaction mixture by the radical-scavenging effect. The addition of formic acid in a reaction mixture brought about another important effect, that is, stabilization of products. Amino acids formed by the reaction in aqueous formic acid were less decomposed in the later part of the reaction, except for methionine sulfoxide, since the sulfur atom is not fully oxidized and was still labile to radicals (Fig. 3).

Conclusion: (1) A hydrogen atom was found to be extracted from hydrogen-rich flames into the water phase and to react with radicals in solution. This fact would be a rare example of a liquid-phase homogeneous reaction in which the hydrogen atom is made to react. We can utilize, of course, only a hydroxyl radical as the active species by employing the hydrogen(50%)-oxygen(50%) flame. (2) Conversion reactions of methionine or its sulfoxide to homoserine, 2-aminobutyric acid and glutamic acid were discovered. These reactions were applicable to other sulfur-containing amino acids, lanthionine and S-methylcysteine. (3) We successfully controlled the vigorous oxidation of the flame-induced reaction system using formic acid to prevent reaction products from being further converted by hydroxyl radicals. This method could offer a potential new aspect in synthetic technology.[8]

References and Note

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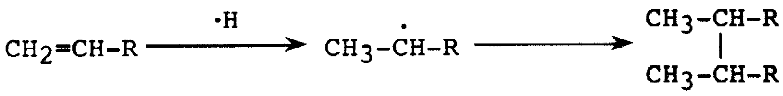
8. In fact, we found a new method for dimerization of unsaturated aliphatic amines and amino acids in a preparative-scale experiment by taking advantage of the reaction of a hydrogen atom from the flame as well as the product-stabilizing effect of formic acid. The reaction pathway is shown in the following scheme, where the starting materials are allylamine, vinylglycine and allylglycine. The details of the dimerization will be reported elsewhere.



$$\mathrm{CH_2{=}CH{-}R} \xrightarrow{\cdot \mathrm{H}} \mathrm{CH_3{-}\dot{C}H{-}R} \longrightarrow \begin{array}{c}\mathrm{CH_3{-}CH{-}R}\\ |\\ \mathrm{CH_3{-}CH{-}R}\end{array}$$