Material Balance and Additional Effect of Second Organic Solutes in the Radiolysis of Aqueous Methionine Solution[†]

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The gamma-radiolysis of methionine in 10^{-3} M aqueous solution was studied. From the yield *vs.* dose plots, the following values were obtained: $G(-\text{Methionine})=3.0$, $G(\text{Methionine sulfoxide})=0.30$, $G(\alpha\text{-Aminobutyric acid})=0.46$, $G(3\text{-Methylthio propylamine})=0.59$, $G(\text{Methional})=0.08$, $G(\text{Carboxylic acid})=0.24$, $G(\text{Mercaptan} + \text{Disulfide})=0.61$, $G(\text{Ammonia})=1.48$, and $G(\text{Carbon dioxide})=1.45$. From these results, it was concluded that major reaction was deamination and decarboxylation by $\cdot\text{OH}$ radical attack.

Addition of methanol as the second solute at concentration above 4×10^{-3} M could not lower $G(\text{Methionine sulfoxide})$, suggesting the reaction of methionine with secondary radical produced from methanol.

Existence of similar reaction was suggested in the radiolysis of methionine in aqueous pork extract.

In food irradiation, water-soluble food constituents are affected by the attacks of the active species, $\cdot\text{OH}$, e_{aq}^- , and H, primarily produced from water. If an aqueous solution is dilute, thus produced radicals (secondary radicals) from food constituents will disappear mainly by recombination or disproportionation. However, when the solution is concentrated as in foods, the further attacks of the secondary radicals on other solute may become important as radical fate. Cysteine and other thiol may undergo attacks of almost all secondary radicals, thus being expected to show high $G(-\text{thiol})$ value with large existence of other solutes. Furthermore, Loman *et al.* recently reported¹⁾ that secondary radicals produced from ethanol, formate, and methanol by the $\cdot\text{OH}$ radical attack are able to destroy thymine. In previous papers,^{2,3)} we also found that the secondary radicals produced from

methanol were intercepted by added methionine and that the secondary radicals produced from glucose would react with the sulfur-containing amino acids, cysteine and methionine.

In this research, radiolysis of methionine in an aqueous solution with or without methanol was investigated. Methionine was chosen for investigation because its degradation products contribute to the off-flavor in irradiated foods,⁴⁾ and of interest in radiation chemistry by its high reactivity toward $\cdot\text{OH}$. Methanol was used as the second solute; this compound is reactive toward $\cdot\text{OH}$ *via* $\text{CH}_3\text{OH} + \cdot\text{OH} \rightarrow \cdot\text{CH}_2\text{OH} + \text{H}_2\text{O}$.

To construct models related to food, radiolysis of methionine in aqueous pork extract was also investigated.

EXPERIMENTAL

Materials. Triply distilled water was prepared as described elsewhere.⁵⁾ L-Methionine and methanol used for the radiolysis were GR grade reagents of Kantō Chemical Co., Inc. Aqueous pork extract was prepared as follows: commercially available pork

[†] Radiation Chemistry of Foods. Part V. Part IV; see reference.¹⁶⁾

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was crushed in a blender with a fixed volume of triply distilled water and the extract was collected by filtration. pH value of the extract was 5.6~5.8.

Irradiation. Irradiation was as described previously.³⁾ The dose rate, 1.8×10^4 rad/hr used throughout, was measured with a Fricke dosimeter.

Methods for analysis of degradation products. Ninhydrin-positive products were determined with an amino acid analyzer (Hitachi Corp., Model KLA-3A). The destruction of methionine was also estimated with an amino acid analyzer.

Amines were detected as 2,4-dinitrophenyl derivatives. Thin-layer chromatography and spectrophotometric method⁶⁾ were used for identification. To determine total amount of amines quantitatively, tracer technique was used. Methionine-methyl-¹⁴C was added to the solution to be irradiated and amines produced were separated by means of ion-exchange column chromatography. Radioactivity measurement was done by liquid-scintillation method using a liquid-scintillation counter (Hitachi-Horiba Corp., Model LS-500).

Total amount of carboxylic acids was also determined quantitatively by tracer technique using methionine-carboxyl-¹⁴C.

Ammonia was determined by the Conway's method. Carbon dioxide was measured as follows: irradiated ampule was opened under vacuum (below 10^{-4} mmHg) and gaseous products were collected through dry ice/ethanol and liquid nitrogen traps by an oil diffusion pump. Carbon dioxide condensed at the liquid nitrogen temperature was evicted by He gas and analyzed by a gas-chromatograph (Hitachi Corp., Model K-23).

Mercaptan was measured as described previously.³⁾ Disulfide was measured after reduction to mercaptan by the method of Kolb *et al.*⁷⁾ Methional was measured as their 2,4-dinitrophenylhydrazones.

RESULTS AND DISCUSSION

Radiolysis of methionine in aqueous solution

Radiolysis of methionine in aqueous solution was studied by some workers.⁸⁻¹⁰⁾ However, the mechanism of radiation degradation has not been completely understood. In this study, we attempted to determine the initial *G*-values* of products produced from aqueous solution

* Number of molecule produced per 100 eV absorbed energy.

FIG. 1. Yields of the Products from Methionine Solution.

○—○ Ammonia, ●—● Methionine sulfoxide, ●—● α-Aminobutyric acid, ◻—◻ 3-Methylthio propylamine, ◻—◻ Carbon dioxide, ■—■ Carboxylic acid, △—△ Methional, ▲—▲ Mercaptan + Disulfide.

TABLE I. *G*-VALUES OF PRODUCTS FROM DEAERATED METHIONINE SOLUTION

Functional group	Product	<i>G</i> -value
-NH ₂	—Methionine	3.0
	Methionine sulfoxide	0.30
	α-Aminobutyric acid	0.46
	3-Methylthio propylamine	0.59
	Ammonia	1.48
	Total	2.83
-COOH	Amino acid	0.76
	Carboxylic acid	0.24
	Carbon dioxide	1.45
	Total	2.45
-S-	Methionine sulfoxide	0.30
	3-Methylthio propylamine	0.59
	Carboxylic acid	0.24
	Mercaptan + Disulfide	0.61
	Methional	0.08
	Total	1.82

under deaerated conditions.

Solution of L-methionine in water (10^{-3} M) was exposed to ⁶⁰Co γ-rays and the yields of

all products identified were measured at various doses between 1.6×10^4 rad and 6.4×10^4 rad. The results are shown in Fig. 1. The yields measured were linear with dose up to 4×10^4 rad. The initial G -values obtained from the straight lines through the yield *vs.* dose plots are given in Table I. In the table, the G -values of products are shown with classifying by their functional groups.

The results show that yield of carbon dioxide amounts to 50% of the decomposition of carboxylic group and that of amino acids and of carboxylic acids amounts to 25% and 10%, respectively. The results also show that $G(\text{ammonia})$ amounts to 50% of the decomposition of amino group and $G(\text{Amine})$ amounts to 20%. Furthermore, the agreement between total yields of these products and $G(-\text{Methionine})$ indicates that all degradation products were determined quantitatively. Two figures calculated from the material balance of decarboxylation and deamination, *viz.*,

$$G(\text{Carbon dioxide}) - G(\text{Amine}) = 0.86$$

$$G(\text{Ammonia}) - G(\text{Carboxylic acid}) = 1.24$$

indicate that yield of product resulting from deamination and decarboxylation amounts to 30~40% of total yield of degradation products. However, G -value of methional, one of the deaminated and decarboxylated products, is only 0.08, suggesting the existence of other unknown products. From the result of calculation on sulfur balance, *viz.*,

$$G(-\text{Methionine})$$

$$- \text{total } G(\text{Sulfur containing product}) = 1.18$$

it was suggested that non-identified compounds would contain a sulfur atom.

This result that major degradation products are formed by the reaction at C_1 -carbon, but not at the sulfur, is interesting from the consideration that high reactivity of methionine toward $\cdot\text{OH}$ would be caused by its sulfur atom. Possibly, the radical produced at sulfur by the attack of $\cdot\text{OH}$, which is detected by ESR,¹¹⁾ will be transferred to C_1 -carbon by

intramolecular radical transfer.

Radical scavenger was employed to examine the mechanism of the radiolysis. Experiment was carried out by using deaerated solution with nitrous oxide (10^{-2} M) which scavenges e_{aq}^- and produces an $\cdot\text{OH}$ radical. From the results listed in Table II, it can be seen that

TABLE II. G -VALUES OF PRODUCTS FROM DEAERATED METHIONINE SOLUTION (WITH NITROUS OXIDE)

Functional group	Product	G -value
-NH ₂	-Methionine	4.8
	Methionine sulfoxide	0.50
	α -Aminobutyric acid	0.20
	3-Methylthio propylamine	0.72
	Ammonia	2.72
	Total	4.14
-COOH	Amino acid	0.70
	Carboxylic acid	0.20
	Carbon dioxide	3.4
	Total	4.3
-S-	Methionine sulfoxide	0.50
	3-Methylthio propylamine	0.72
	Carboxylic acid	0.20
	Mercaptan + Disulfide	1.24
	Methional	0.19
	Total	2.85

FIG. 2. Competition between Methanol and Methionine toward $\cdot\text{OH}$.

the yields of methionine sulfoxide, carbon dioxide, and ammonia increased with nitrous oxide present. From this fact, it was concluded, in agreement with the results previously reported by us,³⁾ that oxidation to methionine sulfoxide, decarboxylation and deamination are caused by $\cdot\text{OH}$ radical attack. On the other hand, the fact that the yield of α -aminobutyric acid was reduced by nitrous oxide present indicates that the cleavage to α -aminobutyric acid is caused by e_{aq}^- attack.

Effect of methanol on the radiolysis of methionine solution

Effect of methanol concentrations on the yield of methionine sulfoxide from 10^{-3} M methionine solution is shown in Fig. 2. Solutions were irradiated under deaerated conditions and the total dose given was 4×10^4 rad. The yield of methionine sulfoxide decreased with the addition of methanol and plots of $1/G(\text{Methionine sulfoxide})$ vs. $[\text{Methanol}]$ gave a straight line up to $[\text{Methanol}] = 4 \times 10^{-3}$ M. It is assumed that there is simple competition toward $\cdot\text{OH}$ between methanol and methionine sulfoxide, the relation should be expressed by the following equation:

$$\frac{1}{G(\text{Methionine sulfoxide})} = \frac{1}{G_0(\text{Methionine sulfoxide})} \times \left\{ 1 + \frac{k_1[\text{Methanol}]}{k_2[\text{Methionine}]} \right\} \quad (1)$$

where k_1 and k_2 are the rate constants of methanol and methionine toward $\cdot\text{OH}$, respectively, and $G_0(\text{methionine sulfoxide})$ is $G(\text{methionine sulfoxide})$ without additional methanol. The straight line in Fig. 2, fitted the relation (1) and k_1/k_2 was calculated to be 0.13 from the slope. This value agrees reasonably well with 0.11 calculated from the constants* previously reported by us.¹²⁾

* $k_{\text{CH}_3\text{OH}+\cdot\text{OH}} = 8.7 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$, $k_{\text{methionine}+\cdot\text{OH}} = 8.1 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$.

TABLE III. YIELD OF METHIONINE SULFOXIDE FROM METHIONINE SOLUTION (10^{-3} M)

	Methanol concentration (M)	G-value	
		Found	Calculated ^{a)}
Deaerated	10^{-1}	0.10	0.02
	1	0.12	0.00
with nitrous oxide	1	0.19	

^{a)} From equation (1)

However, further addition of methanol could not depress the yield of methionine sulfoxide as shown in Fig. 2 and Table III. The experimental values are higher than that calculated by equation (1). An explanation of this fact is that methionine sulfoxide is also produced by e_{aq}^- attack under such conditions. To examine this possibility, experiment using nitrous oxide as e_{aq}^- scavenger was carried out. As $k_{e_{\text{aq}}^- + \text{N}_2\text{O}} = 8.7 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}$,¹³⁾ $k_{e_{\text{aq}}^- + \text{Methionine}} = 3.5 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$,¹⁴⁾ $\text{N}_2\text{O} = 10^{-2}$ M, it follows that in a solution containing 10^{-3} M methionine, most of e_{aq}^- are converted to $\cdot\text{OH}$ radicals. Therefore, if methionine sulfoxide is produced by e_{aq}^- attack, yield of methionine sulfoxide from solution containing nitrous oxide must be lower than that without nitrous oxide. The result listed in Table III does not agree with this assumption and denies surely that possibility.

The second explanation is that methionine sulfoxide is produced by the reaction with secondary radicals produced from methanol. Under this condition, the G -value of $\cdot\text{OH}$ radicals attacking methionine and methanol is given approximately 5, by $G(e_{\text{aq}}^-) \simeq 2.9$ and $G(\cdot\text{OH}) \simeq 2.4$.¹⁵⁾ G -value of $\cdot\text{OH}$ radicals which attacks methionine is obtained by equation,

$$\frac{\text{number of radicals attacking methionine}}{\text{number of radicals attacking methanol}} = \frac{k_2[\text{Methionine}]}{k_1[\text{Methanol}]} \quad (2)$$

and under the experimental conditions listed

in Table III, it is calculated as $5 \times (8.1 \times 10^9 \times 10^{-3}) / (8.7 \times 10^8 \times 1) \simeq 0.05$. Even if all the molecules of methionine which were attacked by $\cdot\text{OH}$ radicals were converted into methionine sulfoxide, the yield could not exceed 0.05. The experimental values listed in Table III are higher than this value. From this fact, it would be concluded that methionine sulfoxide is produced by the reaction with secondary radicals from methanol; *e.g.*, $\text{Methionine} + \cdot\text{CH}_2\text{OH} \rightarrow \text{Methionine sulfoxide}$.

Radiolysis of methionine in aqueous pork extract

Water extract of pork was diluted with triply distilled water and methionine was added at a concentration of 10^{-3} M. After irradiation (4×10^4 rad), the yields of α -aminobutyric acid and methionine sulfoxide were determined. The yields of such products should be depressed by addition of pork extract containing substances which scavenge primary species.

In a previous paper,¹⁶⁾ we found that pork extract containing many substances can be regarded kinetically as a solution containing one e_{aq}^- scavenger. In this paper, we elucidate that α -aminobutyric acid is produced by e_{aq}^- attack. If simple competition exists between methionine and the extract toward e_{aq}^- to give α -aminobutyric acid, a linear relation should be obtained between the reciprocal of $G(\alpha\text{-Aminobutyric acid})$ and concentration of the extract. As shown in Fig. 3, the experimental data satisfy perfectly this relation. From this result, it may be concluded that, in pork extract, α -aminobutyric acid is produced by e_{aq}^- attack only.

However, the result of determination on methionine sulfoxide is complicated. In this case, high G -value was observed in high concentration of extract was shown in Fig. 3. This finding is similar to that obtained in the experiment using methanol as second solute. It may be concluded that, in pork extract, methionine sulfoxide is produced not only by $\cdot\text{OH}$ radical attack, but also by the reaction with secondary radicals derived from sub-

Fig. 3. Depression of the Yields of Products from Methionine by Water Extract of Pork.

○—○ α -Aminobutyric acid, □—□ Methionine sulfoxide.

stances in the extract.

In food irradiation, sulfur-containing compounds contribute to many parts in production of off-flavor. Generally, oxidation and/or reduction at sulfur atom influence considerably their odor. The result reported in this paper that oxidation of sulfur-containing compounds to sulfoxide is caused by secondary radical attack to large extent maybe important from this point of view.

In this research, the authors studied the reaction of methionine with secondary radicals produced from methanol and substances in pork extract. This type of reaction may contribute to not small part of degradation of water-soluble food constituents when foods are irradiated.

REFERENCES

- 1) H. Loman, S. Voogd and J. Blok, *Radiation Res.*, **42**, 437 (1970).
- 2) M. Morita, M. Tajima and M. Fujimaki, *Agr. Biol. Chem.*, **33**, 250 (1969).
- 3) M. Tajima, M. Morita and M. Fujimaki, *ibid.*, **33**, 1277 (1969).

- 4) E. L. Wick, M. Koshika and J. Mizutani, *J. Food Sci.*, **30**, 433 (1965).
  - 5) M. Morita and M. Fujimaki, *Bull. Chem. Soc. Japan*, **41**, 1109 (1968).
  - 6) S. Kato, T. Kurata and M. Fujimaki, *Agr. Biol. Chem.*, **33**, 1144 (1969).
  - 7) J. J. Kolb and G. Toennies, *Anal. Chem.*, **24**, 1164 (1952).
  - 8) F. Shimazu, U. S. Kumta and A. L. Tappel, *Radiation Res.*, **22**, 276 (1966).
  - 9) J. Kopoldova, J. Liebster and E. Cross, *ibid.*, **30**, 261 (1967).
  - 10) A. Fojtik, J. Kopoldova and R. Brdicka, *Collection Czechoslov. Chem. Commun.*, **34**, 3623 (1969).
  - 11) H. Hatano, *Food Irradiation, Japan*, **3**, 85 (1968).
  - 12) M. Fujimaki and M. Morita, *Agr. Biol. Chem.*, **32**, 574 (1968).
  - 13) G. Scholes and M. Simic, *J. Phys. Chem.*, **68**, 1731 (1964).
  - 14) R. Braams, *Radiation Res.*, **27**, 319 (1966).
  - 15) A. O. Allen, *Radiation Res., Suppl.*, **4**, 54 (1964).
  - 16) M. Morita, H. Eguchi, M. Tajima and M. Fujimaki, *Agr. Biol. Chem.*, **34**, 547 (1970).
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