Decomposition of Linear Dodecylbenzenesulfonate by Simultaneous Treatment with Ozone and Ultraviolet Irradiation: Rapid Disappearance of Formed Mutagens

Kazuya MURAKAMI,[a,b,1)] Hisao MATSUMOTO,[a] Takemi KINOUCHI[b] and Yoshinari OHNISHI*,[b]

Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University,[a] Yamashiro-cho, Tokushima 770, Japan and Department of Bacteriology, School of Medicine, The University of Tokushima,[b] Kuramoto-cho, Tokushima 770, Japan. *Received August 23, 1991*

The decomposition products and mutagenic activity in *Salmonella typhimurium* strains TA98, TA100 and TA104 in the presence and absence of S9 mix of linear dodecylbenzenesulfonate (DBS) in aqueous solution after ozone treatment alone or simultaneous treatment with ozone and ultraviolet (UV) irradiation (ozone/UV treatment) were investigated. The decomposed DBS solutions after these treatments for 4 h were mutagenic for strains TA98, TA100 and TA104 both with and without S9 mix, but this mutagenicity disappeared rapidly during further ozone/UV treatment. Mutagenicity of the decomposed solution of DBS, however, was not substantially decreased by treatment with ozone alone. Formaldehyde and glyoxal were identified as the decomposition products of DBS in water by high-performance liquid chromatography after treatment with 2,4-dinitrophenylhydrazine. Although these two compounds were mutagenic for strain TA104 both with and without S9 mix, they disappeared after further ozone/UV treatment but not after ozone treatment alone. These results indicate that ozone/UV treatment is an effective procedure for purifying drinking water.

Keywords decomposition; dodecylbenzenesulfonate; ozone; ozone-UV irradiation; mutagenicity; Ames assay

Introduction

Linear alkylbenzenesulfonates (LAS) have been widely used as synthetic detergents for more than ten years. Although LAS are known to be decomposed easily by bacteria in aqueous environments, they have been detected in various environmental waters and sediments.[2-4)] Ozone has been used for the purification of drinking water since 1906.[5)] Today, throughout the world, there are more than 1000 drinking water treatment plants in which ozonation is used for disinfection and other purposes.[5)] Ozone oxidizes a large number of substances, including color and odor substances as well as bacteria found in raw water.[5,6)] However, it is difficult to decompose organic materials completely by ozone treatment alone,[7,8)] and, moreover, mutagens are formed as decomposition products. Sayato *et al.*, for example, reported mutagenic substances following the ozonation of naphthoresorcinol, a constituent of humic substances.[9)] Similarly, Heming *et al.* reported that the chlorination of drinking water produced a strong mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX).[10)] These research findings indicate that advanced purification systems for drinking water are needed.

It has been shown that ozone treatment in combination with ultraviolet (UV) irradiation (ozone/UV treatment) is a more powerful oxidizing procedure than ozone treatment alone, because the simultaneous treatment method involves the formation of excited organic species which subsequently react with formed hydroxyl radicals.[11-13)] In previous papers, we reported that LAS in water and in various river waters were decomposed completely by ozone/UV treatment.[8,14)] In the present study, we report that the mutagenic products formed from linear dodecylbenzenesulfonate (DBS), one type of LAS, disappeared more rapidly after ozone/UV treatment than they did after ozone treatment alone.

Materials and Methods

Materials Sodium linear DBS with purity above 95% was purchased from Wako Pure Chemicals, Osaka. Dow-Corning silicone antifoam DB-110N emulsion, 2,4-dinitrophenylhydrazine, acetaldehyde and formaldehyde were purchased from Nacalai Tesque Inc., Kyoto. Glyoxal was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and all other materials were of reagent grade or better.

Decomposition Procedure The reaction apparatus and procedures for decomposition by ozone/UV treatment were described previously.[8)] Ozone was generated from oxygen using a Nippon Ozon O-1-2 ozonizer, and a 15 W low-pressure mercury lamp was used to UV irradiate 200 ml of 1×10^{-2} M aqueous DBS solution from the interior of a vessel. We used high concentration of DBS to assay mutagenicity of the decomposed DBS and to measure concentration of the decomposed products. During the treatment, 70 mg of the antifoam reagent was added to the solution ten times at appropriate intervals to suppress foaming. Ozone production was adjusted to high concentration (11.5 mg/ml) at a flow rate of 833 ml/min because the concentration of DBS was high.

Analytical Methods During decomposition, samples were taken from the reactor and DBS was separated by high-performance liquid chromatography (HPLC) using the procedure described in Matsumoto *et al.*[8)] HPLC determinations were carried out using a liquid chromatograph (Shimadzu LC-6A), equipped with a fluorescence detector (Shimadzu RF-530). DBS was separated on a Cosmosil $5C_8$ column (4.6 mm i.d. × 150 mm) at 40 °C with a mobile phase of methanol–water (3 : 1) containing 0.1 M sodium perchlorate at a flow rate of 1.5 ml/min. The column effluent was monitored with a detector adjusted to $\lambda_{ex.}$ 228 nm and $\lambda_{em.}$ 298 nm.

The decomposition products, and standard aqueous solutions of formaldehyde, acetaldehyde and glyoxal were treated with aqueous dinitrophenylhydrazine solution under acidic conditions, and their dinitrophenylhydrazone derivatives were extracted with dichloromethane.[15-17)] The dinitrophenylhydrazones were separated by HPLC (Tosoh CCPD) using a Cosmosil octadecylsilane (ODS) column (4.6 mm i.d. × 100 mm) at 40 °C with a mobile phase of acetonitrile–water (4 : 3) at a flow rate of 1.0 ml/min. The column effluent was monitored with a variable-wavelength UV-visible detector (Tosoh UV-8000) adjusted to 360 nm. The decomposition products were identified by their retention times.

Mutagenicity Test The mutation frequency was assayed by the pre-incubation method (37 °C for 20 min) described in Yahagi *et al.*,[18)] using *Salmonella typhimurium* strains TA98, TA100 and TA104.[19,20)] Strain TA104 is more sensitive than other strains for identifying mutagenic carbonyl compounds.[20)] Liver S9 fraction (50 μl) that was obtained from Sprague-Dawley rats pretreated with phenobarbital and 5,6-benzoflavone was used as a component of S9 mix for exogenous metabolic activation.[21)] All determinations were made in duplicate plates in at least two independent experiments.

Results

Decomposition Products of DBS Aqueous solutions of DBS (1×10^{-2} M) were decomposed by ozone/UV treatment or ozone treatment alone. During decomposition,

© 1992 Pharmaceutical Society of Japan

NII-Electronic Library Service

70 mg of the antifoam reagent was added ten times at appropriate intervals to suppress foaming in the reactor. Figure 1 shows a typical HPLC chromatogram of the dinitrophenylhydrazone derivatives of DBS products following 4 h of ozone/UV treatment. Using retention times of the dinitrophenylhydrazones, two decomposition products were identified as formaldehyde and glyoxal,

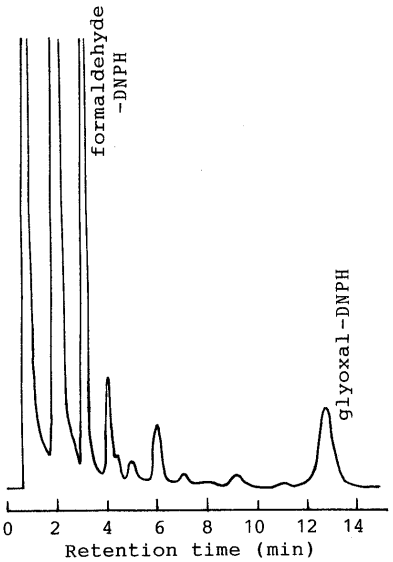

Fig. 1. High-Performance Liquid Chromatogram of the Dinitrophenylhydrazones (DNPHs) of Formaldehyde and Glyoxal Produced from DBS by Ozone/UV Treatment for 4 h

Retention time of acetaldehyde–DNPH: 3.9 min.

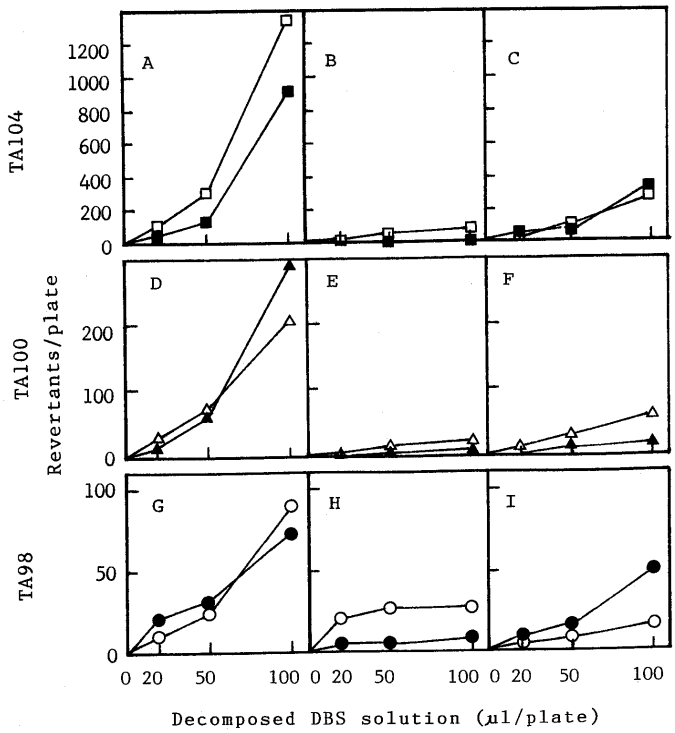

Fig. 2. Dose–Response Curves of Mutagenic Activity of Decomposed DBS

A, D, G: by ozone/UV treatment for 4 h, B, E, H: by ozone/UV treatment for 8.5 h, C, F, I: by ozone treatment alone for 16 h. TA104; □ (−S9), ■ (+S9): TA100; △ (−S9), ▲ (+S9): TA98; ○ (−S9), ● (+S9). The number of spontaneous revertants per plate of 407 (TA104, −S9), 521 (TA104, +S9), 135 (TA100, −S9), 165 (TA100, +S9), 36 (TA98, −S9) and 46 (TA98, +S9) was subtracted.

following the 4 h exposure of DBS to ozone/UV. Both compounds were also formed after ozone treatment alone, although acetaldehyde was not detected as a decomposition product. Since formaldehyde and glyoxal were not produced from the antifoam reagent itself during ozone/UV treatment or ozone treatment alone (data not shown), it was clear that both compounds were formed from DBS in the two procedures.

Mutagenicity of Decomposed DBS in Water The mutagenicity of decomposed DBS in water after ozone/UV treatment for 4 and 8.5 h, and after ozone treatment alone for 16 h, was assayed in strains TA98, TA100 and TA104 both with and without S9 mix (Fig. 2). The mutagenicity could not be assayed at a concentration of more than 10^{-4} M DBS because of its bacteriocidal action. Judging from the dose–response curves shown in Fig. 2A, D and G, the decomposed DBS solution after ozone/UV treatment for 4 h was mutagenic for strains TA98, TA100 and TA104 both with and without S9 mix. Strain TA104 was most sensitive to the decomposed products among these strains. Since antifoam reagent and decomposed antifoam reagent in water by ozone/UV or ozone treatment was not mutagenic (data not shown), and DBS solution was also not mutagenic at a concentration of less than 10^{-4} M, it was clear that the mutagenicity was due to

Table I. Mutagenicity of Decomposed DBS Solution for Strain TA104 after Ozone/UV Treatment

Treatment time (h)	Mutagenicity (His$^+$ revertants/plate)[a]	
	(−)S9	(+)S9
4.0	1364	918
5.5	197	156
7.0	83	16
8.5	92	0

a) The number of spontaneous revertants per plate shown in Fig. 2 was subtracted.

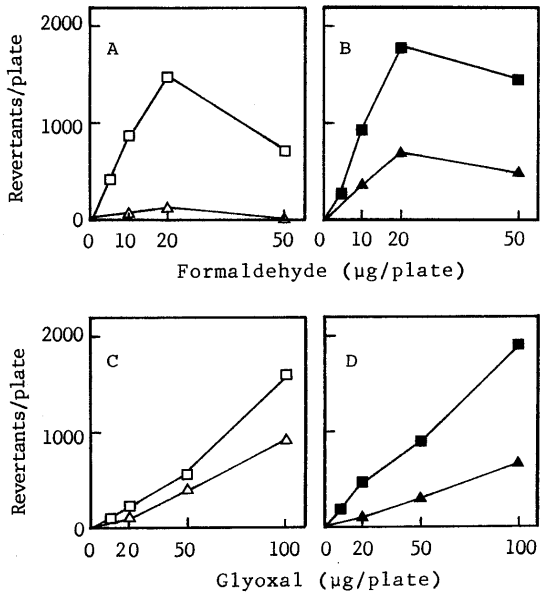

Fig. 3. Dose–Response Curves of Mutagenic Activity of Formaldehyde (A, −S9 and B, +S9) and Glyoxal (C, −S9 and D, +S9)

TA104; □ (−S9), ■ (+S9): TA100; △ (−S9), ▲ (+S9). The number of spontaneous revertants per plate shown in Fig. 2 was subtracted.

NII-Electronic Library Service

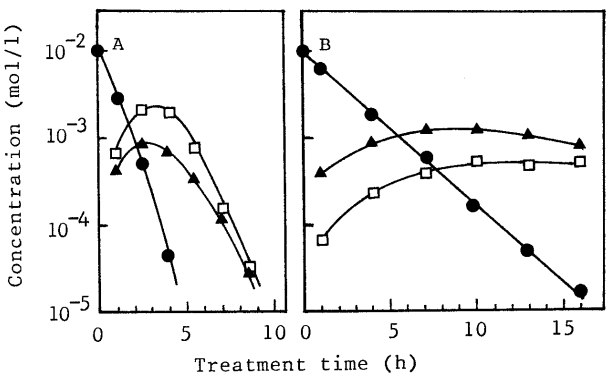

Fig. 4. Decomposition of DBS and Production of Formaldehyde and Glyoxal by Ozone/UV Treatment (A) and by Treatment with Ozone Alone (B)

DBS (●), formaldehyde (□), glyoxal (▲).

decomposed DBS products following ozone/UV treatment. Moreover, almost all of the mutagenicity disappeared after the treatment for 8.5 h (Fig. 2 B, E and H, and Table I). However, the mutagenicity of the DBS solution decreased slowly by treatment with ozone alone (Fig. 2 C, F and I).

In order to study the possibilities of the interaction of formaldehyde, glyoxal, decomposed DBS and decomposed antifoam reagent, and of the effect of DBS on the mutagenic activity of decomposed DBS solution, the mutagenic activities of the following solutions were measured by strain TA104 with and without S9 mix: a solution containing the same concentration of formaldehyde and glyoxal as in the reactor after ozone/UV treatment for 4 h, solutions containing the same concentration of formaldehyde, glyoxal and various concentrations of DBS, and solutions containing the same concentration of formaldehyde, glyoxal, DBS and antifoam reagent as in the reactor. There was no interaction and no effect (except its bacteriocidal action) observed in these experiments (data not shown).

Mutagenicity of Formaldehyde and Glyoxal As shown in Fig. 3, formaldehyde was mutagenic as indicated by the dose–response curves for strain TA104 both with and without S9 mix and strain TA100 with S9 mix. Glyoxal was also mutagenic for strains TA104 and TA100 both with and without S9 mix. These results indicate that the mutagenic activity of decomposed DBS solution, at least part of it, could be due to formaldehyde and glyoxal formed from the DBS.

Time Required for Disappearance of DBS and Decomposed Products Figure 4 shows the time required for DBS decomposition and the formation of by-products after ozone/UV treatment and ozone treatment alone. DBS was decomposed and decreased to less than 0.1% of the initial concentration for 5.5 h during ozone/UV treatment, but remained at a concentration of more than 10^{-5} M even 16 h after ozone treatment alone. The decomposition rate of DBS during ozone/UV treatment was almost 3-fold greater than during ozone treatment alone.

Formaldehyde and glyoxal formed rapidly and disappeared during ozone/UV treatment. The maximum concentration of the formed glyoxal was almost the same in the treatments with ozone/UV and with ozone alone. However, the maximum concentration of the formed formaldehyde was different in the two procedures: 2.2×10^{-3} M at 2.5 h during ozone/UV treatment and 5.6×10^{-4} M at 10 h during treatment with ozone alone.

Discussion

Alkylbenzenesulfonates have been used as synthetic detergents and have caused water pollution problems by contaminating drinking water. Advanced purification systems for drinking water are being developed in many parts of the world to safeguard human health. One of these, ozone treatment, is an effective procedure for purification of raw drinking water containing color and odor substances. However, the effects on human health of organic materials decomposed by ozone treatment are not clear. It was recently reported that ozonated organic materials in water have mutagenic activity.[9] Furthermore, decomposition products such as the carbonyl compounds formic acid, oxalic acid, glyoxal, glyoxylic acid and maleic acid are formed from aromatic compounds in water after ozone treatment.[17,22,23] In this study, we used two procedures, ozone and ozone/UV treatment, to decompose aqueous DBS solution and promote degradation of mutagenic products, formaldehyde and glyoxal.

Ozone treatment to degrade DBS produced low concentration of formaldehyde (Fig. 4), however, ozone/UV treatment produced it in high concentration. The concentration of glyoxal produced from DBS by the two treatments was almost the same. These observations indicated that the benzene ring of DBS was decomposed easily by both procedures, but that decomposition of the alkyl chain of DBS was accomplished more easily by the ozone/UV treatment, because glyoxal is formed from the benzene ring and formaldehyde is formed from the alkyl chain.[17]

This study showed that the mutagenic products, formaldehyde and glyoxal, were formed from synthetic detergent DBS in water following ozone/UV treatment and treatment with ozone alone. However, the mutagens formed disappeared more rapidly after the combined treatment than with ozone alone. These results clearly indicate that mutagenic substances formed from organic materials are decomposed by ozone/UV treatment, and that this treatment is, therefore, a helpful procedure for the purification of drinking water.

Acknowledgments This work was supported in part by Grants-in-Aid for cancer research from the Ministry of Health and Welfare and the Ministry of Education, Science and Culture of Japan.

References and Notes

1) Present address: *Department of Bacteriology, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima 770, Japan.*
2) M. Kikuchi, A. Tokai and T. Yoshida, *Water Res.*, **20**, 643 (1986).
3) J. McEvoy and W. Giger, *Environ. Sci. Technol.*, **20**, 376 (1986).
4) H. Yokoyama, K. Kawata and N. Shibuya, *Eisei Kagaku*, **34**, 291 (1988).
5) R. G. Rice, C. M. Robson, G. W. Miller and A. G. Hill, *J. Am. Water Works Assoc.*, **73**, 44 (1981).
6) W. H. Glaze, *Environ. Sci. Technol.*, **21**, 224 (1987).
7) A. S. C. Chen, V. L. Snoeyink and F. Fiessinger, *Environ. Sci. Technol.*, **21**, 83 (1987).
8) H. Matsumoto, K. Murakami and R. Matsumoto, *Eisei Kagaku*, **35**, 408 (1989).
9) Y. Sayato, K. Nakamuro and H. Ueno, *Mutat. Res.*, **189**, 217

NII-Electronic Library Service

(1987).
10) J. Heming, B. Holmbom, M. Reunanen and L. Kronberg, *Chemosphere*, **15**, 549 (1986).
11) G. R. Peyton, R. Y. Huang, J. L. Burleson and W. H. Glaze, *Environ. Sci. Technol.*, **16**, 448 (1982).
12) H. W. Prengle, *Environ. Sci. Technol.*, **17**, 743 (1983).
13) R. A. Sierka and G. L. Amy, *Ozone; Sci. Eng.*, **7**, 47 (1985).
14) K. Murakami, T. Kinouchi, S. Akimoto, Y. Ohnishi and H. Matsumoto, *Eisei Kagaku*, **36**, 542 (1990).
15) M. Jarret, A. Bermond and C. Ducauze, *Analysis*, **11**, 185 (1983).
16) Y. Sayato, K. Nakamuro and H. Ueno, *Eisei Kagaku*, **34**, 451 (1988).
17) K. Murakami and H. Matsumoto, *Eisei Kagaku*, **36**, 62 (1990).
18) T. Yahagi, M. Nagao, Y. Seino, T. Matsushima, T. Sugimura and M. Okada, *Mutat. Res.*, **48**, 121 (1977).
19) D. M. Maron and B. N. Ames, *Mutat. Res.*, **113**, 173 (1983).
20) L. J. Marnett, H. K. Hurd, M. C. Hollstein, D. E. Levin, H. Esterbauer and B. N. Ames, *Mutat. Res.*, **148**, 25 (1985).
21) T. Matsushima, M. Sawamura, K. Hara and T. Sugimura, "*In Vitro* Metabolic Activation in Mutagenesis Testing," ed. by F. J. de Serres, J. R. Fouts, J. R. Bend and R. M. Philpot, Elsevier/North-Holland Biomedical Press, Amsterdam, 1976, pp. 85—88.
22) Y. Yamamoto, E. Niki, H. Shiokawa and Y. Kamiya, *J. Org. Chem.*, **44**, 2137 (1979).
23) B. Legube, S. Guyon, H. Sugimitsu and M. Dore, *Water Res.*, **20**, 197 (1986).

NII-Electronic Library Service