Mutagenicity of Activated Carbon Adsorbate of Drinking Water in the Ames Assay

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Biochemical Laboratory, Niigata College of Medical Technology, Niigata 950-21, *Department of Biology, Niigata College of Pharmacy, Niigata 950-21, and †Department of Hygiene and Preventive Medicine, Niigata University School of Medicine, Niigata 951

SHIBUYA, N., OHTA, T., NAKADaira, H., MANO, H., ENDOH, K. and YAMAMOTO, M. Mutagenicity of Activated Carbon Adsorbate of Drinking Water in the Ames Assay. Tohoku J. Exp. Med., 1993, 171 (1), 89-95 —— Mutagenicity of activated carbon adsorbate from drinking water collected in Niigata City was assayed by the Ames assay. Adsorbate was extracted from activated carbon with benzene, and then with ethanol. Although the benzene extract was not mutagenic, the ethanol one showed the mutagenic activity for Salmonella typhimurium strains TA98 and TA100 with and without S9 mix. The ethanol extract was much more mutagenic on TA100 than TA98 both with and without S9 mix. The mutagenic activity per liter of water was found to be the strongest in winter and the weakest in summer.

——— drinking water; mutagenicity; Ames assay; activated carbon adsorption

Genotoxic activity, especially mutagenicity by the Ames assay, of organic chemicals in drinking water has been investigated by a number of investigators, as Meier (1988) reviewed. In these studies, Amberlite XAD resins were widely used as adsorbents. XAD resins generally adsorb the directly acting mutagens, e.g., organic halogens such as 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone referred to as MX (Hemming et al. 1986; Kronberg and Vartiainen 1988), but not the indirect mutagens. In the case of activated carbon (AC), however, various kinds of chemicals are known to be adsorbed. According to the report by Kool et al. (1982), AC adsorbed not only the direct mutagens, but also the indirect ones from drinking water. Therefore, we adopted AC instead of XAD resins for the extraction of the mutagenic chemicals from drinking water. In addition to the study on the Ames mutagenicity of AC adsorbate, the seasonal changes in mutagenic activity of drinking water are examined in the present report.

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MATERIALS AND METHODS

Adsorption from water and elution

In Niigata City, the raw water for drinking is taken from the lower reaches of the Shinano and Agano rivers and treated by the conventional method at waterworks: alum flocculation followed by clarification, sand filtration and chlorine disinfection, and supplied. In this experiment, drinking water was sampled from an elevated water tank located in the western part of Niigata City where water from the Sinano river is used.

Granular AC (100 g), purchased from Wako Pure Chemical Industries, Ltd. (Osaka), was washed with 500 ml of distilled water by using ultrasonication for 30 min and packed into a chromatographic column (26 mm I.D.). One liter of the drinking water for one experimental day was passed through the column at a flow-rate of ca. 500 ml/min at room temperature and the procedure was repeated 80 times during four months period: Oct. 13, 1987-Feb. 13, 1988. The treated AC was placed on filter paper (No. 2) obtained from Toyo Roshi Inc., (Tokyo) to remove excess water and then lyophilized. The AC (20 g) was placed in a Soxhlet for 48 hr with 500 ml of benzene, and then for 48 hr with 500 ml of ethanol. These solvents were evaporated to dryness under reduced pressure and the residue was dissolved in 3 ml of dimethylsulfoxide (DMSO). The DMSO solution was stored at -80°C and assayed in the Ames mutagenicity test.

In order to study the seasonal changes in mutagenicity of drinking water, we carried out the procedure described above in four seasons: Feb. 13, 1988-May 17 (Spring), May 19-Sep. 6 (Summer), Sep. 8-Dec. 2 (Autumn) and Dec. 5-Feb. 27, 1989 (Winter). The extraction was performed with 500 ml of ethanol for 24 hr, and the total amount of lyophilized AC were about 101 g. Benzene extraction was not undertaken because of its the low mutagenic activity as compared with ethanol one.

Mutagenicity assay

The assays with Salmonella typhimurium strains TA98 and TA100 were performed according to Yahagi et al. (1977) using the preincubation method. S9 preparations from livers of Sprague-Dawley rats induced with Aroclor 1254 (Maron and Ames 1983) were purchased from Litton Bionetics, Inc. (Kensington, MD, USA). To each plate, 20 μg of S9 was added. Assays were conducted with duplicate plates in different 4–7 doses in the range of about 3 μg–5 mg extract per plate. Mutagenic activity, expressed as revertants per mg extract, was calculated from the linear part of dose-effect curve. Revertants per liter of water was calculated from the revertants per mg extracts and weight of extract per liter of water. DMSO was used as a negative control and the background level of reversion was determined. As positive control mutagens, 4-nitroquinoline N-oxide (4NQO) and 2-aminoanthracene (2AA), and the ethanol extract from AC control were used.

In the absence of S9 (−S9), 0.025 μg 4NQO gave 256 ± 43 (mean ± S.D.) net revertants per plate with strain TA100 and 0.2 μg 4NQO did 264 ± 78 net revertants per plate with strain TA98. In the presence of S9 (+S9), 2 μg 2AA gave 531 ± 56 net revertants per plate with strain TA100 and 393 ± 55 net revertants per plate with strain TA98. The numbers of spontaneous background revertants per plate were 106 ± 17 for TA100−S9, 93 ± 18 for TA100+ S9 18 ± 8 for TA98−S9 and 32 ± 7 for TA98+ S9. In the case of ethanol extract from the untreated AC, no mutagenic activity was demonstrated in the four test systems described above.

The samples showing a mutagenicity level of more than 2-fold spontaneous revertants, and showing the dose-effect relationship were evaluated to be mutagenic.

RESULTS

The benzene extract from 20 g of AC was 1.13 mg per one liter of water, but
it did not show mutagenicity regardless of the strains, with and without S9 mix, even though doses expanding from 4.53 to 597 µg per plate were applied. The weights of ethanol extract were 5.15 mg per one liter of water and 4.6 times heavier than the benzene extract. The ethanol extract showed mutagenicity in all the four test systems (Table 1 and Fig. 1). The bactericidal toxicity appeared in the TA98-S9 group, when the maximum dose (2.72 mg/plate) was applied to the plate (Fig. 1). The mutagenic activity of ethanol extract was about three times stronger for TA100 than for TA98 both with and without S9 mix. Regardless of the type of the tester strain, the effect of the presence of S9 mix on the mutagenic activity was not found clearly.

Fig. 2 shows the seasonal changes in weights of extracts and mutagenic

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<tr>
<th>Ethanol extract (mg/l)</th>
<th>Revertants per mg extract</th>
<th>Revertants per liter water</th>
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<tr>
<td></td>
<td>TA100</td>
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<td>−S9 +S9</td>
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<tr>
<td>5.15</td>
<td>201 188</td>
<td>71 64</td>
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<td>1035 968</td>
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Benzene extract weighted 1.13 mg per liter water, but did not show mutagenicity. Results were from linear dose-effect curves, as shown in Fig. 1.
activity of drinking water. From 20 g of AC treated with drinking water, the extracts weighted 4.97, 2.48, 1.65, 2.33 mg per liter of water in spring, summer, autumn and winter, respectively.

Mutagenicity for TA100 with and without S9 mix was observed in every season. When seasonal changes of the mutagenic activity are described in terms of per mg extract, there is a tendency to increase from spring to winter in the TA100-S9 test system. In the TA100+S9 test system, however, the mutagenic activity was almost constant and relatively low level in spring, summer and autumn, but high in winter. When the mutagenic activities of per liter of water are compared among four seasons, these in spring, summer and autumn did not change greatly as compared with the mutagenic activity in winter in the TA100-S9 system. In the TA100+S9 system, the activity was the strongest in winter and tended to decrease gradually from spring to autumn.

For the strain TA98, only weak mutagenic activity was found in three seasons.

Fig. 2. Weight and mutagenicity of activated carbon adsorbate from seasonal drinking water (Niigata City) on Salmonella typhimurium tester strain TA100 with or without S9 mix. Adsorbate was extracted with ethanol from activated carbon, through which the seasonal water was passed (top-left). Mutagenicity was scored from the results showing the linear dose-effect curves and expressed in numbers of revertant colonies per mg extract (top-right) or per liter of water (bottom). The number of spontaneous revertants was subtracted. Spring, Feb. 13, 1988–May 17; Summer, May 19–Sep. 6; Autumn, Sep. 8–Dec. 2; Winter, Dec. 5–Feb. 27, 1989.
besides winter. Especially, in the test system without S9 mix, test samples were not evaluated to be mutagenic. The extract of drinking water collected in winter, however, showed strong mutagenicity; 124 and 113 revertants per mg extract and 228 and 268 per liter of water in the treatment groups with and without S9 mix, respectively.

As a preliminary experiment, histidine content in AC treated with drinking water was measured by means of high performance liquid chromatography, since the presence of histidine affects the growth of *Salmonella typhimurium* tester strains. But histidine was not detected.

**DISCUSSION**

Nearly 1100 organic compounds were identified in a survey of drinking waters from five cities in the USA (Lucas 1985). Moreover, studies from countries throughout the world have documented the widespread presence of genotoxic activity, especially Ames mutagenicity, in organic concentrates of drinking water (Meier 1988). In these studies, XAD resins have been extensively used for the extraction of mutagenic chemicals. For the evaluation of these experimental results, the distinction of adsorbent materials is important, since XAD resins adsorb mainly direct mutagens and AC adsorbs both direct and indirect mutagens. XAD resins have been known as the effective adsorbent for direct mutagens, e.g., chlorinated butenoic acids such as MX in drinking water (Tikkanen and Kronberg 1990). In this case, however, an inference should be carefully made, since the mutagens lose their activity after S9 treatment in the Ames assay. As long as we use XAD resins alone, the lack of information concerning whether or not the mutagens in drinking water have carcinogenic potential in humans after metabolism may be a serious problem. Therefore we adopted AC instead of XAD resins.

In a preliminary experiment, the extraction from AC was carried out with benzene followed by ethanol extraction. In this procedure, only the extract with ethanol showed mutagenic activity. It is generally said that AC adsorbs hydrophobic compounds more effectively than hydrophilic ones. In order to extract aromatic-organics such as polycyclic aromatic hydrocarbons, which are characterized as hydrophobic compounds, benzene may be the best solution. It is likely that the drinking water collected in Niigata City has little aromatic-organics mutagens, but much polar and hydrophilic ones. Although any mutagenic chemicals in drinking water have not been identified in our laboratory, they are characterized as basechange type mutagens rather than frameshift ones, judging from the findings in the Ames mutagenicity test by using *Salmonella typhimurium* strains TA98 and TA100.

A number of investigators observed the mutagenic activity in drinking water. Little has been, however, studied about the seasonal change of the mutagenicity of drinking water. Sayato et al. (1991) assayed XAD-2 adsorbate of chlorinated drinking water by using *S. typhimurium* TA100 without metabolic activation and
found that its mutagenicity was strong in the samples collected in autumn and winter and weak in summer. In the present study, we analyzed the seasonal change of the mutagenicity of AC adsorbate by using the Ames mutagenicity test. Strongest mutagenic activity was obtained in winter, but weak mutagenicity or negative result was observed in summer. It is suspected at first that the seasonal changes were due to the difference of adsorption temperature, i.e., water temperature. In a preliminary study, drinking water was treated with AC at two different degrees of temperature (1°C and 26°C), but the mutagenicity of AC adsorbate was not affected by the temperature (data not shown).

It has been documented that the mutagenic compounds, detected by use of TA100-S9 test system, are probably the products of reaction of chlorine with humic substances in water (Meier et al. 1983; Kronberg et al. 1985). In drinking water, the mutagenic activity found by TA100-S9 test system does not correlate with the amount of total organic carbon, but with the total organic halogen (Monarca et al. 1985). As another likely explanation is that the seasonal change of the mutagenic activity for the TA 100-S9 test system appears to be due to the difference in the quality, not in the quantity, of the organic matter in source water. For example, the winter water may contain many humic substances and the drinking water may have more reaction products with chlorine in spite of the small amount of extract. However, chlorination itself does not explain the increase of mutagenicity in water, because chlorination occurs more extensively in summer, when the germs vigorously propagate, than winter at Niigata waterworks.

The last explanation is that the mutagens extracted from AC may be biotransformation products from microbial action in the AC, especially in winter season. A further analytical study should be carried out so as to explain the reason why the mutagenic activity increase in winter.

References


