Validity of Mutagenic Activity as an Indicator of River Water Pollution

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Abstract

Objectives: To investigate if mutagenicity could be expressed by known water pollution indicators, we determined the mutagenic activity of blue rayon extracts from sampled river water with the Ames test utilizing new strains of bacteria, and compared the results with those of known indicators of water pollution.

Methods: Water samples were collected by the blue rayon adsorption method at sixteen sites in six rivers in the North Kyushu district. The assay of mutagenicity was carried out using the Ames test. The test strains were *Salmonella typhimurium* TA100, YG1024, YG1041 and YG1042. B(a)P, Trp-P-1 and Trp-P-2 were quantified by HPLC. Determinations of SS, BOD, COD, T-N, T-P, DOC, and A₂₆₀/DOC were performed.

Results: The extracts from five sampling sites showed higher mutagenicity toward strain YG1024 with or without S9mix, and the extracts from two of these five sites showed higher mutagenicity toward strain YG1041 with and without S9mix. However, the water pollution indicators did not show specific trends that were consistent with the mutagenic activity.

Conclusions: Since the mutagenic activity of river water could not be predicted using known water pollution indicators, we recommend that biological examinations such as mutagenicity tests be added to the indicators that are currently in use.

Key words: mutagenicity; Ames test; YG strains; river water; sewage

Introduction

Numerous organic pollutants have been found in the water environment (1–4). Their individual concentrations are very low, but certain unidentified substances may be mutagenic or carcinogenic. Detection of the combined effects of different chemicals of low concentrations is very difficult. Therefore, a mutagenicity test that is able to evaluate river water synthetically has been developed (1–5).

New strains of bacteria that show high levels of enzyme activity and that are sensitive to the mutagenic effects of some nitro-aromatic compounds have been established (6). Further, an adsorbent specific for some kinds of mutagens has been developed (7). Therefore, it is possible to determine mutagenicity in river water more sensitively and efficiently. To confirm the importance of the mutagenicity test as a water pollution indicator, it is necessary to perform the mutagenicity test

repeatedly comparing the results with those of known water pollution indicators.

Blue rayon is a rayon preparation using copper phthalocyanine trisulfonate as a covalently linked ligand (7). It adsorbs compounds with three or more fused rings. Although this method does not yield quantitative results, it is a simple method for extracting chemical compounds and can be applied to a greater number of samples for river water pollution tests than other methods (8).

To certify the validity of the mutagenicity test as a water pollution indicator, we determined the mutagenic activity of blue rayon extracts from the sampled river water and examined whether the mutagenicity could be detected from the results using known water pollution indicators.

Materials and Methods

Sampling

Water samples were collected by the blue rayon adsorption method as described below, at sixteen sites in six rivers (Tatara River, Naka River, Mikasa River, Hae River, Doumen River, and Siragane River) in the North Kyushu district.

Blue rayon in plastic mesh bags (1 g per bag) was hung in

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the river. Three bags were attached to a wooden float, and the depth of the hanging bag was about 20 cm. After 24 h, the blue rayon was taken out, washed with distilled water, dried with paper towels, and, later on, processed for mutagenic assay and chemical analysis by HPLC.

While the blue rayon was being hung in the river, we took water samples from each site and chemically analyzed them to estimate the condition of river water pollution (water pollution indicators). The analysis included suspended solid (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved organic carbon (DOC), absorbance at 260 nm/DOC (A_{260}/DOC), total nitrogen (T-N), and total phosphorus (T-P). The analyses of SS, BOD, COD, T-N and T-P for waters of rivers or lakes are provided by the water pollution prevention law in Japan (1970). A_{260}/DOC is useful as a predictor of trihalomethane (THM) formation potential, which was defined as the amount of THM formed in the water by chlorination (9).

The sampling was carried out in March 1997.

Solvent extraction

The dried blue rayon was eluted with methanol-concentrated ammonia (50:1, 160 ml/g blue rayon) for 30 min by gentle shaking. The elution was repeated, and eluents were combined. The solvent was removed by an evaporator. The samples were then dissolved in dimethylsulfoxide (DMSO) and sterilized through a 0.45 μm filter.

Mutagenic assay

The Assay of mutagenicity was carried out with the Ames test (10). The test was performed in duplicate with or without S9mix. An S9 fraction of phenobarbital/5,6-benzoflavone-pretreated male Sprague-Dawley (SD) rat liver was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). The S9mix contained 50 μl of S9 in a total volume of 500 μl . The test strains were *Salmonella typhimurium* TA100 and YG1024 for extracts from all sampled river waters, and YG1041 and YG1042 for extracts from the Mikasa River. The TA strain was kindly supplied by Dr. Bruce N. Ames, University of California, Berkeley, and the YG strains by Dr. T. Nohmi of the National Institute of Health Sciences. YG1024 is an *O*-acetyltransferase-overproducing derivative of strain TA98, strain YG1041 is a nitroreductase- and *O*-acetyltransferase-overproducing derivative of strain TA98, and strain YG1042 is the same derivative of TA100 (6). Revertant colonies were counted using an automatic colony counter (Colony Analyzer CA-9, Toyo sokki Co. Ltd., Tokyo, Japan). When the dose-response curve showed a 2-fold or greater increase over the spontaneous revertants, the mutagenicity of the sample was judged to be positive. Numbers of revertants per plate of positive controls were 960 (TA100 -S9mix, AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide 0.01 μg), 731 (TA100 +S9mix, B(a)P: benzo(a)pyrene 1.25 μg), 662 (YG1024 -S9mix, AF-2 0.05 μg), 990 (YG1024 +S9mix, B(a)P 2.5 μg), 37 (YG1041 -S9mix, AF-2 0.025 μg), 360 (YG1041 +S9mix, B(a)P 5 μg), 419 (YG1042 -S9mix, AF-2 0.05 μg), and 523 (YG1042 +S9mix, B(a)P 1.25 μg).

Quantification of B(a)P, Trp-P-1, and Trp-P-2 by HPLC

Chemical substances of extracts from the Mikasa River were quantified by HPLC with fluorescence detection with a Waters LC-Module 1, Mightysil ODS column (5 μm particle size, 250 $\times$ 4.6 mm i.d., Kanto Chemical Co., Inc.), and a Waters 470 scanning fluorescence detector, with mobile phases of 90% methanol+10% water for benzo(a)pyrene(B(a)P) and 20% acetonitrile+80% 20 mM phosphoric acid (pH 2) for 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). The analysis was performed with a column temperature of 40°C, flow rate of 0.8 ml/min, injection volume of 20 μl , and wavelengths (Ex, Em) for B(a)P of 370 nm, 420 nm and for Trp-P-1 and Trp-P-2 of 266 nm, 397 nm.

Determination of water pollution indicators

Determinations of SS, BOD, COD, T-N and T-P were performed according to the testing methods for industrial wastewater of Japanese Industrial Standard (JIS) K0102 (11). DOC was determined after passing samples through a 0.45 μm filter according to the total organic carbon (TOC) method of JIS K0102. A_{260}/DOC was determined by measuring absorbance at 260 nm through a 50 mm cell, and dividing the value by DOC.

At each sampling site, the determination of each item was repeated twice, and the results were averaged.

Results

Water pollution indicators

The results of the determination of river water pollution indicators are shown in Table 1. SS values were higher for the Hae River than other samples, while BOD and COD values were highest for the Siragane River. T-N values were higher for the Mikasa River than the other sites, and T-P values were highest for the Mikasa River and the Siragane River. There were no remarkable differences in the DOC and A_{260}/DOC parameters among the sixteen sampling sites.

Mutagenic activity assessed by the Ames test

The mutagenic activity of the extracts from the sixteen sites in strains TA100 and YG1024 with and without S9mix is shown in Table 2. The extracts from sites 3 and 4 of the Tatura River and site 1 of the Naka River showed mutagenic responses toward strain YG1024 without S9mix. This result indicates that these sites were more polluted with frame-shift type direct mutagens such as nitroarenes. The extracts from sites 2 and 3 of the Mikasa River showed higher mutagenic responses especially toward strain YG1024 with S9mix. This result indicates that these sites were more polluted with frame-shift type indirect mutagens such as aminoarenes. No sample showed mutagenic responses toward strain TA100, indicating that the extracts from blue rayons in the river were weakly polluted by base-substitution type mutagens.

The mutagenic activity of the extracts from the Mikasa River in strains TA100, YG1024, YG1041, and YG1042 with and without S9mix is shown in Table 3. The extracts from all the sampling sites of the Mikasa River showed higher mutagenic responses toward strains YG1041 without S9mix

Table 1 River water pollution indices at all sampling sites

Sampling site		SS (mg/l)	BOD (mg/l)	COD (mg/l)	DOC (mg/l)	A ₂₆₀ /DOC (50 mm) ⁻¹	T-N (mg/l)	T-P (mg/l)
Tatara River	1	10	2.2	2.6	2.02	0.054	2.15	0.03
	2	17	1.9	4.0	2.41	0.075	2.35	0.07
	3	19	1.7	4.2	3.20	0.058	2.47	0.08
	4	46	2.2	2.8	3.70	0.019	1.96	0.06
Naka River	1	17	2.4	3.8	2.52	0.063	3.17	0.08
	2	13	1.9	3.4	3.39	0.047	2.00	0.07
Mikasa River	1	3	1.9	9.9	5.89	0.083	19.12	0.73
	2	5	2.2	10.2	6.17	0.081	19.98	0.90
	3	4	2.5	10.4	5.52	0.090	20.16	0.82
	4	8	2.7	9.5	5.40	0.087	17.79	0.81
Hae River	1	88	6.3	11.1	2.93	0.064	4.10	0.28
	2	86	4.2	8.6	3.07	0.055	4.30	0.28
Doumen River	1	17	5.6	7.9	4.31	0.060	2.64	0.11
	2	13	7.4	11.1	4.16	0.070	2.80	0.14
Siragane River	1	79	35.6	23.7	7.17	0.042	4.81	0.59
	2	13	12.8	16.5	6.25	0.077	9.23	0.85

Table 2. Mutagenic activity of extracts from blue rayons in strains TA100 and YG1024

Sampling site		Mutagenic activity (net revertants/g blue rayon)			
		TA100		YG1024	
		–S9mix	+S9mix	–S9mix	+S9mix
Tatara River	1	— ^a	—	—	—
	2	—	—	—	—
	3	—	—	862	—
	4	—	—	1,068	—
Naka River	1	—	—	543	—
	2	—	—	—	—
Mikasa River	1	—	—	—	—
	2	—	—	—	4,571
	3	—	—	—	2,764
	4	—	—	—	—
Hae River	1	—	—	—	—
	2	—	—	—	—
Doumen River	1	—	—	—	—
	2	—	—	—	—
Siragane River	1	—	—	—	—
	2	—	—	—	—

^a The revertants were less than twice those of the control.

Spontaneous controls: TA100 –S9mix, 157; TA100 +S9mix, 161; YG1024 –S9mix, 214; YG1024 +S9mix, 240 revertants per plate.

Table 3 Mutagenic activity of extracts from the Mikasa River in strains TA100, YG1024, YG1041 and YG1042

Sampling site	Mutagenic activity (net revertants/g blue rayon)							
	TA100		YG1024		YG1041		YG1042	
	–S9mix	+S9mix	–S9mix	+S9mix	–S9mix	+S9mix	–S9mix	+S9mix
1	— ^a	—	—	—	3,278	—	2,404	—
2	—	—	—	4,571	2,115	3,185	—	—
3	—	—	—	2,764	1,297	2,693	1,731	—
4	—	—	—	—	2,482	1,661	—	—

^a The revertants were less than twice those of the control.

Spontaneous controls: TA100 –S9mix, 157; TA100 +S9mix, 161; YG1024 –S9mix, 214; YG1024 +S9mix, 240; YG1041 –S9mix, 12; YG1041 +S9mix, 34; YG1042 –S9mix, 105; YG1042 +S9mix, 92 revertants per plate.

Table 4 Quantitative determination of B(a)P, Trp-P-1 and Trp-P-2 in extracts from the Mikasa River

Sampling site	ng/g blue rayon		
	B(a)P	Trp-P-1	Trp-P-2
1	n.d.	6	4
2	7	n.d.	13
3	6	n.d.	14
4	7	n.d.	13

n.d.=not detected.

and, at sites 1 and 3, toward YG1042 without S9mix. This indicates that sites 1 to 4 were polluted with frame-shift and base-substitution types of direct mutagens such as nitroarenes. The extracts from sites 2 to 4 showed higher mutagenic responses toward strain YG1041 with S9mix, indicating that these sites were more polluted with frame-shift type indirect mutagens such as aminoarenes.

Quantification of B(a)P, Trp-P-1, and Trp-P-2

Table 4 shows B(a)P, Trp-P-1 and Trp-P-2 concentrations in the extracts from the Mikasa River measured by fluorescence HPLC. B(a)P is produced by imperfect combustion and distrib-

uted throughout the environment, therefore B(a)P is regarded as one of the representative chemicals of environmental pollution (12, 13). It has been reported that Trp-P-1 and Trp-P-2 are contained in sewage (4). In the present study, the concentrations of B(a)P and Trp-P-2 were higher in the extracts from sites 2, 3 and 4 than site 1. This result seems to be caused by the outfall of a sewage treatment plant located between sites 1 and 2. However, the chemicals we detected accounted for less than 17% of the total mutagenicity in strains YG1024 and YG1041. These low rates of contribution would indicate that other chemicals are contained in the extracts from blue rayons.

Discussion

The mutagenic activity and the seven water pollution indicators are shown in Fig. 1. The indicators did not show specific trends that were consistent with the mutagenic activity. The extracts from sites 2 and 3 of the Mikasa River showed higher mutagenic responses especially toward strain YG1024 with S9mix, and the extracts of sites 3 and 4 of the Tatara River and site 1 of the Naka River showed mutagenic responses toward strain YG1024 without S9mix. This result would indicate that sites 2 and 3 of the Mikasa River were more polluted

Fig. 1 Chemical characteristics and mutagenic activity of sixteen river water samples.

Fig. 2 Location of sampling sites in the Mikasa River. ①, ②, ③ and ④ are sampling sites.

Fig. 3 Correlation between mutagenic activity and chemical characteristics of Mikasa River water samples.

especially with frame-shift type indirect mutagens such as aminoarenes. Therefore, to investigate the main cause of the mutagenicity of the extracts of the Mikasa River, we measured the mutagenicity toward YG1041 and YG1042 with and without S9mix, and B(a)P, Trp-P-1 and Trp-P-2 concentrations.

The Location of sampling sites in the Mikasa River is shown in Fig. 2. This river flows through an area furnished with sewerage, and a domestic and commercial area is located downstream. There is outfall from a sewage treatment plant located between sites 1 and 2 (the capacity of sewage disposal is 4,170,000 m³/day, the area of sewage disposal is 9,294 ha, and the population it serves is 687,000). The extracts from sites 2 to 4 showed high mutagenic responses toward strain YG1041 with S9mix. The extracts from all the sampling sites showed high mutagenic responses toward strains YG1041 without S9mix and, at sites 1 and 3, toward YG1042 without S9mix. The concentrations of B(a)P and Trp-P-2 in the extracts from sites 2, 3 and 4 were higher than those of site 1. The main source of mutagenic activity in strains YG1024 and YG1041 with S9mix of the extracts from sites 2 to 3 appeared to be the water discharged into this river from the sewage plant located between sites 1 and 2 with the possible main causes being the frame-shift type indirect mutagens such as aminoarenes: Mutagenic activity in strain YG 1024 with S9mix decreased from sites 2 to 3, and mutagenic activity in strain YG1041 with S9mix decreased from sites 2 through 4 in that order. The extracts from site 1 did not show mutagenic responses toward strains YG1024 and YG1041 with S9mix. Furthermore, the concentration of Trp-P-2 was higher in the extracts from sites 2, 3 and 4 than site 1, and Trp-P-2 is mutagenic toward strains YG1024 and YG1041 with S9mix (4). Mutagenic responses

toward strain YG1041 without S9mix, however, were found in the extracts from all sites, the possible main cause of mutagenicity being nitroarenes. Nitroarenes are ubiquitous environmental pollutants found in diesel emission and airborne particulates (14–16). Manabe et al. (1984) reported the presence of 1-nitropyrene in wastewater from oil-water separating tanks at gasoline stations as well as in used crankcase oil, and its mutagenicity (17). They also reported that the wastewater in the tank flows out to drains and finally into the wastewater treatment plant or directly into the river. Therefore, the main cause of the wide-spread mutagenic activity toward strains YG1041 and YG1042 without S9mix in the extracts of the samples appeared to be direct-acting mutagens, i.e., nitroarenes.

Mutagenic activity and the four water pollution indicators are correlated in Fig. 3. It is clear that there were no remarkable differences in the four parameters among sites 1 to 4 of the Mikasa River. Also, the other three parameters did not show clear differences (Table 1). In contrast, the extracts from sites 2 and 3 showed higher mutagenic responses especially toward strains YG1024 and YG1041 with S9mix, and the extracts from all the sampling sites showed high mutagenic responses toward strain YG1041 without S9mix and, at sites 1 and 3, toward YG1042 without S9mix. The mutagenicity determined in this report would not affect human health. However, water pollution in small amounts by many kinds of chemicals has been an important problem. The difference of the results between the water pollution indicators and the mutagenic activities indicates that the known water pollution indicators do not reflect the mutagenic activity of river water. Detection of the combined effects of different chemicals of low concentration is very difficult using known water pollution indicators, however, the

mutagenicity test is able to evaluate river water synthetically. Furthermore, it is possible to predict the main cause of the mutagenic activities by conducting mutagenicity tests in many kinds of bacteria. Therefore, the evaluation of river water mutagenicity seems to be useful and necessary as one of the indicators of water pollution.

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