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Metabolic Enzyme Induction in the Rat by Organic River Sediment Pollutants

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Among various organic pollutants in environments, the lipophilic substances which possess the property of resistance to microbial attack, e.g., insecticides and polychlorinated biphenyl (PCB), are the most important and hazardous substances because of the toxicity and the accumulative property. Such substances are also known to induce drug metabolizing enzymes such as microsomal mixed-function oxidases in liver of animals.

Payne and Penrose (1975) and Lee and Singer (1980) have shown that a mixed-function oxidase system can be induced in fish and polychaetes, respectively, exposed to petroleum or its components. On the basis of these phenomena, they and Kurelec et al. (1977, 1979, 1981) have reported that the activities of mixed-function oxidases in the liver of fish can be a tool for monitoring oil pollution.

Fish and other aquatic animals, however, are migratory and difficult to sample uniformly. Therefore, we attemped to evaluate the enzyme inducing ability of sedimentary organic matters in river using conventional rat which is uniformly available.

MATERIALS AND METHODS

PCB (Kanechlor 500) was a gift from Dr Takeshita of the Institute of Public Health, Tokyo. Ethyl acetate, n-hexane, methanol, benzo(a)pyrene and aminofluorene were purchased from Kanto Chemical Co. Cytochrome c (from horse heart, Type II) was purchased from Sigma Chemical, quinine sulphate from Nakarai Chemicals Ltd., NADPH and glucose-6-phosphate (disodium salt) from Oriental Yeast Co. All other chmicals were of analytical grade.

Sediment was collected at near the mouth of the Tama River (at Daishibashi, June 1983) (Figure 1). Domestic

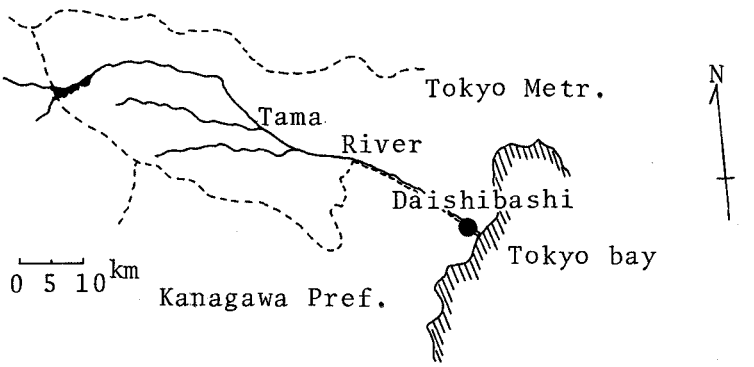

Figure 1. Location of sampling station in the Tama River

sewage (190t/day) and industrial waste water (60t/day) flow into the river (Japan River Society 1982). The sediments were dried at room temperature and passed through a 20 mesh screen. Organic matters in the dry sediment were extracted successively every 8 hr with n-hexane, ethyl acetate and methanol as reported previously (Tabata et al. 1984). The amounts of the respective extracts per one kilogram of dry sediment were as follows; n-hexane extract, 225 mg: ethyl acetate extract, 317 mg: methanol extract, 3434 mg.

The animals used were male Sprague-Dawley rats weighing 180-210 g. For treatment of rat, each extract was dissolved in olive oil to a concentration of 250 mg/mL and a single i.p. injection of 500 mg/kg was administrated to two rats 5 days before sacrifice. Only olive oil and PCB (olive oil solution) of 500 mg/kg were administrated in a similar manner as negative control and positive control, respectively. The rats were given drinking water and commercial rat feed ad libitum until 12 hr before sacrifice when the food (but not the water) was removed. After 5 days of the administration the rats were killed by clubbing, the liver was immediately perfused with ice cold KCl solution (0.15 M) through the hepatic portal vein using a syringe and then excised. After weighing, the liver was minced, and homogenized with 3 volumes of 0.15 M KCl (3 mL/g wet liver) in a Potter-Elvehjem homogenizer. The homogenates were centrifuged for 10 min at 9000g and the supernatant fraction (S-9) was used for determination of enzyme activities. The protein contents of the S-9 fractions were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Enzyme activities of S-9 fraction were assayed as follow: benzo(a)pyrene monooxygenase (BPMO) activity,

that is, the conversion of benzo(a)pyrene to 3- hydroxy-benzo(a)pyrene, was determined with a Shimadzu RF-520 spectrophotofluorometer (excitation at 396 nm and emission at 520 nm) according to the method of Nebert and Gelboin (1968). The activity was expressed in arbitary units (a.u.) per mg of protein per 1 min of incubation time using the quinine sulphate standard (1 ng/mL as 1 a.u.) (Kurelec et al. 1977). Cytchrome c (Cyt. c) reductase activity, that is, the conversion of oxidized Cyt. c to its reduced form, was determined with a Hitachi 181 spectrophotometer (detection at 550 nm) (Omura and Takesue 1970). The activity was expressed in μmol of Cyt. c reduced per mg of protein per 1 min of incubation time. Metabolic activation capability for premutagens, benzo(a)pyrene and aminofluorene, was determined by the mutation assay according to the Ames' method (Moron and Ames 1983) using Salmonella typhimurium TA 98. The activities were expressed in the number of revertant colonies per 5 μg of benzo(a)pyrene and aminofluorene per mg of protein per plate, which was estimated from the initial linear part of the dose-response curve, that is, the plot of revertant colonies vs. mg of protein per plate.

RESULTS AND DISCUSSION

Table 1 shows the BPMO and Cyt. c reductase activities of the S-9 fractions obtained from the rat liver treated with the solvent extracts of sedimentary organic pollutants in the river. The BPMO activities of the S-9 fractions from the rats treated with n-hexane, ethyl acetate and methanol extracts, as well as PCB as positive control inducer, were higher than that of olive oil treated rat. The activity of the ethyl acetate extract-treated rat was the highest among the three solvent extracts. This indicates that the sedimentary organic matters which could induce BPMO possessed relatively high polarity. The monooxygenation of benzo(a)pyrene, that is, aromatic ring, is well known to be due to cytochrome P-450 and/or P-448 (Jakoby 1980), and hence the above-mentioned results implied that the sedimentary organic matters induced cytochrome P-450 or P-448 in rat liver.

On the other hand, Cyt. c reductases which participate in electron transport system for oxidation-reduction of drugs was scarcely induced by any solvent extracts except for PCB. Even the reductase activity of the PCB-treated rat as positive control was only 2 times higher than that of the negative control rat. Cyt. c reductases might be hard to be induced, or else the feed or the drinking water (tap water) used might contain some contaminated inducers.

Table 1. Induction of BPMO and NADPH-Cyt. c reductase in the rat liver S-9 fraction by solvent extracts of the Tama River sediment.

Inducer	BPMO		NADPH-Cyt.c reductase	
	activity[1] (au/mg protein/min)[2]	relative activity	activity[1] (μmol Cyt.c reduced/mg protein/min	relative activity
Control (olive oil)	10.1	1.0	0.015	1.0
PCB	389.4	38.5	0.028	1.9
n-hexane extract	56.1	5.6	0.018	1.2
ethyl acetate extract	131.1	13.0	0.017	1.1
methanol extract	19.3	1.9	0.018	1.2

1) Each value is the average of duplicate experiments using 2 rats
2) Arbitary units (au) corresponds to fluorescence intensity of qunine sulfate solution (1 ng/mL of 0.1 M H_2SO_4)

It is well known that inducers of drug metabolizing enzymes can fall into two groups represented by phenobarbital (PB type) and methylcholanthrene (MC type), based on the feature of inducible enzymes (Jakoby 1980; Conney 1967). The former can induce many enzymes such as cytochrome P-450, NADPH-P-450 reductase (Cyt. c reductase), cytochrome b_5, benzphetamine N-demethylase etc. The later, MC type, can specifically induce only cytochrome P-448 which catalyzes monooxygenation of aromatic ring (Jakoby 1980; Conney 1967). The induction of these enzymes results in not only an enhancement of metabolic detoxication of the corresponding substrate but also an enhancement of metabolic activation. That is to say, it is known that the treatment with the inducer of MC type enhances the metabolic activation of poly aromatic hydrocarbons such as benzo(a)pyrene and that the treatment with the inducer of PB type enhances the metabolic activation of many kinds of chemicals

Table 2. Metabolic activation capability of rat liver S-9 fraction by solvent extracts of the Tama River sediment.

Inducer	benzo(a)pyrene (5 μg/plate)		aminofluorene (5 μg/plate)	
	revertant/mg protein[1)]	relative activity	revertant/mg protein[1)]	relative activity
Control (olive oil)	41	1.0	159	1.0
PCB	259	6.3	4212	26.5
n-hexane extract	33	0.8	716	4.5
ethyl acetate extract	58	1.4	1050	6.6
methanol extract	30	0.7	139	0.9

1) Each value is the average of duplicate experiments using 2 rats

such as aflatoxin B_1 and aromatic amines (Jakoby 1980).

Therefore, we attempted also to evaluate the induction of metabolic enzymes by treatment with the sedimentary extracts on the basis of the effects of S-9 fractions on the mutagenesis of benzo(a)pyrene and aminofluorene. Table 2 shows the metabolic activation capability of each S-9 fraction in terms of the number of revertant colonies per 5 μg of benzo(a)pyrene or aminofluorene per mg of protein per plate and of the activity ratio of the PCB- and the extract-treated rats to control one. The metabolic activation of benzo(a)pyrene was confirmed to increase by the treatment with the ethyl acetate extract besides PCB of positive control. The n-hexane and methanol extracts could not enhance the metabolic activation for mutagenesis of benzo(a)pyrene. However, the both extracts could induce BPMO as mentioned above. This means that the BPMO activities induced by treatment with the n-hexane and methanol extracts were too low or that the BPMO activities were due to cytochrome P-450 but not P-448.

On the other hand, the activation of aminofluorene was significantly enhanced even by the treatment with the n-hexane and ethyl acetate extracts, as well as PCB. This suggests that cytochrome P-450, which catalyzes N-hydroxylation of aminofluorene, was induced by the treatment with the sedimentary n-hexane and ethyl acetate extracts.

On the basis of the above-mentioned results, it was concluded that the Tama River sediment contained organic matters which can induce cytochrome P-450 and P-448. In other words, the river sediment contained some inducers of MC and PB types. Accordingly, the sedimentary extracts must have induced also various metabolic enzymes other than cytochrome P-450 and P-448. The above-mentioned results indicate also that the evaluation of metabolic activating capability of S-9 fraction on premutagens is useful for detection of various enzymes induced by samples comparing the measurement of individual enzyme activity of S-9 fractions.

It is noteworthy that the relatively polar ethyl acetate extract exhibited higher induction activity than the non-polar n-hexane extract. We reported previously that polyaromatic hydrocarbon and chlorinated aromatic hydrocarbon such as PCB among sedimentary organic matters were extracted into the n-hexane fraction (Suzuki et al. 1982). Therefore, the present experimental results suggested that the sediment of the Tama River contained inducers of MC and PB types other than polyaromatics and PCB.

As mentioned above, the present study substantiated that rat is useful to evaluate organic pollutants which can induce drug metabolizing enzymes in river. The use of fish or other aquatic animals as proposed by Kurelec et al. (1977) is surely useful as a tool for monitoring bioactive pollutants in the habitat. The use of rat, however, seems to be more useful in terms of the uniformity of monitoring bioactive pollutants in a wide range of aquatic environments.

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