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Differences in the Drug-Metabolizing Enzyme Activities among Fish and Bivalves Living in Waters near Industrial and Non-Industrial Areas

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Fish and shellfishes, which live in the coastal areas receiving agricultural, industrial and domestic wastewaters, have been exposed to various chemicals including degradation products. It is quite difficult, however, to identify and determine the various harmful chemicals which have been distributed in the environments and also accumulated in aquatic organisms. Even though the concentrations of pollutants in the environments are low so that no mortality of fish and shellfishes occurs, the pollutants may affect the biochemistry and physiology of aquatic organisms.

It has been demonstrated recently that the activities of some drug-metabolizing enzymes, especially the cytochrome P-450 dependent monooxygenase (MO) in fish livers, increase when fish are exposed to environmental pollutants such as polycyclic aromatic hydrocarbons, halogenated organic chemicals (Lech *et al.* 1982). Since Payne (1976) applied the induction of the aryl hydrocarbon hydroxylase activity in fish to monitor marine petroleum pollution, a number of studies have been subsequently reported in this field (Payne *et al.* 1987). However, most of the studies have been done on the field evaluation only by MO induction in fish as a monitor for marine pollution with crude-oil and halogenated organic compounds, without regard for other chemicals such as phenolic compounds.

In our previous paper (Kobayashi *et al.* 1987a) concerning the induction of MO activity in carp (*Cyprinus carpio*) by administration of a PCB-diet for 16 wk, we demonstrated that the activity of benzo(a)pyrene hydroxylase (AHH) was induced by 22 times at 2-wk, although the cytochrome P-450 content increased only twice. We also reported (Kobayashi *et al.* 1987b) that the activity of phenol-sulfate transferase in the mid-gut gland of short-necked clam was induced by exposure to some phenolic compounds, especially pentachlorophenol (PCP), resulting in the increase of the enzyme activity by approximately 7 times that in the control after 5 wk exposure. The induced activity was maintained at least for 3 wk, even after the clam had been transferred

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to running clean sea water, although the PCP accumulated in its body is rapidly excreted (Kobayashi *et al.* 1969). Although the activity of this enzyme in the clam is easily induced by exposure to phenols, the induction of this enzyme activity in fish is very low as compared with that of clam (Kobayashi 1979). Therefore, we examined the activities of drug-metabolizing enzymes of fish and bivalves living in waters near industrial and non-industrial areas to elucidate the applicability of the sulfate transferase activity in bivalves as a monitor for marine pollution, as well as the MO activity in fish.

MATERIALS AND METHODS

Short-necked clam (*Ruditapes philippinarum*) was selected as a suitable species for this investigation in northern Kyushu, because the clam commonly lives in seashore, and should reflect local environmental conditions since it is relatively stationary. In non-tidal coasts such as reclaimed lands and harbors, however, mussel (*Mytilus edulis*) was employed as another test bivalve instead of clam. The average body wt of the clams and mussels used in this study were *ca.* 12 and 15 g with shell, respectively. Flatfish (*Limanda yokohamae*) was also selected as a test animal, because the fish migrate in fairly restricted areas and may reliably reflect their living environment. The fish were collected mostly by fishing with a rod and partly by a fixed shore net. The flatfish collected from sampling sites were of similar size, having the average body wt of 191 g. No significant difference was observed among them. Test animals used in this investigation were not gonadally mature.

Test animals were collected between June 1984 and January 1985 from the sampling sites shown in Figure 1. Five polluted sites were selected as follows: Hakata Harbor where oil tanks are set up and occasionally leak a small amount of oil which is visible on the surface of water, and also the outfall of a sewage disposal plant is located; Oomuta and Miike Harbor area where a coal chemical industry is situated; Otozu Harbor and Otozu River mouth where oil refining and oil chemical plants, a pesticide factory and other industries are located; Ube where an oil refining plant and chemical factories are located. Reference sites were set up nearby the respective polluted sites as follows: the mouths of Hakata Bay, Midori River and Oono River, and also off the coasts of Fukuma and Buzen.

Bivalves collected from each sampling site were carried back to the laboratory alive and kept in sea water until dissection within 24 hr after catching. Approximately 30 bivalves from each site were dissected, and their mid-gut glands were pooled. The pooled mid-gut glands were homogenized in 0.25*M* sucrose-10 m*M* tris-HCl buffer solution (pH 7.5) and subjected to cell fractionation by a conventional method, using an ultracentrifuge. The microsomal and cytosol fractions were frozen in liquid nitrogen and kept in a deep freezer at -80 °C. The cytosol fractions obtained were subjected to the assay for phenol-sulfate transferase

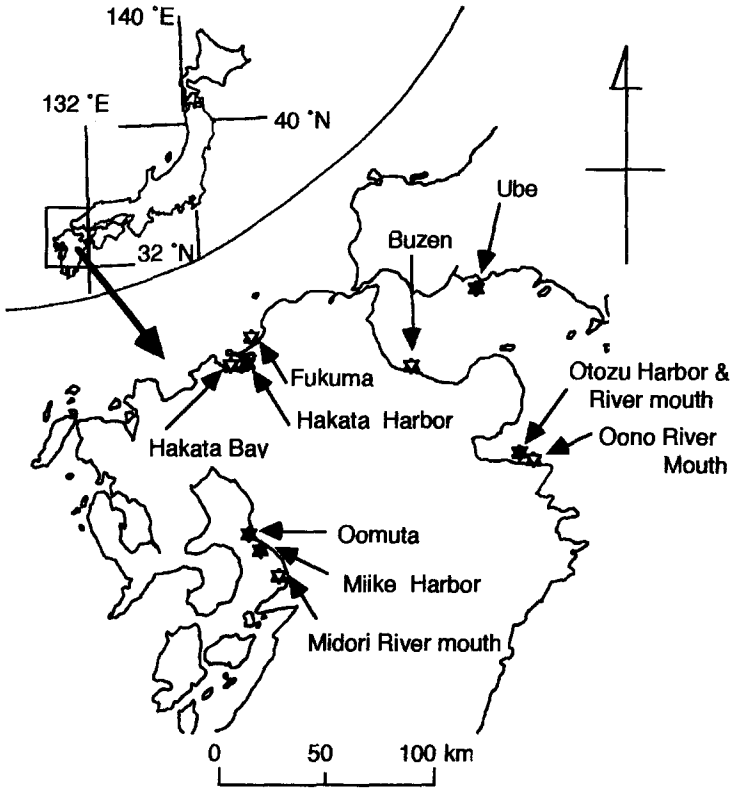

Figure 1. Map of Kyushu Island, Japan showing sampling sites.
✱ Sites (polluted) in industrial area.
✲ Sites (reference) nearby the respective polluted sites.

activity by the method of Kobayashi *et al.* (1987b), as follows. An aliquot of the cytosol fraction was incubated for 1 hr at 35°C with the appropriate amounts of KCl, $MgCl_2$, ATP-Na_2, phenol and $[^{35}S]K_2SO_4$. After 1-hr incubation, free $^{35}SO_4$ remaining in the medium was removed by precipitation as $BaSO_4$ with $BaCl_2$. The supernatant was subjected to the measurement of radioactivity by a liquid scintillation counter. The amount of conjugated sulfate was calculated from the radioactivity on the basis of the specific activity of $[^{35}S]K_2SO_4$ used in the experiment.

The livers of fish at each sampling site were immediately removed from the fish bodies, frozen in liquid nitrogen and then transferred to the laboratory. The livers were subjected to the cell fractionation as mentioned above. The microsomal fractions obtained were frozen in liquid nitrogen and stored in a deep freezer at -80 °C. The microsomal fractions prepared from fish livers were subjected to the determination of the cytochrome P-450 content by the method of Omura and Sato (1964). The activities of NADPH-cyt. *c* reductase, AHH, *p*-nitroanisole *O*-demethylase, and *p*-nitrophenol-UDPglucuronyltransferase were

Table 1. Regional differences in the activity of phenol-sulfate transferase in the mid-gut gland of short-necked clam (Oct. ~ Nov. 1984).

Sampling site	Enzyme activity (nmol/min/g-tissue)
Hakata Bay (Central)	2.72
Oomuta	4.80
Miike Harbor	6.57
Midori River (Mouth) *	1.25
Otozu River (Mouth)	5.52
Oono River (Mouth)	3.13
Buzen *	1.15

* Reference site.

measured at 30°C for 20 min following the methods of Omura and Takesue (1970), Cantfort *et al.* (1977), Netter and Seidel (1964), and Illing and Benford (1976), respectively. The protein content of fractions were also determined by the method of Lowry *et al.* (1951). Data were analyzed by the Mann-Whitney U-test.

RESULTS AND DISCUSSION

Table 1 shows regional differences in the phenol-sulfate transferase activity in clams. The clams collected from the tidelands off the coast of Oomuta and the outer Miike Harbor nearby coal chemical factories, had the activities of 4.80 and 6.57 nmol/min/g-tissue, corresponding to *ca.* 4 and 5 times that in the clams from the reference site (Midori River mouth), respectively. The clams from the Otozu river mouth near the oil refining plant and chemical factories, also showed a high sulfate transferase activity corresponding to 5 times that in the clams from the reference site (Buzen). The clams from the Oono River mouth at a distance of about 3 km from the Otozu River mouth, showed approximately half the activity in those from the Otozu River mouth. The clams from Hakata Bay showed approximately twice the activity of the clams from the reference site.

As shown in Table 2, both of the mussels collected from the petroleum polluted site (Hakata Harbor) and the chemical industry site (Otozu River mouth), showed a similar high activity corresponding to *ca.* 10 times that in the mussels from the clean open sea (Fukuma). The mussels from Hakata Bay and Otozu Harbor also showed the activities corresponding to *ca.* 1/3 and 1/2 that in the mussels from the respective polluted sites.

The high activity of phenol-sulfate transferase in bivalves collected

Table 2. Regional differences in the activity of phenol-sulfate transferase in the mid-gut gland of mussel (June 1984).

Sampling site	Enzyme activity (nmol/min/g-tissue)
Hakata Harbor	1.88
Hakata Bay (Central)	0.54
Fukuma*	0.19
Otozu River (Mouth)	1.77
Otozu Harbor	0.96

* Reference site.

from the polluted sites corresponding to 2 ~ 10 times those in the reference ones, suggests that their living areas might be contaminated with phenolic compounds. Stegeman (1985) reported the presence of AHH activity in the mid-gut gland of mussel. In our study, however, any AHH activity was not detected in the mid-gut glands of clams and mussels collected from both of the polluted and non-polluted sites.

Table 3 shows the cyt. P-450 contents and the activities of NADPH-cyt. *c* reductase, AHH, *p*- nitroanisole *O*- demethylase and *p*-nitrophenol-UDPglucuronyltransferase in flatfish collected from the six sampling sites divided into two areas. Among Hakata Harbor and Bay sites, Hakata Harbor (polluted) showed the highest cyt. P-450 content and enzyme activities corresponding to 2 ~ 4 times those in the Hakata Bay mouth (non-polluted), while fish from the central part of Hakata Bay had the intermediate values of those in the Harbor and Bay mouth. In another survey area, Otozu River mouth (polluted) fish had the highest values (2 ~ 5 times those in non-polluted Buzen). Ube where the oil refining and chemical factories are located, showed the intermediate values of those in the Otozu River mouth and Buzen, as well as in the central part of Hakata Bay.

As mentioned above, the induction of AHH and *O*- demethylase activities was observed in the livers of flatfish caught from the polluted sites, as reported by Stegeman *et al.* (1987). The flatfish from the polluted sites also showed the high activities of NADPH-cyt.*c* reductase and UDPglucuronyltransferase, as well as those in carp treated with long-term dietary administration of PCB (Kobayashi *et al.* 1987a).

Thus, we recommend the phenol-sulfate transferase activity in bivalves as a biochemical monitor for the evaluation of marine pollution, as well as the cyt. P-450 dependent MO activity in fish.

Table 3. Regional differences in the cytochrome P-450 content and the activities of NADPH-cyt. *c* reductase, benzo(a)pyrene hydroxylase, *p*-nitroanisole *O*-demethylase and *p*-nitrophenol-UDPglucuronyltransferase in the hepatic microsomes of flatfish (Nov. 1984 ~ Jan. 1985).

Sampling site	Fish		P-450 content	NADPH-cyt. *c* reductase	Benzo(a)pyrene hydroxylase	*p*-Nitroanisole *O*-demethylase	*p*-Nitrophenol-UDP-glucuronyltransferase
	N	Body wt (g)					
Hakata Harbor	7	172 ± 12	0.51 ± 0.21	17.7 ± 4.9**	1.03 ± 0.48**	0.83 ± 0.40**	0.53 ± 0.21
Hakata Bay (Central)	5	192 ± 15	0.33 ± 0.08	17.3 ± 6.6	0.45 ± 0.43	0.27 ± 0.07	0.48 ± 0.20
Hakata Bay * (Mouth)	6	192 ± 20	0.27 ± 0.19	5.9 ± 3.5	0.27 ± 0.16	0.23 ± 0.26	0.25 ± 0.18
Otozu River (Mouth)	12	185 ± 12	0.62 ± 0.14***	14.9 ± 6.4**	1.02 ± 0.42***	0.69 ± 0.39***	0.45 ± 0.13***
Ube	9	194 ± 100	0.41 ± 0.33	9.4 ± 6.7	0.64 ± 0.52	0.49 ± 0.36	0.19 ± 0.09
Buzen*	7	212 ± 30	0.21 ± 0.16	7.5 ± 3.1	0.19 ± 0.19	0.20 ± 0.10	0.17 ± 0.14

The P-450 content and each enzyme activity are expressed as the mean ± SD in nmol/mg-microsomal protein and nmol/min/mg-microsomal protein, respectively. Each body wt (g) is expressed as the mean ± SD.

* Reference site.

** Significantly different from the respective reference sites, at $P<0.05$.

*** Significantly different from the respective reference sites, at $P<0.01$.

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