Biological Evaluation of Pollution in the Sagami River Using the 7-Ethoxycoumarin *O*-Deethylase Activity Induced by River Sediment Extracts in HepG2 Cells

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The arylhydrocarbon receptor (AhR)-dependent induction of 7-ethoxycoumarin *O*-deethylase (ECOD) activity in HepG2 cells was employed in a longitudinal study of pollution in the Sagami River flowing into Sagami Bay using river sediment extracts. The six sampling points for river sediment were Ogurabashi, Shimomizo, Atsugi, Kurami, Shinomiya, and Hiratsuka (in descending order of distance from the estuary). In samples obtained at the four midpoints along the river, ECOD activity was induced almost to the same extent. Ogurabashi, the point farthest from the estuary, seemed less polluted than downstream areas based on ECOD activity, while a marginal increase in the activity above the baseline was found at the highest concentration in the sample from Hiratsuka. The points where the river sediment caused a marked induction of ECOD activity in HepG2 cells were surrounded by inland industrial areas scattered along the river.

Key words —— environmental pollution, river sediment, 7-ethoxycoumarin *O*-deethylase, HepG2 cells

INTRODUCTION

For the most part, environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-*p*-dioxins and -frans (PCDDs/Fs) are known to induce cytochrome P450s (CYPs), especially CYP1A1 both *in vivo* and *in vitro*.[1–3] Although CYP in cultured cells cannot generally be induced, the human hepatoma cell line HepG2 retains this feature and has been applied to a bioassay for environmental pollutants in combination with 7-ethoxycoumarin as a substrate with satisfactory sensitivity.[4]

In our series of experiments to monitor environmental pollution, HepG2 cells have been used as a biosensor for arylhydrocarbon receptor (AhR)-activatating compounds. The rivers flowing into Tokyo Bay were studied comparatively by examining the extent of pollution with AhR-agonists such as PCDDs/Fs, polychlorinated biphenyls (PCBs), and PAHs using surface sediment extracts sampled at points near their estuaries.[5,6] Among them, the Tsurumi River ranked first in terms of potential to induce AhR-dependent 7-ethoxycoumarin *O*-deethylase (ECOD) activity. To determine the cause of the heavy pollution at the estuaries, the pollution of the Tsurumi River was studied longitudinally, showing the primary effects of nearby industrial complexes. Given the resemblance of the inverted U-shape dose–response curve produced by the river sediment extracts to that of 3-methylcholanthlene (3-MC), but not to the ones reported for PCDDs/Fs, PAHs such as 3-MC and benzo[*a*]pyrene seemed to be responsible for the pollution.

In the present study, a geographical expansion beyond the rim of Tokyo Bay was attempted. The Sagami River runs north to south through central Kanagawa Prefecture, Japan, and flows into Sagami Bay at Hiratsuka, with an inland industrial district located in the river basin. To date, few data have been reported concerning the chemical analysis of dioxin pollution in the branch streams of this river (http://www.k-erc.pref.kanagawa.jp/oldrelease/200012/08-01-05.htm).

MATERIALS AND METHODS

Sediment Samples —— Surface sediment samples were collected at several points on the Sagami River in Kanagawa Prefecture in central Japan at the sampling points indicated in Fig. 1. River sediments were collected from the river bed at a depth of 5 cm within 1 m from the bank. Sampling dates for individual points were as follows: Ogurabashi, September 2, 2002; Shimomizo, June 28, 2002; Atsugi, June 28, 2002; Kurami, April 6, 2002; Shinomiya, April 6, 2002; and Hiratsuka, August 29, 2002.

Reagents —— Dichloromethane and ethylacetate of a grade used for the testing of pesticides, acetone,

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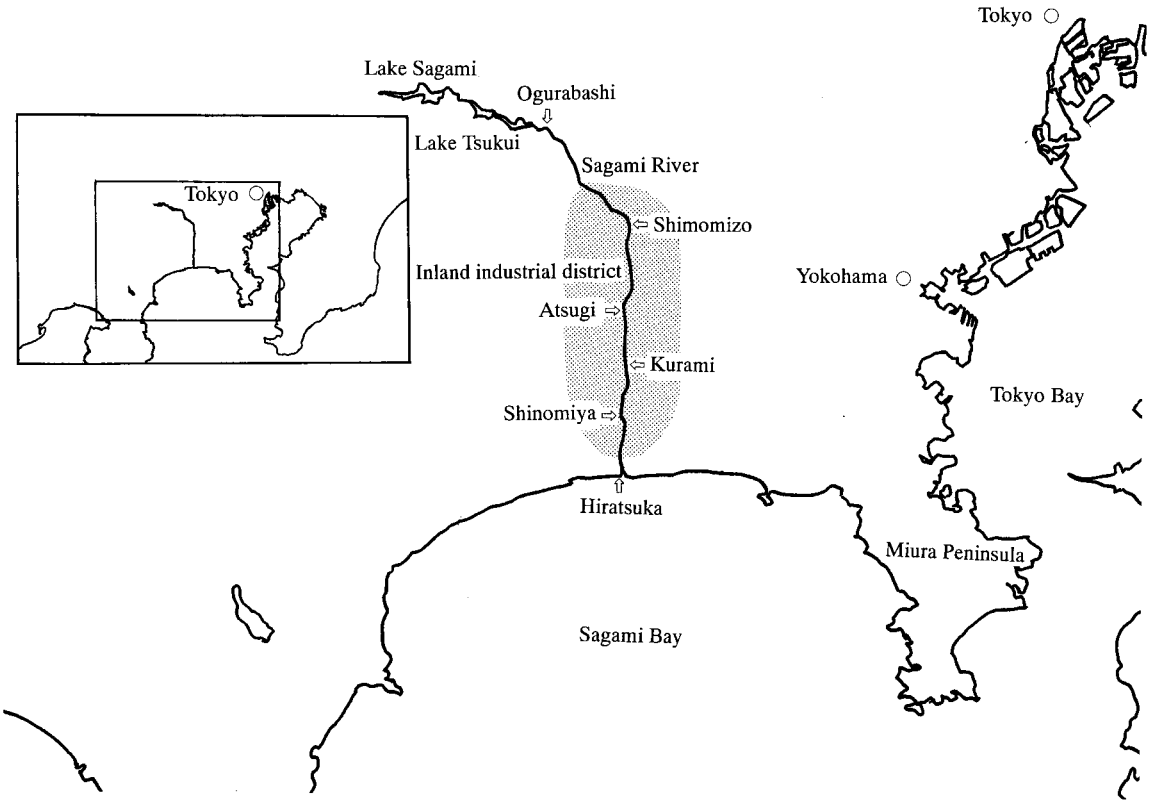

Fig. 1. Location of River Sediment Sampling Points

dimethylsulfoxide (DMSO), and 3-MC of guaranteed reagent grade, and β-glucuronidase of a grade suitable for biochemistry were obtained from WAKO Pure Chemical Industries, Ltd. (Japan) Dulbecco's modified Eagle's medium (DMEM) and phenol red-free Eagle's minimum essential medium (MEM) were obtained from Gibco BRL (U.S.A.).

Extraction —— Sediment samples were air-dried at room temperature and passed through a 10-mesh sieve to remove gravel. Then 20 g of the sieved fraction was extracted successively with dichloromethane using a Soxhlet extractor for 16 hr. The extracts were concentrated to approximately 5 ml with a Kuderna-Danish concentrator, transferred to a 10-ml conical tube, and evaporated to dryness under a N_2 gas stream. The residues were dissolved in 400 μl of DMSO and further diluted 10 and 100 times; each solution of 12 μl corresponds to 600, 60, or 6 mg of sediment.

Cell Cultures and Treatment —— Human hepatoma HepG2 cells were obtained from the Cell Resource Center for Biomedical Research, Tohoku University. The cells were grown in DMEM supplemented with 15% fetal bovine serum and subcultured every 7–10 days at a 1 : 3 split ratio.

HepG2 cells were seeded on 60-mm type I collagen-coated dishes at 1×10^6 cells/dish in 6 ml of culture medium. When the cultures reached confluence, the medium was replaced with 6 ml of fresh medium containing 12 μl of DMSO solution of individual sample diluents or 3-MC (0.5 or 2.5 μM, final concentration). The ECOD induction was carried out at 37°C for 16 hr.

Determination of ECOD Activity —— The determination of ECOD activity was performed as described previously.[5] A Type F-3000 fluorescence spectrophotometer (Hitachi, Japan) was used for fluorescence spectrophotometry.

RESULTS AND DISCUSSION

A longitudinal study on the pollution of the Sagami River was carried out using an intracellular assay based on the induction of ECOD activity in HepG2 cells by river sediment extracts and the results are summarized in Fig. 2. The extent of the pollution of rivers in the metropolitan area flowing into Tokyo Bay was previously compared at their estuaries. The Tsurumi River was found to be the

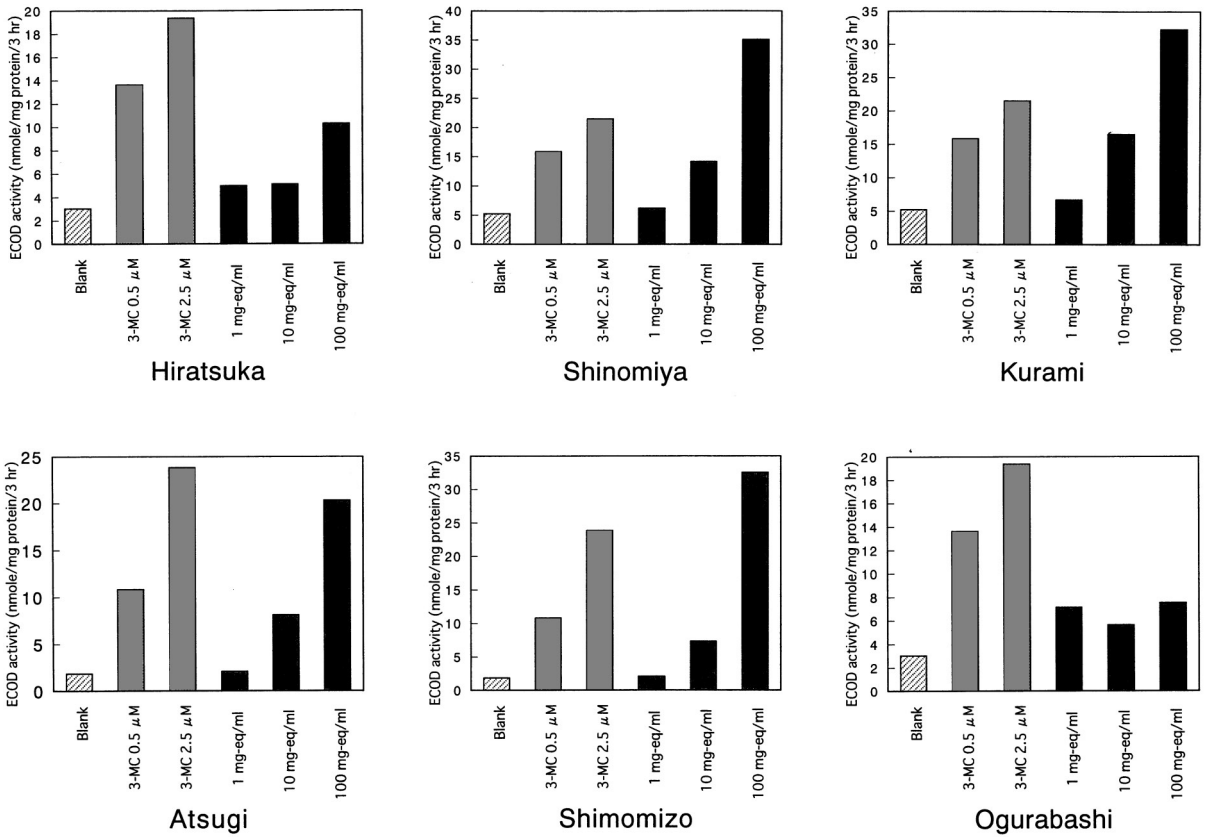

Fig. 2. 7-Ethoxycoumarin *O*-Deethylase Activity Induced by River Sediment Extracts

ECOD activity was measured at 37°C for 3 hr using HepG2 cells after being induced in duplicate at 37°C for 16 hr with sediment extracts at final concentrations of 1 to 100 mg-sediment equivalent (mg-eq)/ml or 3-MC 0.5 and 2.5 μM.

most polluted.[5] More recently, therefore, a longitudinal study on the pollution of the Tsurumi River was conducted, attributing the pollution at the river mouth to the water drainage from industrial areas in its vicinity.[6] The Sagami River, running from north to south in the central area of Kanagawa and flowing into Sagami Bay, separated from Tokyo Bay by the Miura Peninsula, was selected for the first time as a river in the Sagami Bay area, because inland-type industrial areas are located midstream and some of the tributary waters are reportedly contaminated with dioxin-type compounds (http://www.k-erc.pref.kanagawa.jp/oldrelease/200012/08-01-05.htm). Ogurabashi, the uppermost sampling point in the present study, was less polluted with PAHs than the points downstream, *i.e.*, Shimomizo, Atsugi, Kurami, Shinomiya, and Hiratsuka in a geographically descending order, where the induction of ECOD activity was observed dose dependently by the river sediment extracts. At these sampling points, the extracts from 100 mg of soil sediment were estimated to contain *ca.* 2.5 nmol as 3-MC or more in terms of ECOD-inducing ability. Hiratsuka, near the estuary in the center of a residential district, was less polluted than the other points tested except Ogurabashi, with no increase in ECOD activity at 1 and 10 mg-eq sediment/ml. In conclusion, the pollution in the Sagami River is approximately two orders of magnitude lower than that in the most polluted river examined, the Tsurumi River. In general, the release of water pollutants from neighboring areas is responsible for the accumulation of PAHs in river sediment, owing to the potential of rivers to clean up water pollutants biologically, chemically, and physically.

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