1-Nitropyrene-Metabolizing Activities of Fish Liver Preparations

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Nitropolycyclic aromatic hydrocarbons, which are a new class of carcinogenic environmental pollutants, enter waterways by release of urban wastewater into the environment and by atmospheric fallout of airborne particles associated with smog (Wang et al. 1980; Rosenkranz and Mermelstein 1983; Manabe et al. 1984; Hayakawa et al. 1995). It is important to examine their metabolism not only in mammalian species but also in fish species for assessment of possible risk associated with human exposure to the pollutants.

Recently, we examined the in vivo metabolism of 1-nitropyrene, a typical nitropolycyclic aromatic hydrocarbon, in fish focusing on nitroreduction and acylation (Kitamura and Tatsumi 1996). When goldfish were bathed in a solution of 1-nitropyrene or its reduction product 1-aminopyrene, one or two metabolites were isolated from the solution, respectively. The former metabolite was identified as 1-aminopyrene and the latter two metabolites as 1-acetylaminopyrene and 1-formylaminopyrene by comparing their mass and UV spectra, and behaviors in HPLC and TLC with those of authentic samples. In mammalian species, nitro-reduction followed by N-acetylation and N-formulation of nitropolycyclic aromatic hydrocarbons have been demonstrated with their liver preparations (Tatsumi and Amano 1987; Tatsumi et al. 1989). To our knowledge, such metabolic reactions of nitropolycyclic aromatic hydrocarbons have not been studied with fish liver preparations.

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In the present study, nitroreductase activity toward 1-nitropyrene, and N-acetylation and N-formylation activities toward its reduction product, l-aminopyrene, were examined using liver preparations from sea breams and carps.

MATERIALS AND METHODS

Sea bream livers (18 - 26 g) and black carp livers (15 - 23 g) were kindly supplied from a fresh fish shop.

1-Nitropyrene, l-aminopyrene and 2-hydroxypyrimidine were purchased from Tokyo Chemical Industry Co., Ltd. Menadione and xanthine were obtained from Nacalai Tesque, Inc. NADPH and NADH were obtained from Oriental Yeast Co. Acetyl-CoA and N-formyl-L-kynurenine were purchased from Sigma Chemical Co. 1-Acetylaminoptyrene and o-dinitrobenzene were obtained from Aldrich Chemical Co. and Wako Pure Chemical Industries, Ltd., respectively. l-Formylaminopyrene was prepared as described previously (Tatsumi and Amano 1987).

Fish livers were homogenized in 3 volumes of 1.15% KCl. The homogenate was centrifuged for 20 min at 9,000xg, and the supernatant fraction was separated to microsomes and cytosol by its centrifugation for 60 min at 105,000xg. The microsomes were washed by resuspension in 2 volumes of the KCl solution and by resedimentation for 60 min at 105,000xg.

Silica gel plates (Kieselgel 60 GF, Merck; 0.25 mm thick) were developed in benzene - acetone (7:3, v/v). Spots were visualized under UV light (254 nm). Rf values of authentic 1-nitropyrene, l-aminopyrene, 1-acetylaminoptyrene and 1-formylaminopyrene were 0.64, 0.55, 0.35 and 0.39, respectively.

HPLC was performed in a Hitachi L-6000 chromatograph fitted with a 130 x 4 mm column of LiChrosphere 100 RP-8(e). The mobile phase was CH₃CN - H₂O (1:1, v/v). The chromatograph was operated at a flow rate of 1 mL/min at ambient temperature and at a wave length of 254 nm. Elution times (min) of authentic 1-nitropyrene, l-aminopyrene, 1-acetylaminoptyrene and 1-formylaminopyrene were 31.8, 10.3, 3.9 and 4.8, respectively.

The incubation mixture consisted of 0.1 µmol of 1-nitropyrene,