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Isolation and sequence of cDNA encoding a 3-methylcholanthrene-inducible cytochrome P450 from wild red sea bream, Pagrus major

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Abstract We have isolated a cDNA clone of mRNA for the cytochrome P450 from a 3-methylcholanthrene (MC)-treated red sea bream, Pagrus major, using a cDNA fragment for rat P4501A2 as a probe. The cloned cDNA is ca. 1.8 kb long and contains an open reading frame of 1545 nucleotides for polypeptides of 515 amino acids. The deduced N-terminal amino acid sequence of the cDNA is very similar to that for purified cytochrome P450 protein from the marine fish scup, which was reported previously (Klotz et al. 1983). A conserved amino acid sequence containing a putative heme-binding cysteine is present in the equivalent position proximate to the C-terminus of the molecules. The deduced amino acid sequence shows more than 50% positional identity with known members of the mammalian aromatic hydrocarbon-inducible P450 family. RNA blot analysis indicates that P450 mRNA(s) is expressed in the liver, kidney, gill and gut of the MC-treated P. major.

Introduction

The cytochrome P450 family consists of hemoproteins that catalyze the oxidative metabolism of a variety of substrates from endogenous steroids and fatty acids to numerous exogenous compounds including carcinogens, mutagens and other environmental contaminants (e.g. Sato and Omura 1978; Melancon et al. 1981; Klotz et al. 1983; Williams and Buhler 1984; Stegeman et al. 1985). Stegeman and co-workers have demonstrated high similarity between mammalian and fish aromatic hydrocarbon-inducible cytochrome P450s in drug-inducibility, catalytic profiles and immunochemical characteristics (Stegeman et al. 1981; Klotz et al. 1983; Klotz et al. 1986; Park et al. 1986; Kloepfer-Sams et al. 1987).

Since the cDNA and genomic gene for rat phenobarbital-inducible cytochrome P450 (P4502B1) were cloned (Fujii-Kuriyama et al. 1982; Mizukami et al. 1983) and the complete amino acid sequence was deduced from this cDNA and gene, many genes for various forms of mainly mammalian P450s have been isolated and analysed. These gene analyses have provided much information useful for an elucidation of the structure and function of cytochrome P450s. However, as for marine fish cytochrome P450, little is known as yet about gene structure, precise amino acid sequence or mechanisms of gene expression.

It is of great interest that the syntheses of some forms of fish cytochrome P450 are induced by such marine environmental pollutants as polychlorobiphenyl (PCB) (Stegeman et al. 1987; Gooch et al. 1989), polycyclic aromatic hydrocarbons (Stegeman and Kloepfer-Sams 1987; Van Veld et al. 1990) and dioxins (Cooper et al. 1990),
which has been demonstrated by quantifications of catalytic activity, P450 protein (by immunodetection using monoclonal antibody) and P450 mRNA (by RNA hybridization, Kraemer et al. 1991; Haasch et al. 1992). Quantifications of these fish P450 gene products have been used in pollution studies as biomarkers for exposure of fish to organic xenobiotics (reviewed in Payne et al. 1987; Goksøyr and Förlin 1992).

In the present study, we analyze a cDNA for the cytochrome P450 molecule from 3-methylcholanthrene (MC)-treated marine fish, red sea bream, Pagrus major, and demonstrate the putative complete amino acid sequence of this enzyme. In addition, using this cDNA as a probe, we examine the expression of cytochrome P450 mRNA(s) in various organs of MC-treated fish. This cDNA should prove to be a useful tool in future studies on the induction of cytochrome P450 mRNA by various chemical drugs and environmental pollutants in the aquatic environment. Such studies are now in progress in our laboratory.

Materials and methods

Materials

Restriction enzymes, T4 polynucleotide kinase, terminal transferase, ribonuclease H, DNA polymerase (Klenow) and AMV reverse transcriptase were obtained from Takara Shuzo Co. (Kyoto, Japan). Nylon filters for hybridization experiments and a random priming DNA labeling kit were purchased from Amersham (UK).

Fish and treatments

Living marine fish, wild red sea bream, Pagrus major (mixed sex, body weight of 600 to 800 g), were obtained from the fish market, after having been caught in the sea near Nagasaki in Japan. Red sea bream were held in flowing, filtered seawater in a 500-liter tank at 15 to 16 °C for 2 d prior to use. Fish were not fed at all during the holding and experimental periods. Fish were intraperitoneally (ip) injected with 20 mg kg⁻¹ of MC in corn oil (10 mg ml⁻¹) and sacrificed after 7, 22 and 48 h for Northern blot analysis, or after 48 h for the RNA dot hybridization experiment. Control fish were ip injected with 1.2 ml of corn oil and sacrificed after 48 h. To examine the effect of phenobarbital on the induction of fish P450 mRNA by various chemical drugs and environmental pollutants in the aquatic environment. Such studies are now in progress in our laboratory.

cDNA synthesis and screening procedures

Total RNA was extracted by the guanidium thiocyanate method (Chirgwin et al. 1979) from the livers of red sea bream at 48 h after administration (ip) of 20 mg kg⁻¹ body weight of MC. Poly (A)⁺RNA was purified with Oligo (dT)-cellulose column chromatography (Maniatis et al. 1982) and fractionated with sucrose density gradient centrifugation (Fuji-Kuriyama et al. 1982) and transferred to a nylon filter in 20xSSC. The RNA was coelectrophoresed in another lane of the identical gel with total RNAs as a size marker. For dot analysis of RNAs, total RNAs (1 to 10 μg dot⁻¹) were denatured in the same manner as described above. droplet on the nylon filter in 10xSSC using a filtration apparatus. Prehybridizations and hybridizations were carried out as described (Fujii-Kuriyama et al. 1981) using cloned red sea bream cytochrome P450 cDNA as a hybridization probe.

DNA sequencing

Nucleotide sequence analysis was performed mainly in accordance with the Maxam-Gilbert method (1977) and partially with the dideoxy method (Sanger et al. 1980) in both orientations.

Results and discussion

Identification and nucleotide sequencing of cDNA clones

To obtain cDNA encoding MC-inducible cytochrome P450 of red sea bream, we carried out cDNA colony hybridization using a 1.0-kb DNA fragment of rat cytochrome P4501A2 cDNA as a probe. The 1.0-kb DNA fragment of rat P4501A2 cDNA contains nucleotide sequences corresponding to amino acid sequences of heme-binding region of cytochrome P4501A2 protein, which is conserved among various forms of cytochrome P450 (Yoshioka et al. 1986). Out of approximately 1.2×10⁸ clones screened, we obtained nine cDNA clones which hybridized with probed DNA more intensively than other clones. The cross hybridization experiments and the comparison of restriction maps (data not shown) indicated that four out of nine clones were homologous. A clone named RSB-154, involved the long fragment of rat P4501A2 cDNA as a hybridization probe. The positive clones were rescreened following the same procedures mentioned above.

RNA blot analysis

Total RNAs were extracted from the liver, kidney, gill and gut of red sea bream in accordance with the method described above. For gel blot analysis, total RNAs (10 μg lane⁻¹) were denatured in formamide/formaldehyde, electrophoresed in agarose gel containing formaldehyde/formaldehyde (Maniatis et al. 1982) and transfered to a nylon filter in 20xSSC. The RNA was coelectrophoresed in another lane of the identical gel with total RNAs as a size marker. For dot analysis of RNAs, total RNAs (1 to 10 μg dot⁻¹) were denatured in the same manner as described above. droplet on the nylon filter in 10xSSC using a filtration apparatus. Prehybridizations and hybridizations were carried out as described (Fujii-Kuriyama et al. 1981) using cloned red sea bream cytochrome P450 cDNA as a hybridization probe.

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