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Identification of cytochrome P450 1B-like sequences in two teleost fish species (scup, *Stenotomus chrysops* and plaice, *Pleuronectes platessa*) and in a cetacean (striped dolphin, *Stenella coeruleoalba*)

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Abstract

The cytochromes P450 (CYP) constitute a multigene family of enzymes playing a critical role in the oxidation of many endogenous and xenobiotic substrates. The CYP1 family is of particular interest in environmental toxicology because its members are dominant in the metabolism of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and aryl amines. Three members of the CYP1 family, CYP1A1, CYP1A2, and CYP1B1, have been identified in mammals. We report here on the identification and cloning of cytochrome P4501B-like sequences from two teleost fish species and a marine mammal. Sequences clustering with CYP1B1 in phylogenetic analysis were obtained from liver cDNA of scup (*Stenotomus chrysops*), genomic DNA of plaice (*Pleuronectes platessa*), and liver cDNA of striped dolphin (*Stenella coeruleoalba*). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cytochrome P450; Gene sequence; Dolphin; Cloning; Scup

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Until the recent identification and characterization of CYP1B1 in mammals, CYP1A1 and CYP1A2 were mainly held responsible for the oxidation and activation of polycyclic aromatic hydrocarbons (PAHs) and planar halogenated aromatic hydrocarbons (PHAHs) and of aryl amines, respectively. Metabolism and carcinogenesis studies (Buters et al., 1999; Shimada et al., 1996) have now shown CYP1B1 to be a critical and, in some cases, necessary enzyme in the activation of several of these pollutants, notably the PAH 7,12-dimethylbenz[a]anthracene. CYP1B1 is also prominent in estradiol hydroxylation, forming catechol estrogens, which can undergo redox cycling generating reactive oxygen species, and thus potentially playing a role in estrogen-induced carcinogenesis (Hayes, Spink, Spink, Cao, Walker & Sutter, 1993).

CYP1As have been identified in most vertebrate groups (amphibians, fish, birds, mammals) and CYP1A induction is used widely as a biomarker when assessing exposure to contaminants in environmental systems. In contrast to CYP1As, CYP1B1 has been characterized and sequenced in only three species: human (*Homo sapiens*), rat (*Rattus norvegicus*), and mouse (*Mus musculus*). The role of CYP1B1 in the metabolism of many xenobiotics and in PAH- and estrogen- induced carcinogenesis prompted our investigation of CYP1B presence in aquatic species. Phylogenetic analysis of CYP1 family sequences indicating that the mammalian CYP1A and CYP1B genes diverged before the evolutionary emergence of mammals suggests the likely existence of CYP1B in fish species (Nelson et al., 1996). In this study, we sought CYP1B sequences in fish and in cetacean species.

Degenerate inosine-containing primers for CYP1B were designed based on the aligned sequences of the three known CYP1B1 genes: human, rat and mouse (Savas, Bhattacharyya, Christou, Alexander & Jefcoate, 1994; Sutter et al., 1994; Walker, Gastel, Costa, Clark, Lucier & Sutter, 1995). Liver tissues were obtained from a male scup and a freshly stranded male striped dolphin and frozen upon collection. Total RNA was prepared using RNA STAT-60 (Tel-test) and poly(A)⁺ RNA was isolated with a mini-oligo(dT)-cellulose spin column kit (5 prime-3 prime Inc.). Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the Gene-Amp RNA-PCR kit (Perkin-Elmer) and a Gene-AMP 2400 thermocycler. Reverse transcription was primed with random hexamers. PCR products of the expected size were cloned in pGEM-T Easy (Promega) and sequenced in both directions using a Sequitherm Excel long-read cycle sequencing kit (Epicentre Technologies) and an automated DNA sequencer (LI-COR). A minimum of three clones were sequenced in both forward and reverse directions for each fragment. A plaice genomic DNA library in Lambda FIXII (Stratagene) was screened at low stringency with plaice CYP1A cDNA and positive clones were sequenced. All but one weakly positive clone were CYP1A. This non-CYP1A clone displayed exon/intron boundaries and sequence characteristics of mammalian CYP1B genes.

Mammalian CYP1B1 and CYP1A1 (human, rat, and mouse), fish CYP1A (scup, plaice, toadfish *Opsanus tau*) and spiny lobster CYP2L1 (*Panulirus argus*) sequences were retrieved from the Genbank database and used in the phylogenetic analysis conducted on the fragments isolated from the scup, plaice and dolphin tissues. CYP2L1 was chosen as an outgroup because of its evolutionary position and of the

possibility of its alignment with the other sequences used in the analysis. All sequences were aligned (Clustalw version 1.7) and truncated to the size of the dolphin 110-AA long fragment. This fragment corresponds to approximately one fifth of the mammalian CYP1B1 sequences. Phylogenetic trees were constructed using both the maximum parsimony method with a Branch and Bound search setting, with and without an outgroup, and the Neighbor-Joining method (PAUP, Version 4.0b2 for Macintosh).

Results of phylogenetic analysis were similar for all cases and the maximum parsimony tree is displayed in Fig. 1. The scup, plaice and dolphin fragments cluster with the known CYP1B1 sequences, the dolphin fragment being closer to the other mammalian sequences than the fish fragments, as expected. This clustering indicates

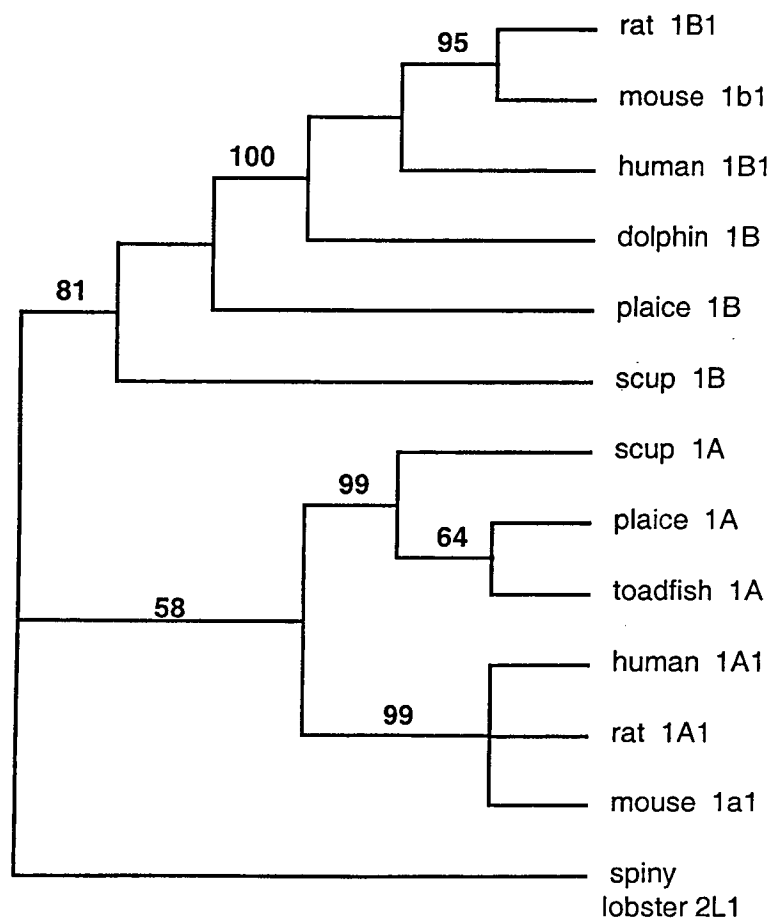


Fig. 1. Phylogenetic analysis of novel CYP1B-like sequences. Maximum parsimony tree was constructed with Branch and Bound search setting and CYP2L1 as an outgroup (PAUP, Version 4.0b2 for Macintosh). Bootstrap values above 50% are indicated at the nodes.

that these sequences are members of the CYP1B subfamily. Sequence comparisons and alignments between the three fragments and the mammalian CYP1B1s and the fish and mammalian CYP1As suggest some intriguing features of the fish CYP1Bs. For both the scup and plaice, the CYP1B fragments have less than 52% amino acid (AA) identity with the three CYP1A sequences used in this analysis and more than 54% and 61% AA identity with mammalian CYP1B1s, respectively. Striped dolphin CYP1A sequence is not available, but the striped dolphin CYP1B fragment shows more than 86% AA identity with the other mammalian CYP1B1s. Surprisingly, the two fish CYP1B-like fragments share only 63% AA identity, less than what was anticipated based on the 85% AA identity between the CYP1As from these species. If this pattern is confirmed in the full-length sequences, it could suggest that CYP1B functions and sequences may be rapidly diverging in fish. Full-length sequences and expression studies are underway, and should reveal the full nature of the similarity between fish and mammalian CYP1Bs.

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