



Cloning a new cytochrome P450 isoform (CYP356A1) from oyster *Crassostrea gigas*

Guilherme de Toledo-Silva^a, Marília N. Siebert^a, Igor D. Medeiros^{a,b}, Thaís C.M. Sincero^c, Milton O. Moraes^d, Jared V. Goldstone^e, Afonso C.D. Bainy^{a,*}

^aLaboratório de Biomarcadores de Contaminação Aquática, Departamento de Bioquímica, CCB, UFSC, Florianópolis, SC 88040-900, Brazil

^bLaboratório de Ciências Marinhas, Universidade do Sul de Santa Catarina, Av. Colombo Sales, 89, Laguna, SC 88790-000, Brazil

^cLaboratório de Protozoologia, Departamento de Microbiologia e Parasitologia, CCB, UFSC, Florianópolis, SC 88040-900, Brazil

^dLaboratório de Hanseníase, Fundação Oswaldo Cruz, Cruz, Instituto Oswaldo Cruz, Av. Brasil, 4365, 21040-360 Rio de Janeiro, RJ, Brazil

^eBiology Department, Redfield 3-42 MS#32, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

ARTICLE INFO

Keywords:

Cytochrome P450
Pacific oyster
Crassostrea gigas
Biotransformation enzymes
CYP356A1
CYP17

ABSTRACT

We have cloned the full-length cDNA of the first member of a new cytochrome P450 (CYP) family from the Pacific oyster *Crassostrea gigas*. This new CYP gene was obtained based on an initial 331 bp fragment previously identified among the list of the differentially expressed genes in oysters exposed to untreated domestic sewage. The full-length CYP has an open reading frame of 1500 bp and based on its deduced amino acid sequence was classified as a member of a new subfamily, CYP356A1. A phylogenetic analysis showed that CYP356A1 is closely related to members of the CYP17 and CYP1 subfamilies. Semi-quantitative RT-PCR was performed to analyze the CYP356A1 expression in different tissues of the oyster (digestive gland, gill, mantle and adductor muscle). Results showed slightly higher CYP356A1 expression in digestive gland and mantle, than the other tissues, indicating a possible role of the CYP356A1 in xenobiotic biotransformation and/or steroid metabolism.

© 2008 Elsevier Ltd. All rights reserved.

The cytochromes P450 (CYP) superfamily is one of the largest and functionally most diverse protein families. CYP enzymes are associated with xenobiotic biotransformation and other processes, including homeostasis, hormone biosynthesis and degradation, and oxidative stress (Stegeman and Hahn, 1994). CYP enzymes are the most important oxidative (phase I) biotransformation enzymes in terms of catalytic versatility and breadth of xenobiotic biotransformations carried out (Guengerich, 1987). In this study we cloned the full-length cDNA of a member of a new CYP subfamily from the gill of Pacific oyster *Crassostrea gigas* which has been previously identified in the list of up-regulated genes in oysters exposed to domestic sewage (Medeiros et al., this volume).

An initial fragment of 331 bp was previously identified among the list of the differentially expressed genes in oysters exposed to untreated domestic sewage (Medeiros et al., this volume). Amplification of 5' and 3' cDNA ends were performed by SMART RACE (Clontech), using specific primers (forward 5'-CCAGAAGAATTTGACCCACTTCG-3' and reverse 5'-TTTGTAAATCGGACGGAAGCTC-TAC-3'). Reactions were set to 25 cycles of: 30 s at 94 °C, 30 s at 51 °C, and 2 min at 72 °C. PCR products were analyzed in 1.2% agarose gel and the 550 bp and 400 bp expected products were puri-

fied, cloned and sequenced on ABI3730 (Applied Biosystems). The results were analyzed using BioEdit software. Amplification of the internal region was carried out using the primers, forward 5'-GAAAGGCTCTCAGGCATTATCT-3' and reverse 5'-CCTCTTGACATTTGCTTGG-3'. Amplification conditions were initial denaturation for 2 min at 94 °C, followed by 30 cycles: 30 s at 94 °C, 45 s at 47 °C, and 60 s at 72 °C. PCR product was directly sequenced on MEGABACE 1000 (GE Healthcare). Phylogenetic studies were carried out using Bayesian techniques as implemented in the software MrBayes (version 3.1.2), which estimates posterior probabilities using Metropolis-Hastings coupled Monte Carlo Markov chains (MC³). Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out in order to analyze the CYP expression in different tissues (digestive gland, gill, mantle and adductor muscle) using the forward and reverse primers (forward: 5'-CCAGAAGAATTTGACCCACTTCG-3', reverse: 5'-TTTGTAAATCGGACGGAAGCTCTAC-3'). In order to avoid individual variability, each tissue was pooled from 5 oysters and the total RNA was used for this analysis. The densitometry of products was quantified using Scion Image software.

The full-length sequence of the new CYP gene has 1500 bp (Fig. 1, Genbank access no. ABR45717). The deduced amino acid sequence shows conserved motifs typical of CYP enzymes, such as the heme group binding region, helix-C, helix-I and helix-K motifs (Fig. 1). This sequence was classified by the CYP

* Corresponding author.

E-mail address: bainy@mbx1.ufsc.br (A.C.D. Bainy).

1	TAAGCAGTGGTATCAACGCAGAGTACGCGGGGGCATTTCGATGAGAGAAAAACAGTAGAGA
	M L K L S M N T Q T V L A G
61	ACACCAAAGAGAGCAATCATGTTGAAGTTGTCCATGAACACCCAGACCGTTTTAGCGGGA
	I C V G L L V Y Y V I K R M R Y R L G P
121	ATATGCGTGGTCTTTTGGTATATTACGTTCATCAAACGGATGCGGTATCGTCTGCCACCC
	G P W C I P L V G H Y K I Y S S P E M H
181	GGGCCATGGTGTATCCCTCTTGTGGTCATTATAAAATTTATTCATCTCCCGAGATGCAC
	K K I A A L S K D Y G P V V R I S F G P
241	AAGAAAATCGCAGCGCTGTCCAAGGACTACGGCCCTGTCTCCGAATTTCTGTTTGGCCCC
	Q T W V V L N D I N T V V E A M V K R K
301	CAAACCTGGGTTGTGCTTAATGACATCAACACCGTGGTGAAGCCATGGTCAAAGGAAG
	A D F A G R P H F T S G D V F T E G G K
361	GCTGATTTTGCCTGGAGGCGCACTTTACATCGGGTGTGTTCACAGAAGGAGGAAAG
	D I A F S N Y S A S W K F H R K I A G K
421	GATATAGCCTTCAGCAATTTACAGCTTCTGGAAATTCATAGGAAAATAGCCGGAAAG
	A L R H Y L Q G D L L E N M I Q E N M N
481	GCTCTCAGGCATTATCTACAAGGAGATTTACTGGAAAACATGATTCAAGAGAACATGAAT
	K F L N K M A E E K E P F M F K E Y V D
541	AAATTTTGAACAAGATGGCCGAGGAAAAGAGCCGTTTATGTTTAAAGAATACGTCGAT
	L M V F H Q L Y T I C F G E K R P T D D
601	CTGATGGTTTTTCATCAACTATACACAATATGCTTTGGAGAAAAGCGTCCACAGATGAC
	P E V N K L L K I D N D L I D K L G T G
661	CCGGAAGTGAATAAATGCTTAAAGATAGACAATGATTTGATTGACAAGCTAGGTACCGGG
	L F E D I I P Y F K D I Y P T K K W Q M
721	CTTTTTGAGGATATAATCCCCATTTTAAAGACATCTATCCAACGAAAAAATGGCAGATG
	F L S M V D E M L T V L R R K F R E H V
781	TTTCTCTCCATGGTGGACGAAATGCTCACAGTTCTTAGAAGAAAATTTAGAGAGCATGTT
	E T F Q P G V N R D F I D S M L I A K Q
841	GAAACCTTCCAGCCAGGAGTCAACAGGACTTCATTGACAGCATGTTAATCGCTAAACAG
	E A K D E G D E A A L E V M D D T H L V
901	GAAGCGAAGGATGAGGGCGATGAGGCGCCCTGGAGGTCATGGATGATACGCACCTCGTT
	Q T I S D I F F A G V D T T R F T M D W
961	CAGACCATATCTGATATCTTCTTTCGCGGGGTAGACACTACTCGTTTCAATGGATGG
	F V Y F M T R F P E F Q A K C Q E E I D
1021	TTTCGTTTATTTTCATGACAGATTTCCGGAATTCGAAGAAAATGTCAAGAGGAAATTGAC
	R V V G S E Q P S M K D R S K L D Y T E
1081	AGAGTTGTTGGATCAGAACAACCTTCAATGAAGGACAGAAGCAAATTTGATTACACCGAG
	A C L F E S M R L S N V V G I G L P H M
1141	GCCTGTCTGTTTGAATCGATGCGGCTTTCGAATGTTGTAGGCATAGGGCTCCACACATG
	T I C D S Q V G G Y D V P K G T T V V I
1201	ACAATTTGTGATTCACAAGTTGGTGGATACGATGTCCCAAAGGTACCCTGTATCATC
	N H W A L H H D P K Y W K D P E E F D P
1261	AACCACTGGGCGCTTCCCATGACCCTAAATATTTGAAGGACCAGAAGAAATTTGACCCA
	L R Y L D E N G K M K P A K P D S W L P
1321	CTTCGCTATCTCGATGAAAACGGTAAATGAAACCCGGAACAGATAGCTGGCTTCCC
	F S A G R R V C L G E S L A K P E I L L
1381	TTCTCAGCCGGACGTAGAGTTTGCTTGGGAGAAAGTTTGGCCAAACCAGAAATCTACTG
	M C A N L L Q R F E I S L P E G V K P N
1441	ATGTGTGCCAATCTTCTACAGCGATTTGAAATAAGTCTCCAGAGGGCGTGAAGCCGAAT
	L E H R L P G F G V E L P S D Y K I V V
1501	TTAGAGCACCAGCTTCCGGGCTTGGCGTAGAGCTTCCGTCGGATTACAAAATCGTGGTG
	K E R N R D -
1561	AAAGAGAGAAAATAGAGATTAAAGAATAATGGCGCTTGGCATCTTTTTTGTATTACAGACA
1621	CCACTAACACAACACTGACTATTTAATATTAATACAATGT

Fig. 1. Nucleotide and deduced amino acid sequences of CYP356A1 from *Crassostrea rhizophorae*. Some motifs of CYP signatures are indicated, including the C, K, and I helices, and the heme-binding region.

Nomenclature Committee as a member of a new subfamily, CYP356A1. The phylogenetic analysis demonstrates a close relationship between the CYP356A1 and CYP1 and CYP17 subfamilies (Fig. 2a). CYP356A1 may be classified as an invertebrate CYP Clan 2 (CYP17-like) gene, sharing 32–36% amino acid identity (masking out regions of alignment uncertainty) with vertebrate CYP17s. In contrast, CYP356A1 shares a lower percentage identity (30–33%) with CYP1 and CYP2 genes. The CYP17 family is associated with steroid metabolism, while CYP1A is classically used as biomarker of exposure to polycyclic aromatic hydrocarbons (Hahn, 2002). No CYP17 genes have been found in non-chordate invertebrates, although CYP17 is present in amphioxus (Mizuta and Kubokawa,

2007), and CYP17-like genes were identified in the genome of the purple sea urchin, *Strongylocentrotus purpuratus* (Goldstone et al., 2006). Similarly, CYP1 and CYP1-like genes have been detected in tunicates (CYP1E and CYP1F subfamilies; Goldstone et al., 2007) and sea urchins (Goldstone et al., 2006, 2007), but no CYP1 sequences have been found in non-deuterostome invertebrates.

Semi-quantitative RT-PCR results showed that higher expression of CYP356A1 was observed in digestive gland and mantle when compared to gill and adductor muscle (Fig. 2b). The digestive gland and mantle are important tissues for both biotransformation and steroid metabolism.

Download English Version:

<https://daneshyari.com/en/article/4551529>

Download Persian Version:

<https://daneshyari.com/article/4551529>

[Daneshyari.com](https://daneshyari.com)