Expression Profiles of CYP1-4 Isozymes and Accumulation of dioxin-like and non-dioxin-like organochlorine compounds in the Liver of Common Minke Whales (Balaenoptera acutorostrata)

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Introduction

Cytochrome P450 (CYP) enzyme, a superfamily of hemoproteins, participates in classical phase I metabolic reaction in which the substrate is oxidized. The CYP enzyme is primarily responsible for bioactivation and detoxification of a variety of endogenous and xenobiotic compounds. CYP1-4 families are highly inducible by xenobiotic chemicals and are thought to be important in their metabolisms.[1] Certain marine mammals accumulate high levels of organochlorine contaminants such as PCBs (polychlorinated biphenyls) and DDTs (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane and its metabolites) that have generated serious concern because of their ubiquitous distribution, toxicity, and bioaccumulation potential.[2] However, available information on CYP genes and their expression in association with organochlorine compounds (OCs) and dioxin related compounds are limited in marine mammals. We previously reported the partial sequence of some CYP genes, CYP1A, 2C, 2E, 3A, 4A and 4 isozyme[3], and the full-length sequence of CYP 1A1 and 1A2 [4],cloned from a hepatic cDNA library in common minke whale. To understand metabolic and signaling pathways of the CYPs in the liver of common minke whale, the present study investigated the full-length cDNA sequences of CYPs (CYP2C, 2E, 3A, 4A and 4) using the clones from a hepatic cDNA library. Additionally, the relationships between mRNA expression of individual CYPs and accumulation of classical OCs including PCBs, DDTs, hexachlorocyclohexanes, chlordanes and hexachlorobenzene, and dioxin-like compounds (PCDDs/DFs and coplanar PCBs) were also examined in the liver of common minke whales.

Materials and Methods

Nineteen males of common minke whales were collected from the western North Pacific in 2001 by JARPN II (Japanese Whale Research Program under Special Permit in the Western North Pacific-Phase II). Livers whichwere immediately removed after collection, were frozen in liquid nitrogen and stored at -80°C until preparation for total RNA isolation. The liver samples for chemical analysis were stored at –20°C until analysis.

We constructed a cDNA library using oligo-capping methods and pME18SFL3 plasmidsligated with cDNA fragments from the liver. A total of 6930 clones randomly selected in the library were further screened for the isolation of cDNA clones encoding novel members of CYP subfamily. The CYP cDNAs were sequenced using ABI PRISM 310^{TM} genetic analyzer.

Total RNAs from 19 livers were extracted using TRIzol reagent (Invitrogen). Following DNase treatment, mRNA expression level of each CYP isozyme was quantified using a real-time RT-PCR which were performed with TaqMan One-Step RT-PCR Master Mix Reagent Kit (Applied Biosystems).

Chemical analysis for organochlorine compounds and dioxin-like compounds were carried out following the method previously described. [5,6]

The Mann-Whitney *U* test was employed to detect the differences in OC concentrations, and CYPsmRNA expression levels between immature and mature whales. Spearman's rank correlation test was used to examine the relationships between CYP mRNA levels and OCs concentrations. This statistical analysis was performed by StatView for Windows (Version 5.0, SAS Institute Inc., USA).

Results and Discussion

Characteristics of CYPs amino acid sequences

Table 1 Characteristics of CYP cDNAs isolated from common minke whale

	Amino acid (No.)	Calculated M.W. (kDa)	5'-UTR (bp)	3'-UTR (bp)
CYP1A1*	516	58.3	135	1,321
CYP1A2*	516	58.1	62	367
CYP2C78	504	57.8	46	433
CYP2E1	495	56.6	29	207
CYP3A72	503	57.4	88	444
CYP4A35	510	58.3	33	847
CYP4V6	525	61.1	36	258

* cited after ref. 4

As a result of screening of 6930 clones randomly selected in a hepatic cDNA library, we obtained full-length cDNA sequences of seven CYPs, CYP1A1, CYP1A2, CYP2C78, CYP2E1, CYP3A72, 4A35 and CYP4V6, for which names were given by the P450 Nomenclature Committee (Table 1). In all CYPs identified, the amino acid sequences including the heme-binding motif (FXXGXXXCXG) in the vicinity of C-terminus, conserved threoninein the distal helix I, K-region (ExxR) in the helix K and proline rich sequences near the N-terminal were highly conserved.

The amino acid sequences and their molecular characteristics of CYP1A1 and CYP1A2 have already been reported elsewhere.[4] The deduced amino acid sequence of commonminke whale CYP2C78 showed 80% identity with pig 2C33, and 52-59% with other terrestrial mammals.CYP2C78 showed an Asn at position 293 amino acid residue. It is known that Asp-293 in human CYP2C9 plays a key role in substrate recognition and catalytic activity.[7] Considering the preservation of Asp-293 in other mammalian species, the replacement of Asn-293 in common minke whale CYP2C78 implies the decline of binding activity to substrate. Common minke whale CYP2E1 showed high identities with those of pig (85%) and cattle 2E1 (84%).The CYP3A72 belonged to the same group as cattle 3A28 (81%) and pig 3A subfamilies (77-80%). To our knowledge, this is the first report on full-length of CYP2E and 3A amino acid sequence in aquatic mammals, although the presence of CYP3A-like protein has already been reported in pinniped.[8] Twoisozymes of CYP4 family, CYP4A35 and CYP4V6 were also obtained. CYP4A35 amino acid sequence showed higher identities with those of pig 4A (80%). Although the function of CYP4V has not yet been understood, common minke whale CYP4V6 showed 76% identity to human CYP4V2. The distinct sequence of CYP4 family, EVDTFMFEGHDTT located in the position of amino acid residue 314-326 of CYP4A35 and 322 - 334 of CYP4V6 was conserved in these two CYP4 genes.

CYPs expressions and accumulation of OCs and dioxin-like compounds in the liver of common minke whales

To investigate the alteration of CYP expression levels in association with the accumulation of OCs, this study examined the relationship between hepatic OC residue levels and individual CYPs (CYP1A1, CYP1A2, CYP2C78, CYP2E1, CYP3A72, 4A35 and CYP4V6) mRNA expression levels. No clear correlation was found between OCs residue levels and CYPsmRNA expressions in the liver of common minke whales (Figure 1).The residue levels of OCs (e.g., PCBs; 5.5-93 ng/g wet wt., DDTs; 4.1-210 ng/g wet wt.) encountered in this study may be too low to induce CYP isozymes or these OCs do not participate in the induction of these isozymes. TEQ levels in the liver of common minke whales showed a range of 0.64-3.9 pg/g wet wt., and also any very few PCDDs/DFs congeners were detected. The literature survey revealed that the average TEQs (1.9 pg TEQ/g wet wt.) in the common minke whale liver was lower than the EC_{50}values for TCDD in the human hepatoblastoma cell line HepG2 (220 pg TEQ/g wet wt.) and rat hepatoma cell line H4IIE (16 pg TEQ/g wet wt.). [9] No significant relationship between expression of CYP1A1/2 and TEQs or concentrations of individual congeners of PCDDs/DFs and coplanar PCBs was observed (Figure 2). This suggests that the low TEQs in common minke whale livers did not reach the levels to induce CYP1A1 and CYP1A2.

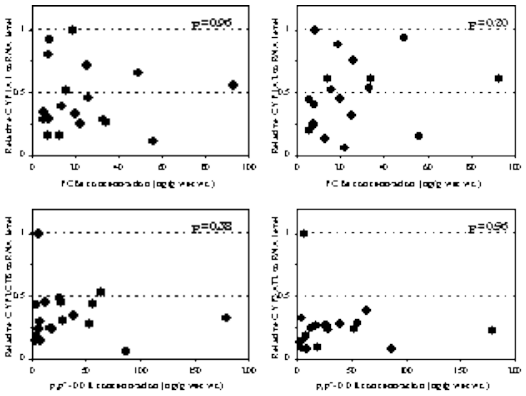

Figure 1 Relationship between OCs and CYPs mRNA expression levels in the liver of common minke whales collected from the North Pacific.

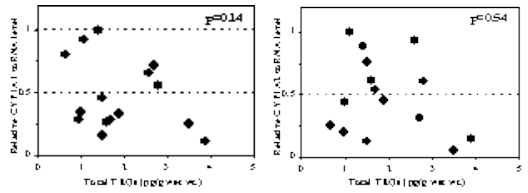

Figure 2 Relationship between TEQs and CYP1A1/1A2 mRNA expression levels in the liver of common minke whales collected from the North Pacific.

Relationships of expression levels among CYPs

Table 2 Spearman's rank correlation among CYP mRNA expression levels in the liver of common minke whales

	1A1	1A2	2C78	2E1	3A72	4A35
1A2	0.64 ***	-				
2C78	0.61 ***	0.78 ***	-			
2E1	0.32	0.61 ***	0.72 ***	-		
3A72	0.54 **	0.80 ***	0.90 ***	0.65 ***	-	
4A35	0.43	0.61 ***	0.77 ***	0.91 ***	0.73 ***	-
4V6	0.44	0.49 **	0.80 ***	0.84 ***	0.75 ***	0.91 ***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

The relationships of mRNA expression levels among individual CYP isozymes are summarized in Table 2. The result revealed a positive correlation between CYP1A1 and 1A2, indicating that the transcription of both CYPs is regulated by a common mechanism such as aryl hydrocarbon receptor signaling pathway in the liver of common minke whales. A strong significant positive correlation between CYP2C78 and CYP3A24 mRNA expression levels was observed, suggesting that these CYPs may share a common mechanism in transcriptional regulation including cross-talk by certain nuclear receptors such as constitutive androstane receptor and pregnane X receptor.[10] Furthermore, positive correlations among CYPsmRNA expressions measured in this study were observed in many cases. This may suggest a shared mechanism through HNF (hepatic nuclear factor) families, which are the transcriptional factors contributing to the liver-specific expression of several genes including CYPs.[10]

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