MOLECULAR CHARACTERIZATION OF AN ARYL HYDROCARBON RECEPTOR IN BAIKAL SEALS (*Phoca sibirica*)

Eun-Young Kim[1], Mark E. Hahn[2], Hisato Iwata[1], Shinsuke Tanabe[1], Nobuyuki Miyazaki[3]

[1] Center for Marine Environmental Studies, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan

[2] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

[3] Marine Research Center, Ocean Research Institute, The University of Tokyo, Akahama, Otsuchi-cho, Iwate 028-11, Japan

Introduction

Dioxin and planar halogenated aromatic hydrocarbons (PHAHs) have generated serious concern in recent years because of their ubiquitous distribution, toxicity and bioaccumulation potential[1, 2]. These chemical pollutants have been suggested as causal agents of reproductive failure, immunotoxicity and mass mortality in marine mammals[3, 4]. Baikal seals (***Phoca sibirica***), which inhabit freshwater environment, accumulate high levels of PHAHs[5]. This species is thought to be highly sensitive to the toxic effects of these compounds, as suggested by mass mortality. In 1987 and 1988, several thousands of Baikal seals died by morbillivirus infection[6]. Although the direct cause for this outbreak was infectious diseases, chemical pollutants have been suggested as contributing factors in this epizootic[3, 4]. However, the role of these chemicals in Baikal seals remain uncertain, because the lack of direct information concerning the sensitivity to dioxin or other PHAHs.

The aryl hydrocarbon receptor (AHR) is a ligand activated intracellular protein that contains the basic-helix-loop-helix/Per-ARNT (AHR nuclear translocator)-Sim (bHLH-PAS) domains[7, 8]. Most toxic effects of dioxin and PHAHs are mediated by AHR, to which these chemicals bind with high affinity[9]. Although the exact mechanisms underlying many of the receptor-mediated toxic effects are unclear, mice lacking the AHR are insensitive to dioxin, showing that the AHR is an important controlling factor in its toxicity[10, 11, 12]. The cloning and sequencing of AHRs in experimental animals have led to major advances in our understanding of the sensitivity to dioxin toxicity[13, 14]. However, the structural and functional characteristics of AHRs in marine mammals which accmulate high levels of PHAHs and dioxin, are poorly understood[15, 16].

In this study, to investigate the mechanistic basis for dioxin sensitivity in Baikal seals, we cloned the cDNA encoding the AHR, an intracellular protein that is responsible for PHAH effects.

Materials and Methods

The liver sample of Baikal seals were collected in 1992 and stored at -80 °C until total RNA isolation. Total RNA was isolated using RNAgent®Total RNA isolation system (Promega).

Poly(A)$^+$ RNA was purified by PolyATtract® mRNA isolation systems (Promega). The AHR from Baikal seal was cloned using RT-PCR approach. One μg of poly (A)$^+$RNA was reverse transcribed with random hexamers using the Gene-Amp RNA-PCR kit (Perkin-Elmer) following the manufacturer's directions. PCR Primers were designed targeting conserved regions of the AHR (Fig. 1)[17] and synthesized by ASAHI TECHNO-GLASS Inc. After first strand cDNA synthesis, PCR amplification was performed using QF/B2 under the following conditions: 30 cycles of (15 sec. at 94°C, 45 sec. at 50°C, and 1 min at 72°C). The cDNA fragments of the expected size on agarose gels were purified and subcloned into pGEM-T Easy vector (Promega). cDNA samples were sequenced using ABI PRISM ™310 genetic analyzer.

For 5'- and 3'-RACE (Rapid Amplification of cDNA Ends), adaptor-ligated, oligo(dT)-primed, double-stranded liver cDNA was synthesized using a Marathon cDNA Amplification kit (CLONTECH). For 3'-RACE of Baikal seal AHR, gene specific primers (BS-3'3) were coupled with adaptor primers in the PCR reactions and the products were cloned and sequenced. The remainder of the 5' coding sequence was obtained using 5'-RACE with the Bel and BS-5'1 primer pair. AHR amino acid sequences were aligned using CLUSTALW version 1.7.

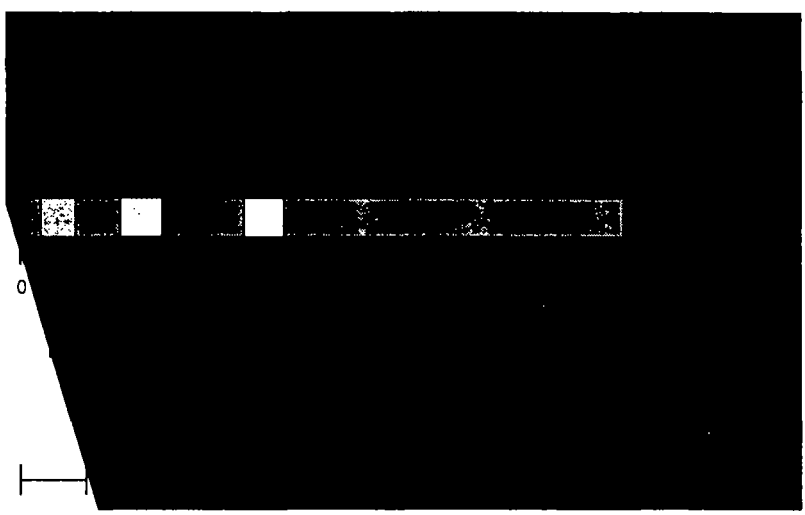

Fig. 1. Cloning strategy for Baikal seal AHR cDNA. RT-PCR and RACE fragments are shown with oligonucleotide pairs used. Translated regions are boxed. BHLH domain, PAS domain A and B repeats are indicated.

Results and Discussion

A full-length AHR cDNA sequence from Baikal seal was obtained using the RT-PCR approach described above. The Baikal seal AHR cDNA has an open reading frame of 843 amino acid residues with a predicted molecular mass of 94.6 kDa. The C-terminal sequence includes 105 bp of 3'-UTR with a poly (A)$^+$ tail.

In alignment of the amino acid sequence, the Baikal seal AHR is most closely related to the harbor seal AHR (98%) and shares 82% and 79% overall amino acid identity with beluga and

human AHRs, respectively (Table 1). The Baikal seal AHR cDNA demonstrated strong N-terminal sequence conservation with the harbor seal, beluga and human AHRs. The greatest similarity between the sequences is found within the conserved basic helix-loop-helix and PAS domains. These functional domains of Baikal seal AHR showed the identical amino acid sequences with harbor seal AHR. The high conservation of AHRs between Baikal and harbor seals indicates that these seals express AHR proteins closely related structually. The dioxin-binding affinity of the harbor seal AHR was at least as high as that of the AHR from a dioxin-sensitive strain of mice, suggesting that this seal species may be sensitive to PHAH effects[16]. This implies that Baikal seal may also be sensitive to dioxin effects. The accumulation of high concentrations of PHAHs predicts that certain marine mammals, such as Baikal seal, harbor seal and beluga, may experience a greater threat from dioxin and PHAHs than terrestrial mammals.

Table 1. Amino acid identity(%) in full-length and, N- and C-terminal regions. AHR amino acid sequences were aligned using CLUSTALW 1.7. The boundaries between the N- and C-terminal regions for this table are residues 423, 423, 423, 424, and 418 for Baikal seal AHR harbor seal AHR, beluga AHR, human AHR and mouse AHR, respectively. Full-length comparisons at upper right; N- (N) and C-terminal (C) comparisons at lower left.

	Baikal seal	Harbor seal	Beluga	Human	Mouse
Baikal seal		98	82	79	66
Harbor seal	98 (N)		82	79	66
	98 (C)				
Beluga	90 (N)	91 (N)		83	51
	74 (C)	74 (C)			
Human	87 (N)	88 (N)	91(N)		53
	70 (C)	70 (C)	76 (C)		
Mouse	82 (N)	83 (N)	85 (N)	85 (N)	
	49(C)	49 (C)	51 (C)	53 (C)	

An earlier study[5] suggested from the accumulation pattern of PCB congeners that Baikal seals inhabiting freshwater environments have a lower activity of CYP1A enzymes than marine seals such as harbor seals, while having a higher activity of CYP2B-like enzymes. This difference between Baikal and harbor seals suggested differential regulation or activities of xenobiotic-metabolizing enzymes in these species. Studies on the comparative basis for the dioxin susceptibility between marine and freshwater mammals may provide new clues for understanding the mechanism of toxic action of xenobiotics in the ecosystem. Differences in the characteristics and expression of AHR between Baikal and harbor seals could contribute to the species differences in regulation of xenobiotic-metabolizing enzymes. Future studies will compare the

function and expression of these two proteins.

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