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cDNA cloning of an aryl hydrocarbon receptor from Baikal seals (*Phoca sibirica*)☆

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Abstract

Species differences in sensitivity to related planar halogenated aromatic hydrocarbons (PHAH) add significant uncertainty in assessing the ecological risk to aquatic mammals. To investigate mechanisms of PHAH sensitivity in aquatic mammals, we cloned and sequenced the cDNA of Baikal seal aryl hydrocarbon receptor (AHR), an intracellular protein that initiates PHAH-mediated effects. The Baikal seal AHR cDNA has an open reading frame of 843 amino acid residues with a predicted molecular mass of 94.6 kDa. Comparison of AHR amino acid sequences indicated a high degree of sequence conservation (98%) between Baikal and harbor seals. The high conservation of AHRs between Baikal and harbor seals indicates that these seals express AHR proteins closely related structurally. In our previous report (Kim & Hahn, 2002), 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. This implies that Baikal seal may also be sensitive to dioxin effects.

Keywords: Aryl hydrocarbon receptor; Dioxins; Susceptibility; Baikal seal

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related planar halogenated aromatic hydrocarbons (PHAHs) elicit a wide range of toxic and biological effects in organisms (reviewed in Poland & Knutson, 1982). Baikal seals (*Phoca sibirica*)

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inhabiting freshwater environments exhibit high levels of PHAHs in their tissues (Nakata et al., 1995). This species is thought to be highly sensitive to the toxic effects of these compounds, as suggested by recent cases of mass mortality. Although the direct cause for this outbreak was infectious diseases, chemical pollutants have been suggested as contributing factors. However, the role of these chemicals in Baikal seals remain uncertain, because the lack of direct information concerning the sensitivity to PHAHs.

Most toxic effects of dioxin and PHAHs are mediated by the AHR, a ligand activated intracellular protein, to which these chemicals bind with high affinity (Poland, Glover, & Kende, 1976). The cloning and sequencing of AHRs in experimental animals have led to major advances in our understanding of the sensitivity to dioxin toxicity. However, the structural and functional characteristics of AHRs in aquatic mammals which accumulate high levels of PHAHs, are poorly understood.

Previously, we reported the cloning and characterization of an AHR cDNA from harbor seal (Kim & Hahn, 2002), a marine pinniped, and proposed that the sensitivity of marine mammals to PHAH might be inferred by determining the characteristics of their AHRs using in vitro molecular and biochemical experiments (see also Jensen & Hahn, 2001). The objective of this research is to investigate the potential PHAH sensitivity in Baikal seals. Toward this aim, we initially cloned and sequenced the full length AHR cDNA from the liver of a Baikal seal. Differences in primary structure of AHR sequences among marine, freshwater and terrestrial mammals may provide new clues for understanding the mechanism of toxic action of xenobiotics in these organisms.

Livers of Baikal seals were collected in cooperation with the Limnological Institute of the Russian Academy of Science in 1992. Total RNA was isolated from a liver using RNAgent®Total RNA isolation system (Prormega). Poly(A)$^+$ RNA was purified by PolyATtract® mRNA isolation systems (Promega). The AHR from Baikal seal was cloned using a RT-PCR approach. Primer sequences were: Qf, 5′-aatccttccaagcgacataga-3′; B2, 5′-catgcacaactctgcttcagtatagcc-3′; BS3′3, 5′-caccc-cactgcttgtgatgccaaagg-3′; BS5′1, 5′-aggcagcagttccccctcaagaaacagc-3′; Bel, 5′-ccaagcttgggcaccatgaacagc-3′. PCR amplification was performed using QF/B2 under the following conditions: 30 cycles of (15 s. at 94 °C, 45 s. at 50 °C, and 1 min at 72 °C). For 5′- and 3′-RACE (Rapid Amplification of cDNA Ends), double-stranded 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. The remainder of the 5′ coding sequence was obtained with the Bel and BS-5′1 primer pair. cDNA samples were sequenced using ABI PRISM™310 genetic analyzer. AHR amino acid sequences were aligned using CLUSTALW version 1.7.

The Baikal seal AHR cDNA has an open reading frame of 843 amino acid residues with a predicted molecular mass of 94.6 kDa (Fig. 1). 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 (Fig. 1, Table 1). The greatest similarity between the sequences is found

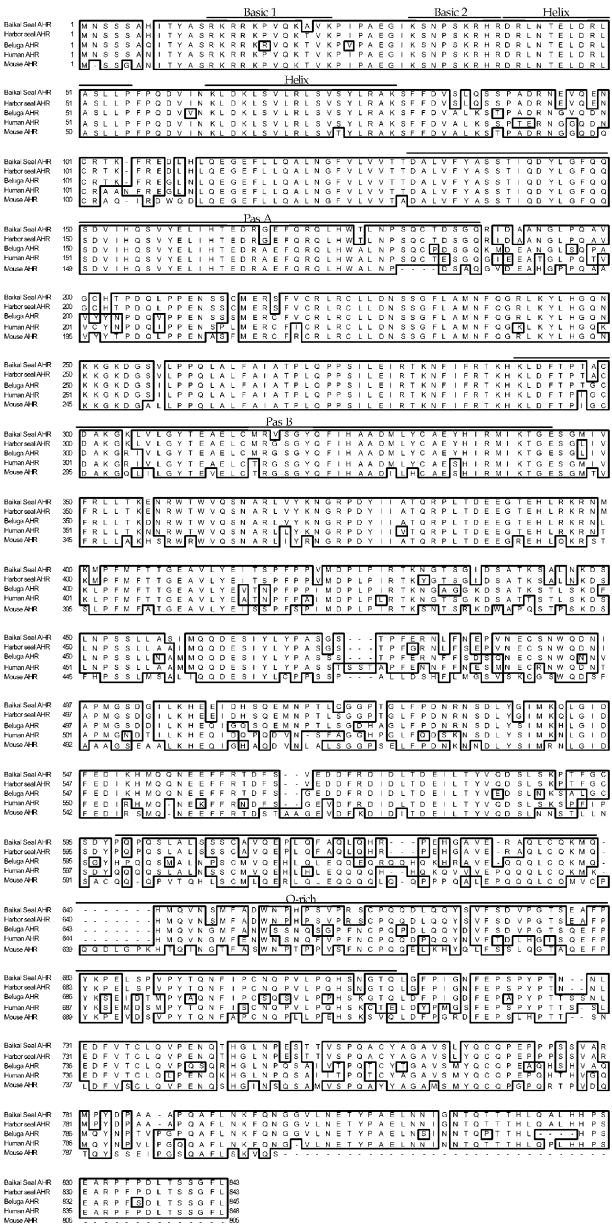

Fig. 1. Alignment of Baikal seal, harbor seal, beluga, human, and mouse AHR amino acid sequences. Sequences were aligned with Clustal W 1.7. Accession numbers and references for sequences used are: Baikal seal (accession number AB072432), harbor seal (Kim & Hahn, 2002) (accession number AB056700), beluga AHR (Jensen & Hahn, 2001) (accession number AF332999), human AHR (Dolwick, Schmit, Carver, Swanson, & Bradfield, 1993) (accession number L19872), and mouse AHR (Ahb-1 allele, Burbach, Poland & Bradfield, 1992) (accession number M94623). The functional domains labeled were identified by homology to other mammalian AHRs (see text). Identical amino acids are shaded and boxed.

Table 1
Amino acid identity (%) in full-length and, N- and C-terminal regions

	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)	

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.

within the conserved basic helix-loop-helix and PAS domains, which are important in DNA binding, AHR/ARNT dimerization and ligand binding (reviewed in Hahn, 1998). The high conservation of AHRs between Baikal and harbor seals indicates that these seals express structurally related AHR proteins.

Studies in experimental mammals have shown that AHR binding affinity is highly correlated with susceptibility to PHAH toxic effects. In our previous report (Kim & Hahn, 2002), 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 (C57BL/6), suggesting that this seal species may be sensitive to PHAH effects. This implies that Baikal seals may also be sensitive to dioxin effects due to the high similarity in the full-length and functional domains of their AHR amino acid sequences. Korkalainen, Tuomisto, and Pohjanuirta (2001) reported that there was a distinct correlation across published mammalian species between the number of glutamine residues in the Q-rich subdomain and sensitivity to the acute lethality of TCDD. The number of glutamine residues in the Q-rich subdomain of guinea pig AHR was 23, which is only half that of the more resistant hamster (McConnell, Moore, Haseman, & Harris, 1978). The number of glutamine residues in the Q-rich subdomain of Baikal and harbor seal AHR was 18, implying that seals might be sensitive to dioxin effects.

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