# THYROID HORMONE TRANSPORT IN GREAT LAKES HERRING GULL AND SVALBARD GLAUCOUS GULL: COMPETITIVE TRANSTHYRETIN (TTR) BINDING OF SELECTED ORGANOHALOGENS

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**Introduction**

Chlorinated and brominated aromatics, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE) flame retardants, and their hydroxylated (OH) PCBs and OH-BDE analogues or metabolites are being found with increasing frequency in mainly the blood of wildlife from various environments including fish, mammals and birds.[1-5] Certain congeners of the organohalogen contaminant classes have be reported to influence, and the potential to affect the thyroid hormone (TH) system. For example, decreased serum concentrations of thyroxine (T4) have been negatively correlated with PCB and PBDE concentrations in humans, seals and birds such as glaucous gull (*Larus hyperboreus*), which is the top avian Arctic predator.[3,4,6-8] Recent studies suggest that several persistent environmental contaminants, including PCBs, PBDEs, and their OH and possibly methyoxylated (MeO) analogues interfere with T4 binding to thyroid hormone transport protein, transthyretin (TTR), and are neurotoxic.[9-11]

THs are lipid soluble and this solubility allows for permeation into membranes. However, the distributions of THs in an organism, which are generally hydrophobic, require certain proteins to increase their hydrophilicity for transport via the blood. Therefore, higher evolved vertebrates like mammals and birds possess a number of T4 transport proteins to serve this function. In mammals and birds three T4 transport (carrier) proteins are synthesized in the liver, i.e., T4-binding prealbumin (or TTR), T4 binding globulin (TBG) and albumin (ALB). In birds, albumin and TTR serve as thyroid hormone carrier proteins. From an avian toxicology perspective, there are limited reports on the effects of environmentally relevant organohalogen and metabolites on TH gene expression in the brain and in other tissues. This is especially true for wild birds. Thyroid hormones (TH) are essential for the development and continued function of many organs and tissues, including the central nervous system, as well as being necessary for proper growth and metabolism in vertebrates. In birds both TTR and ALB are carriers. TTR has two main origins of secretion in birds, the liver and brain. In the brain the TTR is secreted by choroid plexus. All TTR produced in the brain is secreted into the cerebrospinal fluid, where it becomes the major thyroid hormone-binding protein. During ontogeny, the maximum TTR synthesis in the choroid plexus precedes that of the growth rate of the brain and occurs during the period of maximum neuroblast replication.[10]

Lake Ontario is one of the Laurentian Great Lakes of North America, and has numerous colonial populations of herring gulls (*Larus argentatus*).[12] As their populations are found across the Great Lakes basin, among other reasons, herring gulls are avian sentinel species for ecosystem contamination of persistent organic pollutants (POPs), e.g., PCBs and PBDEs.[13] In Norway, the Svalbard area is a key breeding area for seabirds such as the predatory glaucous gull. Glaucous gulls are at a high trophic level on the Svalbard marine food web, and have been found to contain high levels of chlorinated and brominated organochlorines.[3,4,7] In addition to PCBs and PBDEs, recently a number of OH-PCB (e.g., 4-OH-CB187) and OH-PBDE (e.g., 6-OH-BDE47) congeners were reported in the blood, and to a lesser extent in egg, of breeding glaucous gulls from Bear Island.[3,4] OH-PCBs have also been detected, but

not confirmed or reported, in the blood of herring gull from various breeding colonies in the Great Lakes (G.A Fox, personal communication).

In the present study we cloned, sequenced and expressed the nucleotide sequence of TTR from the brain and liver from a Lake Ontario herring gull and from a Svalbard glaucous gull. These gull species are also exposed to different levels and profiles of chlorinated and brominated contaminants that have been shown to have or have potential thyroidogenic properties. We compared the phylogenic differences and similarities of these TTR amino acid sequences between gull species and tissues. Using these TTR sequences, competitive binding assays we performed for T4 and T3 and selected contaminants and metabolites that are relevant contaminants in these species.

**Material and Methods**

Liver and brain tissues were sampled from adult herring gull during the summer of 2004 from Chantry Island, Ontario, as part of the herring gull monitoring program carried out by the Canadian Wildlife Service. Liver and brain tissue were sampled from a glaucous gull that was collected from Bear Island near Svalbard in the Norwegian Arctic, and provided via the Norwegian Polar Institute in Tromsø, Norway.

TTR is hypothesized to be the major transport protein for THs in these avian model predator species. TTR sequencing was accomplished using fractions of brain and liver tissues, primers were designed from published information for chicken TTR. The partial sequence (~600 base pairs) of TTR of gull livers and brains was cloned. The cloning of the TTR sequence was performed using an adapted methodology from Arukwe *et al.*[14] The cloning of the TTR sequence was performed using a RACE cDNA amplification technique. The sequences of primers used were: TTR chicken FWD (5'-3') [d(CTCCCATGGCTCTGTTGATT) (n = 20)] and [TTR chicken REV (5'-3') d (TTGTCTGAATTTTTGCCAGGT) (n = 21)]. A comparison of nucleotide sequence among inter-genus was performed to observe any divergence between species.

Competitive TTR binding was investigated using a protein assay that utilizes radio-labelled $^{125}$I-T3 and $^{125}$I-T4. The methodology of TTR competitive binding was adapted from Meerts *et al.*[15] The selected and thyroidogenic organohalogens that were screened were: 4-OH-CB187 (2,2',3,4',5,5',6-$Cl_7$); 4-MeO-CB187 (2,2',3,4',5,5',6-$Cl_7$); CB187 (2,2',3,4',5,5',6-$Cl_7$); BDE47 (2,2',4,4'-$Br_4$); 6-OH-BDE47 (2,2',4,4'-$Br_4$); 4'-OH-BDE49 (2,2',4,5'-$Br_4$) and 6-MeO-BDE47 (2,2',4,4'-$Br_4$). The competitive binding assay is generally described as follows. A contaminant substrate competes for labelled versus unlabelled TH ligand. Following the separation of free and bound TH, the TH is quantified by comparing the bound and unbound ratios to known standards. Separation from bound THs were determined using a Beckman 5000 scintillation counter. Specific binding was calculated using the following method. A regression equation was fit to the UPL data, where the x-axis represents "free" L-$^{125}$I-T3 and L-$^{125}$I-T4 (nM) the y-axis represents bound L-$^{125}$I-T3 or L-$^{125}$I-T4 (DPM). The regression equation was used to calculate the non-specific binding at each concentration of free L-$^{125}$I-T3 or L-$^{125}$I-T4. Subtracting the calculated non-specific binding from the actual total binding gave the specific binding. The specific binding was fit to the following equation: $B=B_{max}[L]/[L]+K_d$ where B is specific binding of L-$^{125}$I-T3 or L-$^{125}$I-T4, $B_{max}$ is maximum bound receptor (DPM), L is the concentration of free L-$^{125}$I-T3 or L-$^{125}$I-T4, and $K_d$ is the dissociation constant. The T3 and T4 $K_d$ mean values for each species was calculated based on three separate experiments.

**Results and Discussion**

*Larus argentatus* from Lake Ontario in Great lakes and *Larus Hyperboreus* from Bear Island in Norway are important gull species in their respective aquatic ecosystems. The former inhabits a freshwater system (Lake Ontario), and is located in a highly urbanized watershed. The Bear Island glaucous gulls inhabit a marine system, and are located in a remote Arctic location where POPs enter the ecosystem and food web via atmospheric transport. Thus, the concentrations and profiles of exposure to known or potentially thyroidogenic contaminants (i.e., high affinity for TH transport proteins) are hypothetically different for individual gulls from these two distinct populations.

The complementary DNA (cDNA) cloning showed a large degree of nucleotide and amino acid sequence similarity (> 95%) between the brain and liver, and between the tissues from the two gull species. Specifically, we observed a one nucleotide difference in the TTR sequence in the brain of herring gull, compared to glaucous gull. The comparison between them a profile of evolutionary conservation of nucleotide composition of TTR transport protein. The amino acid sequences obtained from avian models are presented in Table 1.

Table 1. Transthyretin (TTR) amino acid sequence obtained for brain and liver tissues of glaucous gull and herring gull.

| TISSUE | TTR amino acid sequence |
|---|---|
| Herring gull Liver | SHGSVDSKCPLMVKV**L**DAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEY GEIHELTTEEQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYT IAALLSPFSYSTTAVVSDPQE |
| Glaucous gull Liver | SHGSVDSKCPLMVKV**L**DAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEY GEIHELTTEEQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYT IAALLSPFSYSTTAVVSDPQE |
| Herring gull Brain | SHGSVDSKCPLMVKV**P**DAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEY GEIHELTTEEQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYT IAALLSPFSYSTTAVVSDPQE |
| Glaucous gull Brain | SHGSVDSKCPLMVKV**L**DAVRGSPAANVAVKVFKKAADGSWQDFATGKTTEY GEIHELTTEEQFVEGIYRVEFDTSSYWKGLGLSPFHEYADVVFTANDSGHRHYT IAALLSPFSYSTTAVVSDPQE |

Using the cloned and expressed plasmids, competitive binding assays were conducted with TTR-T3/T4 and chlorinated and brominated POPs with known or hypothetically high binding affinity for TTR. In addition to the binding affinity, using a training set of POP ligands, we also examined the influence of key structural features on the molecules, and how the affinity and efficacy of binding was affected. The competitive binding assay showed higher competitive binding affinity for 4-OH-CB187 relative to CB187. The 4-OH-CB187 has been shown to be a metabolite of CB187 via enzyme-mediated, direct OH-insertion. Similarly, 6-OH-BDE47 and 4'-OHBDE49 had higher binding affinity for the TTRs relative to BDE47. This demonstrated that TTR binding relative to T3 and T4 favours PCB and PBDE substrates that contain an OH function group (Figure 1).

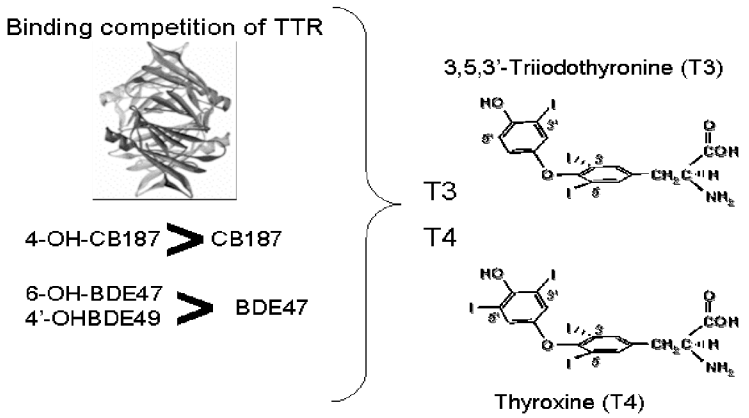


Figure 1. Competitive TTR binding affinity of PCB and PBDE and related OH-containing substrates relative to T3 and T4.

In birds the transport of THs via binding with TTR is very important, and more so than with ALB. We cloned and expressed TTR proteins from both liver and brains from these gull species, and found little inter-genus divergence in the nucleotide sequence for TTR from these species and tissues. Using these cloned TTR proteins, we demonstrated that key thyroidogenic, PCB and PBDE congeners (and especially OH-PCBs and OH-PBDEs), which are environmentally relevant in the herring and glaucous gulls under study, can modulate T3- and T4-TTR binding *in vitro*, and possibly *in vivo* in POP exposed birds.

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**References**

1. McKinney MA, Martineau D, Dallaire AD, Béland P, De Guise S, LeBeuf M, Letcher RJ. 2006. *Environ Toxicol. Chem* 2006;25:1246.
2. Li H, Bennett ER, Drouillard K, Haffner GD Letcher RJ. *Environ Sci Technol 2003;*37:832.
3. Verreault J, Gabrielsen GW, Chu S, Muir DCG, Andersen M, Hamaed A, Letcher RJ. *Environ Sci Technol* 2005;39:6021.
4. Verreault J, Gabrielsen GW, Muir DCG, Chu SG, Gebbink W, Letcher RJ, *Environ Toxicol Chem* 2005; 24:2486.
5. Valters K, Li H, Alaee M, D'Sa I, Marsh G, Bergman Å, Letcher RJ. *Environ Sci Technol* 2005;39*(*15):5612.
6. Rolland RM. *J Wildlife Dis* 2000;36:615.
7. Verreault J, Skaare JU, Jenssen BM, Gabrielsen GW. *Environ Health Pespect* 2004;112: 5.
8. Fernie KJ, Mayne G, Shutt JL, Pekarik C, Grasman KA, Letcher RJ, Drouillard K. *Environ Pollut* 2005;138(3):485.
9. Dawson A. *Ecotoxicology* 2000;9:59.
10. Leatherland JF. In: *Environmental Endocrine Disrupters*, Guillette L, Crain DA (eds.), Taylor & Francis, New York, 2000:155.
11. Schreiber GB. *J Endocrinol*, 2002;175(1):61.
12. Weseloh DV, Pekarik C, De Solla SR. *Environ Monit Assess* 2006;113(1-3):265.
13. Norstrom RJ, Simon M, Moisey J, Wakeford B, Weseloh DV. *Environ Sci Technol* 2002;36:4783.
14. Arukwe A, Kullman SW, Berg K, Goksoyr A, Hinton DE. *Comp Biochem & Phisiology* 2002; 315-326
15. Ilonka ATMM, van Zanden JJ, Luijks EAC, van Leeuwen-Bol I, Marsh G, Jakobsson E, Bergman Å, Brouwer A. *Toxicol Sci* 2000;56:95.