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Molecular and thyroid hormone binding properties of lamprey transthyretins: The role of an N-terminal histidine-rich segment in hormone binding with high affinity

Kentaro Kasai [a], Norihito Nishiyama [b], Kiyoshi Yamauchi [a, *]

[a] Department of Biological Science, Graduate School of Science, Shizuoka University, Shizuoka 422-8529, Japan
[b] Department of Biology, School of Medicine, Fukushima Medical University, Fukushima 960-1295, Japan

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ABSTRACT

Transthyretin (TTR) is a plasma thyroid hormone (TH) binder that emerged from an ancient hydroxyisourate hydrolase by gene duplication. To know how an ancient TTR had high affinity for THs, molecular and TH binding properties of lamprey TTRs were investigated. In adult serum, the lipoprotein LAL was a major T3 binder with low affinity. Lamprey TTRs had an N-terminal histidine-rich segment, and had two classes of binding sites for 3,3′,5-triiodo-L-thyronine (T3): a high-affinity and a low-affinity site. Mutant TTR$\Delta_{3\text{-}11}$, lacking the N-terminal histidine-rich segment, lost the high-affinity T3 binding site. [^{125}I]T3 binding to wild type TTR and mutant TTR$\Delta_{3\text{-}11}$, was differentially modulated by Zn^{2+}. Zn^{2+} contents of wild type TTR were 7–10/TTR (mol/mol). Our results demonstrate that lamprey TTR is a Zn^{2+}-dependent T3 binder. The N-terminal histidine-rich segment may be essential for neofunctionalization (i.e., high-affinity T3 binding activity) of an ancient TTR after gene duplication.

1. Introduction

More than 99% of thyroid hormones (THs) circulating in plasma are protein bound, which accomplishes a uniform distribution of THs in the bloodstream to peripheral tissues (Mendel et al., 1987). Plasma TH distributor proteins (THDPs) in large eutherians consist of thyroxine-binding globulin, transthyretin (TTR) and albumin (Farer et al., 1962; Larsson et al., 1985; Tanabe et al., 1969). Because the loss of one THDP had no adverse effects on the thyroid function of rodents and humans (Bartalena, 1993; Mendel et al., 1989; Palha et al., 1994), plasma THDPs are thought to be a functionally redundant network system. Plasma THDPs differ among vertebrate groups (Richardson et al., 1994; Yamauchi and Ishihara, 2009). Thyroxine-binding globulin has been detected in plasma from eutherians and some marsupials (Richardson et al., 1994). Albumin has been detected in plasma from all vertebrate groups (Farer et al., 1962; Larsson et al., 1985; Tanabe et al., 1969), although TTR was detected as a THDP in several species of reptiles, amphibians, fish and agnatha (Power et al., 2009; Richardson et al., 2005; Suzuki et al., 2017; Yamauchi and Ishihara, 2009). Lipoproteins also act as major THDPs with low binding affinity for THs in some fish (Benvenga, 1997; Cyr and Eales, 1992; Richardson et al., 2005).

Agnathan cDNA clones encoding TTR were first isolated from the liver of the lampreys *Petromyzon marinus* and *Lampetra appendix* (Manzon et al., 2007). However, the functions of these TTRs remain to be clarified. In gnathostomes, THs are key hormones that induce post-embryonic development, e.g., metamorphosis in amphibians (Leloup and Buscaglia, 1977) and smoltification in salmonids (Dickhoff et al., 1978), in which elevated TH concentrations correlated with increased TTR concentrations in plasma (Richardson et al., 2005; Yamauchi et al., 1993). However, an inverse relationship between TH concentrations and the induction of metamorphosis exists in lampreys (Holmes et al., 1999; Leatherland et al., 1990; Lintlop and Youson, 1983; Youson et al., 1994; Wright

Abbreviations: BSA, bovine serum albumin; CBB, Coomassie Brilliant Blue; EDTA, ethylenediaminetetraacetic acid; HIC, hydrophobic interaction column chromatography; HIU, 5-hydroxyisourate; HIUHase, 5-hydroxyisourate hydrolase; HPLC, high performance liquid chromatography; IC_{50}, half maximal (50%) inhibitory concentration; K_d, dissociation constant; MBC, maximum binding capacity; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA end; rT3, reverse T3 or 3,3′,5′-triiodo-L-thyronine; RT, reverse transcription; SDS, sodium dodecyl sulfate; T3, 3,3′,5-triiodo-L-thyronine; T4, L-thyroxine; TBS, Tris-buffered saline; TH, thyroid hormone; THDP, TH distributor protein; TTR, transthyretin.

* Corresponding author.
E-mail addresses: mitsuya59451192@gmail.com (K. Kasai), norihito@fmu.ac.jp (N. Nishiyama), yamauchi.kiyoshi@shizuoka.ac.jp (K. Yamauchi).

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and Youson, 1977). Thyroid hormone concentrations are high in plasma at larval stages, and decrease markedly with the onset of metamorphosis. Furthermore, precocious metamorphosis can be induced in lampreys by goitrogen treatments (Hoheisel and Sterba, 1963). Interestingly, the abundance of TTR transcript peaks in the liver during metamorphosis (Manzon et al., 2007), suggesting that TTR may play a physiological role in the TH signaling system that is negatively involved in the progress of lamprey metamorphosis. Despite these circumstances, analysis of the lamprey sera by autoradiography of native polyacrylamide gels following incubation with [^{125}I]-labelled THs did not show the presence of TTR as a THDP (Gross and Manzon, 2011). Instead, at least four distinct THDPs were detected in a developmentally regulated manner: albumin-like glycoproteins AS and SDS-1, glycolipoprotein CB-III and Spot-5 of low molecular mass. As TTR emerged from an ancient 5-hydroxyisourate hydrolase (HIUHase) by gene duplication at an early stage of chordate evolution (Zanotti et al., 2006), we investigated if the lamprey TTR has TH-binding activity with a loss of 5-hydroxyisourate (HIU) hydrolysis activity.

In this study, TTR cDNAs were cloned from the liver of the Far Eastern brook lamprey *Lethenteron reissneri* and expressed in *Escherichia coli*. The recombinant TTR (lrTTR) was purified from the bacterial extract, whereas native TTR (ljTTR) was purified from sera of the Japanese lamprey *L. japonicum*. We investigated their molecular properties, and TH binding and HIU hydrolysis activities. Through this study, it becomes evident that the lamprey TTR is a functional THDP and that an N-terminal histidine-rich segment is essential for forming the high-affinity TH-binding sites.

2. Materials and methods

2.1. Reagents

[^{125}I]-3,3′,5-Triiodo-L-thyronine ([^{125}I]T3; 122MBq/mg, carrier-free) was purchased from PerkinElmer (Waltham, MA, USA). Unlabeled T3, L-thyroxine (T4) and 3,3′,5′-triiodo-L-thyronine (rT3) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Co-resin was obtained from Clontech (Talon Metal Affinity Resin, Mountain View, CA, USA) and Ni-resins were from Bio-Rad (Ni-IMAC Profinity, Hercules, CA, USA) and Invitrogen (Probond Nickel-Chelating Resin, Carlsbad, CA, USA). Uricase (*Candida* sp.) and urate were purchased from Wako (Tokyo, Japan). Isopropyl-1-thio-β-D-galactopyranoside was obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals used in this study were the highest grade available and were purchased from Wako, Nacalai Tesque, and Kanto Chemical (Tokyo, Japan).

For the binding assay, unlabeled iodothyronines were dissolved in dimethyl sulfoxide to the concentration of 2 mM, and cations and ethylenediaminetetraacetic acid (EDTA) were dissolved in an assay buffer to the concentration of 100 mM. Iodothyronines were diluted with the assay buffer to give less than 0.3% (v/v) solvent. Although a control assay without unlabeled iodothyronines was conducted in the presence of dimethyl sulfoxide at the same concentration, the solvent did not affect the binding assay.

2.2. Animals

The care and treatment of animals were in accordance with the guidelines for the care and use of laboratory animals of Shizuoka University (permit #29F-7) under the international guidelines “Act of Welfare and Management of Animals” (Ministry of Environment of Japan).

Adult Far Eastern brook lampreys (*L. reissneri*), 15–18 cm in length ($n = 2$), were caught in a local river in Fukushima Prefecture, Japan, in July 2010. Pooled livers ($n = 2$) were harvested from the lampreys following anesthesia by submersion in a 0.1% solution of ethyl 3-aminobenzoate methanesulfonic acid (Sigma-Aldrich), and were immediately frozen in liquid nitrogen and stored at −84 °C until used. As this species is too small to collect blood, we used it for cDNA cloning and characterization of recombinant TTR. Post-metamorphic adult Japanese lampreys (*L. japonicum*), approximately 50 cm in length, which were collected in Ishikari River, Hokkaido, Japan, were commercially obtained from Kojima in May 2012 ($n = 3$) and Akafuku in October 2013 and 2014 ($n = 4$). These lampreys were upstream migrants from the sea. Blood was collected by cardiac puncture from anesthetized animals. As *L. japonicum* was bigger in size than *L. reissneri*, we used *L. japonicum* to investigate [^{125}I]T3 binding to serum proteins or TTR purified from the serum, and transcript amounts. Chicken blood was also collected at a local abattoir in Shizuoka. Serum was separated from blood cells by centrifugation at 400 × *g* for 15 min at 4 °C, pooled each time, and then immediately used or stored at −35 °C. Tissues (liver, kidney, ovary, intestine, brain, skeletal muscle and eye) were separately harvested from two female Japanese lampreys, and were immediately frozen in liquid nitrogen and stored at −84 °C until used.

2.3. cDNA cloing

Total RNA was prepared from the pooled liver of adult *L. reissneri* ($n = 2$) using a kit (QIAamp RNA Blood Mini kit, Qiagen, Hilden, Germany). To confirm its integrity, RNA (50 ng per lane) was electrophoresed in a 1% agarose gel containing 2.6 M formaldehyde, and 28S and 18S rRNAs were visualized by ethidium bromide staining in an image analyzer (LAS-4000, GE Healthcare, Chicago, IL, USA). Single stranded cDNAs were generated using a reverse transcriptase (TaqMan Reverse Transcription Reagents, Applied Biosystems, Foster City, CA, USA) and oligo $(dT)_{16}$ primer (2.5 μM) according to the manufacturer's instructions. Polymerase chain reaction (PCR) of cDNAs was done using Taq DNA polymerase (TaKaRa Ex Taq, Takara, Otsu, Shiga, Japan) with the primers F3 and R3-2 (each 0.5 μM), which were designed based on the cDNA sequences of *P. marinus* and *L. appendix* TTR cDNAs (Manzon et al., 2007). Nested PCR was subsequently conducted using amplicons as templates with the primers F3 and R5 (each 0.5 μM) (Table 1). A 0.53 kbp PCR product was ligated into a pT7Blue T-vector (Novagen, Madison, WI, USA).

To obtain the 3′ region of lrTTR cDNA, 3′ rapid amplification of cDNA end (RACE) was carried out using 5′/3′ RACE Kit 2nd Generation (Roche, Basel, Switzerland). In this experiment, poly(A)$^+$ RNAs were prepared as starting materials using PolyA Tract System (Promega, Madison, WI, USA) according to the manufacturer's instructions. After reverse transcription of the poly(A)$^+$ cDNAs, 3′ RACE was done with the oligo d(T)-anchor primer in the kit and the specific primer F4 (12.5 μM) and then the anchor primer in the kit and the primer F4 (Table 1). A 0.75 kbp amplicon was ligated into a pMD20 T-vector (Takara). The 0.53 kb and 0.8 kb insert DNAs (clones #6 and #138) (Fig. 1A) were sequenced (accession no. LC331616).

2.4. Real-time reverse transcription (RT)-PCR

Total RNA was isolated from liver, kidney, heart, ovary, intestine, brain, skeletal muscle and eye of each adult female *L. japonicum* ($n = 2$) using the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). RNA concentration was determined using a spectrophotometer (BioSpec-nano, Shimadzu, Kyoto, Japan) (A_{260}/A_{280} = 1.90–2.10). The integrity of RNA was confirmed by 1% agarose gel electrophoresis in 2.6 M formaldehyde. DNase (RQ1, Roche) treated RNA (0.2 μg) was

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