Study of Lamprey Transthyretin as a Model for Binding Site Function of Transthyretin-Like Proteins.

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Introduction

Human transthyretin (TTR) is 55kDa tetrameric carrier protein responsible for the transport of thyroid hormones and retinol-binding-protein (Fig 1a). Thyroid hormones are involved in the regulation of growth and development, including vertebrate metamorphosis[1].

In mammals, TTR is not the main thyroid hormone transporter, but is still necessary for the transport of vitamin A. The majority of thyroid hormones are transported by thyroxin-binding-globulin (TBG). TBG does not exist in lower vertebrates. Also, human TTR binds thyroxine, while lower vertebrate TTR binds triiodothyronine[2].

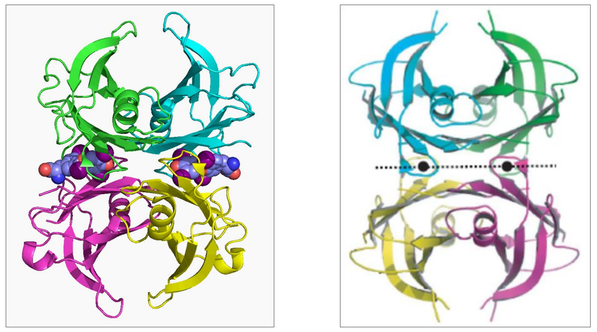

Fig. 1. Comparison of structures between (a) human TTR bound with T4 (left) and (b) S. Dublin TLP (right).

TTR is only found in vertebrates. Open reading frames have been identified in sequenced genomes of invertebrate organisms that were predicted to encode a putative transthyretin-like protein (TLP) that shares a 60% sequence similarity with human TTR (Fig. 3)[3].

These observations suggest that the TTR gene may have evolved from the duplication of the TLP gene in early vertebrate evolution[3]. Structural data for TLP reveal a tetrameric structure similar to TTR (Fig. 1b). Also like TTR, TLP has a central channel that forms the ligand binding domain (Fig. 2)[3,4].

TLP does not bind thyroid hormones, but is a 5-hydroxyisourate hydrolase (5-HIUase), involved in uric acid metabolism[5].

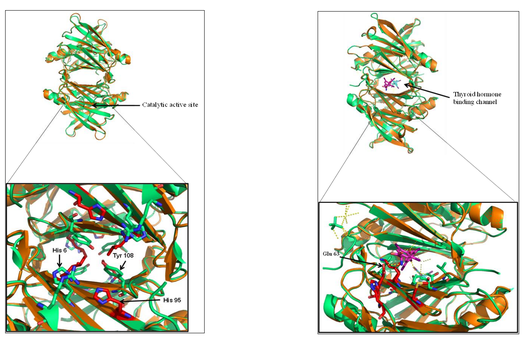

Fig. 2 Comparison of the binding channels of hTTR (left) with TLP (right). Conserved residues are in red, those that are not conserved are green.

Purpose

The purpose of this research is to form a crystal of Lamprey TTR in order to obtain its structure via X-ray diffraction. A structure of LTTR allows an understanding of the 3D structure of LTTR, hTTR and TLP, which can be used validate the hypothesis that LTTR is the closest TTR to diverge from TLP. This research supplements ongoing research with collaborators Dr. Richardson in Australia and Dr. Yamauchi in Japan, studying the activity and structure of LTTR and related TTRs and TLPs.

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hTTR  MASHRLLLL--------CLAGLVFVSEAGPTGTGESKCPLMVKVLDAVRGSP
LTTR  MTRFLCLLVLIASSSLLCRADDDHKSHESHEGGVKDSCPLMVKAIDSVQGKP
TLP   -------------------------------------MILSVHILDQQTGKP
                                             * *: :*   *.*
hTTR  AINVAVHVFRKAADDTWEPFASGKTSESGELHGLTTEEEFVEGIYKVEIDTK
LTTR  AAGVKLSVMKKQADASWKEVATGVTGKTGESHHLIGDKDFTEGTYKVRFETQ
TLP   APGVEV-VLEQKKDNGWTQLNTGHTDQDGRIKALWPEKAAAPGDYRVIFKTG
      * .* : *:.:  *  *  . :* *.: *. : *  ::  . * *:* :.*
hTTR  SYWKALGISPFHEHAEVVFTANDSGPRRYTIAALLSPYSYSTTAVVTN-PKE
LTTR  AYWTKAGITPFHEAAEVVFMAHDAGHKHYHIPMLLSPYFYATGAIVGDAEGH
TLP   QYFESKKLDTFFPEIPVEFHISKT-NEHYHVPLLLSQYGYSTY------RGS
       *:    : .*.    * *   .:  .:* :. *** * *:*
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Fig. 3. Alignment of human TTR, lamprey TTR, and S. dublin TLP. Conserved residues are denoted by (*), highly conserved denoted by (:), and slightly conserved denoted by (.).

Lamprey TTR

Lamprey, an organism considered to be one of the most primitive extant vertebrates, has been identified as a crossover species of TTR. Lamprey TTR (LTTR), a 63.5kDa protein, was found to have characteristics of both TTR and TLP, containing 3 of the 6 key residues required for thyroid hormone binding (Fig. 3, blue) and 2 of the 5 key residues required for 5-HIUase activity (Fig. 3, red)[6,7].

Fig. 4. Examples of Lamprey

Interestingly, lamprey metamorphosis is driven by a decrease in thyroid hormone, whereas amphibian metamorphosis is driven by an increase in thyroid hormone (Fig 3). Also, the thyroid in lamprey originates as an exocrine gland, changing to an endocrine gland during metamorphosis.

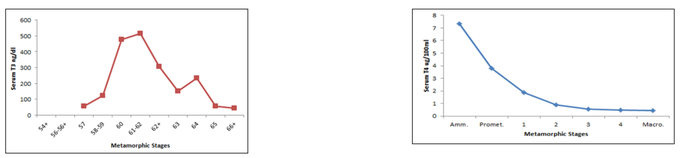

Fig. 5. Thyroid hormone binding during metamorphic stages for amphibians (left) and lamprey (right).

Methods/Results

Previously transformed E. coli BL21(DE3) cells containing the LTTR sequence were used for expression and purification. Expression was carried out using cold shock induction of cells at $O.D._{600}$ 0.6-0.8 with 1mM IPTG for 16-18hrs. Typical yield for 1L of culture is 16-18mg/mL. Cells were then harvested and lysed. LTTR was purified via a three step purification scheme of HisTrap then ULP1 digest, a second HisTrap and finally size exclusion chromatography.

Purified LTTR was set up for crystallization in complex with T4 or VCP6. Protein was concentrated to ~10mg/mL. Crystallization was setup using hanging drop vapor diffusion in 24 well plates using siliconized coverslips. Two microliters of protein and 2 microliters of reservoir solution were placed on coverslips and sealed with vacuum grease over wells. Trays were then placed in 14°C incubation room.

Expression of LTTR by E. coli cells was confirmed by SDS-PAGE. Electrophoresis shows LTTR exists as a tetramer and weighs ~60kDa, with its monomers weighing ~15kDa .

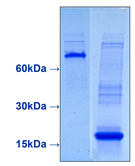

Fig. 6. Electrophoresis shows LTTR weighs ~60kDa (left), and can be separated into monomers that weigh ~15kDa (right).

Crystals appeared in a sample using Procomplex 34 screen. However, X-ray diffraction of these crystals failed to produce interpretable results.

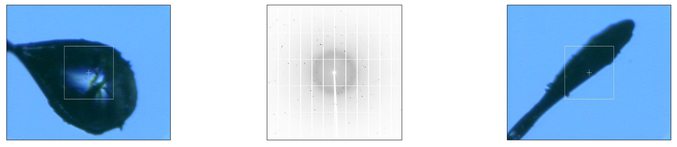

Fig. 7. Crystal and diffraction pattern for LTTR set up with Procomplex 34 screen. Diffraction suggests a peptide instead of a full protein or salt crystal.

References

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