

GREAT LAKES FISHERY COMMISSION

2003 Project Completion Report¹

Molecular cloning of petromyzonol sulfotranferase of *Petromyzon marinus* and enzymatic synthesis of petromyzonol sulfate

by:

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GLFC-Final Report (Year-2003)

We have isolated, partially purified and characterized the petromyzonol sulfotransferase (PZ-SULT) from the larval lamprey liver. The details of this work has been described, in the attached online published manuscript, which was accepted for publication in the Journal of Lipid Research (**JLR**). The print version of this article will appear sometime in march of next year (2004). JLR is a well reputed journal and the impact is even greater especially after it became part of the American Society of Biochemistry and Molecular Biology (**ASBMB**) journal, similar to the Journal of Biological Chemistry (**JBC**).

The exact title of the journal article is “**Isolation, Partial Purification and Characterization of a Novel Petromyzonol Sulfotransferase from Petromyzon marinus, Lamprey (Larval) Liver[#]**”

K. V. Venkatachalam, Domingo E. Llanos, Kristophe J. Karami, and Vladimir A. Malinovskii

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J. Lipid Res. published 1 December 2003, 10.1194/jlr.M300346-JLR200
<http://www.jlr.org/cgi/content/abstract/M300346-JLR200v1?ct>

**I have included the abstract of this accepted crucial JLR paper. For detailed information kindly refer to the attached full paper below.*

***ABSTRACT**

We have isolated, partially purified and characterized the 5 α -petromyzonol (5 α -PZ), (5 α -cholan- 3 α , 7 α , 12 α , 24-tetrahydroxy-) sulfotransferase (PZ-SULT) from larval lamprey liver. Crude homogenates of liver extracts exhibited a PZ-SULT activity of 0.9120 pmol/min/mg in juvenile and 12.62 pmol/min/mg in larvae. Using concentrated crude larval liver extracts and 5 β -cholan substrates like cholic acid and its various derivatives; there was only background level of sulfonated products. The extracts were then tested for sulfotransferase activity using 5 α -cholan substrates, 5 α -PZ and 3-keto-5 α -PZ which exhibited an activity of 231.5 pmol/min/mg and 180.8 pmol/min/mg respectively. With allocholic acid there was negligible sulfotransferase activity. This established that the sulfotransferase present in the lamprey larval liver extracts prefers (5 α) substrates and it is

selective for hydroxyl at the C-24, for sulfonation. Partially purified PZ-SULT exhibits a pH optimum of 8.0; a temperature optimum of 22°C and the stability of the activity was linear for one hour. Using optimal conditions, PZ-SULT activity was then purified by DEAE ion exchange, gel filtration and PAP affinity column chromatography. PZ-SULT exhibited a Km of 2.5 μM for PAPS and a Km of 8 μM for PZ. The affinity purified peak PZ-SULT fraction exhibited a specific activity of 2038 pmol/min/mg. The peak activity fraction while subjected to SDS-PAGE, correlated to a protein with a molecular weight of 47 kDa. Photoaffinity labeling with PAP³⁵S cosubstrate, specifically crosslinked the 47 kDa protein, further confirming the identity of PZ-SULT. Partial amino acid sequencing of the putative 47 kDa PZ-SULT protein, yielded a peptide sequence of (M)SISQAVDAAFXEI, which possessed an overall ~ 35-40% homology with mammalian SULT2B1a.

Abbreviations: SULT, sulfotransferase; PZ, petromyzonol; 3-keto-PZ, 3-ketopetromyzonol

We have obtained tremendous amounts of information on the biosynthesis of the crucial lamprey pheromone PZ and the enzyme PZ-SULT, which catalyzes the formation of this compound, which will be of great value and asset to the GLFC. However our strategies on cloning the cDNA corresponding to PZ-SULT, has not been accomplished yet. Also now I feel that I might have been over ambitious in accomplishing everything to successful completion within the proposed time frame. Nevertheless I have briefly described our tireless efforts in isolating the cDNA corresponding to the PZ-SULT.

Degenerate oligonucleotide probes:

PAPS binding motif is highly conserved among various sulfotransferases (SULT) from different organisms. Using this amino acid information, oligonucleotide probes were designed to isolate the cDNA corresponding to the PZ-SULT by RT-PCR method. Subsequent molecular cloning and DNA sequencing revealed the identity of the RT-PCR products. This process yielded lamprey cDNA's that are unrelated to PZ-SULT. Though may not be relevant directly, perhaps at a later time these informations will be of use in understanding the molecular biology of the lamprey.

These cDNA informations were deposited into GenBank.

1. **Petromyzon marinus KIAA0542-like protein mRNA, partial sequence.**
GenBank ACCESSION: AY090634
2. **Petromyzon marinus vitellogenin-like protein mRNA, partial cds.**
GenBank ACCESSION: AY077582

Thus our attempts to isolate the PZ-SULT cDNA using degenerate primers, yielded only 2 other unrelated lamprey cDNA's. However during the early years of this project (year 2002) one of the undergraduate student won the ASBMB travel award, which is a feather on the cap for the GLFC undertakings.

Isolation of PZ-SULT cDNA using gene specific primers:

Using the amino terminal peptide sequence corresponding to the PZ-SULT protein (for exact sequence, please refer to the attached accepted JLR article), oligonucleotide primer was designed. Using this primer RT-PCR was performed. Though being gene specific primer it did not yield PCR products, perhaps due to improper PCR conditions. We are varying the annealing temperature, Mg_2^+ concentration etc., to obtain the PZ-SULT cDNA. Touchdown PCR is a another method that is being tested for obtaining specific products. We have also raised peptide antibodies, in hope for screening cDNA library. I have proposed all these in the forthcoming pre-proposal to make it a dream come true. With the available tools (gene specific primers, peptide antibodies) and better strategies proposed in my 2004 pre-proposal the chances of obtaining PZ-SULT cDNA is great. With the much wanted cDNA for PZ-SULT a lot can be done in the way of gene expression and regulation of PZ-SULT.

Again I am looking forward to explore more on this sea of biochemistry/molecular biology of lamprey world, in answering questions that are crucial to the GLFC, so that strategies are available to control lamprey over-population in the Great Lakes. With our knowledge on the biochemistry of PZ-SULT it is only going to be better for future successful paths in understanding the molecular biology of this pheromone synthesis.