Identification of mitochondrial enzymes mRNA in the rectal gland of *Squalus acanthias*

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All cells require fuel to perform their metabolic work. The cellular engine that converts fuel to energy via oxidative phosphorylation is the mitochondrion. This report shows the rectal gland cells have the necessary enzymes to use fatty acids, sugars, and the carbon skeleton of amino acids as fuel for their metabolic work.

Isolated perfused rectal glands continue to secrete chloride in the absence of any exogenous substrate although at a reduced rate.[1] This observation suggests that the rectal gland cells have endogenous sources of energy capable of sustaining their metabolic work. The rectal gland cells contain glycogen a natural source of glucose and lipid bodies from which fatty acids could be derived.[2] We had previously shown that the rectal gland cells express the mRNA of both glycogen synthase and phosphorylase, both necessary for the synthesis and hydrolysis of glycogen, respectively.[2] We examined in this report whether the rectal gland cells have the mRNA for carnitine palmitoyl transferase, the enzyme that catalyzes the rate limiting step in the oxidation of fatty acids.

Two rectal glands, from two dogfish were homogenized in lysis buffer from Qiagen using a Tekmar tissue homogenizer. The homogenate was passed through a Qiagen shredder column, and messenger RNA was prepared using Qiagen RNAeasy minikit and treated with DNase. Single strand cDNA was then prepared using an Invitrogen First-Strand synthesis kit. PCR amplification was done using RedTaq ready mix from Sigma and the primers shown in Table 1. The amplified products were separated using 2% agarose gel in TAE. The products were eluted from the gel using MinElute Gel extraction kit from Qiagen, purified and sequenced at the MDIBL DNA Sequencing Core.

Table 1			
		Primer sequence	Predicted number of bases
Carnitine Palmitoyl Transferase	Forward	5'- gtaggcttccagttcact -3'	700
	Reverse	5'- actcctcccaccagtcgct -3'	
	Forward	5'- gttggcttccagttcacc -3'	700
	Reverse	5'- actcttcccaccagtcact -3'	
	Forward	5'-gtggcatttcagttcac-3'	319
	Reverse	5'-tggcaaacaggatggcactc-3'	
	Forward	5'-gagtgccatcctgtttgcca-3'	400
	Reverse	5'-tggcaaacaggatggcactc-3'	
Citrate synthase	Forward	5'- cagcataccagagtgccaga -3'	642
	Reverse	5'- aacgtgggttctgtgtagcc -3'	
	Forward	5'-ccgtccgtactcatggactt-3'	479
	Reverse	5'-aacgtcagtgctcacaccag-3'	
	Forward	5'-tgacccttacctgtgtttcg-3'	400
	Reverse	5'-ctccttcattccgtaatactgc-3'	

There are no published sequences of *S. acanthias* carnitine palmitoyl transferase. The published amino acid sequences of the enzyme from several mammalian species, *Ailuropoda melanoleuca, Loxodonta africanus, Macaca fascicularis, Odoberus rosmanus,* and *Papio anubis*, and the teleost species *Oncorhyncus mykiss, Larimichthys crocea, Sparus aurata, Lateolabrax japonicus, Oreochromis niloticus, Maylandia zebra, Takifugu rubripes, Oryzias latipes,* and *Danio rerio* were aligned and the consensus sequence used to design PCR primers. The primers used are shown in Table 1. Three left primers and two right primers were used in different combinations.

Since carnitine palmitoyl transferase is a mitochondrial enzyme, we used as a control for mitochondrial enzymes, citrate synthase. The published sequence for citrate synthase from *Callorhinchus milli* was used to design primers. The primers are also shown in Table 1.

Figure 1 shows the partial sequence obtained for carnitine palmitoyl transferase. Based on the consensus for published sequences of several mammalian and teleost species, the sequence is 75% homologous to the sequence for another elasmobranch *Leucoraja erinacea* that was recently published, after the primers were designed and results obtained in the present experiments. The sequence is considerably shorter than that published for *L. erinacea*, which is 2402 bases long. The sequence does not contain an open reading frame that corresponds to the open reading frame for the enzyme from *L. erinacea* or that of other species.

TTGCGCTCGTTGGCAATTTTCAGTCACGGTCACTCCCCGCTGGGCCCGTCCCCCACCTGAGCCATGACG
CCCTGAGACGAGTCTTCCTGTCAGGCCTGCGCTCCGGGAAGGAGCGGGTTCGGCGGTCCAAGAATGGT
GTTCTGACCGGGGTTTTCCCGGCCAAGCCCCTCCACCTGGCTCTTTGGGGGGGTACCGGTGATCGCGTC
GATGTACGCCCGGGTCGATCCCTCCGTGGGAGTGGTCGAGAAAATCCGGGGCCCTCTGCCCTCCAAGG
GGTTCCTGTCCAATCAGAGCGAGAGTATCCGGGGGGGGGGTGCTCTTTGGCACCGGGCTGTGGGTCTCC
CTGGTCTTCACCGTGCGCCCGATGCTCAAGCTGCTCCTGTCCTTCCCCGGCTGGATGGTTAGTCGCCCC
GGCAAGCCGCCCCCCACCCTCAAGATCTGGATGTTGTTGGGGAAGATCTTCTCTGGCCGCAAGCCGCT
GACGTTCCGGTTCCCGGACTTCCCTCCCCCGGTTACCGGGGCCGGCTGGGAAGGGCCCCTTGAGGAGG
TACCTGGGGTCCGGGCAGCCTCTTGCTGGATGACGAGCAGTTCAAGCGAACGCAGGCTCTGGCTCAAG
GCCTTGAGCGGAGCGTGGGGCCTAGGCTGCAGTGGTATCTTATAACTCCATGCCTGGGGGCCCTCCAA
CTATGTCAGCGACTGGGGGGGGGAGTAGTGCGTTGAA

Figure 1. Partial nucleotide sequence of carnitine palmitoyl transferase in the rectal gland of *S. acanthias*. The sequence contains 720 bases.

CGTTTCCGTGGGTATAGCATACCAGAGTGTCAGAAGGTTTTACCTAAGGGCCCAGGTGGAGATGAGCC
TCTTCCTGAAGGGCTCTTCTGGCTGCTTGTGACCGGACAGATCCCCACCCCAGAACAGGCGGCCTGGCT
GTCCACAGAGTGGGCAAAGCGGGCCGCCCTGCCCTCCCACGTCGTCACCATGCTGGACAACTTCCCCA
ACAACCTGCACCCCATGTCCCAGCTCAGCGCCGCCGTCACCGCACTCAACAGCGAGAGCAGCTTCGCC
CGCGCCTACGCGGAGGGAGTCAGCAAGGCCAAGTACTGGGAGTTTGTTTATGAAGATTCGATGGACCT
GATCGCCAAACTGCCCTGCGTGGCGGCTAAGATCTACCGGAACCTGTACAGAGAGGGCAGCAGTATCG
GGGCCATCGACCCCGACCTGGACTGGTCTCACAACTTCACCAACATGCTGGGCTACACTGAACCCCCGT
TTACTGAGCTTATGAGACTTTACCTCACTATTCACAGTGATCATGAGGGTGGGAATGTCAGTGCTCACA
CCAGCCACCTCGATGGGGAGCGCTCTCTCCGACCCCTACCTGTGCTTCGGGGCTGCAATGAACGGACTG
GCTGGACCTCTTCACGGACTTGCAAATCAGGAGGTGCTGGGCTGGCTGACCAACCTTCAGGCTGAGCTC
GGCGGTGAGGTGTCCGATGAGCAACTGCGGGACTTCATTTGGAACACGCTGAATTCCGGCAGGGTGGT
TCCTGGTTACGGCCACGCCGTTCTCCGCAAGACGGACCCCCGCTACACCTGCCAGAGGGAGTTCGCCCT
CAAGCACCTGCCCAACGACCCTTTGTTCAAGCTCGTCGCCCAACTCTACAAGATCGTACCCGGGGTGCT
CCTCGACCAGGGCAAAGCCAAGAACCCCTGGCCCAACGTGGACGCGCACAGCGGTGTCCTGCTGCAGT
ATTACGGAATGAAGGAGATGAACTACTACACGGTGCTGTTTGGCGTCTCCCGAGCCCTCGGCGTCCTCG
CGCAGCTTATCTGGAGCCGTGCCCTGGGCTTCCCCCTGGAGAGACCCAAGTCCATGAGCACTGACGGC
CTCATACATTTGGTGGGAGACAAGTCTGGTTGAGCCGCCCTGGTTGCGGACGTTAATATTAAAAACACA
TCTGTGCTTCTACGCATCCAGTGTAAATGTTGCCACCTTGAGGCAAGTCGCTGAACGTGGCCATTCAGA
ATCACCTGATGACTGCGATGCTTTATATATTTTTGTGTTTTGGGTTGAAAACTTGCAGAGTGAAAAGAA
TTTAATTTCCTTATTAACATTTCAATTTTTTTTTCTGTGCCAGACGGTTTGTCCATCCGGTTTGGACAGGT
AAACCCTCCTTGGGCAGCGACTAGACCGCTCTTTCTTTACC

Figure 2. Partial sequence for citrate synthase.in the rectal gland of *S. acanthias*. The sequence contains 1415 bases.

Figure 2 shows a partial sequence for citrate synthase. This sequence was obtained using primers based on the published sequence for *Callorhinchus milii*, the elephant shark. At 1415 bases long it is 250 bases shorter than that of *C. milii*, and both sequences are 91% homologous. The sequence is also highly homologous to the citrate synthase of several different species including teleosts, reptiles and mammals.

The present report shows that the rectal gland expresses the mRNA for carnitine palmitoyl transferase, the rate-limiting enzyme in the oxidation of fatty acids, located in the outer membrane of the mitochondria. Rectal gland cells should be capable of utilizing long chain fatty acids as a source of fuel. This report shows that rectal gland cells also express citrate synthase another mitochondrial enzyme. Both carnitine palmitoyl transferase and citrate synthase are mitochondrial enzymes; the first is located in the outer membrane of the mitochondria and the second is located in the mitochondrial matrix. Although both are mitochondrial enzymes, citrate synthase is encoded by the nuclear DNA, rather than the mitochondrial DNA.

1. **Kinne RKH, Spokes KC, Silva P.** Secretion of chloride and mechanism of transport of glucose in the rectal gland of *Squalus acanthias*. *Bull. Mt Desert Isl. Biol. Lab*. 49:44, 2010.
2. **Doyle WL.** Tubule cells of the rectal salt-gland of *Urolophus. Am J Anat* 111: 223-237, 1962.