Open reading frame of glycogen phosphorylase in the rectal gland of *Squalus acanthias*

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Many cells store glucose, the most common exogenous fuel, to maintain a fuel source necessary for their work. Glucose is stored in the form of glycogen. Glycogen is built up when there is enough glucose and taken down when it is scarce. This report shows that rectal gland cells have glycogen phosphorylase, the enzyme that breaks down glycogen to form glucose.

Glycogen is the endogenous source of fuel in the rectal gland[1]. Glycogen can be synthetized and broken down in the cells of the rectal gland to either store glucose when this is abundant or to provide glucose when it is needed. We have already described a 664 base fragment of glycogen phosphorylase in the rectal gland of *S. acanthias*[2]. In the present report we describe a longer sequence of the glycogen phosphorylase that includes an open reading frame that codes for the catalytic center.

Two rectal glands and kidneys from a two dogfish were homogenized separately in lysis buffer from Qiagen using a Tekmar tissue homogenizer. The homogenates were passed through a Qiagen shredder column; 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 I. 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 sequences used to amplify Glycogen phosphorylase and Na-K-ATPase.

Glycogen phosphorylase		Primer sequence	Predicted # bases
	Left	5'-gcacctttctcctctgtcg-3'	462
	Right	5'-caagacctccattgccaagt-3'	
	Left	5'-gcacctttctcctctgtcg-3'	534
	Right	5'-agcgaattccatagccgtaa-3'	
	Left	5'-cgctatcggctctcagtcc-3'	654
	Right	5'-gttaccatagcgcagccaat-3'	
	Left	5'-caatacgggcctgtgcttat-3'	719
	Right	5'-gtcctgggaacaaagggttt-3'	
	Left	5'-ctgtaccccaccgacagtct-3'	735
	Right	5'-agctgagtgcaggtctgtga-3'	
	Left	5'-tggcargtngargargcngay-3'	1596
	Right	5'-tcytcngccatytcnacrtt-3'	
	Left	5'-gcacctttctcctctgtcg-3'	1910
	Right	5'-agccatrtggtarccaggwg-3'	
Na-K-ATPase	Left	5'-gacagctctttggtggcttc -3'	657
	Right	5'-gcttcaagccagctgtatcc -3'	

The first two primers were designed using the published sequence for glycogen phosphorylase from normalized cDNA EST for *Squalus acanthias*. The remaining primer pairs were a combination of the first two primer pairs and primers designed from published amino acid sequences of glycogen phosphorylase from *Salmo salar, Rattus norvegicus, and Xenopus tropicalis*. The primer pairs yielded results of the expected size, and the results from the first two pair, 462 and 534 bases long, are shown in Figure 1. The results for the other primer pairs are not shown. The control primer pair for Na-K-ATPase, also shown in Figure 1, resulted in a product of the expected number of bases.

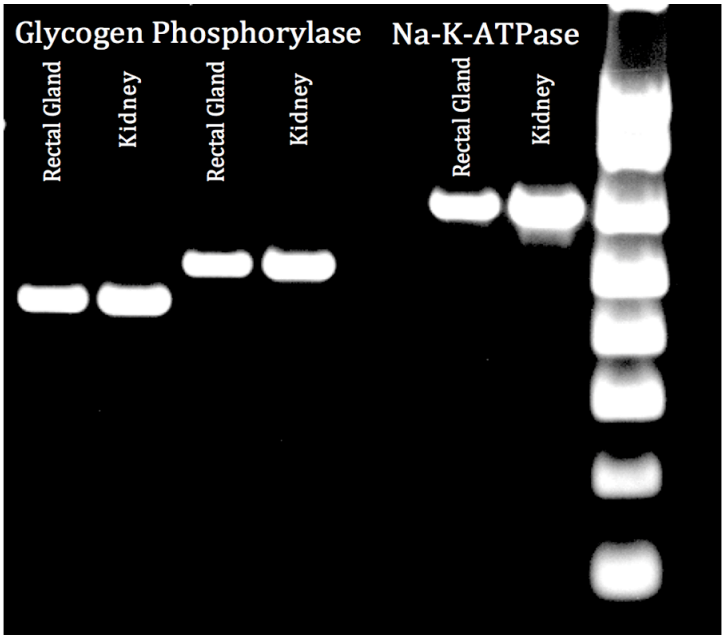

Figure 1. PCR amplification of glycogen phosphorylase from *S. acanthias* rectal gland and kidney. All primer pairs yielded products of the expected size. Only the results for the smaller of the primer pairs, 462 and 534 bases are shown in this figure. The control Na-K-ATPase, also shown here, resulted in products of the expected size.

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ATCGCGTCCAACCAACCAACGCGCGCTATCGGCTCTCAGTCCGGACCTGAGCCG
GAGTCCTGTGCACCTTTTCTCCTCTGTCGCGGAACCACCTCCCCACTGCCGGGG
ACACCATGGCCACCCCACTGACAGACTCCGAGAGGAGGAAGCAGATCACGCGTG
AGGGGCATCGCCGAGCTCGGGGACGTGGTGGAGTTGAAAAAAAGCTTCAACAGG
CATCTGCATTTCACACTGGTCAAAGACAGGAATGTCGCCACCCCCCGGGACTAC
TACTTCGCTCTGGCTCACACCATCCGCGACCACCTGGTGGGACGGTGGATCCGC
ACCCAGCAGTATTACTACGAGAAAGATCCCAAGCGTATCTACTACCTGTCTCTG
GAGTTCTACATGGGCCGGACCCTGCAGAACACTATGGTGAACCTGGGGCTGGAG
AATGCCAGTGATGAGGCCATATATCAGCTGGGCTTGGACATTGAGGAACTGGAA
GAAATCGAAGAAGATGCTGGACTTGGCAATGGAGGTCTTGGTCGACTGGCAGCG
TGTTTCCTTGATTCATTGGCCACACTGGGTTTGGCAGCTTACGGCTATGGAATT
CGCTATGAATTTGGTATTTTTAATCAGAAGATTCAGAATGGCTGGCAGATAGAA
GAAGCCGATGACTGGCTGCGCTACCGGTAACCCTTGGGGAACAGCGCTGGCCGG
AACAGATGGACGAAGTTCACCTGCCGAGGCACTGGCTGCGCATCTAGGATGCAA
TCAACCAGTTCGAACCCTTGGGAAACGGCGCCATGCCCGGTATCACTCCAGTGC
CGGCTCCCTGGTACACTTCTATGGGCACCGTGGCGCATACCGAGGACGGTGCCC
CCACAAGGGTCGCTATGACGGTCCTGAGCGTCGTCGAATCGCTCGCCATCCCCG
TGGTCGCTCGCCTGCACTCCGGATTCACGACCAACACTGTTTTCAAGCGACGAT
TTCTACGACCTTGAGGAGCTCTGTCGTGTTCCAGTCCGGACCGCGGTATTTGAA
GCTCTCCAAGACATGGCTGCCATTCAGCTGAGTGACTCTCACCCTGCCGAGGCC
ATAGCTGAGCGGCTTGGAGACGGGCTTCATCAAAGACCTGGAGCAGCTGGACAA
TCTTAAGGACTATGCCGCCTTCGACGCTTTCATTACAGGTCTGGCGCTCCGTAT
ACCACGACATTACAGGTCTGGCGCTCCGTATACCACGACAAGTCTCTCTGCCGC
GGATCCACGCGTCCAACCGGCATCATTTGGACCGTTCCCAGACAAGGACACCAT
ACCGCCCAATCAGACCCCCGGCGGCCTGGGCCCTCCCTGTGCCGCGCAGGTTCC
TGATCGACGCGGAGGAACCCAAAGGTTAGAACATGGCGTGCCTGATCGCAATAC
GGACCAGTCGCTGTTACCGACCTCGGCAACTGTGCTCCCGTCCCTCGCGACAGC
CCCAATATCGCTTTAGTGGTTATCTACGGGCTGGAACTGGCCTTGCTTTAGTCT
TTGTCGTTATTAAACACACCTTGCTGTTGGCACCTGATTAAAACACGTGTTTAA
GTGGATGCCATAGCTGAGAGGATGCTGGAGCGGGTTTCATCACCGACCTGCAGC
AG
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Figure 2. Partial nucleotide sequence of glycogen phosphorylase from the rectal gland of *S. acanthias*. The sequence contains 1622 bases.

All the amplified products were sequenced and the final aligned sequence is shown in Figure 2. The final sequence consisted of 1622 bases, which is significantly shorter than the published complete sequences for glycogen phosphorylase in other species. This sequence yielded an amino acid sequence is shown in Figure 3.

This amino acid sequence codes for an open frame that includes the Rossman folds that bound the cleft in the molecule that contains the catalytic center.

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QTPRGGSRSRVRGIAELGDVVELKKSFNRHLHFTLVKDRNVATPRDYYFALAHTIRD
HLVGRWIRTQQYYYEKDPKRIYYLSLEFYMGRTLQNTMVNLGLENASDEAIYQLGLD
IEELEEIEEDAGLGNGGLGRLAACFLDSLATLGLAAYGYGIRYEFGIFNQKIQNGWQ
IEEADDWLRYGNPWEKARPEYMLPVHFYGHVEHTEDGVRWVDTQVVLAMPYDTPVPG
FKNNTVNTMRLWSAKAPNDFNLKDFNVGDYIQAVSDPSLAENISRVLYPNDNFFEGK
ELRLKQEYFVVAASLQDLIRRFKSSKFGCRDPIRTSFETFPDKVAIQLNDTHPALAI
PELMRILMDVEKLDWDKAWDITIRTCAYTNHTVLPEALERWPVQMFEHLLPRHLQLI
YAINQKHLENVAAQYPGDMDRLRRMSLIEEGDPKRINMAHLSVVGSHAVNGVARIHS
EIVKNTVFKDFYELEPDKFQNKTNGITPRRWLLLCNPGLADAIAERIGEGFITDLQQ
LKKLMDYVDNDAFIRDVANVKQENKPKFAAHLETEYKIKINPSPIFDVHVKRIHEYK
RQLLNCLHIITLYNRIKKQPNKTFVPRTILIGGKAAPGYHMAKMIIKLITSVGNVVN
NDPIVGDRLKVVFLVNYRVSLDEKVIPASDLSEQISTAGTRGLGHGEHEVMLNRVI
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Figure 3. Amino acid sequence deduced from the base sequence shown in Figure 2. This sequence contains the open reading frame for glycogen phosphorylase that encodes the Rossman folds of the molecule and the catalytic center.

The results reported here show that the cells of the rectal gland of *S. acanthias* express a glycogen phosphorylase that is a member of the GT-B structural superfamily of glycogen phosphorylases. Glycogen phosphorylase belongs to a family of oligosaccharide phosphorylases that catalyze the breakdown of oligosaccharides to glucose-1-phosphate. The sequence reported here includes the Rossman folds in the N- and C-terminal domains that enclose the molecular cleft that contains the catalytic center. Thus, the rectal gland cells can break down their stored glycogen to glucose-1-phosphate when glucose is needed to fuel their energy demands.

1. **Kinne R, Spokes KC, Silva P**. Glycogen measurement in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Isl. Bio. Lab*. 50:26-27, 2011.
2. **Silva P, Kinne R, Spokes KC.** Molecular identification of glycogen phosphorylase in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Isl. Bio. Lab*. 51:15-16, 2012.