Molecular identification of glycogen phosphorylase in the rectal gland of *Squalus acanthias*

Patricio Silva,[1] Katherine C. Spokes[2] and Rolf Kinne[3]
[1]Department of Medicine Temple University School of Medicine, Philadelphia, PA 19140
[2]Department of Medicine Beth Israel Deaconess Medical Center,
and Harvard Medical School, Boston, MA 02215
[3]Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany

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 broken down when it is scarce. This report shows that rectal gland cells have glycogen phosphorylase, the enzyme that breaks down glycogen to form glucose.

The secretion of chloride by the rectal gland of *S. acanthias* is supported by exogenous glucose.[2] When glucose is removed from the perfusate in isolated perfused rectal glands, the gland continues to secrete chloride indicating that its cells have an endogenous source of fuel. This source of fuel may be glycogen that has been identified by electron microscopy[1], and biochemical assay.[3] The gland has glycogen synthase signifying that it can synthesize glycogen. The present experiments were designed to ascertain whether the rectal gland cells also have glycogen phosphorylase, the enzyme necessary for the hydrolysis of glycogen and generation of glucose.

A rectal gland, brain, and muscle from a single dogfish were homogenized in lysis buffer from Qiagen using a Tekmar tissue homogenizer. The homogenate was passed through a Qiagen shredder column, messenger RNA was prepared using Qiagen RNAeasy minikit, and treated with DNase. Single strand cDNA 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 a MinElute Gel extraction kit from Qiagen, purified and sequenced at the MDIBL DNA Sequencing Core.

Table I			
		Primer sequence	Predicted # bases
Glycogen phosphorylase	Left	5'-gcacctttctcctctgtcg-3'	462
	Right	5'-caagacctccattgccaagt-3'	
	Left	5'-cgctatcggctctcagtcc-3'	654
	Right	5'-gttaccatagcgcagccaat-3'	
Na-K-ATPase	Left	5'-gacagctctttggtggcttc -3'	657
	Right	5'-gcttcaagccagctgtatcc -3'	

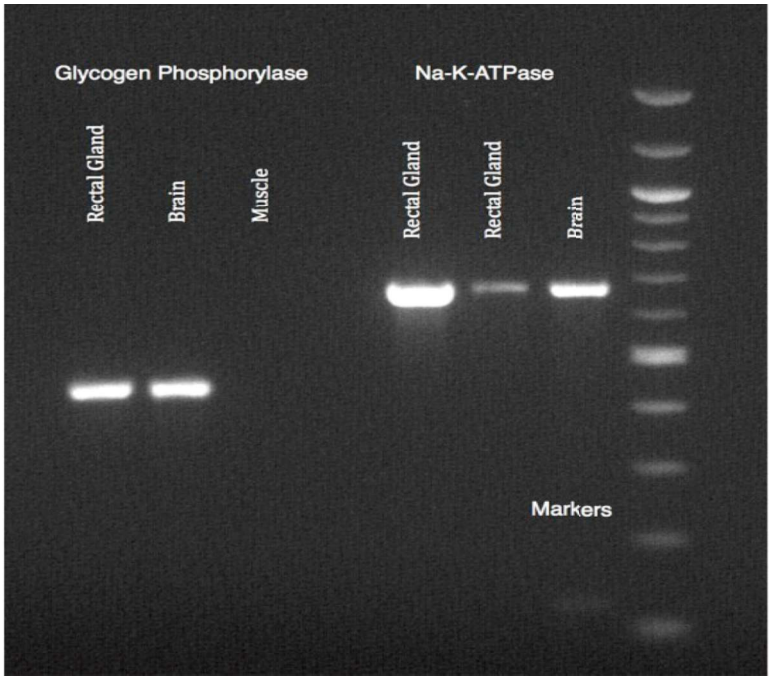

Figure 1. RT-PCR amplification of glycogen phosphorylase from *S. acanthias* rectal gland, brain, and muscle. Both primer pairs yielded products of the expected size, 462 and 654 bases. Only the first primer product (462 bases) is shown in this gel. No product was obtained in muscle. The control Na-K-ATPase resulted in products of the expected size.

The primers were designed using the consensus sequence of the known sequences for glycogen phosphorylase from several different species including normalized cDNA EST for *Squalus acanthias*. Two different primer pairs for the same region of glycogen phosphorylase were used. Both primer pairs yielded products of the expected size, and the result for the first pair, 462 bases long, is shown in Figure 1. The results for the other primer pair 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.

The amplified product of the second primer pair yielded the sequence shown in Figure 2. The sequence is 78% to 82% similar to that of glycogen phosphorylase in a variety of tissues in vertebrate species ranging from fish to man.

```
TCGCTATCGGCTCTCAGTCCNGGCCTGAGCCGGAGTCCTGTGCACCTT
TTCTCCTCTGTCGCGGAACCACCTCCCCACTGCCGGGGACACCATGGC
CACCCCACTGACAGACTCCGAGAGGAGGAAGCAGATCAGCGTGAGGGG
CATCGCCGAGCTCGGGGACGTGGTGGAGTTGAAAAAAAGCTTCAACAG
GCATCTGCATTTCACACTGGTCAAAGACAGGAATGTCGCCACCCCCCG
GGACTACTACTTCGCTCTGGCTCACACCATCCGCGACCACCTGGTGGG
ACGGTGGATCCGCACCCAGCAGTATTACTACGAGAAAGATCCCAAGCG
TATCTACTACCTGTCTCTGGAGTTCTACATGGGCCGGACCCTGCAGAA
CACTATGGTGAACCTGGGGCTGGAGAATGCCAGTGATGAGGCCATATA
TCAGCTGGGCTTGGACATTGAGGAACTGGAAGAAATCGAAGAAGATGC
TGGACTTGGCAATGGAGGTCTTGGTCGACTGGCAGCGTGTTTCCTTGA
TTCATTGGCCACACTGGGTTTGGCAGCTTACGGCTATGGAATTCGCTA
TGAATTTGGTATTTTTAATCAGAAGATTCAGAATGGCTGGCAGATGGA
GGCAGCCGATGATTGGCTGCGCTAATGGTAACAACTGTTT
```

Figure 2. Partial nucleotide sequence of glycogen phosphorylase from the rectal gland of *S. acanthias*. The sequence contains 664 bases.

Muscle glycogen phosphorylase was not amplified by the primers used in these experiments. Controls for muscle, using primers for Na-K-ATPase run simultaneously, resulted in products of the expected number of bases (data not shown). Thus, the primers designed for these experiments do not amplify muscle glycogen phosphorylase. This is not surprising, since there are different types of glycogen phosphorylase in different tissues.

The results reported here show that the cells of the rectal gland of *S. acanthias* express glycogen phosphorylase and may be capable of hydrolyzing glycogen. Thus, glycogen stored in the gland can serve as endogenous fuel when the exogenous sources of energy are not available.

1. **Doyle, WL.** Tubule cells of the rectal gland of Urolophus. *Am. J. Anat.* 111: 223-237, 1962.
2. **Kinne, R, Spokes, KC and Silva, P**. Secretion of chloride and mechanism of transport of glucose in the rectal gland of Squalus acanthias. *Bull. Mt. Desert Isl. Bio. Lab.* 49: 44-46, 2010.
3. **Kinne, R, Spokes, KC and Silva, P**. Glycogen measurement in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Isl. Bio. Lab.* 50:26-27, 2011,