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Fasciola gigantica: molecular analysis of a water channel protein (Aquaporin)

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Abstract

Fasciolosis caused by *Fasciola gigantica* is an important disease of cattle in Thailand. In order to develop new drugs and vaccines we have recently started to conduct research on aquaporins in *Fasciola*. Aquaporins (AQPs) are essential for the maintenance of water homeostasis in all organisms including animals, plants, and bacteria. Structure, function, and pathology of AQPs have been extensively studied in vertebrates but data for AQPs of trematodes is still limited. In the present study, a cDNA encoding an aquaporin (FgAQP-1) was molecular cloned from a metacercarial stage cDNA library of *F. gigantica*. The FgAQP-1 cDNA contained the complete coding sequence for a protein of 299 amino acid residues. Comparison of the deduced amino acid sequence with protein sequences in public databases using NCBI-BLASTP showed highest similarity to aquaporin-1 of *Bos taurus*. Expression and distribution of FgAQP-1 has been characterized at the nucleic acid and protein level in the adult parasite. Functional data of FgAQP-1 will be obtained after transformation of yeast with a plasmid carrying FgAQP-1 DNA by stopped flow analysis. Furthermore, it is planned to introduce mutations into the original FgAQP-1 sequence to analyze the importance of single amino acid residues for the functional integrity of the protein.

Keywords: *Fasciola gigantica*, aquaporin, water, *in vitro* mutagenesis.

Introduction

Fasciolosis caused by *Fasciola gigantica* is an important disease of cattle in Thailand. The successful development of a vaccine for *Fasciola* spp. will require several issues to be addressed [1]. Drug resistance is not a major problem at present, but that is not a reason for complacency, constant vigilance and monitoring are needed to avoid the problems that bedevil the control of *Fasciola* spp. [2]. Aquaporins (AQPs) are essential for the maintenance of water homeostasis in all organisms including animals, plants, and bacteria. Structure, function, and pathology of AQPs have been extensively studied in vertebrates but not in detail in trematodes. Currently, there are two examples of molecules that pass through aquaporins and kill protozoan parasites, hydroxyurea [3] and the hydroxide of tervalent antimony [4]. AQPs could possibly be exploited for the transport of novel drugs into the parasite.

The aim of this study was to identify and characterize the molecular biological properties of a water channel protein (Aquaporin) in *F. gigantica*.

Methods

The following experiments were used in this study:

1. Screening of *F. gigantica* cDNA libraries with a specific AQP probe and sequence analysis of the obtained FgAQP-1 cDNA.
2. Characterization of FgAQP-1 nucleic acids by Southern and Northern analyses.
3. Expression and purification of a C-terminal rFgAQP peptide in a prokaryotic system.

4. Production of polyclonal immune sera against the C-terminal rFgAQP-1 peptide.

Results

1. cDNA Screening, Sequencing and sequence analysis of FgAQP-1

A full-length aquaporin (FgAQP-1) cDNA was isolated from a metacercarial stage *F. gigantica* cDNA library with a partial gene-specific DIG labeled DNA probe. The FgAQP-1 cDNA contained the complete coding sequence for a novel aquaporin. A homology search of the GeneBank database by NCBI-BLASTP showed the highest identity to aquaporin-1 of *Bos taurus*.

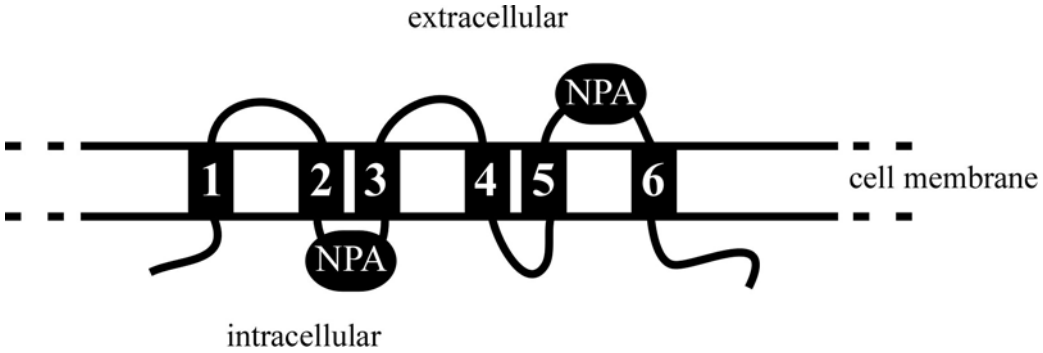

Figure 1 Schematic structure of FgAQP-1. It is a typical aquaporin which contains six-transmembrane segments and cytoplasmic located N- and C-termini. The two family-specific NPA boxes are located in the B- and E-loops.

2. Southern and Northern analysis

Southern and Northern analyses were performed using a FgAQP-1 DNA probe. For Southern analysis, genomic DNA was digested with restriction endonuclease *Hin*d III, *Xho* I and combination of *Hin*d III and *Xho* I. The DIG-labeled FgAQP-1 probe detected several DNA fragments with sizes up to 20,000 bp. For Northern analysis the total RNA of adults was probed with a FgAQP-1 DNA probe and showed a single hybridizing transcript at a size of 1900 nucleotides.

3. Expression and purification of a C-terminal rFgAQP peptide and antibody production against it.

Recombinant C-terminal FgAQP-1 peptide was expressed in *E. coli* BL21pLysS as a 40/41 kDa fusion protein after induction with IPTG. The expressed protein was purified by Zn^{2+}-Sepharose affinity chromatography. The C-terminal FgAQP-1 peptide was released from the fusion protein by chemical cleavage with hydroxylamine and was used for production of polyclonal antisera in mice.

Discussion

FgAQP-1 belongs to the major intrinsic protein (MIP) superfamily. AQPs have two usually conserved NPA motifs that are important for the function of the channel pore [5]. In FgAQP-1 the first NPA box showed an unusual motif while the second box was fully conserved. Aquaporins are encoded by multi-family genes in many organisms, e.g. there are at least 13 human aquaporins [6] and 23 plant aquaporins [7]. More than one DNA fragment was detected by Southern blot analysis, this result may imply that not only a single FgAQP gene exists.

Conclusion

A full length FgAQP-1 cDNA was cloned from a metacercarial cDNA library of *F. gigantica.* The deduced amino acid sequence of FgAQP-1 contains 299 residues and showed the best match to aquaporin-1 of *Bos taurus* when using NCBI-BLASTP. Functional analysis of this protein will be done in *Xenopus* oocytes and yeast systems. Furthermore, it is planned to introduce mutations into the original FgAQP-1 sequence to analyze the importance of single amino acid residues for the functional integrity of the protein.

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