Biochimica et Biophysica Acta 1840 (2014) 1566–1573

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Parasite aquaporins: Current developments in drug facilitation and resistance☆

Jie Song [a], Ellen Mak [b], Binghua Wu [b], Eric Beitz [b,*]

[a] Institute of Physiology, Christian-Albrechts-University of Kiel, Germany
[b] Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Germany

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 2 October 2013
Accepted 6 October 2013
Available online 17 October 2013

Keywords:
Aquaporin
Water
Glycerol
Antimonials
Pentamidine
Parasite

ABSTRACT

Background: Although being situated in a niche, research on parasite aquaporins is a lively field that has provided new insight into basic aquaporin structure–function relationships and physiological roles of water and solute transport. Moreover, it bears the potential to find novel approaches to antiparasitic chemotherapy.
Scope of review: Here, we summarize the current knowledge about the structure and substrate selectivity of aquaporins from protozoan and helminth parasites, review the current views on their physiological roles, and discuss their potency for chemotherapy.
Major conclusions: Parasite aquaporins fulfill highly diverse tasks in the physiology of the various organisms, yet their general protein structure is well conserved. Aquaporins are directly (antimonials) and indirectly (melarsoprol, pentamidine) linked to the uptake of antiparasitic drugs. Unfortunately, drug-like aquaporin inhibitors are still missing.
General significance: Aquaporins expression levels determine the degree of parasite resistance against certain drugs. Further studies on parasite aquaporins may provide data about overcoming drug resistance mechanisms or even spark novel treatments. This article is part of a Special Issue entitled Aquaporins.

1. Introduction

A parasite is an organism that lives in and draws nutrition from a host organism. Parasites are classified into two main categories: ecto-parasites (living in contact with the host but outside of the host organism) and endo-parasites (living inside the host's body, e.g. in the digestive tract, in the blood, or in certain tissues). Endo-parasites can be further divided into protozoa (microscopic, one-celled organisms) and helminths (large, multicelluar organisms). Protozoan parasites are the infectious agents of devastating diseases worldwide, such as malaria, African trypanosomiasis, Chagas' disease, leishmaniasis, and toxoplasmosis. Helminth parasites cause severe tissue infections, such as schistosomiasis and fasciolosis.

The arsenal of chemotherapeutic drugs against parasite infections remains quite limited. This is in part due to the parasite's diverse and complex life styles and their cycling between an invertebrate vector and one or more vertebrate hosts, connected with changing metabolic profiles [1]. Further, parasites develop rapidly with short generation times and, hence, propagate the generation and spreading of drug-resistant strains [2].

The increasing number of parasite genome projects has identified and highlighted various genes coding for proteins of potential value as drug targets, with a special focus on integral membrane proteins at the host–parasite interface [3]. The interface fulfills essential physiological functions for the parasite, including adhesion to a host cell during invasion [4], coping with osmotic stress by rapid water transport [5], uptake of nutrients [6], release of metabolic end-products [7], and secretion of toxic proteins [8]. For antiparasitic drugs, whose targets are located within the parasite cytosol, the parasite plasma membrane forms the final barrier and also can be regarded as a drug delivery system. The selective pressure during parasite–host co-evolution has optimized the interface proteins with regard to their structure and function [9].

Aquaporins (AQPs) constitute an ancient family of transmembrane channel proteins. They are present in virtually all organisms [10] and have been thoroughly characterized with respect to their structure–function relationships since their discovery 20 years ago. However, questions regarding the physiological roles of AQPs or design and discovery of potent and selective inhibitors, remain challenging. The AQP family comprises water-specific channels (orthodox aquaporins), and channels that additionally facilitate transport of glycerol, urea, ammonia or other small, uncharged molecules (e.g. aquaglyceroporins). Water and solute flux through AQPs depends on the presence of osmotic or chemical gradients, which concomitantly define the direction of transport [11].

The elucidation of the physiological roles of mammalian as well as plant AQPs is a thriving topic of research and the work has been

☆ This article is part of a Special Issue entitled Aquaporins.

* Corresponding author at: Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Gutenbergstrasse 76, 24118 Kiel. Tel.: +49 431 880 1809; fax: +49 431 880 1352.
E-mail address: ebeitz@pharmazie.uni-kiel.de (E. Beitz).

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.10.014

Table 1
Parasite AQPs discussed in this review.

	Phylum	Species	AQP
Protozoa	*Apicomplexa*	*Plasmodium falciparum*	PfAQP
		Toxoplasma gondii	TgAQP
	Kinetoplastida	*Trypanosoma brucei*	TbAQP1–3
		Trypanosoma cruzi	TcAQP, TcAQPβ–δ
		Leishmania major	LmAQP1, LmAQPα–δ
Helminthes	*Platyhelminthes*	*Fasciola gigantica*	FgAQP1–2
		Schistosoma mansoni	SmAQP

intensively reviewed (see for instance [12]). The functions of parasite AQPs are less well understood. Here, we summarize the current views and knowledge about parasite AQPs (Table 1) mainly with respect to the structural and functional context and discuss their proven and putative relations to chemotherapeutic treatments, which can be either direct as a drug target or indirect as a facilitator for the uptake or release of drugs.

2. Structural peculiarities of parasite AQPs

AQPs form homotetrameric complexes with each monomer functioning independently as a water/solute channel (see Fig. 1 for visualization of structural details). Each monomer is made of six transmembrane helices and two short half-helices, which dip into the membrane from either side to form a seventh pseudo-transmembrane span. Two highly conserved constrictions in the pore region determine the selectivity of aquaporins (Fig. 1) [13,14]. The constriction at the pore mouth is termed aromatic arginine (ar/R) constriction or selectivity filter [15]. It selects permeants by size and by their electrostatic nature. The ar/R constriction of water specific AQPs is narrow and consists of polar amino acid residues offering optimal hydrogen bond acceptor and donor sites for isolating a water molecule from the bulk. Aquaglyceroporins contain a wider and amphipathic ar/R constriction providing hydrogen bonds only on the hydroxyl side of a glycerol molecule and lipophilic interactions on the alkyl back [16]. The ar/R constriction further co-constitutes the exquisite AQP proton filter together with a second filter region, i.e. the Asn-Pro-Ala (NPA) region [14,16–19]. The NPA motifs are located at the positive ends of two highly conserved half-helices, which act as macro-dipols, and reside in the center of the channel [20]. The Asn residues act as hydrogen donors to the oxygen atoms of passing permeants and also form the filter against inorganic cations [14,17,21].

The aquaglyceroporin from *Plasmodium falciparum* (PfAQP) is the best-characterized parasite AQP. Unlike its close structural homolog from *E. coli* (GlpF), PfAQP not only conducts glycerol but also water at high rates [22–25]. To elucidate the structural peculiarities of PfAQP that are responsible for this functional difference a series of in vivo, in vitro, and *in silico* experiments has been conducted.

Sequence comparison shows almost identical residues in both filter regions of PfAQP and GlpF. While the ar/R constriction of PfAQP and GlpF is composed of the same set of residues (Arg, Trp, and Phe), the NPA motifs of PfAQP are slightly changed to Asn-Leu-Ala (NLA) and Asn-Pro-Ser (NPS). Yet, mutation of the two varying amino acids back to canonical NPA motifs did not alter the water or glycerol permeability of PfAQP [22].

Later, a so-far neglected structural element of the AQPs was linked to the special permeability properties of PfAQP, i.e. the extracellular connecting loop C, which folds into the outer pore vestibule and lies just above the ar/R region. Specifically, a Glu (E125) was identified to be responsible for the high water permeability of PfAQP [23]. The mutation of E125 to Ser, i.e. the residue present at the corresponding position of GlpF, largely abolished water permeability with only little effect on glycerol permeability [23]. The mutation further resulted in an increase of the activation energy by about 3 kcal mol^{-1} and it was suggested that a change in the hydrogen bond network around the pore Arg might cause stronger binding of a passing water molecule and, thus, slower passage. Further evidence for this interpretation came from the 2.05-Å resolution crystal structure of PfAQP (Fig. 1) [25], which shows exactly this: the pore Arg of PfAQP is linked to neighboring residues by four hydrogen bonds, whereas GlpF exhibits only three hydrogen bonds. This free valence in GlpF is thought to enhance binding of a passing water molecule and to considerably increase its residence time in the ar/R filter resulting in the observed low water permeability rate.

The crystal structure also indicated a higher degree of decoration of PfAQP with glycerol molecules, especially within the extracellular vestibule where two glycerol molecules are located in direct vicinity to each other (Fig. 1) [25]. This finding was used to explain discrepant data on water permeability of PfAQP, which was obtained by different research groups and which had confused discussions of the matter. It was found that contrary to GlpF, water permeability of PfAQP strongly depends on the nature of the external osmolyte. PfAQP exhibited high water permeability using salt [22] or saccharose gradients [25], whereas no water permeability above background was seen when sorbitol buffers were used [26]. A screening of various solutes led to the conclusion that sorbitol (effectively representing two linked glycerol molecules) could take the place of the two glycerol molecules bound to the extracellular vestibule that are visible in the crystal structure and, hence, interfere with channel water permeability.

In the in vivo situation, PfAQP is exposed to a mixture of different physiological solutes. A simple functional assay was created to test the permeability of mixtures of glycerol and urea [27]. Under isotonic conditions, any ratio of glycerol and urea passed PfAQP equally well, whereas in a hypertonic buffer where the solute needs to diffuse in a countercurrent against water, glycerol was clearly preferred over urea [27]. Simulation of molecular dynamics and energy calculations of solute permeation through PfAQP equally indicate that PfAQP should conduct glycerol at higher rates than urea [28]. Other theoretical models showed that already micromolar concentrations of glycerol binding to PfAQP should inhibit water permeation [29]. Together, the experimental and theoretical analyses strongly suggest that the structure of PfAQP is particularly optimized for glycerol facilitation and, accordingly, a main task of PfAQP should be in the uptake of glycerol as a precursor for glycerolipid biosynthesis in the malaria parasite [30]. Deletion of the AQP encoding gene in the rodent malaria parasite *Plasmodium berghei* eventually confirmed this hypothesis because this strain has lost its capability of transmembrane glycerol transport [31]. These results further show that individual physiological functions for a parasite cell can be deduced from in-depth in vitro analyses of AQP permeation properties in combination with in vivo studies on the protein localization and expression profiles during development.

Unfortunately, there is no crystal data available of other parasite AQPs. Accordingly, any structural peculiarities must be inferred from sequence comparison. Considering the large effect on PfAQP water permeability of slight changes in the hydrogen bond network around the pore Arg residue, such an attempt can only be crude. In this regard it is notable that the *T. gondii* AQP and one AQP from *T. brucei*, TbAQP2, do not contain an arginine in the ar/R region at all but lipophilic alkyl residues instead, i.e. a valine and a leucine, respectively. Indeed, both showed considerable water permeability. Effects on their proton and cation filter capability are not reported.

3. Physiological functions of parasite aquaporins

Here, we can focus only on certain aspects of parasite physiology. For more detailed information about the biology (morphology, life cycles, metabolism etc.) of the parasites described below, the reader is kindly asked to consult respective literature.