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Molecular dynamics simulations of the transport of reactive oxygen species by mammalian and plant aquaporins

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ABSTRACT

Background: Aquaporins are responsible for water transport across lipid membranes. They are also able to transport reactive oxygen species, playing an important role in redox signaling. Certain plant aquaporins have even the ability to be regulated by oxidative stress. However, the underlying mechanisms are still not fully understood.
Methods: Here, molecular dynamics simulations were employed to determine the activation free energies related to the transport of reactive oxygen species through both mammalian and plant aquaporin models.
Results and conclusions: Both aquaporins may transport hydrogen peroxide (H_2O_2) and the protonated form of superoxide radicals (HO_2). The solution-to-pore transfer free energies were low for small oxy-radicals, suggesting that even highly reactive hydroxyl radicals (HO) might have access to the pore interior and oxidize amino acids responsible for channel selectivity. In the plant aquaporin, no significant change in water permeability was observed upon oxidation of the solvent-exposed disulfide bonds at the extracellular region. During the simulated time scale, the existence of a direct oxidative gating mechanism involving these disulfide bonds could not be demonstrated.
General significance: Simulation results may improve the understanding of redox signaling mechanisms and help in the interpretation of protein oxidative labeling experiments.

1. Introduction

Aquaporins are transmembrane proteins that regulate the passive water transport across phospholipid biomembranes [1,2]. They can be found in all kingdoms of life and are active in many biological functions, including nutrition and signaling [3,4]. Over the past years, great effort has been spent in the elucidation of the structure of aquaporins [5–7], and consequently their water conduction and selectivity mechanisms [8–14].

In the organism, aquaporins exist as tetrameric assemblies in which monomers act as independent water channels. Each monomer is formed by six tilted transmembrane α-helices (H1-H6), two re-entrant short helices (HB and HE) and five interconnecting loops (LA-LE) (Fig. 1). A pathway for water permeation is created by the re-entrant helices HB and HE. This pathway consists of a ~2-nm-long pore that connects the cytoplasmic and extracellular vestibules of the protein. The formation of a narrow water file is assisted by a series of backbone carbonyl groups and hydrophilic side chains placed along the pore. At the pore center, two highly conserved Asn-Pro-Ala (NPA) motifs provide selectivity against the passage of H^+ and other ions [15–18]. Close to the extracellular exit of the channel, the so-called aromatic/arginine (ar/R) constriction region also contributes to selectivity.

Structural differences exist between mammalian and plant aquaporins. In mammals, the formation of the tetrameric complex is driven solely by van der Waals interactions between the monomeric subunits. In plants, monomers are held together by disulfide bonds between conserved cysteine residues at the extracellular loops LA [19]. Most mammalian aquaporins are constitutively open channels, while plant aquaporins are gated channels that can be regulated by various mechanisms [20], including the phosphorylation of specific serine residues [7,21,22]. In the closed, unphosphorylated state, loop LD caps the cytoplasmic exit of the channel and blocks water passage.

Besides their role in keeping water homeostasis, specific aquaporin homologs have also the ability to conduct small metabolic solutes such as glycerol and urea [3]. A complex interplay may exist between aquaporins and biologically relevant reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and oxy-radicals such as hydroxyl (HO), hydroperoxyl (HO_2) and superoxide (O_2^-). The discovery that aquaporins can facilitate the transmembrane diffusion of H_2O_2, thereby acting in redox signaling, shed new light on their biological function [23–27]. In addition, the existence of an oxidative gating mechanism in plant aquaporins was proposed. *In-vivo* experiments in plants and algae showed that aquaporin conductivity can be reversibly inhibited upon exposure to ROS [28–30]. It is tempting to speculate about the role of the highly oxidizable disulfide bonds between monomers because they are completely exposed to solvent at the cytoplasmic side of the membrane.

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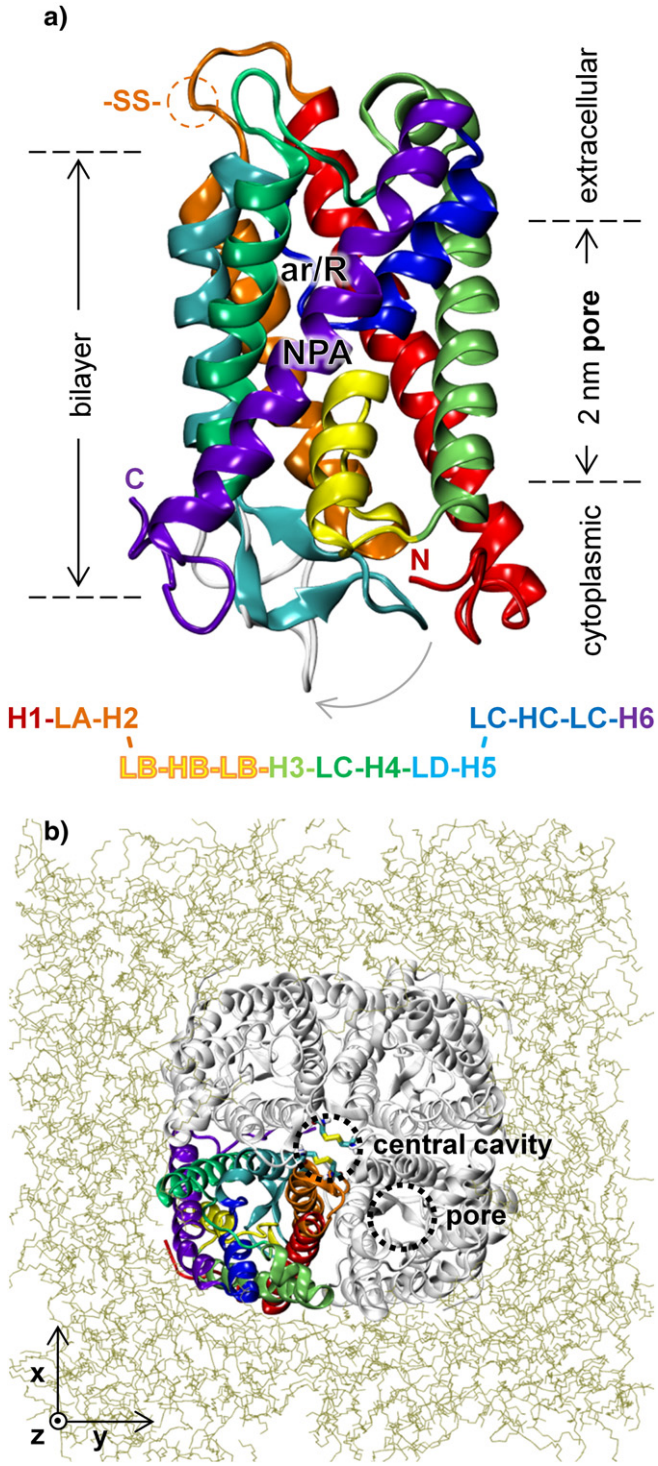

Fig. 1. (a) Structure of the plant aquaporin monomer PIP2;1. The α-helices and loops are colored from the N to the C-terminus according to the legend. The highly conserved NPA region and the ar/R constriction are indicated. The transition of loop LD to the open conformation is represented in grey and the position of the intermonomer disulfide bond (—SS—) is indicated by the dashed circle. (b) Top view of the tetramer (grey) embedded in a phospholipid bilayer (brown). One of the monomers is highlighted in color. The disulfide bonds at the extracellular side are shown in licorice representation. One of the pores and the central cavity are indicated by dotted circles.

There have been several molecular modeling studies aimed at the clarification of the conduction of various non-aqueous substrates by aquaporins [31–35]. However, systematic studies focused on the transport of ROS are still scarce [36]. It was recently found that certain hydrophilic ROS may reside close to the headgroups region of phospholipid bilayers and interact with unsaturated lipid fragments [37]. Still, different ROS had remarkably different tendencies with regards to distribution, mobility and permeation. The presence of aquaporin water channels in the membrane adds a layer of complexity to this issue. Here, classical molecular dynamics simulations were employed to determine the activation free energies related to the transport of ROS through both mammalian and plant aquaporin models. The consequences of disulfide bond oxidation were also investigated in plant aquaporins.

Purely classical models are well suited for the study of partition and permeation phenomena. However, they do not explicitly consider the electronic degrees of freedom needed to describe chemical reactions. It is not a critical issue in the case of relatively stable ROS such as H_2O_2. The lifetime of such species is usually longer than the multi-nanosecond time scale associated with permeation. Contrarily, HO radicals are very reactive [38,39] and classical free energy profiles can only provide a partial representation of their behavior close to aquaporin channels. Still, it might be helpful to know the classical energy barrier that radicals need to overcome in order to gain access to the pore interior. In the so-called "oxidative labeling" experiments, protein topology is inferred from the amino acid oxidation pattern in the presence of HO radicals [40–43]. Oxidized residues are assumed to be located in regions of the protein that are highly exposed to solvent. In the case of aquaporins, the residues that form the channel lumen are exposed to permeating water molecules, but are they also susceptible to direct attack by HO radicals [44,45]? As stated earlier, the ar/R region is located near to the extracellular exit of the pore and contains residues that are essential for selectivity.

2. Simulation methods

Molecular dynamics (MD) simulations [46,47] were performed at single precision with the GROMACS 4.5.1 package [48,49]. Graphical renderings of the simulated systems were produced using the VMD software [50]. In the following, a summarized description of the simulation protocol is provided. Further technical details can be found as supplementary material.

Newton's equations of motion were integrated at intervals of 2 fs. Interatomic interactions were described according to the GROMOS 54A7 force field [51,52], supplemented with parameters for post-translationally modified amino acids [53]. GROMOS had a number of characteristics that were desirable in the present study, for example: *i*) earlier and related versions were successfully employed in the simulation of aquaporins [13–15,54]; *ii*) the 54A7 version has been developed and tested for an improved description of protein structure [51]; *iii*) compatible phospholipid membrane models have been developed and validated [55,56]; and *iv*) water-to-membrane partition of small molecules such as H_2O_2 were correctly described. The GROMOS parameters were used in combination with an updated version of a force field for ROS [37,57] and the SPC water model [58].

The *Bos taurus* aquaporin-1 (AQP1) and the *Spinacia oleracia* plasma membrane intrinsic protein (PIP2;1) were chosen as representative models of mammalian and plant aquaporins, respectively. The initial coordinates of both proteins were obtained from the published crystal structures available in the Protein Data Bank (PDB) under the codes 1J4N [6] and 1Z98 [7], respectively. The tetramers were embedded in pre-equilibrated 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC) bilayers [56] using the g_membed tool [59]. Amino acids were considered at their standard protonation states at pH 7. In plants, aquaporin closure can also be triggered by the protonation of a conserved histidine residue in loop D [60]. Therefore, all histidine side chains were kept at their uncharged (deprotonated) form. The crystallographic water molecules were retained and enough Cl^- counter ions were present at the aqueous phase to keep the systems electrically neutral. The assembled systems had lateral dimensions of ~10 nm parallel to the membrane surface (*xy*-plane) and ~7.5 nm along the bilayer normal