Biophysical Chemistry 192 (2014) 33–40

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Ion-dynamics in hepatitis C virus p7 helical transmembrane domains — a molecular dynamics simulation study

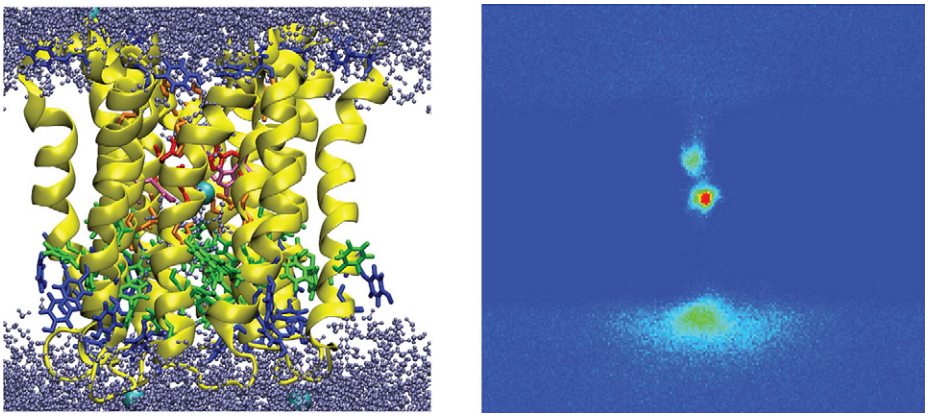
Yi-Ting Wang [a,b], Roman Schilling [c], Rainer H.A. Fink [c], Wolfgang B. Fischer [a,b,*]

[a] Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang-Ming University, Taipei, 112, Taiwan
[b] Biophotonics and Molecular Imaging Research Center (BMIRC), National Yang-Ming University, Taipei, 112, Taiwan
[c] Medical Biophysics Group, Institute of Physiology and Pathophysiology, University of Heidelberg, 69120 Heidelberg, Germany

HIGHLIGHTS

- Generation of hexameric assemblies of ion channel forming p7 of HCV
- Protonation of His-17 allows Cl-ions to enter the pore.
- In unprotonated bundle also Ca-ions are found within the pore.
- Applied voltage identifies large Cl-ion currents from the site of the loop.
- Slight rectification of the current is observed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form 4 June 2014
Accepted 6 June 2014
Available online 16 June 2014

Keywords:
Membrane protein
Ion channels
Computational modeling
Protein structure
Conductance

ABSTRACT

Viral proteins assemble into homopolymers in the infected cells and have a role as diffusion-amplifier for ions across subcellular membranes. The homopolymer of hepatitis C virus, protein p7 of strain 1a, which is known to form channels, is used to investigate the dynamics of physiological relevant ions, Na^+, K^+, Cl^- and Ca^{2+} in the vicinity of the protein bundle. The protein bundle is generated by a combination of docking approach and molecular dynamics (MD) simulations. Ion dynamics are recorded during multiple 200 ns MD simulations of 1 M solutions. His-17 is found to point into the lumen of the pore. Protonation of this residue allows Cl-ions to enter the pore while in its unprotonated state Ca-ions are found within the pore as well. Applied voltage identifies large Cl-ion currents from the site of the loop passing through the pore. Rectification of the current of the Cl-ions is observed.

1. Introduction

Protein p7 from hepatitis C virus (HCV) belongs to the class of viral channel forming membrane proteins (VCPs) [1,2]. Together with other members of this class (e.g. reviewed in Ref. [3]), it shares the role of changing electrochemical and/or substrate gradients across the lipid membrane by forming an oligomeric assembly. The precise role within

* Corresponding author at: Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang-Ming University, 155, Li-Non St., Sec. 2, Taipei, 112, Taiwan. Tel.: +886 2 2826 7394; fax: +886 2 28235460.
E-mail address: wfischer@ym.edu.tw (W.B. Fischer).

http://dx.doi.org/10.1016/j.bpc.2014.06.001
0301-4622/

the life cycle of the virus is still to debate, which is also a fact it has in common with most of the today's known viral channel proteins.

The protein is expressed within a polyprotein precursor from which it is cleaved, together with the other proteins of HCV genome, *via* cellular and viral proteases [4–6]. As a polytopic membrane protein of 63 amino acids with two transmembrane domains (TMDs), its termini are located towards the lumen of the endoplasmic reticulum [7]. Its role within the life cycle of HCV is envisaged to be similar to that of M2 of influenza A, supporting the release of the virion when trapped within the endosome during viral entry process [8,9]. It is proposed that p7 is present in the membrane of the virion [10,11].

It has been shown that p7 conducts ions [12–14] as well as protons [15]. Experiments have mostly been done with full length peptides expressed and purified from *Escherichia coli* [13] or produced from solid phase peptide synthesis [12,14] and reconstituted into artificial lipid bilayers. Proton conductance experiments have been done with vesicles extracted from cells measuring proton permeability as well as cell based imaging of vesicular pH [15].

Structural information has emerged from solid state [10,16] and solution NMR spectroscopy [17–19]. On the monomeric level, the structures either propose two anti-parallel aligned TMDs or a clamp-like overall structure. The fact that parallel aligned models are obtained for proteins with the amino acid sequence according to genotypes, 1a and 1b, and the clamp-like structure from proteins according to genotype 5a [18], drives a debate of structural differences albeit high sequence homology. Genotype dependent results have been derived when generating samples to be used for cryo-electron microscopy (EM) experiments [13,20,21]. Hexameric bundles are found for JFH-1 2a strains [21], while p7 of J4 1b strain is found in both, a hexameric [13] and heptameric formation [20].

Having a highly vital role within the life-cycle of HCV in chimpanzees [22] and in cell cultures [23,24], the protein emerges as a potential drug target. Most recent investigations have identified small molecule drugs to be able to block channel activity of p7 [10,12–14,25], with one used in clinical trials [25]. Poses of the drugs on the monomeric p7 protein are proposed to be within bundle models [10] or at either side when dealing with monomers [19,26].

In a combined bioinformatics and computational modeling approach, an antiparallel aligned model of full length p7 has been proposed ahead of available experimental data as mentioned above and modeled into a hexamer [27]. In the bundle model a histidine at position 17 is suggested to face the lumen of the pore, which is in agreement with the aforementioned NMR data. Extensive molecular dynamics simulations have been performed on both, hexameric and heptameric bundles, suggesting structural plasticity for the mechanism of function of p7 [28]. The model for the bundles based on p7 of genotype 1b has been derived from a monomeric p7 model obtained from NMR spectroscopy [17], copied and centered around a central axis either strictly according to the NMR coordinates or with optimized inter-subunit contacts.

At this stage, the mechanism of ion and/or proton conductance of the p7 bundle is still to be elucidated. With the structural information at hand, the mechanism of function in respect to ions is ready to be investigated computationally.

In this study, a structure based model of p7 monomer is taken [26], copied and assembled into a hexamer using established protocols [29,30]. The protein is embedded into a lipid bilayer. The protein/lipid system is hydrated also in such a way that it is embedded in electrolytes at a concentration of 1 M. The location of ions within the bundle is monitored in dependence of the number of protonated histidines and under applied physiological relevant voltages.

2. Materials and methods

The p7 protein sequence was taken from the HCV genotype 1a, H77 strain [12]: ALENLVILNA^{10} ASLAGTHGLV^{20} SFLVFFCFAW^{30} YLKGRWVPGA^{40} VYAFYGMWPL^{50} LLLLLALPQR^{60} AYA.

2.1. Assembly of the bundles

The monomeric structure of p7 was assembled from the individual TMDs as reported in detail earlier [26]. In brief, each of the TMDs (TMD1: 1–32, TMD2: 36–63) was individually simulated in a fully hydrated lipid bilayer (POPC) for 50 ns. Averaged structures over the backbone atoms of the 50 ns MD simulations of each of the TMDs were derived by fitting the peptide structure of each time frame to the starting structure thereby removing rotational and translational motions. The program g_covar from the GROMACS-4.0.5 packages was used for the calculations. Applying the same assembly protocol as mentioned below, the lowest energy structure of the assembled TMDs was generated. Finally, the monomer is formed by linking the assembled TMDs with a short loop (Lys-33, Gly-34, Arg-35) using the program Loopy [31,32]. The derived monomeric structure was embedded in a fully hydrated lipid bilayer and equilibrated in a 150 ns MD simulation.

Hexameric bundles were generated with the monomer by creating symmetric copies of the monomeric subunit around a central pore axis [29,30]. To sample the whole conformational space of the bundles each of the degrees of freedom was varied stepwise: (i) distance of the monomer to the central axis in steps of 0.25 Å covering 9 to 15 Å; (ii) rotational angle in steps of 5° covering 360°; (iii) tilt in steps of 2° covering −36° to +36°. For each position, the side chains were linked to the backbone. The side chain conformation was chosen to be the most likely one for a given backbone conformation according to the MOE library. A short minimization (15 steps of steepest decent) followed the generation of the side chains. For each conformation the potential energy was evaluated according to the all-atom AMBER94 force field mimicking a bilayer environment ($\varepsilon = 2$). The lowest energy conformer was embedded into fully hydrated POPC lipid bilayer.

2.2. MD simulations of the bundles

Lipid bilayer patches were generated from 16:1 to 18:1 Diester PC, 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (POPC) molecules on the basis of the parameters of Chandrasekhar et al. [33]. The lipid patches were equilibrated for 50 ns. The hexameric bundles system has 512 lipid and 28215 water molecules. The lipid patches were equilibrated for 50 ns.

MD simulations of the bundles/lipid/water system were carried out with GROMACS-4.5.4 using Gromos96 ffG45a3 force field (geometric combination rule). The temperature of the peptide, lipid and the water molecules was separately coupled to a Berendsen thermostat at 310 K with a coupling time of 0.1 ps. Semi-isotropic pressure coupling was applied with a coupling time of 1.0 ps and a compressibility $4.5 \cdot 10^{-5}$ bar^{-1}. Long range electrostatics have been calculated using particle-mesh Ewald (PME) algorithm with grid dimensions of 0.12 nm. Lennard–Jones and short-range Coulomb interactions were cut off at 1.4 and 1 nm, respectively.

The p7 models were embedded into the POPC bilayer system and the overlapping lipid molecules were removed using the g_membed program from GROMACS-4.5.4. The hydrated system underwent 5000 steps of steepest decent and 5000 steps of conjugated gradient minimization to remove unfavorable interactions and equilibration for a total of 7.9 ns. Equilibration was achieved by gradually increasing the temperature from 100 K to 200 K and then to 310 K, while keeping the protein fully restraint applying position restraints with $k =$ 1000 KJ mol^{-1} nm^{-2}. The first two simulations (100 K and 200 K) were run for 200 ps, and the last simulation (310 K) was run for 1.5 ns. Holding the system at 310 K, the restrained protein was released in 4 consecutive 1.5 ns MD simulations reducing the force constant k ($k =$ 500 KJ mol^{-1} nm^{-2}, $k = 250$ KJ mol^{-1} nm^{-2}, $k = 100$ KJ mol^{-1} nm^{-2}, $k = 25$ KJ mol^{-1} nm^{-2}).