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Influence of membrane potentials upon reversible protonation of acidic residues from the OmpF eyelet

Alina Asandei [1], Loredana Mereuta [1], Tudor Luchian *

'Al. I. Cuza' University, Faculty of Physics, Laboratory of Biophysics & Medical Physics, Blvd. King Carol I, No. 11, Iasi, R-700506, Romania

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ABSTRACT

In this research we employed single-molecule electric recording techniques to investigate effects of the transmembrane and dipole potential on the reversible protonation of acidic residues from the constriction zone of the OmpF porin. Our results support the paradigm according to which the protonation state of aspartate 113 and glutamate 117 residues from the constriction region of OmpF is influenced by the electric potential profile, via an augmentation of the local concentration of protons near these residues mediated by increasing negative transmembrane potentials. We propose that at constant bulk pH, pK_a values for proton bindings at these residues increase as the applied transmembrane potential increases in its negative values. Our data demonstrate that the apparent pK_a for proton binding of the acidic aminoacids from the constriction region of OmpF is ionic strength-dependent, in the sense that a low ionic strength in the aqueous phase promotes the increase of the protonation reaction rate of such residues, at any given holding potential. Supplementary, we present evidence suggesting that lower values of the membrane dipole potential lead to an increase in the values of the 'on' rate of the eyelet acidic residues protonation, caused by an elevation of the local concentration of hydrogen ions. Altogether, these results come to support the paradigm according to which transmembrane and dipole potentials are critical parameters for the titration behavior of protein sites embedded lipid membranes.

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1. Introduction

One of the fundamental chemical reactions encountered in the biological world is the proton bindings reaction, which plays critical roles in protein structure and function. That is, reversible proton bindings are elementary steps for the transfer of protons within proteins, which stand as crucial in biochemical catalysis and energy transduction processes. A great deal of both theoretical and experimental approaches has been devoted over the decades to the accurate determination of aminoacid pK_as in proteins [1–6]. This was partly due to the fact that, for instance, pK_a's knowledge of the residues from the active site of an enzyme pinpoints the plausible proton donors and acceptors, and therefore helps in understanding the reaction mechanism [1,7,8]. Equally important, it is well-known that the stability of proteins and of several protein-ligand aggregates varies as a function of the ionization state of titratable aminoacid residues [9–12].

With paramount importance for the biological world, within folded proteins pK_a values of ionizable residues are usually shifted with respect to their aqueous solution values. With regard to this, a consensus over major determinants which promote pK_a's shifts has been reached, including here: contributions stemming from the Born solvation energy, the group's interaction with partial charges on the protein such as the peptide dipoles (so-called background charge interactions) and the group's interaction with other ionizable groups in the protein [2,13,14]. From a mathematical standpoint, estimations of pK_a's shifts in proteins are usually computed with the help of the linearized Poisson–Boltzmann equation. Notably, in an experimental attempt towards understanding the effect of the protein environment on the pK_a values of protein residues, protonatable side chains were engineered into the pore domain of the muscle nicotinic acetylcholine receptor, and large negative pK_a shifts for basic aminoacid residues in nonaqueous environments were reported [15].

In addition to the arguments presented above and taking into account that local electric fields in proteins alter the ion binding energy as compared to the bulk solution, it is very plausible that lipid membranes electric profile possesses the potential to influence via long-range interactions the equilibrium constant of hydrogen ions interaction with various residues, and implicitly the pK_a values. The most common electrical potentials associated with lipid membranes are the transmembrane potential difference, generated by a gradient of electric charge across the phospholipid bilayer and the membrane surface potential, which stems from the net excess electric superficial charges at the membrane interface in contact with the aqueous medium [16].

To date, electrostatic interactions are known to play extensive role in various aspects of protein structure and function, including here

* Corresponding author. Tel.: +40 232 201191; fax: +40 232201150.
E-mail address: luchian@uaic.ro (T. Luchian).

[1] These authors contributed equally to this work.

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enzyme catalysis, ligand binding, the fine-tuning of redox potentials, and stability of folded proteins. At the microscopic level, the membrane potential was shown to alter the configurational equilibrium and the orientation of membrane-bound proteins [17–19]. On the other hand, the surface potential was proven to play important roles in cell adhesion and spreading, chemotaxis processes, binding of various anesthetics and ion channel antagonists [20–22]. In addition, a component of the electric membrane potential known as the dipole potential was acknowledged to play important roles in peptides–membrane interactions [23]. Its overall value (~300 mV, positive toward the membrane interior) and the correspondingly high electric field associated with it over the aqueous phase–hydrocarbon region interface (10^8–10^9 V m^{-1}), endow the dipole potential with major roles in the modulation of molecular processes which take place within a biomembrane, including protein insertion and functioning, kinetics and electrical conductance of certain aqueous protein pores [24–27].

In a recent contribution, within the framework of continuum electrostatics, authors employed a modified Poisson–Boltzmann equation to include the membrane potential component and presented evidence that the protonation probability of bacteriorhodopsin's aspartate 85 and aspartate 115 which take up protons from different sides of the membrane is differently influenced by the membrane potential [28].

In this work we employed single-molecule electric recording techniques on the OmpF porin inserted in artificial lipid membranes, to investigate effects of the transmembrane and dipole potential on the reversible protonation of acidic residues which are part of the constriction zone of the porin. The outer membrane porin F (OmpF) resembles a β-barrel structure, which possesses sixteen anti-parallel strands connected by turns at the periplasmic side and loops at the extracellular side of the bacteria they reside on [29]. One of the eight extracellular loops (L3) folds back into the pore lumen and about half way down the channel, an aspartate (D113) and glutamate (E117) from the tip of L3 face three arginine groups located at the channel wall (R42, R82 and R132), creating an asymmetrically shaped constriction zone of about 0.7×1.1 nm^2 in area. The transversal electric field on the constriction zone changes its modulus with changes in the solution acidity, fact that is intrinsically linked to the pH-dependent charged state of the residues making up that region [30,31]. As it has been nicely demonstrated, aqueous pH changes alter channel's conductance as a result of the direct interactions of fluctuating residue charges with penetrating ions, and the ensuing stepwise current transients observed mostly close to the pK_a values of the aminoacids located on the constriction zone reveal information about their reversible protonation mechanism [32]. There is plenty of evidence demonstrating that besides the OmpF porin, reversible protonation of ionizable residues induces conductance fluctuations in other ion conducting proteins, including here the α-hemolysin channel [33], Ca^{2+} [34] and mouse nicotinic AChR channels [15,35].

Our work suggests that both transmembrane potential and membrane dipole potential alter the kinetics of the reversible protonation of acidic residues with propensity to alter transport properties of the OmpF porin, with most plausible candidates in this respect being aspartate 113 and glutamate 117 residues, and implicitly modify the pK_as of such acidic aminoacid residues from the constriction site of the protein. We monitored the stepwise current flickering of the OmpF porin inserted in model lipid membranes at a pH value of 2.8, which ensured an optimal resolving accuracy in the power spectra changes of such fluctuations, once the protonation equilibrium of acidic residues, in particular the aspartate 113 and glutamate 117 residues would shift as a result of their electrostatic interactions with lipid bilayer potentials. By approximating power spectra of the current fluctuations with single Lorentzians, our data prove that more negative transmembrane potentials applied with respect to the OmpF addition side of the membrane, promote a faster reversible protonation of such residues. Assuming that such negative transmembrane potential values do little to the energy of the activated complex which characterizes the chemistry of reversible protonation of aspartate 113 and glutamate 117 residues, which plausibly are the preferred candidates to explaining the stepwise current transients through the OmpF porin observed in the acidic range, our results point to an augmentation in the local concentration of protons near these residues, caused by the electric interaction between positively charged protons and the electric potential within the membrane core. As a result of this, it is conceivable that at constant bulk pH, pK_a values for proton bindings at these residues would increase as the applied transmembrane potential increases in its negative values. In addition, we show that the observed phenomenon is ionic strength-dependent; as the Debye–Hückel theory predicts.

We also prove that when added from the cis side of the membrane, phloretin, a known compound for its ability to lower the membrane dipole potential, induces a similar change in the protonation equilibrium of the aspartate 113 and glutamate 117 residues, at any given holding transmembrane potential. This phenomenon comes in good physical agreement with the previously studied herein, since by either modifying the transmembrane holding potential towards negative values or by keeping its value constant but lowering the dipole potential of the membrane, one arrives at circumstances whereby the algebraic value of the electric potential at a point half-way across the hydrophobic core of the membrane decreases.

One very interesting feature of the OmpF porin lies in its change in selectivity with aqueous acidity [30]. In the end, we tested whether charge distribution alterations within the permeation pathway of the OmpF protein, as they are induced by dipole potential alterations and visible in our experiments for acidic aminoacids from the constriction region, lead to any visible changes on its selectivity. Thus far, current–voltage diagrams drawn for the OmpF porin in the absence and presence of phloretin under asymmetrical salt concentration point to no convincing evidence in support of this speculation.

2. Materials and methods

Electrophysiology experiments were carried out in the folded bilayer membranes system obtained with Montal–Mueller technique, as previously described [25,36]. The lipid used was L-α-phosphatidylcholine (Fluka, code 61755), and sodium chloride salt solutions were buffered at a pH value of 2.8 in 5 mM MES buffer (Sigma-Aldrich). Single channel insertion of the OmpF protein was achieved by adding ~1 μl from a dilute stock solution made in 1 M NaCl and 1% (v/v) of octyl POE to the cis chamber only, connected to the ground. Spontaneous channel insertion was usually obtained within a few minutes under stirring. The purified OmpF protein was a precious gift from Prof. Mathias Winterhalter (Bremen, Germany). When employed, phloretin (Fluka) was added to the cis side of the membrane, from a 0.1 M stock solution made in ethanol. Currents from the bilayer chamber were detected and amplified with a resistive headstage patch-clamp amplifier (EPC 8, HEKA, Germany) set to the voltage-clamp mode, via a pair of Ag/AgCl electrodes. Amplified electric signals were low-pass filtered at 10 kHz with the help of an active low-pass filter (LPF-8, Warner Instrument Corp., USA), and data acquisition was performed with a NI PCI 6014, 16-bit acquisition board (National Instruments, USA) at a sampling frequency of 30 kHz within the LabVIEW 8.20 environment. Numerical analysis, including time-domain low-pass filtering, spectral analysis and graphing, were done with the help of the Origin 6 software (OriginLab, USA). Fast Fourier analysis was performed on 2048 Hamming windowed data points, and at least 50 data segments were used to produce an averaged output. All experiments were performed at a room temperature of ~23 °C. When ion selectivity of the OmpF channel was studied, we used a salt gradient of 0.1 M (cis side) KCl // 3 M (trans side) KCl (Sigma-Aldrich), buffered at a pH of 2.87 in 5 mM MES (Sigma-

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