Alamethicin channel conductance modified by lipid charge

Received: 16 November 2000 / Revised version: 25 January 2001 / Accepted: 25 January 2001 / Published online: 12 April 2001
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Abstract The membrane surface charge modifies the conductance of ion channels by changing the electric potential and redistributing the ionic composition in their vicinity. We have studied the effects of lipid charge on the conductance of a multi-state channel formed in planar lipid bilayers by the peptide antibiotic alamethicin. The channel conductance was measured in two lipids: in a neutral dioleoylphosphatidylethanolamine (DOPE) and a negatively charged dioleoylphosphatidylserine (DOPS). The charge state of DOPS was manipulated by the pH of the membrane-bathing solution. We find that at high salt concentrations (e.g., 2 M NaCl) the effect of the lipid charge is below the accuracy of our measurements. However, when the salt concentration in the membrane-bathing solution is decreased, the surface charge manifests itself as an increase in the conductance of the first two channel levels that correspond to the smallest conductive alamethicin aggregates. Our analysis shows that both the salt and pH dependence of the surface charge effect can be rationalized within the nonlinear Poisson-Boltzmann approach. Given channel conductance in neutral lipids, we use different procedures to account for the surface charge (e.g., introduce averaging over the channel aperture and take into account Na\(^+\) adsorption to DOPS heads), but only one adjustable parameter: an effective distance from the nearest lipid charge to the channel mouth center. We show that this distance varies by 0.3–0.4 nm upon channel transition from the minimal conducting aggregate (level L0) to the next larger one (level L1). This conclusion is in accord with a simple geometrical model of alamethicin aggregation.

Keywords Surface charge · Double layer · Lipid titration · Protein electrostatics

Introduction

The limits of applicability of classical continuum electrostatics at the length scales of a protein molecule are among the most vividly discussed issues of modern biophysics (Homig and Nicholls 1995; Eisenberg 1999; Murray et al. 1999; Cardenas et al. 2000; Moy et al. 2000). While Poisson-Boltzmann theory has proved to be a successful tool in studies of proteins and membranes, its potential for a quantitative description is repeatedly questioned. In the present paper we apply this theory to describe the influence of membrane lipid charge on ionic conductance of a transmembrane multi-state channel formed by alamethicin.

The 20-amino acid peptide alamethicin is produced by the fungus Trichoderma veride. It is known that in lipid membranes it self-assembles to form channels which fluctuate between different conductance states, depending on the alamethicin aggregation (Hall et al. 1984; Sansom 1991; Cafiso 1994; Wallace 2000). These channels have been modeled as approximately parallel bundles of transbilayer helices containing at least four helices per bundle. Their expressed sensitivity to the applied voltage makes them an attractive model of voltage-gated channels in neurophysiology (Bezrukov and Vodyanoy 1995, 1998).

Alamethicin channels have been extensively studied both experimentally and theoretically (by using continuum solvent calculations or molecular dynamics), focusing on their selectivity (Borisenko et al. 2000), ionic diffusivity (Smith and Sansom 1999), structure (Sansom 1991), and channel-membrane interaction (Keller et al.
1993; Opsahl and Webb 1994; Kessel et al. 2000), including binding of the peptide to the lipid surface (Tieleman et al. 1999).

Recently, in experiments with planar bilayer membranes formed from charged lipids at varying pH (Bezrukov et al. 1998), it has been found that lipid packing stress modified by changes in electrostatic interaction between the polar lipid heads influences the lifetime of the alamethicin channel “burst” and the probability of alamethicin conductance states. However, there are other effects of lipid charge apart from the change in the equilibrium between differently sized alamethicin aggregates. Accumulation of counterions near the membrane surface also influences the channel electric conductance. There is extensive experimental evidence that the conductance of ion channels can be modified by the fixed charge of lipid polar headgroups (Apell et al. 1979; Bell and Miller 1984; Moczylowski et al. 1985; Coronado and Affolter 1986; Green and Andersen 1991; Rostovtseva et al. 1998). Depending on the channel selectivity, the lipid charge can either increase or decrease the channel conductance.

Here we analyze amplitudes of the first two levels (called L0 and L1, respectively) of alamethicin-induced ion conductance fluctuations in different lipid and electrolyte solution environments. We show that, given the channel conductance in neutral lipids, Poisson-Boltzmann theory allows semi-quantitative description of the surface charge effects with only one adjustable parameter: an effective distance between the nearest lipid charge and the center of the channel. Depending on the calculation details, we obtain that this distance increases by 0.31–0.42 nm when the channel conductance jumps from level L0 to level L1. This observation agrees well with a simple geometrical model of the two minimal alamethicin aggregates.

Materials and methods

Alamethicin channels were inserted into “solvent-free” planar lipid bilayer membranes that had been formed by apposition of two phospholipid monolayers spread on aqueous solutions of sodium chloride (Baker, Phillipsburg, NJ, USA). The monolayers were prepared from a 10% solution of dioleoylphosphatidylethanolamine (DOPS) or dioleoylphosphatidylserine (DOPE) (Avanti Polar Lipids, Alabaster, Ala., USA) in pentane (Burck and Jackson, Muskegon, Mich., USA). The Teflon chamber (Bezrukov and Vodyanoy 1993; after Montal and Mueller 1972) with two compartments of 1 mL was divided by a 15-μm thick Teflon partition (Chemfab, Merrimack, NH, USA) with a 60-μm diameter aperture. The aperture was pretreated with a 1% solution of hexadecane (Aldrich, Milwaukee, Wis., USA) in pentane and dried during 10 min prior to monolayer apposition.

Natural alamethicin (Sigma, St. Louis, Mo., USA) was added only to one side of a membrane from a 10^{-8} M stock solution in ethanol to a final concentration of 1–5x10^{-8} M. All experiments were done at 150 mV, positive from the side of alamethicin addition, and at a room temperature of 23 ± 1 °C. The alamethicin was adjusted to a concentration that gave the first current bursts about 20 min after peptide addition; in this way we were able to monitor single-channel activity (no channel overlapping) for about 10 min. Ion currents, amplified with an Axopatch 200A integrating patch-clamp amplifier (Axon Instruments, Foster City, Calif., USA), were recorded with a sampling rate of 50 kHz into computer memory and, simultaneously, onto recordable compact disks. Conductance data reported here are averages over more than 30 different “current bursts” obtained from at least three different membranes for each lipid composition, pH value, and salt concentration.

Results and discussion

Typical recordings of alamethicin-induced currents through DOPS planar bilayers at pH 6.2 and pH 2.5 are shown in Fig. 1. It is seen that alamethicin channels at these conditions are very different. In 0.1 M NaCl solution the acidity changes the channel “current burst” drastically. Higher proton concentrations favor higher conductance states, corresponding to larger alamethicin aggregates. These pH-dependent changes in channel probabilistic behavior were found recently (Bezrukov et al. 1998) and were attributed to changes in the lipid packing stress. Indeed, at pH 6.2 the proton concentration in solution is so low that practically all lipid headgroups in the membrane are ionized. Consequently, DOPS is almost fully charged and the electrostatic repulsion between headgroups relieves the stress of lipid packing into a planar bilayer membrane (for a novel theoretical treatment, see Li and Schick 2000). The situation is different at high proton concentration, i.e., at pH 2.5. Protonation of negatively charged residues decreases the surface charge density, reduces headgroup repulsion, and promotes higher packing stress. This stress modulates channel expression (Gruner 1985; Keller et al. 1993; Lundbaek and Andersen 1994; Bezrukov 2000).

In the case of DOPE bilayers, channel conductance bursts at pH 6.2 and pH 2.5 are hardly distinguishable, except for the fact that the conductances of each level at pH 2.5 are somewhat higher owing to added protons. Alamethicin channel probabilistic behavior in neutral DOPE is not sensitive to the solution acidity in this pH range (Bezrukov et al. 1998). Figure 2 shows that higher conductance states corresponding to larger alamethicin

![Fig 1 Typical alamethicin channel recordings in DOPS bathed by 0.1 M NaCl at pH 6.2 (left) and pH 2.5 (right). The lipid charge is pH dependent and, as a result, probabilistic behavior of the channel changes dramatically with pH. As the solution acidity is increased, the higher conductance levels corresponding to the larger peptide aggregates become more expressed. The acidity effect on channel conductance is measurable (see below) but less pronounced. The time resolution is 0.1 ms](image-url)