Electrochimica Acta 282 (2018) 213–222

Contents lists available at ScienceDirect

Electrochimica Acta

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

Kinetic mechanism of current-time curves of peptide ion channels in lipid membranes

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ARTICLE INFO

Article history:
Received 26 March 2018
Received in revised form
14 May 2018
Accepted 5 June 2018

Keywords:
Bilayer lipid membranes
Current-time curves
Nucleation and growth
Gating charge
Alamethicin

ABSTRACT

The three-state kinetic model developed in our previous paper (R. Guidelli and L. Becucci, 2016), which explains all the main features of experimental current-voltage curves of peptide ion channels at conventional bilayer lipid membranes (BLMs), is extended to explain some still unsettled features of the corresponding voltage-step current-time (*I*-*t*) curves. To this end, the time dependence of the open probability of peptide ion channels during the first few seconds from the instant of the voltage step, before the attainment of its steady-state value expressed by the well-known one-sided Boltzmann equation, is taken into account. This approach explains the reason for the different behavior of the experimental sigmoidal *I*-*t* curves with respect to the wholly concave downward ones; the frequent occurrence of concave downward *I*-*t* curves characterized by a time course describable by a sum of two exponential functions is also justified. On the other hand, the three-state kinetic model disproves the validity of the experimental procedure aiming at estimating the gating charge of peptide ion channels from the initial stage of the time derivative of ON and OFF ionic currents, commonly justified by an unproved analogy with gating currents of potassium ion channels.

1. Introduction

The biophysical literature is rich with measurements of current-time (*I*-*t*) curves elicited by transmembrane potential steps at conventional bilayer lipid membranes (BLMs) incorporating channel-forming peptides, and with their interpretation. Nonetheless, a number of features of these measurements still remain unexplained. Incidentally, by conventional BLMs we mean bilayer lipid membranes interposed between two bulk aqueous phases and, hence, not supported by a metal.

Peptides are usually added on only one side of BLMs, referred to as the *cis* side. To comply with biophysical usage, the transmembrane potential ϕ_m will be defined as the electric potential on the *cis* side of the BLM with respect to that on the *trans* side, taken as equal to zero. As a rule, the ON ionic current I^i_{on} induced by a positive ϕ_m step tends to a maximum limiting value that increases more than linearly with an increase in step height. While some curves of I^i_{on} against time t tend to their steady-state value by constantly maintaining the concavity of the curve turned toward the time axis [1–6], others [6–11] pass from being concave upward to concave downward, thus exhibiting a clear sigmoidal shape.

Concave downward I^i_{on}-t curves often yield plots of $\ln(I_\infty - I^i_{on})$ against time that exhibit an initial deviation from a straight line, where I_∞ is the maximum limiting value of the current [2,5]. The time course of these current transients can often be described by a sum of two exponential functions, whose time constants differ by about one order of magnitude. In the case of alamethicin, the higher time constant (i.e., that corresponding to the slower process) was reported to increase with an increase in ϕ_m at low ϕ_m values [2], whereas an opposite trend was observed at high ϕ_m values [5].

By definition, a gating current is a current elicited by a ϕ_m step from a transmembrane potential at which the ion channel is certainly closed to one where it progressively opens with time, under experimental conditions preventing permeant ions from flowing along the channel [12]. In practice, these conditions can only be fulfilled with Na^+, K^+ or Ca^{2+} tetrameric ion channels, upon adding suitable inhibitors and/or removing the permeant ions from the appropriate side of the membrane. With these tetrameric ion channels, the gating charge q can also be estimated from the dependence of the decay constants of ON and OFF gating currents upon ϕ_m, on the basis of the absolute reaction rate theory [13,14]. These procedures cannot be applied to channel-forming peptides,

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https://doi.org/10.1016/j.electacta.2018.06.038
0013-4686/

due to the practical impossibility of blocking the flow of permeant ions during ϕ_m steps. In a number of papers [15–21], it was observed that a positive ϕ_m step at BLMs incorporating channel-forming peptides moves cations from the *cis* to the *trans* side, eliciting a positive ionic current density (the ON ionic current density j^i_{on}) that exhibits an initial exponential growth with time; moreover, a subsequent negative ϕ_m step of the same magnitude induces a negative ionic current density (the OFF ionic current density j^i_{off}) that exhibits an exponential decay to zero. On the basis of an unproved analogy with the gating currents of tetrameric ion channels, the initial time derivatives of j^i_{on} and j^i_{off} were expressed by the equations [13,15,19]:

$$dj^i_{on}/dt \propto \exp(\alpha q\phi_m/kT) \quad ; dj^i_{off}/dt \propto \exp[-(1-\alpha)q\phi_m/kT]. \tag{1}$$

Here, $\alpha\phi_m$ is the fraction of the transmembrane potential favoring the *trans*-directed movement of a positive gating charge q for $\phi_m > 0$, whereas $-(1-\alpha)\phi_m$ is the fraction favoring its *cis*-directed movement for $\phi_m < 0$. The transfer coefficient α equals 0.5 if the potential energy barrier is symmetric. The slope of the $\ln(dj^i_{on}/dt)$ vs. ϕ_m plot at $t = 0$ is, therefore, assumed to yield $\alpha q/kT$, whereas that of the $\ln(dj^i_{off}/dt)$ vs. $-\phi_m$ plot provides $(1-\alpha)q/kT$. Hence, q is obtained from the sum of the two initial slopes.

The present note aims at examining the rationale behind the above experimental features of *I-t* curves at BLMs incorporating channel-forming peptides on the basis of the same three-state model already employed to explain the main features of the corresponding current-voltage curves [22]. This approach will also serve to verify the validity of the unproved Eq. (1).

2. Results

2.1. The model

Prior to a ϕ_m step inducing an *I-t* curve, the peptide monomers are considered to be adsorbed on the *cis* side of the membrane bathed by the solution where they were added, parallel to the membrane plane. As a rule, peptides capable of forming ion channels have a helical structure (at least inside the BLM) with one side of the helix lined with hydrophilic amino acid residues. The peptide molecules adsorbed on the *cis* side of the BLM may merely keep the hydrophobic side of their helix in contact with the BLM polar heads, or they may partially penetrate the polar head region. As the transmembrane potential ϕ_m becomes sufficiently positive, the dipole moment of the peptide molecules, distributed along their helical axis, tends to be aligned along the direction of the resulting electric field, which is mainly focused on the hydrocarbon tail region of the BLM. This causes the N-terminal of the peptide helix to reach the polar head region on the *trans* side, causing the helix to span the whole hydrocarbon tail region, about 30–40 Å in thickness for a typical BLM. The dipole moment of the portion or totality of an α-helical peptide spanning the hydrocarbon tail region is estimated at about 70 D, irrespective of the peptide actual length [22]. Hence, the alignment of a peptide molecule along the direction of the electric field is equivalent to the movement of a "gating charge" equal to the ratio of the dipole moment embedded in the hydrocarbon tail region to the thickness of the latter. Once a sufficient number of peptide helices have spanned the BLM, they will tend to aggregate and rotate about their axis, so as to form an ion channel with their hydrophilic side pointing toward the channel lumen. The whole process leading to ion channel formation is depicted in Scheme 1.

The present model assumes that this aggregation proceeds step by step, via a nucleation and growth process. The nucleus, consisting of a number n of monomeric units, has a critical size corresponding to a maximum of Gibbs energy and a higher tendency to shrink than to grow; conversely, peptide clusters greater than the nucleus in size (supercritical clusters) have an irreversible tendency to grow, ultimately yielding an ion channel. The model assumes that the peptide monomeric units exist in any of three possible conformational states: (i) an adsorbed state on the *cis* side of the BLM, parallel to its surface, irrespective of whether the peptide helix is in contact with the *cis* polar head region along its hydrophobic side or is partially embedded in this region; (ii) a non-aggregated transmembrane state spanning the BLM, perpendicular to its surface; (iii) an aggregated transmembrane state. Let us denote by x_0, x_1 and x_2 the mole fractions of the peptide monomeric units adsorbed on the *cis* side of the membrane, those in the transmembrane non-aggregated state and those in the transmembrane aggregated state, respectively. Since only these three states are assumed to exist, the sum $(x_0+x_1+x_2)$ is clearly equal to unity.

In several two-state models available in the literature [10,13,23,24], the probability p of finding one state over the totality of the two states is considered to depend on the transmembrane potential via the well-known one-sided Boltzmann equation. In the case of the present three-state model, the same equation will be applied to express the probability p of finding any of the two transmembrane states, no matter if aggregated or nor, over the totality of the three states:

$$p = \frac{x_1 + x_2}{x_0 + x_1 + x_2} = x_1 + x_2 = \frac{1}{1 + exp[-\Delta m\phi_m/(dkT)]/a}$$
$$\text{with: } a \equiv exp\left(-\frac{\Delta\mu^{\ominus}}{kT}\right); \quad \Delta m/d = q. \tag{2}$$

Here, d is the thickness of the hydrocarbon tail region, Δm is the change in the dipole moment of the peptide in passing from the *cis* polar head region to a transmembrane orientation as a consequence of its alignment along the direction of the electric field, and $\Delta\mu^0$ is the Gibbs energy of the two transmembrane states over that of all three conformational states, in the absence of the electric field (i.e., for $\phi_m = 0$). In view of the previous considerations, the $\Delta m/d$ ratio measures the gating charge q. A positive transmembrane potential ϕ_m moves the positive gating charge q to the *trans* side of the BLM, increasing the mole fraction (x_1+x_2) of the transmembrane dipoles, in accordance with Eq. (2). Since the parameter a is always much less that unity, it practically measures the probability p at zero transmembrane potential.

The mathematical treatment for the kinetics of nucleation and growth provides a solution for the ϕ_m-dependent parameter $S \equiv x_2/(x_1+x_2)$, namely the ratio of the mole fraction of aggregated transmembrane monomers to the sum of the mole fractions of both aggregated and non-aggregated ones. From the definition of S and of the probability p in Eq. (2), it immediately follows that:

$$x_0 = 1 - x_1 - x_2 = 1 - p; \quad x_2 = (x_1 + x_2)S = pS; \quad x_1 = p - x_2 = p(1 - S). \tag{3}$$

Since the current density elicited by a ϕ_m step depends on the number densities of the three conformational states, we must also define the fraction, θ_0, of the unit surface area of the membrane covered by all peptide molecules, irrespective of their state. By so doing, the fractions of the membrane unit surface area covered by the monomers in the *cis* polar head region, by the transmembrane non-aggregated monomers and by the transmembrane aggregated