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The Effect of pH Changes on Individual Permaeabilities in the Ion Channel of Southern Cowpea Mosaic Virus

Reuben D. Budiardja
The Turner Laboratory
Department of Physics
Goshen College
Goshen, IN 46526

Faculty Advisor: Carl S. Helrich

Abstract

A study of the selectivity of the ion channel in Southern Cowpea Mosaic Virus (SCPMV) has been conducted based on measurements of reversal potential variation with acidity. Phospholipid bilayer studies reveal a systematic, irreversible change in the reversal potential of the channel current with pH. In the context of the Goldman-Hodgkin-Katz (GHK) theory this is interpreted in terms of selectivity. An extension of GHK allows the calculation of separate ion permeabilities in addition to the selectivity. Free energy perturbation calculations have also predicted a channel selectivity for potassium ions over Chloride. Analysis of the data in the context of the extended GHK theory yields individual ionic permeabilities consistent with free energy predictions and which are functions of pH. This is not explained in terms of dimensional changes. The IV curves also do not follow the rectification predicted by GHK. This leads to the hypothesis that a more complicated transport mechanisms such as non-constant fields and binding site coupling is involved.

Keywords: Conductivity. Ion Channel. Permeability. Reversal potential. Southern Cowpea Mosaic Virus.

1. Introduction

The experiments reported in this paper are part of a program designed to characterize the Southern Cowpea Mosaic Virus (SCPMV) ion channel activity and to identify its function in SCPMV-infected plants. Southern Bean Mosaic Virus (SBMV) is a member of the sobemovirus group of positive-sense RNA viruses, which has an icosahedral symmetry of triangulation number T=3. This symmetry is determined by the coat protein. The external diameter of SBMV ranges from 285 Å to 328 Å. The structure of SBMV has been determined using X-ray crystallography at 2.8 Å (Abad-Zapatero *et al.*, 1980) and refined using 2.9 Å resolution (Silva and Rossman, 1987).

The coat protein of the SBMV has the properties of a natural protein-ion channel, with pentamers as the structure of the coat protein (Silva, *et al.*, 1987). The ion channel is attractive for cations. Free energy perturbation calculations predict that the channel should be selective for Potassium ions over Chloride.

Some of the first reported artificial phospholipid bilayer studies of the SPCMV ion channel were by P.A. Byler (Byler, 1999). Using Byler's approach P.H. Sprunger conducted experiments on the variation of the channel conductivity with pH (Sprunger, 2000). In these experiments the cis chamber of the bilayer apparatus was very carefully titrated between pH 7.4 and as low as pH 3.5. These experiments have consistently indicated a decrease in conductivity with decreasing pH. The conductivity was also found to be independent of membrane potential for the ranges of membrane potential considered. Reversing the titration after a low value of pH was attained, Sprunger also

has shown that the change in channel conductivity is not reversible. On the basis of the irreversibility Sprunger argues that the change in conductivity is the result of a chemical change and not a simple blockage.

To understand the effect of hydrogen ion concentration on channel conductivity will require studies of both macroscopic diffusive conduction and a statistical mechanical treatment (Roux, 1999), which would include considerations of ion solvation and binding. This paper reports the first step in those studies. In this paper the electrochemical diffusion problem is considered in an extension of the Goldman-Hodgkin-Katz (GHK) theory to the case in which there is both a nonlinear electrical field variation in the channel as well as a spatially dependent diffusivity. If binding of hydrogen ions is the source of the decrease in conductivity this will show up as changes in electrical fields and diffusivities. This is, however, only the first stage of the study and is still macroscopic. This approach will not yield the details of changes in the Gibbs Function.

The paper is organized as follows. In the first section Sprunger's data are discussed in terms of the changes in reversal potential that can be identified experimentally and the basic consequences one can draw from the GHK theory regarding the selectivities based on these data. In the succeeding section the GHK theory is extended to include both variation in internal electrical fields and diffusivities for the individual ions. It is shown that similar equations result. From these equations and experimental measurements of the conductivity at the reversal potential individual (general) permeabilities can be computed. Sprunger's data are then used to compute individual permeabilities. From these permeabilities, and the irreversibility Sprunger reports, it is argued that it is likely that the conductivity decrease results from a hydrogen ion binding that affects the passage of solvated ions through the channel.

2. Experimental Background

In macroscopic terms the flow of ions through a channel is governed by diffusion driven by a gradient in the ionic concentration and the electric field acting on the ionic charge. In general the diffusivities and the electric field in the channel will depend on position in the channel. These dependencies will be determined by the coat protein forming the channel. If one neglects spatial variations in the diffusivities and the electric field the equations for ionic flux in the channel can be integrated to give the Goldman-Hodgkin-Katz equation, given here, for the membrane current due to species a.

$$I_a = \frac{EF^2}{RT\left[1-\exp\left(-EF/RT\right)\right]}\left\{P_a\left[a\right]_i - P_a\left[a\right]_o \exp\left(\frac{EF}{RT}\right)\right\} \tag{1}$$

where the subscripts i and o refer to two sides of the membrane. Here E is the transmembrane potential, P_a is the permeability of the species a , R is the universal gas constant, T is the thermodynamic temperature and F is the Faraday constant. At a particular transmembrane potential the current across the membrane will vanish. The membrane potential resulting for a vanishing current is the reversal potential V_{rev}, which can be obtained from eq (1). It is clear from the form of eq (1) that the reversal potential will depend upon the ionic concentrations and the permeabilities. From an experimental determination of the reversal potential, then, the ratio of permeabilities, which is the selectivity, can be calculated.

A careful analysis of Sprunger's data has revealed that there is a change in the reversal potential with pH. An example of this is shown in Figure 1.

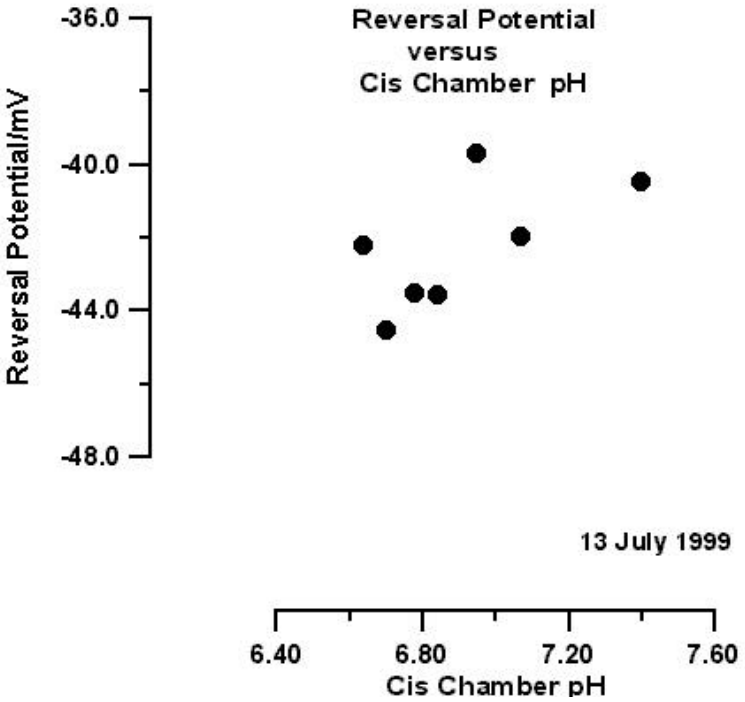

Figure 1. Dependence of reversal potential on cis chamber pH.
In the context of the GHK theory this indicates that the ion selectivity also depends on pH. That is a decrease in the pH asymmetrically affects the ionic species.

Using eq (1) the selectivity can then be obtained from these data for the situation in which the transmembrane field and the diffusivities are constants. In what follows the theory will be extended to encompass spatial dependence in both the field and diffusivity.

3. Theoretical Development of Permeabilities

The Nernst-Planck equation expresses the channel current attributable to the ionic species α under conditions of electrochemical diffusion (Hille, 1992)

$$I_a = -z_a F D_a \left(\frac{dr_a}{dx} + \frac{F z_a r_a}{RT} \frac{d\Psi}{dx} \right) \qquad (2)$$

where, z_α is the ionic charge, D_α is the Diffusivity and ρ_α is the concentration for the species α. Ψ is the electrostatic potential in the channel. Other terms are as in eq (1). Eq (2) may be integrated to yield

$$I_a = -z_a F b_a^* \frac{\left\{ [a]_C \exp\left[\frac{z_a FV}{RT}\right] - [a]_T \right\}}{\int_{x=0}^{x=L} \frac{1}{D_a} \exp\left[\frac{z_a F\Psi}{RT}\right] dx} \tag{3}$$

where the subscripts C and T designate cis and trans and V is the transmembrane potential. In the presence of the two charge carriers potassium, K , and chloride, Cl, the total ionic current is

$$I = \sum_a I_a = -\frac{\left\{ [K]_C - [K]_T \exp\left[-\frac{FV}{RT}\right] \right\}}{\Phi_K \exp\left[-\frac{FV}{RT}\right]} + \frac{\left\{ [Cl]_C \exp\left[-\frac{FV}{RT}\right] - [Cl]_T \right\}}{\Phi_{Cl}} \tag{4}$$

Where

$$\Phi_a = \frac{\int_{x=0}^{x=L} \frac{1}{D_a} \exp\left[\frac{z_a F\Psi}{RT}\right] dx}{F b_a^*} \tag{5}$$

And b_a^* is the water-membrane partition coefficient for the species alpha (Hille, 1992, 339). For variable diffusivities and fields, a general permeability for the a^{th} species may be defined in terms of F_a as

$$P_a = \left(\frac{RT}{FV}\right) \frac{\left[1 - \exp\left(-\frac{FV}{RT}\right)\right]}{F\Phi_a \exp\left[-\frac{FV}{RT}\right]} \tag{6}$$

This definition links that permeability to the transmembrane potential and, through F_a , to the variation in potential and diffusivity along the channel. The ion current, eq (4), then becomes

$$I = \frac{F^2 V}{RT\left[1 - \exp\left(-\frac{FV}{RT}\right)\right]} \left(a \exp\left[-\frac{FV}{RT}\right] - b \right) \tag{7}$$

Where

$$\begin{aligned} a &= \left(P_{Cl}[Cl]_C + P_K[K]_T\right) \\ b &= \left(P_{Cl}[Cl]_T + P_K[K]_C\right) \end{aligned} \tag{8}$$

The reversal potential then has the same general form as that for the GHK theory, provided one remembers the new definition of permeability. That is

$$V_{rev} = \frac{RT}{F} \ln \frac{a}{b} \tag{9}$$

The conductivity, s, $\partial I/\partial V$, is

$$s = \frac{\partial}{\partial V} \frac{F^2 V}{RT\left(1 - \exp\left(-\frac{FV}{RT}\right)\right)}\left(a \exp\left(-\frac{FV}{RT}\right) - b\right) \tag{10}$$

which is a function of the transmembrane potential. At the reversal potential, however, s has a very simple form.

$$s_{rev} = \ln \frac{a}{b} \frac{F a b}{(-a + b)}\left(\frac{F}{RT}\right) \tag{11}$$

Solving this and eq (9) for α and β,

$$a = \left(\frac{RT}{F}\right)\left(\frac{s_{rev}}{F}\right)\frac{\left(1 - \exp\left(\frac{FV_{rev}}{RT}\right)\right)}{\left(\frac{FV_{rev}}{RT}\right)} \tag{12}$$

$$b = \left(\frac{RT}{F}\right)\left(\frac{s_{rev}}{F}\right)\frac{\left(\exp\left(-\frac{FV_{rev}}{RT}\right) - 1\right)}{\left(\frac{FV_{rev}}{RT}\right)} \tag{13}$$

With these the general permeabilities are

$$P_{Cl} = \frac{1}{[Cl]_T} \frac{l a - b}{l^2 - 1} \tag{14}$$

$$P_K = \frac{1}{[K]_T} \frac{l b - a}{l^2 - 1} \tag{15}$$

where $l = [K]_C/[K]_T$. These expressions for the general permeabilities may be evaluated from the experimental data at the reversal potential. The results will include, implicitly, spatial dependence of the diffusivities and electrostatic potential inside the channel.

Equations (14) and (15) can form the basis for a study of details of the electrochemical diffusion within the channel. Here they are used only in the determination of potassium and Chloride ion permeabilities from Sprunger's data.

4. Experimental Results for Permeabilities

Using the data plotted in Figure 1 above and those presented by Sprunger (Sprunger, 2000, Figures 2 and 3), equations (14) and (15) yield the permeabilities as functions of pH. The results are plotted in Figure 2 below.

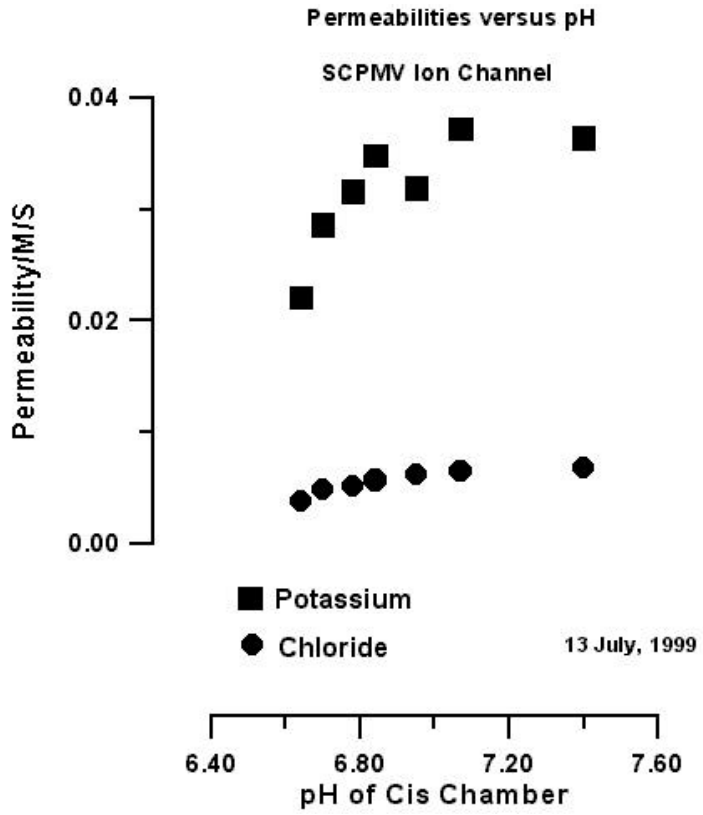

Figure 2 Permeabilities of potassium and chloride ions in the SCPMV ion channel as functions of pH

5. Discussion

Figure 2 is representative of the results of this study. The fact that the permeabilities decrease with decreasing pH reflects the experimental results reported by Sprunger. Figure 2 does, however, show that the permeabilities are affected differently.

It is particularly clear that this dependence of permeabilities on pH cannot be a result of a simple change in channel diameter affected by a swelling or shrinking of the coat protein alone. A change in pore diameter would affect the net flux of ions, but would not affect them selectively.

Because all possible variations in the electrostatic field and diffusivities within the channel have been allowed in the derivation, Figure 2 is a general result dependent only on the assumption that electrochemical diffusion of non-interacting ions determines the ionic current. The electric field within the channel affects each of the ions in the same fashion. The forces on the ions are only in the opposite directions. The contributions to the current from each ionic species depends on the mobility of the charge in the surrounding mileu and the charge on the

ion, which is the same in magnitude for the ions potassium and chloride. Therefore, one must conclude that the difference in permeabilities is a consequence of the differences in diffusivity and not ionic charge.

Experiments based on the extension of the GHK theory presented here could be pursued by attempting to selectively alter one of the diffusivities in the channel. This could be done by site-directed mutagenesis of the coat protein. Such a program would require a more complete understanding of the dependencies of the individual ionic diffusivities than is presently at hand.

The data, of course, already reveal a fundamental difficulty with any confident application of the GHK theory. The IV curves do not exhibit the membrane potential dependence predicted by the GHK theory (Sprunger, 2000, Figure 2). In the present case this does not of itself indicate a failure of the electrochemical diffusion theory. The viral capsid is symmetric along the five-fold axis and ions must cross an identical pore in two directions. This does, however, indicate that an extension of the present theory should be undertaken if an understanding is to be found based on electrochemical diffusion.

Electrochemical diffusion, however, does not exhaust the possibilities for the physics. The passage of ions among binding sites along the channel protein, in this case the viral coat protein, represents an entirely different approach (Laüger, 1987). This has recently been pursued in investigations of the vertebrate potassium ion channel (Roux, 1999). There the discussions are thermodynamically based, which is not the case in the theory presented here, since the only concentration and electrostatic potential gradients are considered.

It is believed that consideration of transport among binding sites will be fruitful in studies of SCPMV. This is based specifically on the finding of Sprunger that the effect of hydrogen ion concentration on the channel is irreversible. It is difficult to understand irreversibility except in the context of chemical bonding. The bonding of hydrogen to part of the coat protein would affect the environment of partially hydrated ions in the channel, which is the basis of transfer among binding sites. Therefore, the results of the present paper, while perhaps exhaustive of the possibilities in electrochemical diffusion theory, are only a first step in the understanding of the SCPMV channel dynamics.

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