Resolving Linear and Non-linear Interactive Kinetic Mechanisms for Ions in Membrane Channels

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Abstract. Thallous ion in gramicidin channels displays the anomalous mole fraction effect and other behavior that suggests its permeation mechanism might be more complicated than the mechanisms for sodium or potassium ion permeation. The permeation is modeled by either multistate first order kinetics where the number of states and the rate constants are modified to fit the data or an ion displacement mechanism that requires higher order rate terms. Although the two classes of mechanism are difficult to distinguish using current-voltage data, the two classes give different responses to a modulated transmembrane potential with frequency comparable to the rate constants for intrachannel ion transitions. The multistate first order kinetics give currents only at the modulation frequency. Information is transmitted in the phase and amplitude of the observed current. The non-linear ion displacement mechanism produces harmonic frequencies. A detailed spectral analysis then distinguishes the two classes of mechanism and provides a range of frequency and phase data that permits determination of the appropriate rate constants.

Key words: Interactions, Non-linear kinetics, Channels, Kinetic Ising models, Multistate kinetic models, Harmonic frequencies

1. Introduction

The accurate determination of reaction order in chemical kinetics is the gateway to the proper molecular mechanism for the reaction. An accurate data fit need not establish a unique mechanism, however, since short-lived transient states can be obscured by the slower rate determining step that matches the reaction time scale. The problem is particularly difficult for the kinetics of ions in channels where the data are the transmembrane currents observed on application of a potential. The currents are predicted from a series of ion transitions between sites within the channel. The kinetic model and transition rate constants are selected to give the best data fit and the steady state approximation is often used to simplify the kinetic analysis. Since the number of fitting parameters is large relative to the experimental data, these data can often be fitted accurately. However, this does not prove that the mechanism is correct or that a second set of kinetic parameters for the mechanism describe the data as accurately.

Thallous ions in gramicidin channels in a lipid bilayer membrane apparently permeate by a more complicated mechanism than that for sodium or potassium ions in these same channels. The ions produce linear current-voltage plots over

an extended potential range suggesting more efficient ion entry or permeation [1]. However, thallous ion at low mole fraction acts to reduce the net current through the channels [2]. This anomalous mole fraction effect is observed for $X_{Tl} < 0.3$ and low transmembrane potentials.

We have observed an increase in conductance above a threshold potential with low X_{Tl} . Such behavior might be modeled with either a multistate first order model or a higher order ion-repulsion model where an incoming ion displaces ions within the channel to eject an ion from the opposite end of the channel. Both models have a number of kinetic parameters available for accurate data fit making them difficult to distinguish using the currents produced by an applied constant transmembrane potential. The two mechanisms are resolved, however, by their response to a modulated potential with frequency comparable to the rate constants for the transitions.

The ion displacement model assumes a rate of displacement proportional to the probabilities that there are ions in neighboring sites. The models are examples of the more general Glauber kinetic Ising models [3] where a species has transition rates that depend on the states of its neighbors. The average of these species/neighbor rates produces rate equations with non-linear, i.e. non first order, rate terms.

The non-linear equations are generated directly using joint probabilities for the states of the species and its neighbors [4, 5]. Such equations give a consistent explanation for the Cole–Moore shift [6, 7], the parallel shift of the rising phase of voltage-clamped potassium currents in squid giant axon following hyperpolarizing preconditioning. The data could not be fitted with first order equations [8] that postulated independent transitions for the four gating molecules of the channels [9].

Ion-ion interactions within the channels are best observed on a time scale comparable with the times of the transitions. Ion inputs must occur with a modulation frequency of approximately 10 MHz. Although low membrane impedance at these frequencies makes electrical modulation input difficult, ion input at these frequencies might be done ultrasonically. The theoretical predictions for currents generated by multistep first order and non-linear ion interaction kinetics models are designed to differentiate the two classes of model.

2. Multistate Linear Kinetics

(a) *Single State Driven Channel*

A sinusoidal ion injection component induces a sinusoidal current response at the same frequency for a model with linear rate terms. A channel with a single ion binding site and irreversible transition kinetics,

generates a current

$$i(t) = eNk_1f_1, \quad (2)$$

where f_1 is the fraction of N channels that have an ion at the binding site. The injection rate includes a constant ion injection rate plus a sinusoidal component that is maximized with a peak to peak amplitude equal to the constant injection rate,

$$j(t) = \frac{j_0(1 + \sin(\omega t))}{2}, \quad (3)$$

so that the ion injection rate is positive at all times.

The irreversible transition of the ion from the binding site to Bath 2 is proportional to the fraction of populated channels,

$$\frac{df_1}{dt} = \frac{j_0[1 + \sin(\omega t)]}{2} - k_1f_1 \quad (4)$$

f_1 evolves to a sinusoidal steady state,

$$\begin{aligned} i^{ss} &= eNk_1f_1^{ss} \\ &= \frac{eNj_0}{2} + eNk_1j_0 \frac{k_1 \sin(\omega t) - \omega \cos(\omega t)}{k_1^2 + \omega^2}, \end{aligned} \quad (5)$$

that is equivalent to a phase shifted sinusoid,

$$i^{ss}(t) = \frac{eNk_1j_0}{\sqrt{k_1^2 + \omega^2}} \sin(\omega t - \phi). \quad (6)$$

The phase delay determines the rate constant as

$$\phi = \tan^{-1} \left(\frac{\omega}{k_1} \right). \quad (7)$$

If reversible transitions to Bath 1 with rate constant k_{-1} are included, the current and phase delay are

$$i^{ss}(t) = \frac{eNk_1j_0}{2} + \frac{eNk_1j_0}{\sqrt{k^2 + \omega^2}} \sin(\omega t - \phi) \quad (8)$$

and

$$\phi = \tan^{-1} \left(\frac{\omega}{k} \right), \quad (9)$$

respectively where

$$k = k_1 + k_{-1}. \quad (10)$$

(b) *Multistate Linear Systems*

A channel with two or more ion binding sites requires rate equations for the fractional populations, $f_i(t)$, of each site. A two site channel with single ion occupancy has a population vector,

$$|f(t)\rangle = \begin{pmatrix} f_1 \\ f_2 \end{pmatrix}. \quad (11)$$

For ion injection only to site 1, the two state ion injection vector is

$$|j\rangle = \begin{pmatrix} j(t) \\ 0 \end{pmatrix}. \quad (12)$$

Irreversible forward transitions for the two state model,

give the rate constant matrix

$$\mathbf{K} = \begin{pmatrix} -k_1 & 0 \\ k_1 & k_2 \end{pmatrix}. \quad (14)$$

The two site model is compatible with the general matrix rate equation for linear kinetics,

$$\frac{d|f(t)\rangle}{dt} = \mathbf{K}|f(t)\rangle + |j(t)\rangle. \quad (15)$$

The formal steady state solution for the population vector,

$$\begin{aligned} |f(t)\rangle &= \mathbf{K}^{-1} \left| \frac{j_0}{2} \right\rangle + (\mathbf{K}^2 + \omega^2 \mathbf{I})^{-1} \\ &\quad \times \left[\mathbf{K} \sin(\omega t) \left| \frac{j_0}{2} \right\rangle - \omega \mathbf{I} \cos(\omega t) \left| \frac{j_0}{2} \right\rangle \right] \end{aligned} \quad (16)$$

is converted to current by applying a summation vector,

$$\langle k| = eN(0, \dots, 0, k_N). \quad (17)$$

The general steady state current

$$\begin{aligned} i^{ss}(t) &= \langle k|f^{ss}(t)\rangle, \\ &= Ne \langle k|(\mathbf{K}^2 + \omega^2 \mathbf{I})^{-(1/2)} \mathbf{K} \sin(\omega t)|j\rangle \\ &\quad - Ne \langle k|(\mathbf{K}^2 + \omega^2 \mathbf{I})^{-(1/2)} \omega \mathbf{I} \cos(\omega t)|j\rangle, \end{aligned} \quad (18)$$

has a phase shift

$$\tan(\phi) = \frac{\langle k | (\mathbf{K}^2 + \omega^2 \mathbf{I})^{-(1/2)} \omega | j \rangle}{\langle k | (\mathbf{K}^2 + \omega^2 \mathbf{I})^{-(1/2)} \mathbf{K} | j \rangle} \quad (19)$$

that can be decomposed into its eigenvalues, λ_i , and projection matrices, $\mathbf{Z}_i$,

$$\tan(\phi) = \frac{\sum (\lambda_i^2 + \omega^2)^{-(1/2)} \omega \langle k | \mathbf{Z}_i | j \rangle}{\sum (\lambda_i^2 + \omega^2)^{-(1/2)} \lambda_i \langle k | \mathbf{Z}_i | j \rangle}. \quad (20)$$

For multistate linear systems, the tangent of the phase shift is the ratio of two quadratic forms.

(c) *Irreversible Permeation*

Although the general expression requires evaluation of eigenvalues and projection matrices for the rate constant matrix, the current for irreversible permeation through the channel is solved sequentially. The initial binding site fractional population

$$\frac{df_1}{dt} = j(t) - k_1 f_1 \quad (21)$$

is solved for f_1 which is then substituted into the rate expression for f_2

$$\frac{df_2}{dt} = k_1 f_1 - k_2 f_2. \quad (22)$$

The rate for binding site i is

$$\frac{df_2}{dt} = k_{i-1} f_{i-1} - k_i f_i. \quad (23)$$

Transitions from the final (i th) state to Bath 2 give

$$i^{ss}(t) = \frac{Ne j_0 k}{(\sqrt{k^2 + \omega^2})^i} \sin(\omega t - \sum (\phi_i), \quad (24)$$

with

$$\phi_i = \tan^{-1} \left(\frac{k}{\omega} \right). \quad (25)$$

Each new state delays the current with respect to the injection phase. The current amplitude decreases as

$$\frac{1}{2^i}, \quad (26)$$

Figure 1. Sinusoidal renormalized currents with phase shifts for multisite channels with independent irreversible transitions involving (a) 2, (b) 3, and (c) 4 intrachannel binding sites. $k_i = \omega$ for $i = 2$ (a), 3 (b) and 4 (c). No ion-ion interactions.

when $k = \omega$. The sinusoidal component effectively injects surges of ions into the channels. The decreased current amplitude with increased intrachannel binding sites reflects the lower probability that an ion traverses the binding sites following the ion injection.

A Runge Kutta fourth order integration of the equations corroborates the analytical predictions when $k = \omega$. The currents of Figure 1 for 2, 3 and 4 site channels are normalized by multiplication by $(k^2 + \omega^2)^{i/2}$ for each case. The phase shifts of 90° , 135° and 180° for 2, 3 and 4 site channels, respectively, are consistent with the expression for irreversible transitions

$$\phi = \sum \phi_i = i \tan^{-1} \left(\frac{\omega}{k} \right). \quad (27)$$

(d) The Two Site Ion Displacement Model

A two site channel follows first order kinetics for single occupancy channels. Non-linear kinetic terms arise in three ways:

- (1) A doubly occupied channel destabilizes the binding of either ion to increase its exit rate.
- (2) An incoming ion displaces an ion from site 1 to site 2 while occupying site 1.
- (3) An incoming ion displaces ions from states 1 and 2 of a doubly occupied channel to produce a net ejection of one ion to Bath 2.

These mechanisms supplement the single ion transitions of the multistate linear model. The two state linear kinetic scheme

$$\frac{df_1}{dt} = j(t) - k_1 f_1, \quad \frac{df_2}{dt} = k_1 f_1 - k_2 f_2 \quad (28)$$

is augmented by the following rates:

- (1) The population of either site is decreased by a rate proportional to the joint probability that both sites are occupied,

$$k_{12}f_1f_2. \quad (29)$$

Either ion might be ejected and the rate is negative in each equation. Currents are defined by ions that actually reach Bath 2 so these two opposing rates do not cancel.

- (2) An ion, driven from Bath 1 by the sinusoidal field displaces an ion at site 1 to site 2 while occupying site 1. The rate, j_1 , is proportional to the injection input and the probability that site 1 is occupied

$$j_1(t) = j_1 \sin(\omega t) f_1. \quad (30)$$

The term does not affect the rate of change of f_1 since an ion replaces the ion at site 1. The rate of f_2 is affected since the displaced ion appears at site 2 as a rate gain.

- (3) The input j_2 displaces the ions in a doubly occupied channel with a rate that depends on j_2 , f_1 and f_2 ,

$$j_2(t) = j_2 f_1 f_2. \quad (31)$$

The populations of sites 1 and 2 do not change during the displacement but an ion is displaced to Bath 2 to contribute to the observed current.

The modified rate equations are

$$\begin{aligned} \frac{df_1}{dt} &= j(t) - k_1 f_1 - k_{12} f_1 f_2 \\ \frac{df_2}{dt} &= k_1 f_1 - k_{12} f_1 f_2 - k_2 f_2 + j_1 f_1 \end{aligned} \quad (32)$$

and the net current is

$$i = eN[k_2 f_2 + k_{12} f_1 f_2 + j_2 f_1 f_2]. \quad (33)$$

The equations reduce to the linear equations for a two site channel as the rate constants for the non-linear rate terms go to zero. With $k_{12} = 2k_1$ and $j_1 = j_2 = 0$, the effects of the non-linear term are imperceptible on either a current versus time or a current versus ion input plot (Figure 2). However, the linear current, generated on Runge Kutta integration with $k_{12} = 0$, is subtracted to expose the currents generated by the non-linear rates with a ‘figure 8’ shape indicating frequency doubling (Figure 2). A fast Fourier transform of the current data also reveals the presence of this harmonic component.

Figure 2. Input/output current plots with irreversible first order transitions for channels having 2, 3 and 4 intrachannel binding sites. (a) horizontal ellipse; (c) minimal b/a .

Figure 3. The small first harmonic current (inner curve) obtained by subtracting the current generated by the linear rate terms from the total observed current (outer loop). $k_1 = k_2 = \omega$, $k_{12} = 2k_1$, $j_0 = 0.8$. Ion repulsion input terms, $j_1 = j_2 = 0$.

Relative enhancement of the non-linear contribution by increasing the non-linear rate constant ($k_{12} = 0.25k_1$, $0.5k_1$ and k_1) increases the magnitude of the harmonic currents (Figure 3). The increase in total current amplitude with the non-linear rate is consistent with the current increase for Tl^+ ions in gramicidin channels at higher potentials.

The magnitude of k_{12} is determined by the magnitude of the frequency doubled currents. These might be extracted directly with a fast Fourier analysis of the observed currents.

Figure 4. Input-output plots of difference currents obtained by subtracting currents from the linear rate terms from the total current generated with a Runge Kutta integration. The first harmonic dominates in all cases with current amplitude increasing with increasing interaction rate constant. $k_1 = k_2 = \omega$, $j_0 = 0.08$, $j_1 = j_2 = 0$; increasing amplitude as $k_{12} = 0.25k_1$, $0.5k_1$ and k_1 .

The interactive driving inputs enhance the frequency doubled amplitude. j_1 and j_2 , increased as $j_1/j_0 = j_2/j_0 = 0.6, 1.2$ and 1.8 respectively (Figure 4) produce both increased current amplitude and a net increase in the average current indicating the enhanced ion injection with these rate terms.

3. Discussion

Modulated ion injection at frequencies comparable to the rate constants for ion transitions within the channel enhances the possibility of establishing the ion transport mechanism. A harmonic analysis provides a clear experimental distinction between mechanisms characterized as multistate linear or multistate with some non-linear (interacting ion) rates. The amplitudes of currents at each frequency provide a measure of the ratios of non-linear and linear rate constants for the system.

Sinusoidal ion injection increases the number of experimental parameters available to determine the kinetic parameters for each model. The amplitude of the ion injection rate is increased by increasing the maximal applied potential and could be used to generate current-voltage plots like those produced by a sequence of constant applied potentials. However, the steady state current amplitude and phase are observable over a range of frequencies and all these data are used to extract a self consistent set of rate constants once the class of mechanism is determined.

A multistate linear system yields populations,

$$|f(t)\rangle = \sum e^{-\lambda_i t} \mathbf{Z}_i |f^0\rangle \quad (34)$$

with λ_i and $\mathbf{Z}_i$ determined by the full set of rate constants. These eigenvalues and eigenvectors appear in both the phase and amplitude of the modulated current. For a first order system, the rate constants determined from these eigenvalues are frequency independent.

Interaction energies are often used to modify the activation energies for the rate constants in a first order multistate system. This alters the rate constants but not the fundamental mechanism that requires independent transitions for each of the ions. Alteration of the activation energies for the first order transitions cannot produce the non-linear rate terms that would be revealed as harmonic frequencies.

With first order kinetic systems, all the rate constants define the eigenvalues and projection matrices even if the states associated with those rate constants are not populated. By contrast, non-linear rate terms approach zero if the population of neighbors approaches zero. The non-linear model contains the linear model as a special limit when conditions favor channel occupancy by single ions. The model evolves smoothly from a linear, independent ion model to a non-linear ion-ion displacement model and can reflect the change in conductance observed for Tl^+ ions at low mole fractions with increasing potential.

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