The gating charge should not be estimated by fitting a two-state model to a *Q-V* curve

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The voltage dependence of charges in voltage-sensitive proteins, typically displayed as charge versus voltage (*Q-V*) curves, is often quantified by fitting it to a simple two-state Boltzmann function. This procedure overlooks the fact that the fitted parameters, including the total charge, may be incorrect if the charge is moving in multiple steps. We present here the derivation of a general formulation for *Q-V* curves from multistate sequential models, including the case of infinite number of states. We demonstrate that the commonly used method to estimate the charge per molecule using a simple Boltzmann fit is not only inadequate, but in most cases, it underestimates the moving charge times the fraction of the field.

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Many ion channels, transporters, enzymes, receptors, and pumps are voltage dependent. This voltage dependence is the result of voltage-induced translocation of intrinsic charges that, in some way, affects the conformation of the molecule. The movement of such charges is manifested as a current that can be recorded under voltage clamp. The best-known examples of these currents are "gating" currents in voltage-gated channels and "sensing" currents in voltage-sensitive phosphatases. The time integral of the gating or sensing current as a function of voltage (V) is the displaced charge Q(V), normally called the *Q-V* curve.

It is important to estimate how much is the total amount of net charge per molecule (Q_{max}) that relocates within the electric field because it determines whether a small or a large change in voltage is necessary to affect the function of the protein. Most importantly, knowing Q_{max} is critical if one wishes to correlate charge movement with structural changes in the protein. The charge is the time integral of the current, and it corresponds to the product of the actual moving charge times the fraction of the field it traverses. Therefore, correlating charge movement with structure requires knowledge of where the charged groups are located and the electric field profile. In recent papers by Chowdhury and Chanda (2012) and Sigg (2013), it was demonstrated that the total energy of activating the voltage sensor is equal to $Q_{max}\ V_M$, where V_M is the median voltage of charge transfer, a value that is only equal to the half-point of activation $V_{1/2}$ for symmetrical *Q-V* curves. V_M is easily estimated from the *Q-V* curve, but Q_{max} must be obtained with other methods because, as we will show here, it is not directly derived from the *Q-V* curve in the general case.

The typical methods used to estimate charge per molecule Q_{max} include measurements of limiting slope (Almers, 1978) and the ratio of total charge divided by the number of molecules (Schoppa et al., 1992). The discussion on implementation, accuracy, and reliability of these methodologies has been addressed many times in the literature, and it will not be discussed here (see Sigg and Bezanilla, 1997). However, it is worth mentioning that these approaches tend to be technically demanding, thus driving researchers to seek alternative avenues toward estimating the total charge per molecule. Particularly, we will discuss here the use of a two-state Boltzmann distribution for this purpose. Our intention is to demonstrate that this commonly used method to estimate the charge per molecule is generally incorrect and likely to give a lower bound of the moving charge times the fraction of the field.

The two-state Boltzmann distribution describes a charged particle that can only be in one of two positions or states that we could call S_1 and S_2. When the particle with charge Q_{max} (in units of electronic charge) moves from S_1 to S_2, or vice versa, it does it in a single step. The average charge found in position S_2, $Q(V)$, will depend on the energy difference between S_1 and S_2, and the charge of the particle. The equation that describes $Q(V)$ is:

$$Q(V) = \frac{Q_{\max}}{1 + \exp\left[\frac{-Q_{\max}(V - V_{1/2})}{kT}\right]}, \quad (1)$$

where $V_{1/2}$ is the potential at which the charge is equally distributed between S_1 and S_2, and k and T are the Boltzmann constant and absolute temperature, respectively. The $Q(V)$ is typically normalized by dividing Eq. 1 by the total charge Q_{max}. The resulting function is frequently

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called a "single Boltzmann" in the literature and is used to fit normalized, experimentally obtained *Q-V* curves. The fit yields an apparent $V_{1/2}$ ($\tilde{V}_{1/2}$) and an apparent Q_{MAX} ($\bar{Q}_{max}$), and this last value is then attributed to be the total charge moving Q_{max}. Indeed, this is correct but only for the case of a charge moving between two positions in a single step. However, the value of $\bar{Q}_{max}$ thus obtained does not represent the charge per molecule for the more general (and frequent) case when the charge moves in more than one step.

To demonstrate the above statement and also estimate the possible error in using the fitted $\bar{Q}_{max}$ from Eq. 1, let us consider the case when the gating charge moves in a series of n steps between $n + 1$ states, each step with a fractional charge z_i (in units of electronic charge e_0) that will add up to the total charge Q_{max}.

$$S_1 \xleftrightarrow{\mu_1} S_2 \xleftrightarrow{\mu_2} \cdots S_i \xleftrightarrow{\mu_i} S_{i+1} \cdots S_n \xleftrightarrow{\mu_n} S_{n+1}$$

The probability of being in each of the states S_i is labeled as P_i, and the equilibrium constant of each step is given by

$$\mu_i = \exp\left[\frac{z_i(V - V_i)}{kT}\right],\ i = 1 \ldots n,$$

where z_i is the charge (in units of e_0) of step i, and V_i is the membrane potential that makes the equilibrium constant equal 1. In steady state, the solution of P_i can be obtained by combining

$$\frac{P_{i+1}}{P_i} = \mu_i,\ i = 1 \ldots n \text{ and } \sum_{i=1}^{i=n+1} P_i = 1,$$

giving

$$P_{i+1} = \frac{\prod_{m=1}^{i} \mu_m}{1 + \sum_{j=1}^{n} \prod_{k=1}^{j} \mu_k},\ i = 1 \ldots n \text{ and } P_1 = \frac{1}{1 + \sum_{j=1}^{n} \prod_{k=1}^{j} \mu_k}.$$

We define the reaction coordinate along the moved charged q as

$$q_i = \sum_{j=1}^{i} z_j,\ i = 1 \ldots n.$$

The *Q-V* curve is defined as

$$Q(V) = \sum_{i=1}^{n} q_i P_{i+1}.$$

Then, replacing P_i yields

$$Q(V) = \frac{\sum_{i=1}^{n}\left[\sum_{j=1}^{i} z_j\right]\left[\prod_{m=1}^{i} \mu_m\right]}{1 + \sum_{j=1}^{n} \prod_{k=1}^{j} \mu_k},$$

or written explicitly as a function of V:

$$Q(V) = \frac{\sum_{i=1}^{n}\left[\sum_{j=1}^{i} z_j\right]\left[\prod_{m=1}^{i} \exp\left[\frac{z_m(V - V_m)}{kT}\right]\right]}{1 + \sum_{j=1}^{n} \prod_{k=1}^{j} \exp\left[\frac{z_k(V - V_k)}{kT}\right]}. \quad (2)$$

Eq. 2 is a general solution of a sequential model with $n + 1$ states with arbitrary valences and V_i's for each transition. We can easily see that Eq. 2 has a very different form than Eq. 1, except when there is only a single transition ($n = 1$). In this latter case, Eq. 2 reduces to Eq. 1 because z_1 and V_1 are equal to Q_{max} and $V_{1/2}$, respectively. For the more general situation where $n > 1$, if one fits the Q(V) relation obeying Eq. 2 with Eq. 1, the fitted $\bar{Q}_{max}$ value will not correspond to the sum of the z_i values (see examples below and Fig. 1). A simple way to visualize the discrepancy between the predicted value of Eqs. 1 and 2 is to compute the maximum slope of the *Q-V* curve. This can be done analytically assuming that $V_i = V_o$ for all transitions and that the total charge Q_{max} is evenly divided among those transitions. The limit of the first derivative of the $Q(V)$ with respect to V evaluated at $V = V_o$ is given by this equation:

$$\left.\frac{dQ(V)}{dV}\right|_{V=V_0} = \frac{Q_{max}(n+2)}{12nkT}. \quad (3)$$

From Eq. 3, it can be seen that the slope of the *Q-V* curve decreases with the number of transitions being maximum and equal to $Q_{max}/(4kT)$ when $n = 1$ (two states) and a minimum equal to $Q_{max}/(12kT)$ when n goes to infinity, which is the continuous case (see next paragraph).

Infinite number of steps

Eq. 2 can be generalized to the case where the charge moves continuously, corresponding to an infinite number of steps. If we make

$$z_i = Q_{max}/n,\ i = 1 \ldots n, \qquad V_i = V_o,\ i = 1 \ldots n,$$

then all $\mu_i = \mu$, and we can write Eq. 2 as the normalized Q(V) in the limit when n goes to infinity:

$$\begin{aligned} Q_{nor}(V) &= \lim_{n\to\infty} \frac{\sum_{i=1}^{n}\left[\sum_{j=1}^{i} \frac{Q_{max}}{n}\right]\prod_{m=1}^{i} \exp\left[\frac{Q_{max}(V - V_o)}{nkT}\right]}{Q_{max}\left[1 + \sum_{i=1}^{n}\prod_{j=1}^{i} \exp\left[\frac{Q_{max}(V - V_o)}{nkT}\right]\right]} \\ &= \frac{\left[Q_{max}(V - V_o) - kT\right]\exp\left[\frac{Q_{max}(V - V_o)}{kT}\right] + kT}{Q_{max}(V - V_o)\left[\exp\left[\frac{Q_{max}(V - V_o)}{kT}\right] - 1\right]}. \end{aligned} \quad (4)$$

Eq. 4 can also be written as

$$Q_{nor}(V) = \frac{1}{2}\left[1 + \coth\left[\frac{Q_{max}(V - V_o)}{2kT}\right] - \frac{2kT}{Q_{max}(V - V_0)}\right], \quad (5)$$

which is of the same form of the classical equation of paramagnetism (see Kittel, 2005).

Examples

We will illustrate now that data generated by Eq. 2 can be fitted quite well by Eq. 1, thus leading to an incorrect estimate of the total charge moved. Typically, the experimental value of the charge plotted is normalized to its maximum because there is no knowledge of the absolute amount of charge per molecule and the number of molecules. The normalized *Q-V* curve, Q_{nor}, is obtained by dividing $Q(\mathrm{V})$ by the sum of all the partial charges.

Fig. 1 shows Q_{nor} computed using Eq. 2 for one, two, three, four, and six transitions and for the continuous case using Eq. 5 (squares) with superimposed fits to a two-state Boltzmann distribution (Eq. 1, lines). The computations were done with equal charge in each step (for a total charge $Q_{max} = 4e_0$) and also the same $V_i = -25$ mV value for all the steps. It is clear that fits are quite acceptable for cases up to four transitions, but the fit significantly deviates in the continuous case.

Considering that experimental data normally have significant scatter, it is then quite likely that the experimenter will accept the single-transition fit even for cases where there are six or more transitions (see Fig. 1). In general, the case up to four transitions will look as a very good fit, and the fitted $\bar{Q}_{max}$ value may be inaccurately taken and the total charge transported might be underestimated. To illustrate how bad the estimate can be for these cases, we have included as insets the fitted value of $\bar{Q}_{max}$ for the cases presented in Fig. 1. It is clear that the estimated value can be as low as a fourth of the real total charge. The estimated value of $\tilde{V}_{1/2}$ is very close to the correct value for all cases, but we have only considered cases in which all V_i's are the same.

It should be noted that if μ_i of the rightmost transition is heavily biased to the last state (V_i is very negative), then the $\bar{Q}_{max}$ estimated by fitting a two-state model is much closer to the total gating charge. In a three-state model, it can be shown that the fitted value is exact when $V_1 \to \infty$ and $V_2 \to -\infty$ because in that case, it converts into a two-state model. Although these values of V are unrealistic, the fitted value of $\bar{Q}_{max}$ can be very close to the total charge when V_2 is much more negative than V_1 (that is, $V_1 >> V_2$). On the other hand, If $V_1 << V_2$, the *Q-V* curve will exhibit a plateau region and, as the difference between V_1 and V_2 decreases, the plateau becomes less obvious and the curve looks monotonic. These cases have been discussed in detail for the two-transition model in Lacroix et al. (2012).

We conclude that it is not possible to estimate unequivocally the gating charge per sensor from a "single-Boltzmann" fit to a *Q-V* curve of a charge moving in multiple transitions. The estimated $\bar{Q}_{max}$ value will be a low estimate of the gating charge Q_{max}, except in the case of the two-state model or the case of a heavily biased late

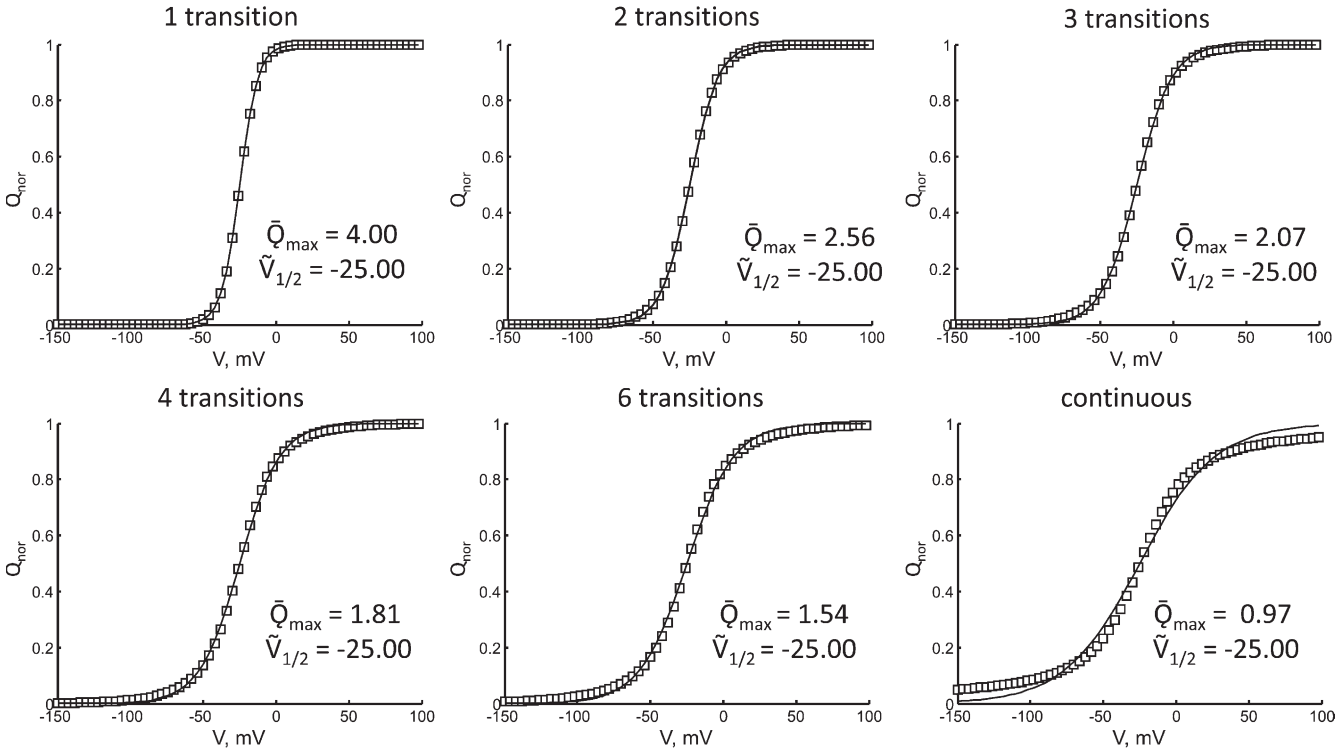

Figure 1. Examples of normalized *Q-V* curves for a $Q_{max} = 4$ computed with Eq. 2 for the cases of one, two, three, four, and six transitions and the continuous case using Eq. 5 (squares). All the *Q-V* curves were fitted with Eq. 1 (lines). The insets show the fitted valence ($\bar{Q}_{max}$) and half-point ($\tilde{V}_{1/2}$).

step, which are rare occurrences. It is then safer to call "apparent gating charge" the fitted $\bar{Q}_{max}$ value of the single-Boltzmann fit.

Addendum

The most general case in which transitions between states include loops, branches, and steps can be derived directly from the partition function and follows the general thermodynamic treatment by Sigg and Bezanilla (1997), Chowdhury and Chanda (2012), and Sigg (2013). The reaction coordinate is the charge moving in the general case where it evolves from $q = 0$ to $q = Q_{max}$ by means of steps, loops, or branches. In that case, the partition function is given by

$$Z = \sum_i \exp\left(\frac{q_i(V - V_i)}{kT}\right). \quad (6)$$

We can compute the mean gating charge, also called the *Q-V* curve, as

$$Q(V) = \langle q \rangle = kT\frac{Z'}{Z} = kT\frac{d\ln Z}{dV} = \frac{\sum_i q_i \exp\left(\frac{q_i(V - V_i)}{kT}\right)}{\sum_i \exp\left(\frac{q_i(V - V_i)}{kT}\right)}. \quad (7)$$

The slope of the *Q-V* is obtained by taking the derivative of $\langle q \rangle$ with respect to *V*:

$$\frac{dQ(V)}{dV} = (kT)^2\frac{d^2\ln Z}{dV^2}. \quad (8)$$

Let us now consider the gating charge fluctuation. The charge fluctuation will depend on the number of possible conformations of the charge and is expected to be a maximum when there are only two possible charged states to dwell. As the number of intermediate states increases, the charge fluctuation decreases. Now, a measure of the charge fluctuation is given by the variance of the gating charge, which can be computed from the partition function as:

$$\left\langle \Delta q^2 \right\rangle = \left\langle q^2 \right\rangle - \langle q \rangle^2 = (kT)^2\left(\frac{Z''}{Z} - \left(\frac{Z'}{Z}\right)^2\right) = (kT)^2\frac{d^2\ln Z}{dV^2}. \quad (9)$$

But the variance (Eq. 9) is identical to the slope of *Q(V)* (Eq. 8). This implies that the slope of the *Q-V* is maximum when there are only two states.

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