Hill Coefficients of a Polymodal Monod-Wyman-Changeux Model for Ion Channel Gating

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ABSTRACT Allosteric transitions of ion channels can be driven by multiple sources of free energies. One class of model for describing such transitions is the multistimulus Monod-Wyman-Changeux model, in which each stimulus interacts with a specific sensor on the protein and activation of the sensor is allosterically coupled to conformational changes of the protein. In general, when a protein is stressed by multiple stimuli, one stimulus can influence the response to another, which can result in both a shift of the midpoint of the dose-response curve and a change of the slope of the curve. Here I show that, for a Monod-Wyman-Changeux model with independent sensors, the different dose-response curves of open probability for one stimulus have the same slope at the same agonist concentration. In the other words, the slope of the dose-response curve for one stimulus is an intrinsic property of the sensors for that stimulus; it is independent of other stimuli or their sensor properties. As the dose-response curve for many receptors can be fit to a Boltzmann or Hill equation, this property provides a practical, usable test for applicability of such models.

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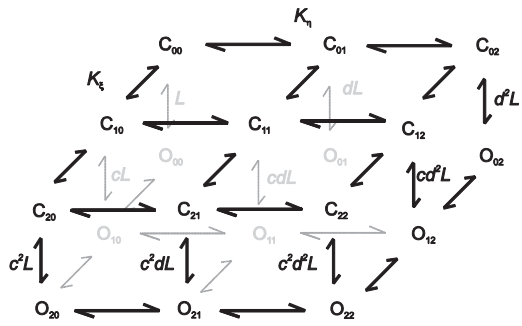
Multistimulus Monod-Wyman-Changeux (MWC) models are useful for describing activation of allosteric proteins by multiple stimuli. In the context of ion channels, for example, they have been applied successfully to explain the gating of BK channels by Ca^{2+} and voltage (1–3). More recently, they have also been exploited for modeling polymodal responses of TRP channels (4,5), which respond to a diverse range of stimuli such as temperature, chemicals, and voltage. A common observation in the analysis of experimental data with these models is that one stimulus influences the response to another and this appears mainly as a shift in the midpoint of the dose-response curve while leaving the slope unaffected (e.g., (1,4,5)). If this observation holds true generally, it would provide a simple yet useful criterion in practice for discerning the applicability and adequacy of such models. This note presents a theoretical analysis of the observation for a strict MWC model (e.g., a homomeric channel with independent subunits each containing single or multiple but independent sensors). The work was prompted by reviewers' questions about applicability of the MWC models to TRPV1 channels (6).



Scheme I

Without loss of generality, consider a MWC model driven by two independent stimuli (e.g., Scheme 1). This model predicts an open probability of (1)

$$P_o = \frac{1}{1 + \frac{1}{L} \cdot \xi^n \eta^n}, \tag{1}$$

where n is the number of subunits of the channel, L is the intrinsic equilibrium constant for the opening of the channel in the absence of stimuli, and ξ and η are functions of the equilibrium constants of the sensors (K_ξ and K_η) between resting and activated states and the allosteric coupling factors (c and d) of these sensors, i.e.,

$$\xi = \frac{1 + K_\xi}{1 + c \cdot K_\xi}, \tag{2}$$

and

$$\eta = \frac{1 + K_\eta}{1 + d \cdot K_\eta}. \tag{3}$$

For BK channels, K_ξ would be the equilibrium constant for Ca^{2+} binding, K_η is the equilibrium constant of a voltage sensor between resting and activated states, and c and d represent the respective increases of the opening equilibrium constant when a single Ca^{2+} ion is bound or a single voltage sensor is activated.

According to Eq. 1, when one stimulus (say η, or voltage) is held constant, the dose-response curve for the other stimulus (ξ, or Ca^{2+}) follows

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$$Y \equiv \frac{P - P_{\min}}{P_{\max} - P_{\min}}, \tag{4}$$

where $P_{\min} = 1/(1+\eta^n/L)$ and $P_{\max} = 1/(1+\eta^n/c^nL)$ are, respectively, the minimal and maximal responses to the stimulus under consideration. The equation normalizes Y to values between 0 and 1.

The slope of the curve, i.e., a generalized Hill coefficient for an agonist (7,8), is defined as

$$n_H \equiv \frac{\partial \log \frac{Y}{1-Y}}{\partial \log S}, \tag{5}$$

where S is the concentration of the agonist. Similar definitions exist for other stimuli such as voltage or temperature by substituting log$S$ with the corresponding gating variable. The gating curve from a MWC model is not exactly sigmoidal (7–9), and the Hill coefficient as defined above is not a constant but varies with the agonist concentration.

For a strict MWC model (i.e., Eq. 1), the generalized Hill coefficient can be represented by (see section S1 in the Supporting Material)

$$n_H = n \cdot \frac{1 - \frac{1}{c^n}}{1 - \frac{1}{c}} \cdot \frac{1 - \xi}{1 - \xi^n} \cdot \frac{1 - \frac{1}{c\xi}}{1 - \frac{1}{c^n \xi^n}}, \tag{6}$$

where ξ is as defined in Eq. 3.

The equation implies that:

1. The slope of the gating curve for stimulus ξ is only dependent on the number of subunits and the properties of the corresponding stimulus sensors (i.e., the equilibrium constant K_ξ and the allosteric coupling factor c). It is independent of the other stimulus (η) or its sensor properties (K_η and d). Nor is it dependent on L, the intrinsic equilibrium constant of the channel.
2. $n_H < n$ (section S2 in the Supporting Material).
3. $n_H > 1$ if $c > 1$ (section S3 in the Supporting Material).
4. $n_H = 1$ at $S = 0$ or ∞ (section S4 in the Supporting Material).

Fig. 1 shows examples of the open probability (P), Y/(1-Y) and the generalized Hill coefficient n_H versus the agonist concentration at several voltages. The simulation was based on a MWC model proposed for BK channels (3). As expected, the plots of Y/(1-Y) are not exactly linear, indicating that the dose-response curves are only approximately fit to a Hill equation. But importantly, while the Y/(1-Y) plots appear differently at different voltages, the n_H plots are exactly overlapping (Fig. 1 *C*), indicating that the different dose-response curves maintain the same slope at the same agonist concentration. Fig. 1 *C* also shows that the n_H plots are bell-shaped and reach an asymptotic value of unity at either $S = 0$ or ∞, which is consistent with the theory. Fig.1 *D* shows similar plots for a nonindependent MWC model (3). In this case, although the plots remain bell-shaped and have asymptonic values of unity, they become non-overlapping when voltage is varied. As further illustrated in section S6 in the Supporting Material, a variable slope was observed over a broad

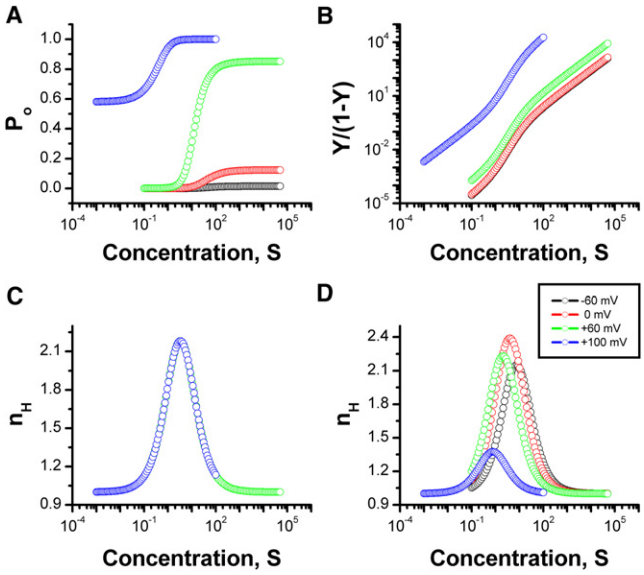

FIGURE 1 Dose-responses and Hill coefficients of a MWC model. (*A*) Open probability versus agonist concentration at indicated voltages. (*B*) *Y*/(1-*Y*) versus concentration. (*C*) Hill coefficient as a function of agonist concentration. (*D*) Hill coefficient for a nonindependent MWC model. Legends for all panels are the same as shown in panel *D*. The models for simulation were from Horrigan and Aldrich (3): Fit *C* of their Table II (*E* = 1, i.e., no coupling between voltage sensors and Ca^{2+} binding domains) for panels *A–C* and Fit *A* (*E* = 31) for panel *D*.

range of values for the coupling strength. Thus, the coincidence of the n_H curves indicates whether the sensors for different stimuli are independent of each other. On the other hand, it is noted that non-MWC models can also produce independent slopes (section S7 in the Supporting Material), indicating that the property of independent slopes is not a sufficient condition for an MWC model.

For a dose-response curve approximately fit to a Hill equation, the slope of the fit is a weighted average across all the concentration. For an MWC model, it can be further demonstrated that the slope for one stimulus is independent of the values of the other stimulus or its sensor properties (K_η and d). For example, consider a least-squares fit of a normalized dose-response curve Y by a Hill equation $Y = 1/[1+(EC_{50}/S)^{nH}]$. As the Hill equation can be rewritten as

$$\log \frac{Y}{1-Y} = n_H \log S - n_H \log EC_{50}, \tag{7}$$

the fitting is equivalent to a linear regression by minimizing the least-squares error,

$$\varepsilon = \sum \left[\log \frac{Y}{1-Y} - n_H \log S - X \right]^2, \tag{8}$$

where n_H and X are the variables, and X is related to EC_{50} by $X = -n_H \log EC_{50}$. Thus, once n_H and X are known, EC_{50} can be determined accordingly. The solutions to Eq. 8 can be obtained analytically as

$$n_H = \frac{\sum \log \frac{Y}{1-Y} \log S - \frac{1}{N}\sum \log \frac{Y}{1-Y} \sum \log S}{\sum \log^2 S - \frac{1}{N}\left[\sum \log S\right]^2}, \quad (9)$$

$$X = \frac{1}{N}\left[\sum \log \frac{Y}{1-Y} - n_H \sum \log S\right], \quad (10)$$

where all summations are over the number of concentrations (total N). By Eqs. 4 and 5, it follows that

$$\frac{Y}{1-Y} = \frac{1 + \frac{1}{L}\cdot\frac{\eta^n}{c^n}}{1 + \frac{1}{L}\cdot\eta^n} \cdot \frac{1-\xi^n}{\xi^n - \frac{1}{c^n}}, \quad (11)$$

which reduces the fitted Hill coefficient in Eq. 9 to

$$n_H = \frac{\sum \log \frac{1-\xi^n}{\xi^n - \frac{1}{c^n}} \log S - \frac{1}{N}\sum \log \frac{1-\xi^n}{\xi^n - \frac{1}{c^n}} \sum \log S}{\sum \log^2 S - \frac{1}{N}\left[\sum \log S\right]^2}. \quad (12)$$

The equation says that the fitted n_H is fully determined by (in this case) the agonist binding properties (i.e., n, K_ξ and c) and independent of the other stimulus or its sensor properties (K_η, d).

For some channels such as BK channels, voltage affects the concerted transitions in addition to the voltage sensors. In such cases, the slope of the conductance-voltage (G-V) curve remains independent of the other stimulus or its sensor properties. The slope, which is related to the total gating charge, can be obtained by generalizing Eq. 4 to include the contribution from the concerted transitions (see section S5 in the Supporting Material), leading to

$$n_H = \frac{F}{RT}\cdot\frac{1 - \frac{L_{\min}}{L_{\max}d^n}}{\left(1 - \frac{L_{\min}\eta^n}{L}\right)\left(1 - \frac{L}{L_{\max}d^n\eta^n}\right)} \cdot \left(nz_J \cdot \frac{(1-\eta)\left(1 - \frac{1}{d\eta}\right)}{1 - \frac{1}{d}} + z_L\right). \quad (13)$$

where z_J is the charge of a single voltage sensor, z_L is the charge associated with concerted transitions, $L_{\min}$ and $L_{\max}$ are the minimal and maximal values of L across voltage, and RT and F have their standard definitions. In the special case where L can be represented by

$$L = L_0 \exp(z_L FV/RT),$$

$L_{\min} = 0$ and $L_{\max} = \infty$, which reduces Eq. 13 to

$$n_H = \frac{F}{RT}\cdot\left(nz_J \cdot \frac{(1-\eta)\left(1 - \frac{1}{d\eta}\right)}{1 - \frac{1}{d}} + z_L\right). \quad (14)$$

Both Eqs. 13 and 14 indicate that the slope is determined only by the properties of the voltage sensors and the concerted transitions.

In summary, a MWC model with independent stimulus sensors predicts a slope for the dose-response curve of one stimulus to be independent of the other stimulus. The other stimulus shifts the midpoint of the curve. Thus, the slope value for a specific stimulus represents a generic property of the sensors for that stimulus. Although our definition of n_H involves the normalized open probability Y (Eq. 4), the latter may be experimentally replaced with the normalized dose-response curve, so that no explicit knowledge of $P_{\min}$ and $P_{\max}$ is required. For temperature as a gating variable, it has broad effects on gating; however, when the effects are dominated by specific temperature sensors as in thermal TRP channels, the conclusions may remain applicable. Because the dose-response curve for many receptors can be adequately fit in practice to a sigmoidal function over a wide range of conditions, the property can serve as a basic and useful test for applicability of such models.

SUPPORTING MATERIAL

Twenty-one equations, one figure, and one scheme are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00618-1.

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