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SINGLE CHANNEL GATING EVENTS IN TRACER FLUX EXPERIMENTS

I. THEORY

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Tracer ion flux measurements are a commonly used method for studying ion transport through membranes of cellular systems, where the rate of ion flow is determined by gating processes which control the opening and closing of transmembrane channels. Due to recent advances in the theoretical analysis of tracer flux from or into closed membrane structures (CMS), the mechanism of gating reactions can, in principle, be derived from flux data. A physically well founded analysis is presented for the dependence of the total tracer ion content of a collection of CMS on the gating processes. For functionally uncoupled gating units a mean single channel flux contribution $\langle e^{-kt} \rangle = \int_0^t e^{-kt} p(\tau,t)\, d\tau$ can be defined, where k is the intrinsic single channel flux coefficient, t the time over which flux is measured, and $p(\tau,t)$ is the probability that a given channel was open for a total period τ during t. This quantity reflects the mean time course of the tracer content due to flux through a single channel. Expressions for $\langle e^{-kt} \rangle$ are derived that explicitly take into account a distribution in the lifetime of open channels. On the basis of the results, kinetic and thermodynamic parameters of multiphasic gating reactions can be determined from the time course of the overall tracer content in a collection of CMS.

1. Introduction

Gated ion transport through cell membranes is a fundamental process in many vital cell functions. In excitable membranes, molecular gating events controlling ion flows have been extensively studied on the basis of tracer ion flux from or into closed membrane structures (CMS) [1–8]. Examples of such CMS are sealed membrane fragments (microsacs [1]), reconstituted vesicular structures, and cells.

Among the instructive results of a recently developed tracer flux analysis applied to the acetylcholine receptor-mediated ion flow is that the ion-transporting conformation of this gating system is a relatively short-lived metastable state, which converts practically completely to nonconducting desensitized states in the presence of neuroactivator molecules [4].

Due to recent technical refinement [9–12], flux processes can now be investigated in the millisecond time range. As a method for quantitatively studying the kinetics of gating processes, the technique is rapidly becoming a viable alternative to the measurement of electrical properties [13–16].

The feasibility of the method rests on the fact that reaction events controlling opening and closing of transmembrane channels modulate ion flux. A general scheme of analysis for determining the time course of gating processes from tracer flux data has been presented [17,18]. It was shown that several physicochemical factors must be explicitly considered before it is possible to extract information about reaction events of single channel gating.

The signal actually determined in tracer flux experiments (e.g., counts per minute, fluorescence intensity) is a direct measure of the total number of tracer ions $X(t)$ inside a collection of CMS at time t. This overall signal is thus a sum of contributions from individual CMS, so that $X(t) = \Sigma_i x_i(t)$, where $x_i(t)$ is the number of tracers ions in the ith CMS.

The time dependence of the tracer content $x(t)$ of a single CMS is determined by several factors

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[18]: diffusion and transient binding of the ions to channel sites, transport through channels, and coupling of tracer flux to flux of other ionic species. In addition, at low tracer ion concentrations, it is necessary to consider explicitly the stochastic nature of the flux process [17,19]. A general mathematical description, accounting for the dependence of flux on all these factors, valid for all possible experimental conditions, would be quite complex. In practice, analysis of flux data can be carried out only if the dependence of $x(t)$ on time is simple. It is particularly important to avoid the complex time dependence that can arise from a coupling of tracer transmembrane transport, and tracer diffusion in the CMS external, or CMS internal medium. Fortunately, several physical factors connected with the experimental conditions underlying tracer flux are accessible to accurate control [18]. It is therefore possible to choose experimental constraints for which a simple exponential dependence of $x(t)$ on time results. Experiments can be carried out under efflux conditions (flux of tracer from CMS into a large bath initially containing no tracer), or under influx conditions (flux of tracer from a large bath into CMS initially containing no tracer). The expressions for the tracer content of a single CMS will then be

$$x(t) = \begin{cases} x(0)e^{-t/\tau_f} & \text{efflux} \quad (1.1) \\ x(\infty)(1-e^{-t/\tau_f}) & \text{influx} \quad (1.2) \end{cases}$$

where τ_f is the time constant for the flux process, and the flux amplitudes $x(0)$ and $x(\infty)$ refer to $t = 0$ and to $t \to \infty$, respectively. Two limiting conditions exist, for which eqs. (1.1) and (1.2) hold:

(1) Transmembrane transport of tracer is a diffusion-controlled process, and the CMS has a uniform high surface density of open channels. Under these constraints the entire CMS surface will constitute a 'sink' for tracer ions. For spherical CMS, flux can then be described in terms of the usual mathematical treatment employed for diffusion-controlled reactions [20]. After an initial transient period of short duration, eqs. (1.1) and (1.2) will hold. The reciprocal flux time constant will equal the rate constant for reactions in the diffusion controlled limit, i.e.

$$\frac{1}{\tau_f} = 4\pi r D \quad (1.3)$$

where r is the radius of the CMS, and D is the diffusion constant.

(2) Transmembrane transport of tracer ions is a stationary flux. The essential features of the flux process can be described by the reaction scheme of minimum complexity [18]:

$$N + R \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} NR \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} N^*R = N^* + R \quad (1.4)$$

where R denotes the unoccupied channel site, N the tracer ions inside the CMS, NR the tracer ions bound to the internal channel sites, and the quantities with asterisks are the corresponding species in the exterior of the CMS. k_t is the rate constant, for a single channel, of tracer transport from the interior to the exterior of the CMS, and k_{-t} is the corresponding rate constant for transport from the exterior to the interior; k_b and k_{-b} are, respectively, the rate constants for binding to and dissociation from the channel sites.

The reciprocal flux time constant in eqs. (1.1) and (1.2) can be expressed as

$$\frac{1}{\tau_f} = mk \quad (1.5)$$

where m is the number of open channels of the CMS, and k the intrinsic flux rate constant of a channel. If the volume of the CMS is very much smaller than the volume of the bath, efflux is essentially a unidirectional process, and influx is an 'equilibration' process. As shown in Appendix C, for steady-state conditions,

$$k = \frac{k_b \cdot k_t}{vN_A(k_b \cdot K' + k_t)} \quad (1.6)$$

where N_A is Avogadro's number, v the internal volume of a CMS, and K' the apparent dissociation constant for tracer binding to channel sites; $K' = k_{-b}/k_b$. Note that influx was previously approximated by a unidirectional flux [18]; this led to separate expressions of k for efflux and influx. For the experimentally more realistic condition of equilibration with a large external bath, eq. (1.6) is valid for both efflux and influx. The dependence

of k on v will be explicitly treated in section 4.

Eq. (1.3) for $1/\tau_f$ under diffusion-controlled conditions does not depend on the number of open channels. Therefore, it cannot be used to obtain information about gating processes. The simple relationships, eqs. (1.1) and (1.2), can be used to study channel gating only when transport is much slower than diffusion.

Both efflux and influx conditions have been used in tracer flux experiments. In principle, they yield the same information about the gating process. However, the requirement that equilibration of ion concentrations by diffusion be rapid is more readily met in the interior of a CMS than in a large bath. In practice, efflux is therefore more suitable for quantitative studies than influx.

In previous theoretical treatments of flux [17,18], the overall tracer content $X(t)$ was represented as a sum $X(t)=\Sigma_n X_n(t)$ of contributions $X_n(t)=\Sigma_i x_i^{(n)}(t)$ from CMS having different total numbers n of functionally intact channels. All CMS of a subpopulation with n channels were modelled by a single representative CMS having a mean number $\bar{m}(t)=n\alpha(t)$ of its channels open, where $\alpha(t)$ is the total fraction of open channels at time t. Integration of the kinetic equation for flux $d[N]/dt=-\bar{m}(t)k[N]$ led to the introduction of an integrated rate coefficient $\kappa(t)$ given by

$$\kappa(t)=k\int_0^t \alpha(t')\,dt' \tag{1.7}$$

where t' is a dummy variable of integration. The mean overall tracer content $\bar{X}(t)$ was found to be

$$\bar{X}(t)=\begin{cases}\sum_n \bar{X}_n(0)e^{-n\kappa(t)} & \text{efflux} \quad (1.8)\\ \sum_n \bar{X}_n(\infty)[1-e^{-n\kappa(t)}] & \text{influx.} \quad (1.9)\end{cases}$$

The mean flux amplitude contributions $\bar{X}_n(0)$ and $\bar{X}_n(\infty)$ can be expressed in terms of a probability P_n, as $\bar{X}_n(0)=\bar{X}(0)P_n$ and $\bar{X}_n(\infty)=\bar{X}(\infty)P_n$. Methods for experimentally determining P_n have been presented [17,18]. Taking into account the dependence of the measured overall tracer content $X(t)$ on P_n, implicit in eqs. (1.8) and (1.9), then permits determination of $\kappa(t)$. From eq. (1.7) it is further possible to determine the fraction of open channels $\alpha(t)$ at time t, which reflects the time course of the overall gating process.

In this article a more fundamental treatment will be presented. A physically more realistic averaging technique will be adopted, to replace the simplifying assumption of a single representative CMS. It will be shown that an experimentally determinable quantity, the mean single channel contribution to the flux amplitude $\langle e^{-kt}\rangle$, can be expressed in terms of the effective kinetic constants for the gating process. The derivation explicitly takes into account a distribution in the lifetimes of open and closed states of a channel. The treatment therefore entails similar fundamental assumptions to those underlying the analysis of electrical current fluctuations [21].

2. The gating process and ion transport

2.1. Definition of gating

No consistent usage of the terms gating, and channel opening and closing has evolved in the literature. In this article the term gating system will denote that portion of an ion transmembrane translocation apparatus that can undergo dynamic changes in state. The term channel will denote that portion through which ions are passively transported across a membrane. To each state G_ν of the gating system there corresponds an intrinsic ion transport rate constant k_ν (in units of ions per unit time) of the associated channel. This will be strictly true only if there is a rate-limiting step in the channel transport of ions, so that coupled transport modes due to multiple activation barriers to transport in the channel interior [22] need not be considered. A consistent interpretation of current fluctuation data has been found possible [21] under the assumption that channels can be either fully open or fully closed. This would imply that k_ν can assume the two values

$$k_\nu=\begin{cases}k & \text{channel open}\\ 0 & \text{channel closed}\end{cases} \tag{2.1}$$

where k is the intrinsic rate constant for the ion transport through a fully open channel. Transi-

tions in state of the gating system are thought to influence only the overall rate constants of opening and of closing of channels; in the following treatment assumption (2.1) will be adopted. In section 4 an extension to more complex cases will be considered. For flux experiments k in eq. (2.1) is given by eq. (1.6).

In the special case of the acetylcholine receptor of fish electric organs, several functionally significant states of the gating system have been identified [23]: a resting state (channel closed), an active state (channel open), an intermediate state (channel closed), and an inactive state (channel closed). Neuroactivator binding to the receptor induces a repopulation among these states, resulting in a transiently varying overall change in the rates of opening and closing of channels. Such a sequence of reaction events may be termed a gating reaction.

2.2. Distribution of fractionally populated CMS

In a collection of CMS, where the gating units are localized on the surface of the CMS, the overall fraction $\alpha_s(t)$ of gating units in state G_s at time t, neglecting localization on the CMS, is given by $\alpha_s(t) = [G_s]/\Sigma_s[G_s]$, where $[G]_s$ is the 'concentration' of G_s at time t, and the summation is over all possible states. When a set of gating units present in more than one state is localized on a collection of CMS, a mixture of fractionally populated CMS results. A given CMS can be assigned a set of occupation numbers $\{m_s\} = m_1, m_2, m_3, \ldots$, where m_s is the number of gating units on the CMS in state G_s. During the course of a gating reaction there will be a change in the fractional population of the CMS. Assuming that the transitions of state of different gating units, whether or not on the same CMS, are statistically uncorrelated, a multinominal distribution will result. The fraction $\mu^{(n)}(\{m_s\}; t)$ of CMS having a total number of gating units n, and occupation numbers $\{m_s\}$ at time t, will be given by

$$\mu^{(n)}(\{m_s\}; t) = P_n \cdot \frac{n!}{\prod_s m_s!} \cdot \prod_s [\alpha_s(t)]^{m_s}. \qquad (2.2)$$

The mean value of $m_s(t)$ for the entire collection of CMS is

$$\bar{m}_s(t) = \alpha_s(t) \sum_n n P_n, \qquad (2.3)$$

and the variance is

$$\sigma_s^2(t) = \alpha_s(t)[1 - \alpha_s(t)] \sum_n n P_n. \qquad (2.4)$$

According to assumption (2.1) the gating unit states G_s can be divided into states $G_s^{(o)}$, associated with a fully open channel, or states $G_s^{(c)}$, associated with a fully closed channel. The overall fraction $\alpha^{(o)}(t)$ of states for which the channel is open is $\alpha^{(o)}(t) = \Sigma_s \alpha_s^{(o)}(t)$, where $\alpha_s^{(o)}(t) = [G_s^{(o)}]/\Sigma_s[G_s]$ and the summations are over all possible states. The overall fraction $\alpha^{(c)}(t)$ for which the channel is closed is $\alpha^{(c)}(t) = 1 - \alpha^{(o)}(t)$.

From eq. (1.4) it follows that, for flux experiments carried out under the constraints stated in section 1, the rate of tracer flux from or into a CMS is directly proportional to the number of open channels. The fraction $\mu_m^{(n)}(t)$ of CMS having a total of n gating units, of which m are in an open channel state, is given by the binomial distribution

$$\mu_m^{(n)}(t) = P_n \cdot \binom{n}{m} \cdot [\alpha^{(o)}(t)]^m \cdot [1 - \alpha^{(o)}(t)]^{n-m} \qquad (2.5)$$

The mean number $\bar{m}^{(o)}(t)$ of open channels per CMS is given by

$$\bar{m}^{(o)}(t) = \alpha^{(o)}(t) \cdot \sum_n n P_n \qquad (2.6)$$

and the variance in the number of open channels by

$$\sigma^2(t) = \alpha^{(o)}(t) \cdot [1 - \alpha^{(o)}(t)] \cdot \sum_n n P_n. \qquad (2.7)$$

A detailed derivation of these results has been presented elsewhere [17]. Eqs. (2.5)–(2.7) are now seen to be a special case of eqs. (2.2)–(2.4).

2.3. Flux and the mechanism of gating

Qualitatively, the effect of the gating reaction on flux may be expressed by eqs. (1.4) and (2.6). The time course of the flux process will be changed by all transitions in state of the gating system kinetically coupled to at least one transition lead-

ing to a change in $\alpha^{(o)}(t)$. In principle, the time dependence of $\alpha^{(o)}(t)$ could be of arbitrary complexity. However, in all physiologically relevant gating reactions so far investigated, the mechanism of gating involves an initial increase, followed by a decrease, in $\alpha^{(o)}(t)$ with time (activation-inactivation sequence).

A particularly well studied case is the gating reaction induced by neuroactivator binding to acetylcholine receptors of fish electric organs [23]. Initially, the gating units are predominantly in the closed channel (resting) states. Ligand binding leads to a large increase in the population of the open channel (active) state. In the absence of acetylcholinesterase activity, this is followed by a slower increase in the population of the closed channel intermediate state. Finally, there is an even slower increase in the population of the thermodynamically most stable state—the closed channel (inactive) state (so-called desensitization).

When the gating reaction occurs in several phases that are well separated on the time scale, a particularly simple analysis of the kinetics of gating is possible. Each phase will then correspond to a distinct reaction mode. Often it is possible to adopt experimental conditions for which such a collection of separate modes results.

For the remainder of this article the following restrictive assumptions will be made about the gating reaction:

(1) There is no interaction between individual channel gating units.

(2) The gating reaction involves a single activation phase, during which $d\alpha^{(o)}/dt > 0$, and one or more inactivation phases, during which $d\alpha^{(o)}/dt < 0$.

(3) Each phase constitutes a separate reaction mode, with a characteristic time constant ('relaxation time').

(4) The activation mode (a) is more rapid than the inactivation modes (i).

(5) The time dependence of the fraction of open channels, $\alpha^{(o)}(t)$ is given by

$$\alpha^{(o)}(t) = \begin{cases} \alpha_a^{(o)}(\infty) + \left[\alpha_a^{(o)}(0) - \alpha_a^{(o)}(\infty)\right] \cdot e^{-t/\tau_a} \\ \qquad\qquad \text{activation} \qquad (2.8) \\ \alpha_i^{(o)}(\infty) + \left[\alpha_i^{(o)}(0) - \alpha_i^{(o)}(\infty)\right] \cdot e^{-t/\tau_i} \\ \qquad\qquad \text{inactivation} \qquad (2.9) \end{cases}$$

where τ_a and τ_i are, respectively, the reaction mode time constants for activation and inactivation. $\alpha_a^{(o)}(\infty)$ is the value of $\alpha^{(o)}(t)$ upon completion of the activation mode, but prior to the onset of inactivation. $\alpha_a^{(o)}(0)$ is the true value of $\alpha_a^{(o)}(t)$ at $t = 0$. $\alpha_i^{(o)}(0)$ is the value of $\alpha^{(o)}(t)$ after completion of all more rapid modes prior to the inactivation mode i. $\alpha_i^{(o)}(\infty)$ is its value after completion of inactivation mode i.

(6) The reciprocal reaction mode time constants are given by

$$\frac{1}{\tau_a} = k_a + k_{-a} \qquad (2.10)$$

$$\frac{1}{\tau_i} = k_i + k_{-i} \qquad (2.11)$$

where k_a and k_{-a} are, respectively, the effective rate constants for the forward and the reverse reaction during the activation phase. k_i and k_{-i} are, respectively, the corresponding forward and reverse rate constants for the inactivation phase i. These rate constants will depend on the rate constants of the rate-limiting step of the respective mode, on the equilibrium constants of more rapid steps, and on ligand concentration, when gating is ligand controlled. When a mode involves a bimolecular step, a pure exponential time course of $\alpha^{(o)}(t)$ will result only under special limiting conditions (e.g., near equilibration of the mode, or for buffering concentrations of one of the reactants in the bimolecular step).

3. Single channel gating events and tracer content of the CMS

3.1. Total period of openness of channels and tracer content

Consider a single CMS with n gating units. During the course of the overall gating reaction each gating unit will pass through a sequence of open channel and closed channel periods. This is shown schematically in fig. 1. The total time, τ_r, for which the rth gating unit was actually in an open state in the time interval from 0 to t is given by

$$\tau_r = \sum_l \Delta\tau_l^{(r)}, \quad 0 \leq \tau_r \leq t \qquad (3.1)$$

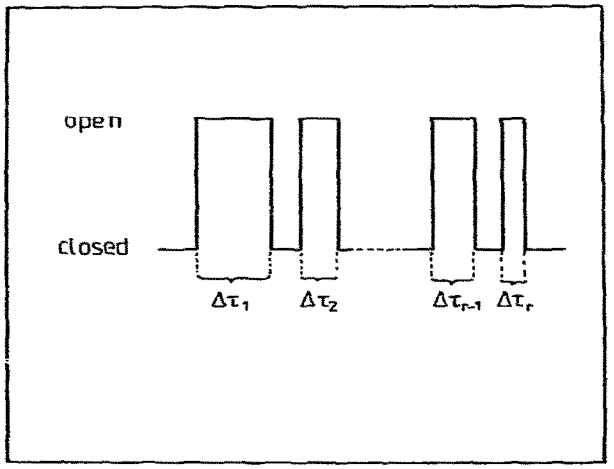

Fig. 1. Schematic representation of successive channel opening and channel closing events of an individual channel. $\Delta\tau_r$ represents the time interval the channel was open during its rth period of opening.

where $\Delta\tau_l^{(r)}$ is the amount of time the rth gating unit was in an open channel state during its lth period of opening. The tracer content $x^{(n)}(t)$ of the CMS at time t is given by eqs. (1.1), (1.2) and (1.4) as

$$x^{(n)}(t) = x^{(n)}(0)\exp\left[-k\cdot\sum_{r=1}^{n}\tau_r\right] \quad \text{efflux} \qquad (3.2)$$

$$x^{(n)}(t) = x^{(n)}(\infty)\left\{1-\exp\left[-k\cdot\sum_{r=1}^{n}\tau_r\right]\right\} \quad \text{influx.} \qquad (3.3)$$

Eqs. (3.2) and (3.3) have important implications. The tracer content at time t is seen to depend only on the sum of the total time during which each of the n gating units was in an open state up to time t. It does not depend on the number of open periods, nor on the overlap of open periods of different gating units.

This suggests that in analogy to eqs. (1.8) and (1.9) the mean overall tracer content $\bar{X}(t)$ of a collection of CMS can be expressed as

$$\bar{X}(t) = \begin{cases} \sum_n \bar{X}_n(0)\cdot\Phi_n(t) & \text{efflux} \quad (3.4) \\ \sum_n \bar{X}_n(\infty)\cdot[1-\Phi_n(t)] & \text{influx} \quad (3.5) \end{cases}$$

with

$$\Phi_n(t) = \sum_{\{\tau_r\}} P_n(\{\tau_r\}) \prod_{r=1}^{n} e^{-k\tau_r} \qquad (3.6)$$

where $P_n(\{\tau_r\})$ is the probability of occurrence of the set of total times $\{\tau_r\} = \tau_1, \tau_2, \ldots \tau_n$ for a CMS with n gating units. For the sake of clarity a summation over all possible sets $\{\tau_r\}$, where $0 \leq \tau_r \leq t$, has been chosen in eq. (3.6); i.e., variation of each τ_r occurs in discrete increments. Actually, $P(\{\tau_r\})$ is a continuously varying function of $\{\tau_r\}$, and should thus be represented by a probability differential.

Note that in contrast to the previously derived eqs. (1.8) and (1.9) for $\bar{X}(t)$, eqs. (3.4) and (3.5) contain a superposition of exponentials. The quantity $\Phi_n(t)$ represents the time course of the change in tracer content of a collection of CMS with n gating units per CMS.

Since the gating units are assumed to be independent, $P_n(\{\tau_r\})$ in eq. (3.6) can be factored to yield

$$\Phi_n(t) = \prod_{r=1}^{n}\left\{\sum_{\tau_r=0}^{t} p(\tau_r)e^{-k\tau_r}\right\} = \langle e^{-kt}\rangle^n \qquad (3.7)$$

with the mean single channel flux contribution $\langle e^{-kt}\rangle$ given by

$$\langle e^{-kt}\rangle = \sum_{\tau=0}^{t} p(\tau)e^{-k\tau} \rightarrow \int_0^t e^{-k\tau}p(\tau,t)\,d\tau \qquad (3.8)$$

where summation has now been replaced by integration. In the integral of eq. (3.8), $p(\tau,t)$ represents a probability density funtion. The probability differential $d\theta = p(\tau,t)\,d\tau$ expresses the probability that a channel was open for a total time τ', where $\tau < \tau' < \tau + d\tau$, in the interval 0 to t.

3.2. Generation and lifetime of open and closed channel states

The constraints expressed by eqs. (2.8)–(2.11) imply that during each phase of the gating reac-

tion the reaction process

$$G^{(c)} \underset{k_c}{\overset{k_o}{\rightleftharpoons}} G^{(o)} \tag{3.9}$$

leads to a change in $\alpha^{(o)}(t)$ and $\alpha^{(c)}(t)$. $G^{(o)}$ and $G^{(c)}$ denote, respectively, all open channel and closed channel states of the gating system that participate in a given reaction mode. The rate constants for net channel opening k_o, and for net channel closing k_c, are given by

$$k_o = k_a \text{ and } k_c = k_{-a} \text{ activation phase} \tag{3.10}$$

$$k_o = k_{-i} \text{ and } k_c = k_i \text{ inactivation phase.} \tag{3.11}$$

For simplicity, general indices u, v and w can be introduced. Each index will denote either an open (o) or a closed (c) channel state, in connection with $\alpha^{(o)}(t)$, $\alpha^{(c)}(t)$, $G^{(o)}$ and $G^{(c)}$, or channel opening (o) or channel closing (c), in connection with k_o and k_c.

The fraction of channels $\alpha^{(u)}(t)$ in state $G^{(u)}$ at time t, given by eqs. (2.8) and (2.9), contains no information about the history of opening and closing of the channels. Each channel that is in a state $G^{(u)}$ at time t was generated in that state at some time t', where $0 \leq t' \leq t$. Subsequently, it remained in that state for an interval $t-t'$. Therefore, an alternative expression for $\alpha^{(u)}(t)$ is

$$\alpha^{(u)}(t) = \alpha^{(u)}(0)\lambda^{(u)}(t) + \int_0^t g^{(u)}(t')\lambda^{(u)}(t-t')\,dt' \tag{3.12}$$

where $g^{(u)}(t')$ is the probability density function for the generation of gating units in state $G^{(u)}$, and $\lambda^{(u)}(t-t')$ is the probability that a gating unit generated in state $G^{(u)}$ at time t' will remain in that state up to time t.

If the reaction event (3.9) is a simple Markov process, an exponential distribution in the lifetime of states $G^{(u)}$ will hold [19]. One then obtains

$$\lambda^{(u)}(t-t') = e^{-k_u(t-t')},\ u \neq v \tag{3.13}$$

where the inequality $u \neq v$ implies that either u denotes o and v denotes c, or alternatlively u denotes c and v denotes o.

$g^{(u)}(t')$ is that fraction of gating units generated in state $G^{(u)}$ in the time period 0 to t which were generated during the interval $t' < t'' < t' + dt'$. From the kinetic equations for reaction process (3.9) one obtains

$$g^{(u)}(t') = k_u \alpha^{(v)}(t'),\ u \neq v. \tag{3.14}$$

3.3. The mean single channel flux contribution during a gating reaction

According to eqs. (2.8) and (2.9), $\alpha^{(u)}(t)$ will vary continously during a given phase of the gating reaction. From eq. (3.14) it follows that $g^{(u)}(t)$ will therefore also vary. $g^{(u)}(t)$ reflects the net rate of generation of gating units in state $G^{(u)}$ at time t. It contains no information about whether a gating unit thus generated was in stage $G^{(u)}$ at any time prior to t. It is possible to express $\alpha^{(u)}(t)$ and $g^{(u)}(t)$ as a sum of component contributions, $\alpha_m^{(u,v)}(t)$ and $g_m^{(u,v)}(t)$, that reflect previous stages of the gating units. One obtains

$$\alpha^{(u)}(t) = \sum_{m=0}^{\infty} \left[\alpha_{2m+1}^{(u,v)}(t) + \alpha_{2m}^{(u,u)}(t)\right],\ u = v. \tag{3.15}$$

Substitution into eq. (3.14) yields

$$g^{(u)}(t) = \sum_{m=0}^{\infty} \left[g_{2m+1}^{(u,v)}(t) + g_{2m}^{(u,u)}(t)\right],\ u = v \tag{3.16}$$

where

$$g_{m-1}^{(u,v)}(t) = k_v \alpha_m^{(u,w)}(t),\ v \neq w. \tag{3.17}$$

In $\alpha_m^{(u,v)}(t)$ and $g_m^{(u,v)}(t)$, the first superscript (u) denotes the state $G^{(u)}$ of the gating unit at time 0, the second superscript (v) the state $G^{(v)}$ at time t. The subscript m denotes the number of times the gating unit underwent a change of state in the time interval from 0 to t. The subscripts $2m$ and $2m+1$ in eqs. (3.15) and (3.16) reflect the fact that an even number of transitions leads to $u = v$, and an odd number of transitions leads to $u \neq v$. Explicit expressions for $\alpha_m^{(u,v)}(t)$, for all possible u, v and m, are derived in Appendix A.

The probability density function $p(\tau,t)$, given by eq. (3.8), can be expanded to yield

$$p(\tau,t) = \sum_{m=0}^{\infty} \left[\alpha_{2m+1}^{(u,v)}(t)\, p_{2m+1}^{(u,v)}(\tau,t) + \alpha_{2m}^{(u,u)}(t)\, p_{2m}^{(u,u)}(\tau,t)\right],\ u \neq v \tag{3.18}$$

where both $p(\tau,t)$ and the component probability density functions $p_m^{(u,v)}(\tau,t)$ are assumed to be normalized over the interval 0 to t. $p_m^{(u,v)}(\tau,t)$ represents the probability that the species of gating unit specified by indices u, v and m was in an open channel state for a period τ, in the interval 0 to t. Substitution of eq. (3.18) into eq. (3.8) yields the expansion

$$\langle e^{-kt}\rangle = \sum_{m=0}^{\infty}\left[\alpha_{2m+1}^{(u,v)}(t)\langle e^{-kt}\rangle_{2m+1}^{(u,v)} + \alpha_{2m}^{(u,u)}(t)\langle e^{-kt}\rangle_{2m}^{(u,u)}\right],\ u \neq v \qquad (3.19)$$

where the component single channel flux contributions are defined as

$$\langle e^{-kt}\rangle_m^{(u,v)} = \int_0^t p_m^{(u,v)}(\tau,t)e^{-k\tau}\,d\tau. \qquad (3.20)$$

Explicit expressions for $p_m^{(u,v)}(\tau,t)$ for all possible u, v and m are derived in Appendix B. Substitution of eqs. (B1), (B2), (B10) and (B11) into eq. (3.20) yields

$$\langle e^{-kt}\rangle_0^{(c,c)} = 1 \qquad (3.21)$$

$$\langle e^{-kt}\rangle_0^{(o,o)} = e^{-kt} \qquad (3.22)$$

$$\langle e^{-kt}\rangle_1^{(o,c)} = \left[\frac{k_c}{k+k_c}\right]\left[\frac{1-e^{-(k+k_c)t}}{1-e^{-k_c t}}\right] \qquad (3.23)$$

$$\langle e^{-kt}\rangle_1^{(c,o)} = \left[\frac{k_o-k_c}{k_o-k_c-k}\right]\left[\frac{e^{-kt}-e^{-(k_o-k_c)t}}{1-e^{-(k_o-k_c)t}}\right] \qquad (3.24)$$

where $\lim_{t\to 0}\langle e^{-kt}\rangle_1^{(o,c)} = \lim_{t\to 0}\langle e^{-kt}\rangle_1^{(c,o)} = 1$. Eq. (3.24) has the special limiting cases

$$\langle e^{-kt}\rangle_1^{(c,o)} = kt\left[\frac{e^{-kt}}{1-e^{-kt}}\right],\ k = k_o - k_c \qquad (3.25)$$

$$\langle e^{-kt}\rangle_1^{(c,o)} = (kt)^{-1}(1-e^{-kt}),\ k_o = k_c. \qquad (3.26)$$

3.4. The equilibrium mean single channel flux contribution

A special limiting case results when nonequilibrium phases of the gating reaction are of negligible duration compared to the period over which flux is measured. When the reaction process (3.9) of a given mode of the gating reaction reaches steady-state or equilibrium, $\alpha^{(o)}(t)$ and $\alpha^{(c)}(t)$ will assume time-independent values. For equilibrium, the relationship $\alpha_{eq}^{(c)} = K\alpha_{eq}^{(o)}$ holds, where $K = k_c/k_o$ is the equilibrium constant. From eq. (3.14) it follows that $g^{(o)}(t)$ and $g^{(c)}(t)$ will then attain the constant equilibrium value $g_{eq}^{(o)} = k_o\alpha_{eq}^{(c)} = k_c\alpha_{eq}^{(o)} = g_{eq}^{(c)}$. Under these conditions channel opening (and closing) will be a purely random process. The probability $\pi_m(t)$, that a given gating unit was generated in an open channel state m times in the interval 0 to t is given by the Poisson distribution

$$\pi_m(t) = e^{-g_{eq}^{(o)}t}\cdot\left(g_{eq}^{(o)}\cdot t\right)^m/m! \qquad (3.27)$$

The equilibrium mean single channel flux contribution $\langle e^{-kt}\rangle_{eq}$ is given by

$$\langle e^{-kt}\rangle_{eq} = \sum_{m=0}^{\infty}\pi_m(t)\left[\langle e^{-kt}\rangle_{so}\right]^m \qquad (3.28)$$

where $\langle e^{-kt}\rangle_{so}$ is the mean contribution during a single opening of the channel. When $t \gg k_c^{-1}$ the limiting value

$$\langle e^{-kt}\rangle_{so} = \lim_{(k_c t)\to\infty}\langle e^{-kt}\rangle_1^{(o,c)} = \left(\frac{k_c}{k+k_c}\right) \qquad (3.29)$$

is obtained from eq. (3.23). Substitution of eq. (3.27) by $g_{eq}^{(o)} = k_o k_c/(k_o+k_c)$, and of eq. (3.29) into eq. (3.28) yields

$$\langle e^{-kt}\rangle_{eq} = e^{-k_{eq}t} \qquad (3.30)$$

where the effective flux rate constant for the equilibrated gating system k_{eq} is given by

$$k_{eq} = k\left(\frac{k_o}{k_o+k_c}\right)\left(\frac{k_c}{k+k_c}\right) \qquad (3.31)$$

4. Discussion

It has been shown that, when gating units controlling channel opening are not functionally coupled, the overall tracer ion content $\bar{X}(t)$ at time t can be rigorously expressed in terms of the mean single channel contribution to flux $\langle e^{-kt}\rangle$. From eqs. (3.4), (3.5) and (3.7) one obtains

$$\bar{X}(t) = \begin{cases} \sum_n \bar{X}_n(0)\langle e^{-kt}\rangle^n & \text{efflux} \quad (4.1) \\ \sum_n \bar{X}_n(\infty)\left[1-\langle e^{-kt}\rangle^n\right] & \text{influx.} \quad (4.2) \end{cases}$$

Previously proposed schemes of analysis [4,17,18] were based on the assumption that the mean number of open channels at a given time determines overall tracer content. In this article a physically more realistic approach was adopted. A distribution in the total time for which a channel is open was explicitly considered. In order to arrive at simple expressions for the mean single channel flux contribution it was necessary to express $\langle e^{-kt}\rangle$ as a weighted sum of component contributions $\langle e^{-kt}\rangle_m^{(u,v)}$ for subspecies of gating unit states. Upon equilibration of the gating reaction $\langle e^{-kt}\rangle$ approaches its equilibrium value $\langle e^{-kt}\rangle_{eq}$.

From eqs. (2.8), (2.9) and (3.15) it is seen that the net change $\Delta\alpha^{(u)}(t)$, in the fraction of gating units in state $G^{(u)}$ up to time t, is given by the expressions

$$\begin{aligned}\Delta\alpha^{(u)}(t) &= \alpha^{(u)}(0) - \alpha^{(u)}(t)\\ &= \alpha^{(u)}(0) - \sum_{r=0}^{\infty}\left[\alpha_{2r}^{(u,u)}(t) + \alpha_{2r+1}^{u,v}(t)\right],\\ &\quad u \neq v \qquad (4.3)\\ &= \Delta\alpha^{(u)}(\infty)\left[1 - e^{-(k_u+k_v)t}\right],\ u \neq v \qquad (4.4)\end{aligned}$$

where $\Delta\alpha^{(u)}(\infty) = \alpha^{(u)}(0) - \alpha^{(u)}(\infty)$.

With increasing m, the maximum value of the component fractions $\alpha_m^{(u,v)}(t)$ decreases in magnitude, and occurs at progressively later times. Truncation of the summation in eq. (4.3) at some value $r = p$, leads to an approximate expression $\Delta\alpha_p^{(u)}(t)$. The error $\epsilon_p(t)$ made in approximating $\Delta\alpha^{(u)}(t)$ by $\Delta\alpha_p^{(u)}(t)$ is given by

$$\epsilon_p(t) = \sum_{r=p+1}^{\infty}\left[\alpha_{2r}^{(u,u)}(t) + \alpha_{2r+1}^{(u,v)}(t)\right] u \neq v. \qquad (4.5)$$

When the reaction process characterized by reaction scheme (3.9) is unidirectional, eq. (4.4) reduces to $\Delta\alpha^{(u)}(\infty) = \Delta\alpha_0^{(u)}(t) = \Delta\alpha^{(u)}(\infty)(1 - e^{-k_v t})$. Therefore, in general, approximation of $\Delta\alpha^{(u)}(t)$ by $\Delta\alpha_0^{(u)}(t)$ leads to an error

$$\begin{aligned}\epsilon_0(t) &= \Delta\alpha^{(u)}(t) - \Delta\alpha_0^{(u)}(t)\\ &= \Delta\alpha^{(u)}(\infty)e^{-k_v t}(1 - e^{-k_u t}),\ u \neq v. \qquad (4.6)\end{aligned}$$

From this result it follows that when $k_v \gg k_u$, a dominant portion of the amplitude change $\Delta\alpha^{(u)}(t)$ will be accurately approximated by $\Delta\alpha_0^{(u)}(t)$. Terms with $r > 0$ in expression (4.3) will constitute a small correction, modifying the time course of $\Delta\alpha_0^{(u)}(t)$ during the later stages of the gating reaction.

This suggests that, when $k_v > k_u$, the expansions for $\alpha^{(u)}(t)$, $g^{(u)}(t)$, $p^{(u)}(\tau,t)$ and $\langle e^{-kt}\rangle$ given by eqs. (3.15), (3.16), (3.18) and (3.19) can be approximated by the terms corresponding to $m = 0$, 1. For $\langle e^{-kt}\rangle$ one obtains

$$\begin{aligned}\langle e^{-kt}\rangle \approx{}& \alpha_0^{(o,o)}(t)\langle e^{-kt}\rangle_0^{(o,o)} + \alpha_0^{(c,c)}(t)\langle e^{-kt}\rangle_0^{(c,c)}\\ &+ \alpha_1^{(o,c)}(t)\langle e^{-kt}\rangle_1^{(o,c)} + \alpha_1^{(c,o)}(t)\langle e^{-kt}\rangle_1^{(c,o)}\end{aligned} \qquad (4.7)$$

where flux contributions due to both channels that are initially open, and channels that are initially closed are explicitly considered. It is useful to examine expressions that result for eq. (4.7) under special limiting conditions.

4.1. Activation phase of the gating reaction

The limiting case of irreversible channel opening is expressed by the reaction scheme $G^{(c)} \xrightarrow{k_o} G^{(o)}$. Initially, all gating units are in the closed channel state. From eq. (2.8) one obtaines $\alpha^{(o)}(t) = \alpha_1^{(c,o)}(t) = 1 - e^{-k_o t}$, $\alpha^{(c)}(t) = \alpha_0^{(c,c)}(t) = e^{-k_o t}$, and $\alpha_0^{(o,o)}(t) = \alpha_1^{(o,c)}(t) = 0$. Substitution of these expressions, and of eqs. (3.21), (3.24) and (3.25) for $\langle e^{-kt}\rangle_0^{(c,c)}$ and $\langle e^{-kt}\rangle_1^{(c,o)}$, into eq. (4.7) yields

$$\langle e^{-kt}\rangle = (k_o - k)^{-1}\left[k_o e^{-kt} - k e^{-k_o t}\right],\quad k \neq k_o \qquad (4.8)$$

$$\langle e^{-kt}\rangle = (1 + kt)e^{-kt},\quad k = k_o. \qquad (4.9)$$

Note that the limit $k_c \to 0$ has to be taken in eq. (3.24) for $\langle e^{-kt}\rangle_1^{(c,o)}$.

Eqs. (4.8) and (4.9) are the exact expressions for $\langle e^{-kt}\rangle$ in the case of an irreversible channel opening process. In the more general case of a reversible net channel opening process with $k_o > k_c$, they will constitute an asymptotic approximation to $\langle e^{-kt}\rangle$. The intrinsic flux rate constant k must then be replaced by the effective constant k_{eq} given by eq. (3.31). Deviations from the purely biphasic time course expressed by eqs. (4.8) and

(4.9) will occur when the activation process approaches equilibrium.

4.2. Inactivation phase of the gating reaction

The limiting case of irreversible channel closing is expressed by the reaction scheme $G^{(o)} \xrightarrow{k_c} G^{(c)}$. Initially, all gating units are in an open channel state. From eqs. (2.9) and (A9) one obtains $\alpha^{(o)}(t) = \alpha_0^{(o,o)}(t) = e^{-k_c t}$, $\alpha^{(c)}(t) = \alpha_1^{(o,o)}(t) = 1 - e^{-k_c t}$, and $\alpha_0^{(c,c)}(t) = \alpha_1^{(c,o)}(t) = 0$. Substitution of these expressions, and of eqs. (3.22) and (3.23) into eq. (4.7) yields

$$\langle e^{-kt} \rangle = (k + k_c)^{-1} \left[k_c + k e^{-(k+k_c)t} \right]. \qquad (4.10)$$

In the limit $t \to \infty$ this expression does not vanish. One obtains the mean single channel flux amplitude contributions

$$\langle e^{-kt} \rangle_\infty = \lim_{t \to \infty} \langle e^{-kt} \rangle = \frac{k_c}{k + k_c}. \qquad (4.11)$$

Eq. (4.10) is the exact expression for $\langle e^{-kt} \rangle$ for an irreversible channel closing process. It will be an approximation to $\langle e^{-kt} \rangle$, in the more general case of a reversible inactivation mode following a rapidly equilibrating reversible activation mode, providing that for the inactivation process $k_c > k_c$ holds. Deviations from the time course described by eq. (4.10) will then occur during the later stages of the inactivation phase. These deviations will vanish as $(k_o/k_c) \to 0$. Since the activation mode is in a steady state during the inactivation phase, k in eqs. (4.10) and (4.11) must again be replaced by the effective constant k_{eq}, given by eq. (3.31), with kinetic constants k_o and k_c for the activation phase.

4.3. Transition between states with different flux rate constants

A special case results when postulate (2.1) does not hold. In the limit of an irreversible transition between states $G^{(1)}$ and $G^{(2)}$ the reaction scheme $G^{(1)} \xrightarrow{k_{12}} G^{(2)}$ will apply, where k_{12} is the rate constant for the transition. The intrinsic single channel ion transport rate constants k_1 and k_2, for states $G^{(1)}$ and $G^{(2)}$, respectively, are assumed to be finite and nonequal. The fractions $\alpha^{(1)}(t)$ and $\alpha^{(2)}(t)$ of gating units in the respective states, are $\alpha^{(1)}(t) = e^{-k_{12}t}$ and $\alpha^{(2)}(t) = 1 - e^{-k_{12}t}$.

In analogy to eq. (3.20) one can write

$$\langle e^{-kt} \rangle_1^{(1,2)} = \int_0^t e^{-k_1 \tau} e^{-k_2 (t-\tau)} p_1^{(1,2)}(\tau, t)\, d\tau, \qquad (4.12)$$

where $p_1^{(1,2)}(t)$ reflects the probability that a gating unit is in state $G^{(1)}$ for a period τ during the interval 0 to t. For a unidirectional transition from $G^{(1)}$ to $G^{(2)}$, the expression for $p_1^{(1,2)}(\tau, t)$ is identical in form to eq. (B10) for $p_1^{(o,c)}(\tau, t)$, with $k_c = k_{12}$. Substitution into eq. (4.12) yields

$$\langle e^{-kt} \rangle_1^{(1,2)} = \left(\frac{k_{12}}{k_{12} + k_1 - k_2} \right) \times \left[\frac{e^{-k_2 t} - e^{-(k_{12} + k_1)t}}{1 - e^{-k_{12} t}} \right]. \qquad (4.13)$$

The mean single channel flux contribution is

$$\langle e^{-kt} \rangle = \alpha^{(1)}(t) \langle e^{-kt} \rangle_0^{(1,1)} + \alpha^{(2)}(t) \langle e^{-kt} \rangle_1^{(1,2)} = (k_{12} + k_1 - k_2)^{-1} \left[k_{12} e^{-k_2 t} + (k_1 - k_2) e^{-(k_{12} + k_1)t} \right] \qquad (4.14)$$

where, in analogy to eq. (3.22), $\langle e^{-kt} \rangle_0^{(1,1)} = e^{-k_1 t}$.

4.4. Approximation by a single exponential

The averaging process implicit in eq. (3.8) can be approximated by the expression

$$\langle e^{-kt} \rangle \approx e^{-k \bar{\tau}(t)} \qquad (4.15)$$

where the mean time $\bar{\tau}(t)$ a gating unit was in an open channel state, in the interval 0 to t, is given by

$$\bar{\tau}(t) = \int_0^t \tau p(\tau, t)\, d\tau. \qquad (4.16)$$

Substituting the expansion (3.18) into eq. (4.16) yields

$$\bar{\tau}(t) = \sum_{m=0}^{\infty} \left[\alpha_{2m+1}^{(u,v)}(t) \bar{\tau}_{2m+1}^{(u,v)}(t) + \alpha_{2m}^{(u,u)}(t) \bar{\tau}_{2m}^{(u,u)}(t) \right], \quad u \neq v \qquad (4.17)$$

where the component mean open times $\bar{\tau}_m^{(u,v)}(t)$ are given by

$$\bar{\tau}_m^{(u,v)}(t) = \int_0^t \tau p_m^{(u,v)}(\tau, t)\, d\tau. \quad (4.18)$$

Explicit expressions for $\tau(t)$ in the case of an irreversible channel opening or channel closing reaction are derived in Appendix B. The resulting eqs. (B16) and (B17) can also be obtained as special cases of the general relationship

$$\bar{\tau}(t) = \int_0^t \alpha^{(\infty)}(t')\, dt' \quad (4.19)$$

where $\alpha^{(\infty)}(t)$ is given by eqs. (2.8) and (2.9). This suggests that the 'integrated rate coefficient' $\kappa(t)$, introduced in previous treatments of flux [17,18], which is given by eq. (1.7), can be written as

$$\kappa(t) = k\bar{\tau}(t). \quad (4.20)$$

A comparison of eqs. (1.8) and (1.9) with eqs. (4.1) and (4.2), leads to the equality

$$\langle e^{-kt} \rangle = e^{-\kappa(t)}. \quad (4.21)$$

Substitution of eq. (4.20) into eq. (4.21) yields eq. (4.15). The assumptions underlying the previous schemes of analysis thus lead to a single exponential approximation of $\langle e^{-kt} \rangle$, with a mean open time $\bar{\tau}(t)$ given by eq. (4.19).

4.5. Inhomogeneities

As discussed elsewhere [17], inhomogeneities in parameters connected with a collection of CMS will influence the flux behavior. A separate distribution of two classes of parameters must be considered: parameters connected with the CMS size (e.g., CMS internal volume v), and parameters connected with the amount of gating units (e.g., number of gating units per CMS n, surface density of gating units $\bar{\rho}$). In general, there may be an arbitrary degree of covariance among the two types of distribution. Expressions (4.1) and (4.2) for $\bar{X}(t)$ are implicitly funtions of the CMS internal volume, since according to eq. (1.6) k depends on v.

The effect of inhomogeneities can be accounted for by averaging $\bar{X}(t)$ over the volume distribution functions $Q_n(v)$; $Q_n(v)$ represents the normalized probability that a CMS with n gating units has a volume v. From eqs. (4.1) and (4.2) one obtains

$$\bar{X}(t) = \begin{cases} \sum_n \bar{X}_n(0) \langle \Phi_n \rangle_v, & \text{efflux} \quad (4.22) \\ \sum_n \bar{X}_n(\infty)[1 - \langle \Phi_n \rangle_v], & \text{influx} \quad (4.23) \end{cases}$$

where $\langle \Phi_n \rangle_v$ represents the volume average

$$\langle \Phi_n \rangle_v = \int_0^\infty Q_n(v) \langle e^{-kt} \rangle^n\, dv. \quad (4.24)$$

Particularly simple expressions result for spherical CMS with a constant surface density $\bar{\rho}$ of gating units. The volume v_n, of a CMS with n gating units, is then given by

$$v_n = \frac{4\pi}{3} \left(\frac{n}{4\pi\bar{\rho}} \right)^{3/2}. \quad (4.25)$$

Substitution of v_n for v in eq. (1.6) for k leads to a flux rate constant k_n dependent on n. Introduction of k_n for k in the expressions for $\langle e^{-kt} \rangle$ derived above yields corresponding expressions $\langle e^{-k_n t} \rangle$ dependent on n. Substituting these into eqs. (4.1) and (4.2) leads to

$$\bar{X}(t) = \begin{cases} \sum_n \bar{X}_n(0) \langle e^{-k_n t} \rangle^n, & \text{efflux} \quad (4.26) \\ \sum_n \bar{X}_n(\infty)\left[1 - \langle e^{-k_n t} \rangle^n\right], & \text{influx.} \quad (4.27) \end{cases}$$

In conclusion it can be said that general expressions describing the time-dependent tracer content of a collection of CMS have been derived. These will apply to tracer flux experiments carried out under restrictive conditions stated in section 1. If there is a heterogeneity in the number of gating units per CMS, and/or in the CMS internal volume, the probability distribution P_n and/or $Q_n(v)$ must be determined experimentally. Special techniques for preparating highly homogeneous CMS are being developed [24]. Thus, the tracer flux method promises to be a general and widely applicable technique for studying the detailed kinetics of ion flux gating processes.

Appendix A

The reaction process characterized by reaction scheme (3.9) can be viewed as a sequence of uni-

directional steps

$$G_0^{(u)} \xrightarrow{k_u} G_1^{(v)} \xrightarrow{k_v} G_2^{(u)} \xrightarrow{k_u} G_3^{(v)} \xrightarrow{k_v} \dots \quad (A1)$$

where $u \neq v$, and where $G_m^{(u)}$ and $G_m^{(v)}$ denote subspecies of gating unit states, which have undergone m transitions in the time interval 0 to t. The kinetic equations for reaction scheme (A1) are

$$\frac{d[G_0^{(u)}]_t}{dt} = -k_v[G_0^{(u)}]_t, \quad (A2)$$

$$\frac{d[G_m^{(u)}]_t}{dt} = -k_v[G_m^{(u)}]_t + k_u[G_{m-1}^{(v)}]_t, \quad m>0 \text{ and } m \text{ even} \quad (A3)$$

$$\frac{d[G_m^{(v)}]_t}{dt} = -k_u[G_m^{(v)}]_t + k_v[G_{m-1}^{(u)}]_t, \quad m>0 \text{ and } m \text{ odd} \quad (A4)$$

where $[G_m^{(u)}]_t$ and $[G_m^{(v)}]_t$ are the respective concentrations of species $G_m^{(u)}$ and $G_m^{(v)}$ at time t. Laplace transformation of these equations yields the corresponding relationships

$$[G_{2r}^{(u)}]_\omega = \left[\frac{k_u k_v}{(\omega+k_u)(\omega+k_v)}\right]^r \left(\frac{[G_0^{(u)}]_0}{\omega+k_v}\right) \quad (A5)$$

$$[G_{2r+1}^{(v)}]_\omega = \left[\frac{k_u k_v}{(\omega+k_u)(\omega+k_v)}\right]^{r+1} \left(\frac{[G_0^{(u)}]_0}{k_u}\right) \quad (A6)$$

where $[G_m^{(u)}]_\omega$ and $[G_m^{(v)}]_\omega$ represent the respective Laplace transforms of $[G_m^{(u)}]_t$ and $[G_m^{(v)}]_t$, and where $r = 0, 1, 2, \dots$. Inverse Laplace transformation leads to the convolution integrals

$$[G_0^{(u)}]_t = [G_0^{(u)}]_0 e^{-k_v t} \quad (A7)$$

$$[G_{2r}^{(u)}]_t = [G_0^{(u)}]_0 \frac{k_u^r k_v^r}{r!(r-1)!} \cdot e^{-k_v t} \int_0^t \tau^{r-1}(t-\tau)^r \times e^{-(k_u-k_v)\tau}\, d\tau, \quad r>0 \quad (A8)$$

$$[G_{2r+1}^{(v)}]_t = [G_0^{(u)}] \frac{k_u^r k_v^{r+1}}{(r!)^2} \cdot e^{-k_v t} \int_0^t \tau^r (t-\tau)^r \times e^{-(k_u-k_v)\tau}\, d\tau. \quad (A9)$$

The fraction $\alpha_m^{(u,w)}(t)$ of gating units in state $G^{(u)}$ at time 0, and in state $G^{(w)}$ at time t, which underwent m transitions of state in the interval 0 to t, is given by $\alpha_m^{(u,w)}(t) = \alpha^{(u)}(0)[G_m^{(w)}]_t / \sum_{r=0}^{\infty}\{[G_{2r}^{(u)}]_t + [G_{2r+1}^{(v)}]_t\}$. Explicit evaluation of expressions (A7)–(A9) for $m = 0$, 1 and 2 yields the equations

$$\alpha_0^{(u,u)}(t) = \alpha^{(u)}(0) e^{-k_v t} \quad (A10)$$

$$\alpha_1^{(u,v)}(t) = \alpha^{(u)}(0)\left(\frac{k_v}{k_v-k_u}\right)[e^{-k_u t} - e^{-k_v t}], \quad u \neq v \quad (A11)$$

$$\alpha_2^{(u,u)}(t) = \alpha^{(u)}(0)\frac{k_u k_v}{(k_v-k_u)^2} \times [e^{-k_u t} - e^{-k_v t} - (k_v-k_u)te^{-k_v t}]. \quad (A12)$$

Appendix B

The functions $p_m^{(u,v)}(\tau,t)$ occurring in eqs. (3.18) and (3.20) can be expressed in terms of the functions $\lambda^{(o)}(\tau)$ and $g_m^{(u,v)}(t)$ given by eqs. (3.13) and (3.14). For the trivial case $m = 0$ one obtains

$$p_0^{(c,c)}(\tau,t) = 0 \quad (B1)$$

$$p_0^{(o,o)}(\tau,t) = \delta(t-\tau) \quad (B2)$$

where $\delta(t-\tau)$ is the delta function. The cases $m>0$ can all be expressed as

$$p_m^{(u,v)}(\tau,t) = \lambda^{(o)}(\tau)F^{(u,v)}(\tau,t) / \int_0^t \lambda^{(o)}(\tau)F_m^{(u,v)}(\tau,t)\, d\tau \quad (B3)$$

where $\lambda^{(o)}(\tau) = e^{-k_c\tau}$. The denominator in eq. (B3) assures that $p_m^{(u,v)}(\tau,t)$ is normalized over the interval 0 to t. The functions $F_m^{(u,v)}(\tau,t)$ for $m = 1, 2$ are given by

$$F_1^{(o,c)}(\tau,t) = \alpha_0^{(o,o)}(0) \quad (B4)$$

$$F_1^{(c,o)}(\tau,t) = g_1^{(c,o)}(t-\tau) \quad (B5)$$

$$F_2^{(c,c)}(\tau,t) = \int_\tau^t g_1^{(c,o)}(t'-\tau)\, dt' \quad (B6)$$

$$F_2^{(o,o)}(\tau,t) = \alpha_0^{(o,o)}(0)\int_0^\tau g_2^{(o,o)}(t-\tau')\, d\tau'. \quad (B7)$$

Expressions for $m>2$ can be generated from the relationships

$$F_m^{(u,c)}(\tau,t)=\int_0^{\tau}F_{m-2}^{(u,c)}(\tau',t-\tau')\times\left[\int_{\tau}^{t-\tau}g_{m-1}^{(u,o)}(t'-\tau')\,dt'\right]d\tau' \quad (B8)$$

$$F_m^{(u,o)}(\tau,t)=\int_0^{\tau}g_m^{(u,o)}(t-\tau')\times\left[\int_0^{t-\tau'}F_{m-1}^{(u,c)}(\tau',t')\,dt'\right]d\tau'. \quad (B9)$$

From eqs. (3.14), (A10), and (B3)–(B5) one obtains, for $m=1$,

$$p_1^{(o,c)}(\tau,t)=k_c e^{-k_c\tau}/(1-e^{-k_c t}) \quad (B10)$$

$$p_1^{(c,o)}(\tau,t)=(k_c-k_o)e^{-(k_c-k_o)\tau}\times[1-e^{-(k_c-k_o)t}]^{-1}. \quad (B11)$$

Substituting the expressions (B1), (B2), (B10) and (B11) into eq. (4.18) for the mean open time one obtains

$$\bar{\tau}_0^{(c,c)}(t)=0 \quad (B12)$$

$$\bar{\tau}_0^{(o,o)}(t)=t \quad (B13)$$

$$\bar{\tau}_1^{(o,c)}(t)=k_c^{-1}-\frac{te^{-k_c t}}{1-e^{-k_c t}} \quad (B14)$$

$$\bar{\tau}_1^{(c,o)}(t)=(k_c-k_o)^{-1}-\frac{te^{-(k_c-k_o)t}}{1-e^{-(k_c-k_o)t}}. \quad (B15)$$

For the special limiting case of irreversible channel opening discussed above, substitution of eqs. (B12) and (B15) into eq. (4.17) yields

$$\bar{\tau}(t)=\alpha_0^{(c,c)}(t)\bar{\tau}_0^{(c,c)}(t)+\alpha_1^{(c,o)}(t)\bar{\tau}_1^{(c,o)}(t)=t-k_o^{-1}(1-e^{-k_o t}). \quad (B16)$$

Similarly, for an irreversible channel closing process, substitution of eqs. (B12) and (B15) into eq. (4.17) yields

$$\bar{\tau}(t)=\alpha_0^{(o,o)}(t)\bar{\tau}_0^{(o,o)}(t)+\alpha_1^{(o,c)}(t)\bar{\tau}_1^{(o,c)}(t)=k_c^{-1}(1-e^{-k_c t}). \quad (B17)$$

Appendix C

A stationary flux of tracer ions will result for the transport process represented by reaction scheme (1.4) when the condition

$$\frac{d[NR]}{dt}=-(k_t+k_{-b})[NR]+k_b[N][R]=0 \quad (C1)$$

holds. One then obtains

$$[NR]=\left(\frac{k_b}{k_t+k_{-b}}\right)[N][R]. \quad (C2)$$

The rate equation for unidirectional efflux of tracer ions is

$$\frac{d[N]}{dt}=-k_b[N][R]+k_{-b}[NR]. \quad (C3)$$

Substitution of eq. (C2) leads to

$$\frac{d[N]}{dt}=-\frac{\alpha k_t}{K'+k_t/k_b}([R_T]-[RN])[N] \quad (C4)$$

where $K'=k_{-b}/k_b$ is the apparent dissociation constant, $[R_T]$ the total 'concentration' of internal channel sites, and α the fraction of open channels. To obtain the corresponding rate equation for influx, assuming the external bath is buffered in tracer ions, a constant rate of influx term must be added to the right-hand side of eq. (C4). The time-dependent tracer content of a CMS, obtained as a solution of the rate equations, is given by

$$x(t)=\begin{cases}x(0)\cdot e^{-mkt}, & \text{efflux} \quad (C5)\\ x(\infty)[1-e^{-mkt}], & \text{influx} \quad (C6)\end{cases}$$

where $m=\alpha\cdot[R_T]\cdot N_A\cdot v$ is the number of open channels on the CMS, N_A Avogadro's number, and v the CMS internal volume. The intrinsic flux rate constant k, that results when $[R_T]\gg[NR]$ (i.e., at low tracer ion concentration), is given by

$$k=\frac{k_t}{N_A\cdot v(K'+k_t/k_b)}. \quad (C7)$$

In the special case $K'\gg(k_t/k_b)$, eq. (C7) becomes identical to the result previously derived for efflux under the more restrictive assumption that tracer ion binding is much more rapid than transport through a channel [18].

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