INTERNAL MOTIONS IN PROTEINS AND GATING KINETICS OF IONIC CHANNELS

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ABSTRACT Single-channel current recordings have revealed a complex kinetic behavior of ionic channels. Many channels exhibit closed-time distributions in which long waiting times occur with a much higher frequency than predicted by a simple exponential decay function. In this paper a model for opening–closing transitions that accounts for internal motions in the protein matrix is discussed. The model is based on the notion that the transition between a conductive and a nonconductive state of the channel represents a local process in the protein, such as the movement of a small segment of a peptide chain or the rotation of a single amino-acid residue. When the blocking group moves into the ion pathway, a structural defect is created consisting in a region of loose packing and/or poor hydrogen bonding. By rearrangements of neighboring groups, the defect may migrate within the protein matrix, carrying out a kind of random walk. Once the defect has moved away from the site where it was formed, a transition back to the open state of the channel is possible only when the defect has returned by chance to the original position. The kinetic properties of this model are analyzed by stochastic simulation of defect diffusion in a small domain of the protein. With a suitable choice of domain size and diffusion rate, the model is found to predict closed-time distributions that agree with experimental observations.

INTRODUCTION

Ionic channels in cell membranes are proteins that provide a low-energy pathway for small ions such as Na^+ or K^+ through the apolar core of the membrane (Hille, 1984). The introduction of the patch-clamp technique (Neher and Sakmann, 1976) has opened the possibility of investigating the stochastic behavior of ionic channels at the level of the single protein molecule. In the active state of a channel the conductance fluctuates between two or more discrete levels in a random fashion. From records of single-channel currents the probability distribution of dwell times in the closed and open states can be evaluated. The observed dwell-time distributions can be compared with theoretical predictions from kinetic models. For a transition $C \rightleftharpoons O$ between a single closed (C) and a single open (O) state, the probability density for the occurrence of a closed time of duration t is predicted to be an exponentially decreasing function of t characterized by a single time constant. Many channels, however, exhibit complex dwell-time distributions in which long-lasting closures occur with a much higher frequency than predicted by an exponential function (Magleby and Pallota, 1983*a*; Horn and Vandenberg, 1984; Sakmann and Trube, 1984; Liebovitch et al., 1987*b*; Horn and Lange, 1983; Horn, 1987; Colquhoun and Sakmann, 1985). On the other hand, open-time distributions usually have a much simpler form. Well-known examples of channels exhibiting nonexponential closed-time distributions are the acetylcholine-activated channel of neuromuscular junctions (Trautmann, 1982; Dionne and Leibowitz, 1982; Colquhoun and Sakmann, 1983; Colquhoun and Sakmann, 1985; Labarca et al., 1985) and the calcium-activated potassium channel (Magleby and Pallota, 1983*a* and *b*; Moczydlowski and Latorre, 1983). In these cases it is commonly assumed that the closed state occurs in several substates C_1, C_2, . . . and that the opening event is preceded by transitions between these substates:

$$C_1 \rightleftharpoons C_2 \rightleftharpoons \cdots \rightleftharpoons C_n \rightleftharpoons O \qquad (1)$$

Kinetic schemes of this kind lead to multiexponential distributions of closed times (Colquhoun and Hawkes, 1977, 1981, 1982; Horn and Lange, 1983; Horn, 1987). The number n of closed states which has to be postulated to obtain a satisfactory fit to the observed closed-time distribution usually depends on the length of the current records used for statistical analysis. For instance, in the case of the calcium-activated potassium channel, n has to be assumed to be larger than three (Magleby and Pallota, 1983*a*). Whereas an analysis based on reaction (1) or on similar kinetic schemes allows a phenomenological description of experimental dwell-time distributions, the nature of the postulated states $C_1, C_2, \ldots, C_n$ remains to be elucidated.

A totally different approach for the analysis of dwell-time distributions of ionic channels has been recently proposed by Liebovitch et al. (1987*a* and *b*). In their treatment an effective rate constant for the transition $C \rightarrow O$ is introduced, whose value depends on the time scale of the measurement. This fractal description is based on the notion that dynamic processes in proteins occur with many

different correlation times. By adjusting the parameters of the model, the observed gating behavior could be described in a wide time range. So far, the nature of the physical processes leading to fractal time behavior of a channel remains unclear, however.

In the following, we discuss a microscopic model for channel gating which takes internal motions in proteins explicitly into account. Numerical simulations of the model yield nonexponential dwell-time distributions that approximately agree with experimental observations.

MOBILE-DEFECT MODEL OF CHANNEL GATING

The model is based on the notion that the transition between a conductive and a nonconductive state of the channel represents a local process in the protein, such as the movement of a small segment of a peptide chain or the rotation of a single amino acid residue. Blocking and unblocking of ion flow may result from the movement of the blocking group into and out of a water-filled pathway traversing the protein. The assumption that small-scale motions (in contrast to a gross conformational changes) are responsible for channel gating is consistent with the fact that in records of single-channel currents, transitions between conductance states appear as instantaneous, step-like events within the time scale (10–100 μs) of the patch-clamp experiments (Hamill et al., 1981; Auerbach and Sachs, 1984; Sigworth, 1986). Molecular dynamics simulations and spectroscopic studies have shown that side-chain reorientations in a protein such as 180° flips of aromatic residues occur within <1 ns, whereas the actual frequency of the transition may be as low as 100 s^{-1} (Williams, 1978; Munro et al., 1979; Gurd and Rothgeb, 1979; Lakowicz and Weber, 1980; Wüthrich et al., 1980; Levitt, 1983; McCammon et al., 1983; Wagner, 1983; Karplus, 1985).

The protein matrix surrounding the ion pathway may be assumed to have a densely packed structure, as is known from globular proteins (Richards, 1977; Schultz and Schirmer, 1979). When a bulky blocking group swings into the ion pathway, a transient packing defect is created within the protein matrix (Richards, 1979). This situation is schematically depicted in Fig. 1. The packing defect resulting from the jump of the blocking group into the "closed" position may be filled by a rearrangement of neighboring residues (McCammon et al., 1983). In this way the packing defect may move around in the protein matrix, carrying out a kind of random walk (Lumry and Rosenberg, 1976; Englander and Kallenbach, 1984).

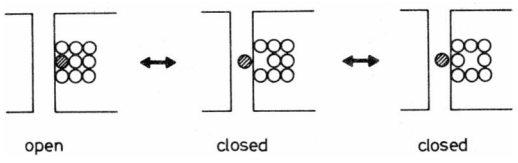

FIGURE 1 Transitions between open and closed states of an ionic channel resulting from the movement of a blocking group (*hatched circle*) into and out of the ion pathway. The packing defect which is created by the jump of the blocking group into the "closed" position may be filled by jumps of neighboring residues into the vacancy. As a consequence, the defect carries out a random walk within a finite domain of the channel protein.

It is feasible that the transition of the blocking group from the "open" to the "closed" position involves transient breaking of one to several hydrogen bonds. In this case the defect chiefly consists in a region of poor bonding. The bonding defect may migrate through the protein matrix by breaking and making of new hydrogen bonds (Lumry and Rosenberg, 1976). For the following analysis it is irrelevant whether the defect consists in poor packing, poor hydrogen bonding, or a combination of both.

Once the defect has moved away from its site of origin (position (0, 0, 0) in Fig. 2), a transition back to the open state of the channel becomes possible only when the defect has accidentally returned to position (0, 0, 0). As will be shown below, this mechanism leads to a distribution of closed times in which long waiting times occur with increased frequency. At the same time, the model predicts a purely exponential distribution of open times, in agreement with many experimental observations.

Similar mobile defect models have been used for the treatment of diffusion of small molecules inside proteins (Woodward and Hilton, 1979; Weber, 1975; Austin et al., 1975; Lumry and Rosenberg, 1976; Richards, 1979; Englander and Kallenbach, 1984). Furthermore, mechanisms involving mobile defects have been proposed in theories of dielectric relaxation in polymeric systems and glasses (Glarum, 1960; Shlesinger and Montroll, 1984; Klafter and Blumen, 1985; Klafter and Shlesinger, 1986; Jäckle, 1986). In such systems it is often found that after switching off the electric field, the polarization decays much slower than predicted by an exponential time law.

STOCHASTIC SIMULATONS

The predictions of the model can be analyzed by stochastic simulation. For this purpose we consider an assembly of

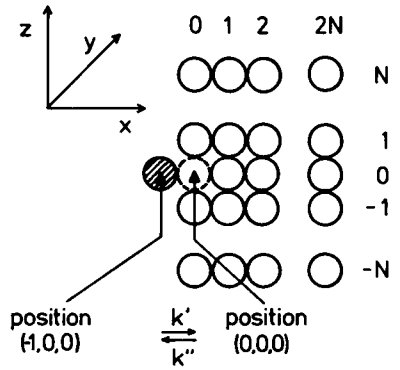

FIGURE 2 Cubic lattice containing $(2N + 1)^3$ sites. k' and k'' are the rate constants for jumps of the blocking group (*hatched circle*) between positions $(-1, 0, 0)$ (channel closed) and (0, 0, 0) (channel open).

$(2N + 1)^3$ residues arranged in a cubic lattice (Fig. 2). Lattice positions are denoted by (x, y, z) where x, y, and z are Cartesian coordinates. The blocking residue is allowed to jump back and forth between positions $(-1, 0, 0)$ and $(0, 0, 0)$, the frequency of jumps being given by rate constants k' and k''.

$$\underset{\text{(channel closed)}}{(-1, 0, 0)} \underset{k''}{\overset{k'}{\rightleftharpoons}} \underset{\text{(channel open)}}{(0, 0, 0)} \qquad (2)$$

k' is the probability per unit time that the blocking residue moves to site (0, 0, 0) when the site is empty. When the blocking residue has moved to site $(-1, 0, 0)$, a packing defect is left behind at position (0, 0, 0). By a jump of adjacent residues into the cavity, the defect may migrate in x-, y-, or z-direction. The rate constants for jumps within the lattice are assumed to be the same in all directions and are denoted by l. At the surface of the lattice, rate constants corresponding to outward-directed jumps are set equal to zero.

The introduction of a regular, quasi-isotropic lattice for the description of defect motion inside a protein represents a strong simplification. In reality, the rate constants for jumps are anisotropic and vary from place to place. Furthermore, the meaning of a "lattice site" is somewhat ambiguous, because the entities that actually move are not specified; they may be single amino acid residues or small groups of residues. Depsite these simplifications, the model is thought to describe, in principle, how defect motion in the protein matrix may influence gating kinetics of a channel.

The minimal version of the model described above contains three rate constants (k', k'', and l) as parameters, as well as the quantity N determining the size of the domain in which the defect is allowed to move. However, since the closed-time distribution is independent of k'' and since k' (or l) merely defines the absolute time scale of the distribution, the model is essentially characterized by two parameters, k'/l and N, which may be adjusted to fit experimentally observed dwell-time distributions.

The distribution of closed times may be described by the probability $P(t)$ that a closed event has a duration longer than t. Instead of $P(t)$ it is convenient to evaluate the probability density $f(t)$ which is defined by the relation $dn = f(t)dt$ where dn is the number of events with dwell times between t and $t + dt$ (Colquhoun and Hawkes, 1977, 1981, 1982). $P(t)$ and $f(t)$ are simply related by

$$f(t) = -dP/dt. \qquad (3)$$

Predictions on the shape of $P(t)$ or $f(t)$ may be obtained by stochastic simulation of the random walk of the defect in the lattice. For the simulations two different methods have been used which are described in Appendix A and which yield (within the limits of statistical error) identical results.

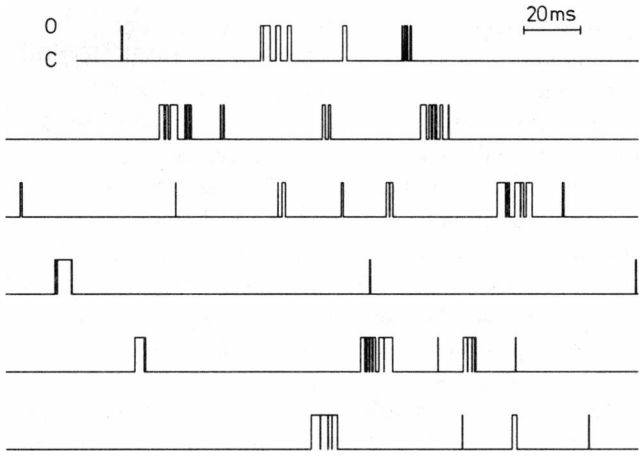

FIGURE 3 Simulated transitions between closed (C) and open (O) states of a channel. Cubic lattice of $3^3 = 27$ sites ($N = 1$). The rate constants for opening (k') and closing (k'') of the channel and for defect migration in the lattice (l) have been assumed to be $k' = k'' = 10^3\ s^{-1}$ and $l = 300\ s^{-1}$, respectively.

RESULTS

An example of simulated single-channel conductance fluctuations is represented in Fig. 3 for a cubic lattice of $3^3 = 27$ sites ($N = 1$). The rate constants for channel opening and closing and for defect diffusion in the lattice have been assumed to be $k' = k'' = 10^3\ s^{-1}$ and $l = 300\ s^{-1}$, respectively. As seen from Fig. 3, the current exhibits "bursting" behavior characterized by the occurrence of groups of densely spaced openings and closings which are separated by long closed periods. This behavior is fre-

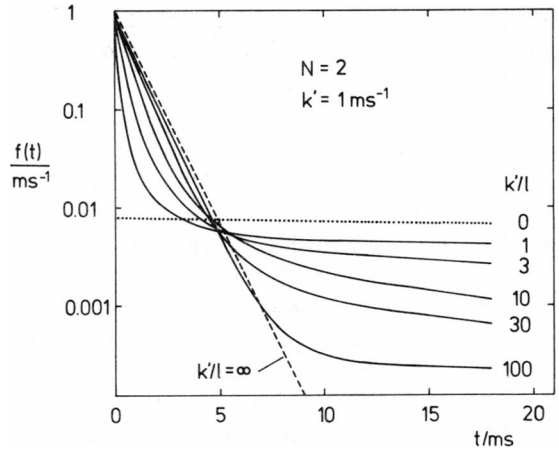

FIGURE 4 Closed-time distribution $f(t)$ for a lattice with $N = 2$ and different values of k'/l. The rate constant k' for the opening step (Fig. 2) has been assumed to be 1 ms^{-1}. l is the rate constant for jumps of the defect within the lattice. $f(t)$ is independent of the closing-rate constant k'' (Fig. 2). In the absence of defect diffusion ($l = 0$, $k'/l = \infty$), $f(t)$ is given by $k' \exp(-k't)$ (*dashed line*). In the limit of fast diffusion ($k'/l = 0$), $f(t)$ is again a monoexponential function, $k'_{eff} \exp(-k'_{eff}/t)$ with $k'_{eff} = k/(2N + 1)^3 = 0.008\ ms^{-1}$ (*dotted line*). The simulations have been carried out on a MINC/PDP11 as described in Appendix. For each parameter set between 10^5 and 10^7 closed events have been analyzed for the evaluation of $f(t)$.

quently observed in records of single-channel currents (Colquhoun and Hawkes, 1982). In the model discussed here, bursting occurs because the blocking group may jump back and forth many times between positions $(-1, 0, 0)$ and $(0, 0, 0)$ before the defect leaves position $(0, 0, 0)$ to another lattice site. Furthermore, the defect created at position $(0, 0, 0)$ by a channel-closing event may visit this site again from neighboring lattice sites before it moves away to more distant positions. In agreement with expectation, bursting behavior is found to become more and more pronounced with increasing values of the ratio k'/l (not shown).

Closed time distributions $f(t)$ are represented in Fig. 4 for a lattice with $N = 2$ and different values of the ratio k'/l. The rate constant k' for the opening step (Fig. 2) has been fixed at 1 ms^{-1}. In the absence of defect diffusion ($l = 0$, $k'/l = \infty$), the closed-time distribution is a simple monoexponential function $f(t) = k' \exp(-k't)$ which is represented by the dashed line in Fig. 4. When the defect is allowed to move in the lattice ($l > 0$), $f(t)$ decays much slower at long times than a monoexponential function. As just discussed, this behavior results from the fact that a closed channel can open only when the defect which has moved away from position $(0, 0, 0)$ visits this site again after a random walk of variable duration.

When defect migration in the lattice is fast compared with the rate of opening–closing transitions of the blocking group ($k'/l \approx 0$), the defect may visit site $(0, 0, 0)$ many times during the lifetime of the closed state of the channel. The frequency of channel opening may then be described by an effective rate constant $k'_{eff} = p_0 k'$, where $p_0 = 1/(2N + 1)^3$ is the probability of the defect being located at position $(0, 0, 0)$. In this case the closed-time distribution is again given by a simple exponential decay function $f(t) = k'_{eff} \exp(-k'_{eff} t)$, as indicated by the dotted line in Fig. 4.

In Fig. 5, A and B, closed-time distributions are shown for different sizes of the domain ($N = 1, 2, 3$) at fixed values of the rate constants ($k' = 1$ ms^{-1}, $k'/l = 3$). At short times, $f(t)$ becomes independent of domain size. This has to be expected because for small t the closed-time distribution is governed by local motions of the defect in the vicinity of the blocking group. At intermediate times, $f(t)$ becomes smaller with increasing value of N, whereas at longer times the curves intersect and the dependence of $f(t)$ on N is reversed (Fig. 5 *B*). This agrees with the expectation that the probability of very long waiting times increases with increasing size of the domain available to defect diffusion.

Comparison with Experimental Findings

In the following we compare predictions of the model with the kinetic behavior of the nicotinic acetylcholine–activated channel in the neuromuscular junction, which has been studied in considerable detail (Dionne and Leibowitz, 1982; Colquhoun and Sakmann, 1983, 1985; Auerbach and Sachs, 1984; Labarca et al., 1985). The closed-time distribution of the channel, as observed by Colquhoun and Sakmann (1985), is represented in Fig. 6 as the number of closed events, $\Delta n/\Delta t$, per unit time interval (circles). Because very brief closures may escape detection due to limitations in the experimental time-resolution, the total number n of events cannot be determined from the single-channel current record. Accordingly, in comparing $\Delta n/\Delta t$ with the predicted distribution $n \cdot f(t)$, n has to be treated as an adjustable parameter, corresponding to an upward or downward shift of $n \cdot f(t)$ in the logarithmic plot of Fig. 6. The full line in Fig. 6 represents the predicted closed-time distribution obtained by simulation of the model with $N = 2$, $k' = 1.8 \cdot 10^4$ s^{-1}, and $k'/l = 30$. The total number of events was assumed to be $n = 3{,}230$. It is seen that the predicted distribution approximately agrees with the experimental data in the time range between 50 μs and 500 ms. Attempts to fit the distribution with $N = 1$ gave distinctly inferior results, whereas $N = 3$, $k'/l = 20$ led to comparable fits in the experimental time range.

As a further example we consider the ionic channel from

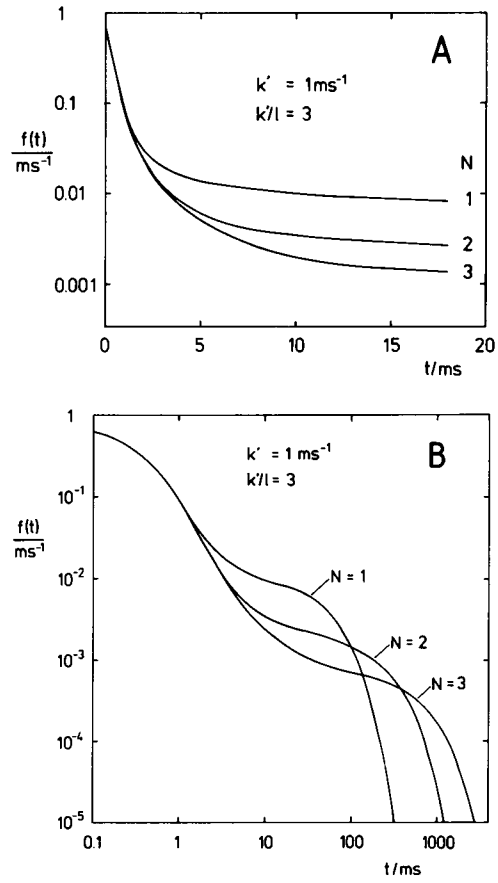

FIGURE 5 (*A*) Closed-time distribution $f(t)$ for different sizes of the lattice ($N = 1, 2, 3$) at fixed values of the rate constants ($k' = 1$ ms^{-1}, $k'/l = 3$). (*B*) $f(t)$ for the same parameter values as in *A*, but plotted in more extensive time range.

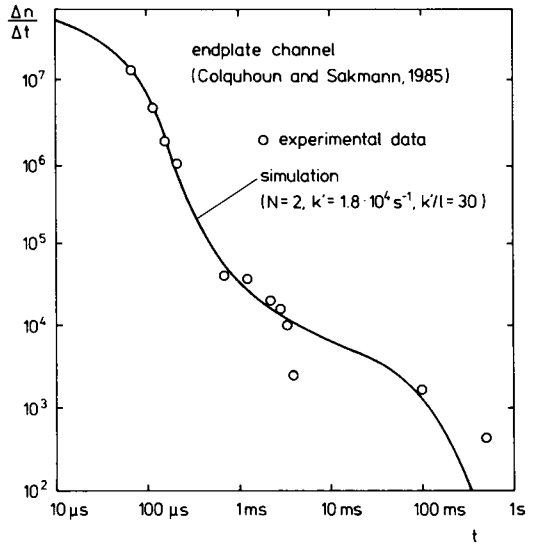

FIGURE 6 Closed-time distribution of the frog endplate channel in the presence of 100 nM suberylcholine. $\Delta n/\Delta t$ is the number of closed events per second. The experimental data (*circles*) have been taken from Fig. 2 of Colquhoun and Sakmann (1985). The data points between 100 ms and 1 s are uncertain and subjected to the assumption that the membrane patch contained only a single agonist-activated channel. The theoretical distribution $n \cdot f(t)$ (*solid line*) was obtained by simulation of the model using the following parameter values: $N = 2$, $k' = 1.8 \cdot 10^4\ s^{-1}$, $k'/l = 30$. The total number of closed events was assumed to be $n = 3{,}230$.

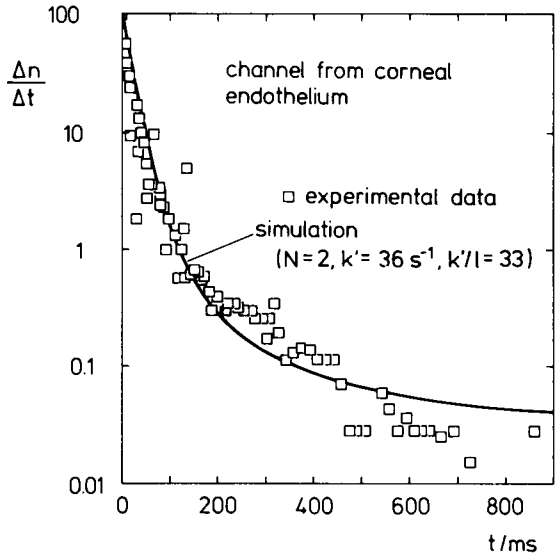

FIGURE 7 Closed-time distribution of a channel from corneal epithelium. The experimental data (*squares*) have been taken from Liebovitch et al. (1987*b*). The theoretical distribution (*solid line*) was obtained by numerical simulation, using the following parameter values: $N = 2$, $k' = 36\ s^{-1}$, and $k'/l = 33$.

corneal endothelium studied by Koniarek et al. (1986). Liebovitch et al. (1987*b*) have shown that the distinctly nonexponential distribution of closed times of this channel can be represented by a fractal law. In Fig. 7 the experimental data are compared with the result of a simulation with $N = 2$, $k' = 36\ s^{-1}$, and $k'/l = 33$. Again, the model is found to describe approximately the observed dwell-time distribution. The rate constant for the opening of the channel ($k' = 36\ s^{-1}$) is much smaller than in the case of the endplate channel ($k' = 1.8 \cdot 10^4\ s^{-1}$), but the values of N and k'/l which had to be chosen for fitting the experimental data are remarkably similar. It is pertinent to note, however, that values of k' are uncertain because of limitations in the experimental time resolution. It is feasible that recordings carried out at increased band width would lead to fits with larger values of k' (and probably also larger values of k'/l).

Fractal Description

A fractal description of gating kinetics has recently been proposed by Liebovitch et al. (1987*a* and *b*). In their treatment the transition closed → open is described by a time-dependent (nonmarkovian) rate constant:

$$k_C = A/t^{D-1} \qquad (1 < D < 2). \tag{4}$$

t is the time after the channel has entered the closed state, D the so-called fractal dimension, and A a constant. According to Eq. 4, the probability $dp = k_C dt$ for the channel to reopen in the time interval dt is very large at the beginning, but decreases with time. From the general relations $dp/dt = -d\ln P/dt$ and $f = -dP/dt$, the closed-time distribution corresponding to Eq. 4 is obtained as (Liebovitch et al., 1987*a* and *b*).

$$f(t) = \frac{A}{t^{D-1}} \exp\left(-\frac{A}{2-D}\, t^{2-D}\right). \tag{5}$$

For the discussion of Eq. 5 it is convenient introducing a dimensionless time $\tau \equiv k_0 t$, where k_0 is a scaling factor which may be chosen to be, for instance, $k_0 = 1\ ms^{-1}$. Eq. 5 then assumes the form

$$f(\tau) = \frac{A^* k_0}{\tau^{D-1}} \exp\left(-\frac{A^*}{2-D}\, \tau^{2-D}\right), \tag{6}$$

where $A^* \equiv A \cdot k_0^{D-2}$ is a dimensionless constant.

A strict comparison between the fractal formalism and the vacancy diffusion model is not possible, because the fractal description predicts an infinitely high transition rate constant k_C at small times, so that $[f(0) = \infty]$, whereas the vacancy diffusion model contains a characteristic rate constant k' which causes $f(0)$ to remain finite. It is possible, however, to choose the parameters A^* and D in such a way that the simulated closed-time distribution $f(t)$ is approximately described by Eq. 6 in a certain time range. For example, for $N = 2$, $k' = 1\ ms^{-1}$, and $k'/l = 3$, $f(t)$ can be approximated by Eq. 6 with $A^* \approx 0.08$ and $D \approx 1.92$ in the time range between 0 and 20 ms ($k_0 = 1\ ms^{-1}$). A straightforward interpretation of the quantities A^* and D in terms of the parameters of the model, N, k', k'/l seems difficult, however.

DISCUSSION

Many patch-clamp studies over the last years have revealed a complex kinetic behavior of ionic channels. Nonexponential dwell-time distributions indicate that observable conductance states often contain multiple substates. Single-channel current records thus may yield valuable information on internal motions of proteins that cannot be easily obtained by other methods. In this paper a model has been discussed which is derived from current concepts of protein dynamics and is able to reproduce the shape of experimental dwell-time distributions. The model is based on the assumption that the open-closed transition creates a transient structural defect which may migrate through the protein matrix. The existence of mobile structural defects in proteins has been previously proposed in the treatment of diffusion of water molecules in proteins. It has been shown to account for the complex time dependence of hydrogen–deuterium exchange of amide groups (Lumry and Rosenberg, 1976).

The random-walk model for the description of defect migration in the protein has been introduced here in a simple form to keep the number of free parameters small. The shape of the dwell-time distribution $f(t)$ is determined by two parameters, the quantity N describing the size of the domain available to defect diffusion, and the ratio k'/l of jumping frequencies; the absolute value of k' defines the time scale of the distribution. The model may be generalized, for instance by introducing the possibility that, on the average more than one mobile defect is present in the lattice. Furthermore, fitting the model to observed dwell-time distributions may require the introduction of position-dependent rate constants for defect migration. The rate constant for escape of the defect from a certain site may be low so that the site acts as a trap. Trapping of defects may create longlived closed states, such as the "desensitized" or "inactivated" state in agonist-dependent channels.

An essential assumption of the model is the notion that open-closed transitions of the channel result from local motions of a small blocking group. Such small-scale motions can exhibit only a limited voltage dependence. Activation of a voltage-sensitive channel may require a preceding conformational transition in which charges move over a substantial part of the transmembrane electric field. For instance, Finkelstein and Peskin (1984) and Edmonds (1987) have discussed models in which a voltage-dependent rearrangement of charged groups precedes the electrically silent channel-opening event. In a similar way, a chemically activated channel may become competent for carrying out opening–closing transitions only after binding of an agonist molecule. Consistent with this picture is the finding that in the acetylcholine-activated channel the frequency of open-closed transitions within a "burst" period is virtually independent of voltage and of agonist concentration (Colquhoun and Sakmann, 1985).

APPENDIX

Evaluation of $P(t)$ and $f(t)$ by Stochastic Simulation

The random walk of the defect in the cubic lattice (Fig. 2) depends on the probabilities per unit time, $s_1, s_2, \ldots, s_6$, for jumps in a given direction. Index 1 refers to the negative x-direction, index 2 to the positive x-direction, index 3 to the negative y-direction, and so forth. The probability for a jump in negative x-direction in the time interval dt is given by $dp_1 = s_1 dt$. The values of the probability densities p_i ($i = 1, 2, \ldots, 6$) vary with the location of the defect in the lattice: $s_i \equiv s_i(x, y, z)$. Because we assume isotropic diffusion, the relation $s_1 = s_2 = \ldots = s_6 = l$ holds for an internal lattice point, where l is a rate constant. If the defect is at a surface position, some of the s_i vanish; if the defect is at position (0, 0, 0), the relations $s_1 = k'$, $s_2 = s_3 = s_4 = s_5 = s_6 = l$ hold (Fig. 2).

The probability q_i that the next jump of the defect occurs in a given direction i ($i = 1, 2, \ldots, 6$) may be expressed by

$$q_i(x, y, z) = s_i(x, y, z) \Big/ \sum_1^6 s_j(x, y, z). \qquad \text{(A1)}$$

For any lattice position (x, y, z) we introduce the probability density $g(x, y, z, t)$ that a defect at this position has a dwell time of duration t (Colquhoun and Hawkes, 1981):

$$g(x, y, z, t) = \exp\left[-t/\tau(x, y, z)\right]/\tau(x, y, z) \qquad \text{(A2)}$$

$$\tau(x, y, z) \equiv 1 \Big/ \sum_1^6 s_j(x, y, z). \qquad \text{(A3)}$$

It is pertinent to note that Eq. A2 not only holds for the overall distribution of dwell times at (x, y, z) but also for any subpopulation of events terminated by a jump in a given direction. Thus, when the defect is located at position (0, 0, 0), the dwell-time distribution of sojourns ending in a jump to $x = -1$ (rate constant k') is identical with the dwell-time distribution of sojourns ending in a jump to $x = 1$ (rate constant l).

For stochastic simulations of random walks two different methods have been used.

Method I. The random walk is started at time $t = 0$ with the defect at a random position (x, y, z). The probability that the defect jumps within a small time interval Δt in a given direction i is assigned to be $\Delta p_i = s_i(x, y, z)\Delta t$. Thereafter, six random numbers $R_i \in (0, 1)$ are generated by the random number generator of the PDP11-FORTRAN library. If $R_1 < \Delta p_1$, the defect is allowed to jump to position $x - 1$. Otherwise R_2 is compared with Δp_2, and so forth. At the end the time is set to $t = 2\Delta t$ and the procedure is started again. This procedure is based on the fact that the probability of a random number $R \in (0, 1)$ being smaller than a given number $Z \in (0, 1)$ is equal to Z. The time interval Δt must be small enough so that the condition $R_i < \Delta p_i$ is very rarely met.

Method II. With the defect at position (x, y, z), in a first step a random dwell-time t is assigned according to

$$t = -\tau(x, y, z) \cdot \ln R, \qquad \text{(A4)}$$

where $\tau(x, y, z)$ is the mean dwell-time in position (x, y, z) given by Eq. A3, and $R \in (0, 1)$ is a random number (Clay and DeFelice, 1983; Blatz and Magleby, 1986). This procedure leads to an exponential dwell-time distribution corresponding to Eq. A2 (Cox and Miller, 1977). In a second step the direction of the jump is assigned according to Eq. A1. For this purpose a new random number R is generated; when R lies in the interval $(0, q_1)$, the defect jumps in direction 1 ($x \rightarrow x - 1$); when R lies in the interval $(q_1, q_1 + q_2)$, the defect jumps in direction 2 ($x \rightarrow x + 1$), and so forth.

Within the limits of statistical error, both methods gave identical

results. Method II requires much less computer time, because in method I many small time-steps Δt are needed for a single jump of the defect. For this reason mostly method II was used.

The function $P(t)$ was obtained by statistical analysis of the closed-times $t_1^c, t_2^c, \ldots$ which were recorded in the course of the simulation. (In general, a given closed time is the sum of many dwell times of individual substates). The function $f(t)$ was calculated from $P(t)$ by numerical differentiation according to Eq. 3.

The procedure for the calculation of $P(t)$ and $f(t)$ was checked by applying simulation methods I and II to a simplified system exhibiting transitions between three states (x is the position of the defect).

$$(x = -1) \underset{k'}{\overset{k''}{\rightleftharpoons}} (x = 0) \underset{l'}{\overset{l''}{\rightleftharpoons}} (x = 1). \tag{A5}$$

In this case a simple analytical expression for $f(t)$ can be obtained (Colquhoun and Hawkes, 1981):

$$f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \tag{A6}$$

$$1/\tau_1 = r + w; \qquad 1/\tau_2 = r - w \tag{A7}$$

$$A_1 = \frac{k'}{2w}(w + r - l'); \qquad A_2 = \frac{k'}{2w}(w - r + l') \tag{A8}$$

$$r \equiv (k' + l' + l'')/2; \qquad w \equiv \sqrt{r^2 - k'l'}. \tag{A9}$$

The values of $f(t)$ obtained by numerical simulations were found to agree with the predictions of Eq. A6.

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