Closed-time distribution of ionic channels

Analytical solution to a one-dimensional defect-diffusion model

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ABSTRACT A one-dimensional version of the model recently proposed by Läuger (1988) to explain the closed-time distribution of ionic channels in cell membranes is solved analytically. While the probability density $f(t)$ for closed-time lengths may show a well-defined exponential behavior at short times, a power-law decay is predicted at long times. The influence of an additional random distribution of defects in the current-conducting protein is investigated and found to be dominating at long times. Explicit expressions that may be used for fitting experimental data are given for the closed-time distribution. Some of the available data are discussed and shown to be in good agreement with the predictions of the model.

INTRODUCTION

Since the introduction of the patch clamp method (Neher and Sakmann, 1976), it has been possible to analyze in detail the properties of single ionic channels in proteins embedded in cell membranes. The fluctuations of the electrical current through such a channel can be characterized by assuming that the channel oscillates randomly between a "closed" (nonconducting) state and "open" (conducting) state, in which the value of the electrical current is well defined. In most cases the distribution $f(t)$ of closed times is not a single exponential (which would correspond to a single rate constant for transitions between these states), but exhibits a more complex behavior. This observation suggests that a closed channel can exist in different substates, in which the structure of the channel protein is slightly different. In other words, the reopening of a closed channel seems to be coupled to time-dependent variations of the protein structure. Therefore, the measurement of a nonexponential distribution of closed times yields indirect information about internal motions in the protein molecule. The general question is how this information may be extracted by modeling the dynamics of the structural fluctuations that influence the channel behavior.

A straightforward procedure is to fit the measured distribution of closed times by a superposition of a certain number of exponentials, and to interpret the result in terms of a kinetic model containing the same number of protein substates. It is often assumed that the "open" state and the n substates of the "closed" state of a channel form a chain-like sequence,[1] in which transitions are allowed only between nearest-neighbor states (Fig. 1*a*). In this case, the model parameters—the $(2n - 1)$ rates for the transitions between the open state and the n substates of the closed state—are uniquely determined by the fit parameters—the n relaxation times plus $(n - 1)$ parameters for the relative weights of the n exponential terms (see, for example, Colquhoun and Hawkes, 1981, and Hille, 1984). This procedure, which is commonly referred to as the Markov model, is meaningful only if the fit of the closed-time distribution in the time range of the measurement is unambiguous. This is the case if the doubly logarithmic plot of the experimental closed-time distribution shows well separated humps, each of which corresponds to a different relaxation time. However, the decomposition of the measured distribution into a sum of exponentials may be not at all unique, and even the number of exponentials to be used in a fit may not be sharply defined. This is particularly true in the cases where the measured distribution function resembles a power law (McGee et al., 1988) or a fractional exponential (Liebovitch et al., 1987). In view of such ambiguities one may question the basic assumption of the Markov model, according to which only a small number of well-defined substates of the protein molecule are involved in the channel kinetics.

In fact, the total number of configurational substates of a protein molecule is very large. The evidence comes from a variety of sources. We mention the experiments on flash photolysis of CO-liganded myoglobin (Frauenfelder, 1984; Ansari et al., 1987; Frauenfelder, 1988*a*), the measurement of the Debye-Waller (Frauenfelder et al. 1979; Artymiuk et al., 1979; Frauenfelder, 1988*b*) and

[1]Many few-site Markov models with a structure different from that of Fig. 1 a have been used in the literature. Although we focus here on a linear sequential structure, our conclusions remain valid for the other variants.

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the Lamb-Mössbauer factors (Parak et al., 1981; Parak and Knapp, 1984), and the results of computer simulation (see, e.g., Elber and Karplus, 1987).

The existence of the substates is due to the fact that some degrees of freedom of the molecular structure are governed by relatively weak forces, such as van der Waals forces or hydrogen bonds, which allows certain intramolecular rearrangements to take place at normal temperatures (Frauenfelder, 1984, 1988*a*). Simple examples are the reorientation of molecular side groups and the shift of hydrogen bonds. A more general type of rearrangement can be visualized as the diffusion of mobile packing defects which occurs in the otherwise close packed protein structure (Lumry and Rosenberg, 1975; Richards, 1979; McCammon et al., 1983; Englander and Kallenbach, 1984). Building on this knowledge about conformational substates, models of the kinetics of protein channels may be constructed which describe the participation of a large number of substates, without introducing a large number of model parameters at the same time. A model of this kind is Läuger's defect-diffusion model (Läuger, 1988). This model describes the following physical picture of channel closing and reopening. A channel is closed when a molecular group "swings away" from the channel wall into the ionic pathway, which leaves behind a "hole" in the channel wall. The hole can propagate into the interior of the protein. Inside the protein the hole may be visualized as a packing defect in the nearly close-packed structure, to which a certain amount of free volume can be assigned. The molecular group blocking the channel can swing back into the channel wall only if the hole is still there, or has returned from its random walk inside the protein. In more general terms, the idea is that the molecular group can return from its position in the ionic pathway to the channel wall only when the channel wall is in exactly the same state which it occupied when the molecular group swung away. The processes in which the channel wall makes a transition from the original state to other states and back are modeled by the diffusion of defects in the protein molecule. Läuger made a simulation of the random walk on a finite 3-d lattice, obtaining good agreement with experimental data.

An empirical ansatz, which is referred to as a "fractal model" in the literature (Liebovitch et al., 1987) should be mentioned in this context, because it also builds on the existence of a large number of substates. The ansatz uses Kohlrausch's fractional exponential function (Kohlrausch, 1854), which corresponds to a time-dependent transition rate following a power law. It is argued that such a behavior of the transition rate, which is invariant under a change of time scale, can only occur if a continuum of substates is involved in the channel kinetics. A kinetic model leading to Kohlrausch's function, however, is not given. In two recent publications (Korn and Horn, 1988; Mc Manus et al., 1988) a critical comparison of the "fractal model" with the "Markov model" was made for data on a number of different channels.

The model proposed here is based on Läuger's defect-diffusion mechanism. Our first objective is to work out an analytical solution; this is feasible if the random walk is performed on a one-dimensional lattice, and permits the explicit analysis of parameter dependence and asymptotic forms. An argument in support of the one-dimensional model is that the chain structure of the protein molecule may render the defect diffusion very anisotropic, favoring one-dimensional motion along the main chain. Our second objective is to explore the influence of other defects pre-existent in the protein at the moment the channel shuts. This possibility can be related to the Glarum model for dielectric relaxation in glasses (Glarum, 1960; Jäckle 1986). With these goals in mind we now proceed to describe the initial condition for the closure problem and the dynamics of the random walk.

THE MODEL

Consider a semi-infinite one-dimensional lattice (see Fig. 1 *b*). Its site number 1 corresponds to the location at the pore wall where a defect is created by the channel-blocking displacement.

Initial condition

A composite initial condition is assumed. At $t = 0$, the time when the channel closes, a defect (the Läuger defect) is created at site $s = 1$. In the rest of the lattice ($s > 1$) we add a random distribution of defects (the Glarum defects), with a specified concentration c.

We note that the assumption that the channel closes instantaneously is consistent with the experimental obser-

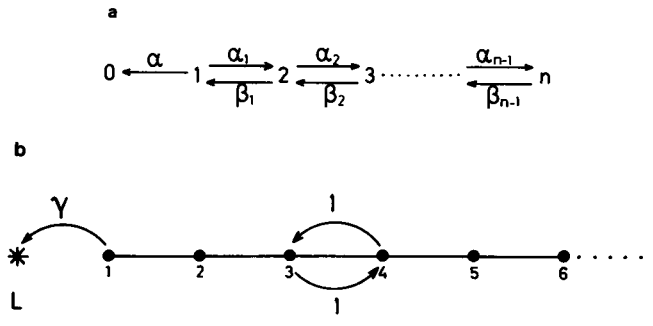

FIGURE 1 (*a*) Kinetic scheme for the Markov model. (*b*) Random walk on a semi-infinite chain with an absorbing boundary. The absorbed walker may be visualized as jumping into a limbo state *L*.

vation that it must occur over an extremely short time scale.

Kinetics

The defects are supposed to be noninteracting (which is probably the most reasonable assumption if the defects are indeed free volumes), and any of them can reopen the channel upon arrival at site $s = 1$. Inside the protein molecule a defect is assumed to diffuse with a constant hopping rate given by Γ. It is convenient to formulate the model using a time scale for which this rate is unity. The relation between the usual, experimental, time t_{exp} (measured in seconds) and the dimensionless scaled time t is then

$$t = \Gamma t_{exp}. \tag{1}$$

The scaled rate at which a closed channel is reopened by a defect at $s = 1$ will be denoted by γ. A defect that reopens the channel can be thought of as leaving the lattice and jumping into a limbo state L. When one of the defects goes into L the channel reopens and the process is considered terminated.

The distribution of open times is usually simpler than that of closed times. In Läuger's picture (Läuger, 1988), the channel closing is described by a rate γ' associated with the displacement of the blocking group into the channel. It is not necessary to consider explicitly the closing process in what follows.

The single defect kinetics can be described in terms of a master equation for the continuous-time random walk:

$$\dot{p}_{ns}(\gamma, t) = p_{n+1,s} + p_{n-1,s} - 2p_{ns} \qquad (n > 1) \tag{2a}$$

and

$$\dot{p}_{1s}(\gamma, t) = p_{2s} - (1 + \gamma)p_{1s}. \tag{2b}$$

Here we have defined $p_{ns}(\gamma, t)$ as the probability that a defect located at site s at time $t = 0$ is at site n at time t, given the absorption rate γ at the boundary.

SOLUTION OF THE MODEL

To solve the model it is convenient to compute the probability $\Phi(\gamma, c; t)$ that a channel that closed at $t = 0$ is still closed at time t. Upon realizing that Φ is the survival probability for all the defects, we can easily get a formula suitable for its evaluation because the defects are assumed to be independent of one another (Tachiya, 1981; Shlesinger and Montroll, 1984)

$$\Phi(\gamma, c; t) = P_1(\gamma, t) \exp\left\{-c \sum_{s=2}^{\infty} [1 - P_s(\gamma, t)]\right\}, \tag{3}$$

where $P_s(\gamma, t)$ is the probability that a walker that started from site s at time $t = 0$ is still somewhere in the lattice at time t, i.e., the probability that such a defect has not reopened the channel. It can be computed from the solution to Eq. 2 as

$$P_s(\gamma, t) = \sum_{n=1}^{\infty} p_{ns}(\gamma, t). \tag{4}$$

It is clear that $P_s(\gamma, 0) = 1$ and $P_s(\gamma, t) < 1$ for all γ, $t > 0$.

From Eq. 3 we can find the probability density $f(\gamma, c; t)$ that the waiting time for the reopening of the channel has a length t by differentiating with respect to the time:

$$f(\gamma, c; t) = -\frac{\partial \Phi}{\partial t}(\gamma, c; t). \tag{5}$$

It should be noted that on the usual time scale of t_{exp} the probability density is obtained by multiplying f by the defect hopping rate, i.e.,

$$f_{exp}(\gamma, c; t_{exp}) = \Gamma f(\gamma, c; t). \tag{6}$$

The noninteracting nature of the walkers permits the calculation of the $p_{ns}(\gamma, t)$'s and, consequently, the solution to the problem, using the normal modes method of Van Kampen and Oppenheim (Van Kampen and Oppenheim, 1972; Van Kampen, 1981). The full details will be presented in a forthcoming publication (Condat, 1989). It is useful to mention, however, that, while for $\gamma \leq 2$ only a continuous spectrum of eigenvalues is present, for $\gamma > 2$ an isolated point, representing a surface state, must be added. This surface state plays a crucial role in the determination of the short-time behavior of the solution.

RESULTS

Next we summarize the results obtained for $f(\gamma, c; t)$.

(*a*) An explicit solution of the model equations in terms of known functions can be given if $\gamma = 1$ or $\gamma = 2$. In these cases f can be expressed by the modified Bessel functions $I_0(2t)$ and $I_1(2t)$ as follows:

$$f(1, c; t) = e^{-2t}\{t^{-1}I_1 + ce^{-2t}[(I_0 + I_1)^2 - t^{-1}I_1(I_0 + I_1)]\} \cdot \exp\left(c\left\{\frac{3}{2} - e^{-2t}\left[\left(\frac{3}{2} + 2t\right)I_0 + (1 + 2t)I_1\right]\right\}\right), \tag{7}$$

$$f(2, c; t) = 2e^{-2t}[I_0 - I_1 + ce^{-2t}I_0I_1] \cdot \exp\{c[1 - e^{-2t}(I_0 + 2tI_0 + 2tI_1)]\}. \tag{8}$$

The appearance of the factor $\exp(-2t)$ multiplying each of the Bessel functions is characteristic of the solutions to the model under consideration. The Glarum defects con-

tribute the elements containing the factor c in Eqs. 7 and 8. Setting $c = 0$ we are left with the contribution of Läuger's defect. There is no surface state component in Eqs. 7 and 8. (*b*) Approximate analytical solutions can be obtained from expressions for large γ and for $|\gamma - 1| \ll 1$. These can also be written in terms of $e^{-2t}I_0(2t)$ and $e^{-2t}I_1(2t)$. The large γ solution is presented in Appendix B. (*c*) The long term behavior of $f(\gamma, c; t)$ is given by

$$f(\gamma, c; t) = \left[\frac{c}{\pi\gamma t} + \frac{1}{2\gamma\pi^{1/2}t^{3/2}}\right] \cdot \exp\left\{c\left[-\frac{2t^{1/2}}{\pi^{1/2}} + \frac{1}{2} + \frac{1}{\gamma} - \left(\frac{1}{8} + \frac{1}{\gamma^2}\right)\frac{1}{(\pi t)^{1/2}}\right]\right\} \quad (9)$$

($t \gg |1 - \gamma|\gamma^{-2}$, $t \gg 1$). The $t^{-3/2}$ term in the prefactor is the contribution of Läuger's defect. This is the general form for a single defect performing a random walk on a semi-infinite chain with an absorbing boundary. The proportionality with γ^{-1} is reasonable, because for large γ there is an enhanced probability of early pore opening; obviously this reduces the chances of a late opening.

The first term in the exponential of Eq. 9 is dominant at very long times. It corresponds to Bordewijk's solution of the Glarum model on a continuum (Bordewijk, 1975). As noted by Blumen et al. (1986), the $t^{1/2}$ stretched exponential should be a general feature of this type of model. The $t^{1/2}$ term is also independent of γ (except through the prefactor, of course), which means it is controlled by the walk dynamics and not by the absorption process at the pore; it represents the contribution of defects originally located at distances of the order of $t^{1/2}$ from the channel.

The following term in the exponential is time-independent but not γ-independent. It becomes important at small values of γ, for which there will be on the average many passages of the defects through site 1 before the channel is opened. For small values of γ one must wait very long times before the Bordewijk asymptotic form takes over.

(*d*) For arbitrary values of γ and t, the result may be expressed in terms of integrals that are well-suited for rapid numerical evaluation (see Appendix A).

ANALYSIS

The infinite one-dimensional and the finite three-dimensional models have an important feature in common. In both cases the Läuger defect must return to the origin. Hence, the channel will be reopened at $t < \infty$ for any $\gamma > 0$, even in the absence of Glarum defects. In our description this is expressed through the equation

$$\int_0^\infty f(\gamma, c; t)dt = 1. \quad (10)$$

Note that in an infinite three-dimensional lattice the Läuger defect can migrate to infinity, and thus Eq. 10 can be valid only for $c > 0$.

Since the site $s = 1$ is known to be occupied by a defect at $t = 0$, then the initial value of f is given by

$$f(\gamma, c; 0) = \gamma. \quad (11)$$

This equation can also be verified by an explicit evaluation of the solution.

At large values of γ, the Läuger defect is strongly privileged, the Glarum defects becoming qualitatively important only at very long times. The large γ solution is presented in full in Appendix B. However, given the weak dependence on c at short and moderate times, it is useful to look at the much simpler $c = 0$ case, for which Eq. B1 reduces to:

$$f(\gamma, 0; t) = \frac{e^{-2t}}{\gamma t}\left\{-\frac{2}{\gamma}I_0(2t) + \left[1 + \frac{2}{\gamma}\left(1 + \frac{1}{t}\right)\right]I_1(2t)\right\} + \left(\gamma - \frac{1}{\gamma} - \frac{2}{\gamma^2}\right)\exp\left[-\gamma^2 t/(\gamma - 1)\right]. \quad (12)$$

The last term in this equation, which is due to the surface state, is at $t = 0$ roughly proportional to γ, and at times $t < \gamma^{-1}$ its contribution is much larger than that of all the continuum states taken together. Therefore, we have initially a well-defined exponential decay. This behavior is clearly seen in the curves for $\gamma = 10$ and $\gamma = 30$ in Fig. 2

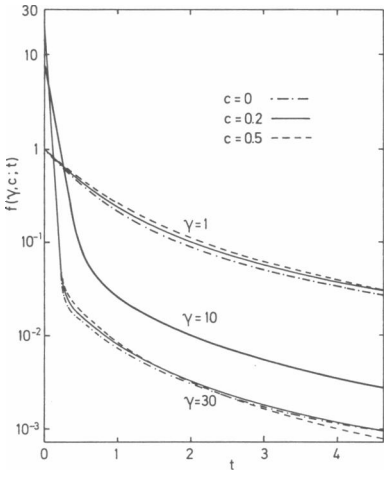

FIGURE 2 Closed-time distribution as a function of time for several values of the parameters γ (rate constant for channel reopening) and c (density of Glarum defects). Note the well-defined short-time exponential decay in the $\gamma = 10$ and $\gamma = 30$ curves.

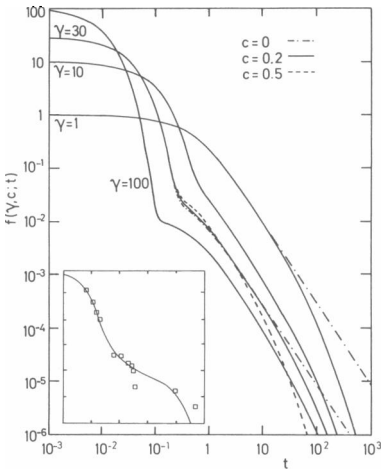

FIGURE 3 Closed time-distribution on a log–log scale. The different regimes can be observed. The long-time behavior is given either by a power law ($c = 0$) or by a stretched exponential ($c > 0$). In the inset the data for an end-plate channel (Colquhoun and Sakmann, 1985) are plotted on a log–log scale together with the results of Läuger's simulation (from Läuger, 1988). See text.

In the last case, $f(t)$ decays by three orders of magnitude almost as a pure exponential. The exponential regime can be thought of as describing the intraburst region observed in many experiments. (For example see Colquhoun and Sakmann, 1985).

At longer times, the continuum solution dominates and a long tail results. This is consistent with the often observed fact that long closed times appear far more frequently than it would be expected from a purely exponential distribution.

In Fig. 3 we show $f(\gamma, c; t)$ on a log-log scale, which allows us to follow its behavior over several time decades. In the large γ curves a shoulder is seen to appear beyond the exponential region; this shoulder becomes more marked with increasing γ. The Läuger-dominated $f(\gamma, 0; t) \sim t^{-3/2}$, and Glarum-dominated, $f(\gamma, c \neq 0; t) \sim \exp(-ct^{1/2})$, asymptotic regimes become evident at long times.

COMPARISON WITH EXPERIMENTS

Rabbit corneal endothelium channel

To test the predictions of the theory it is necessary to look at single-channel experimental data that extend over a relatively long time scale. An appropriate experiment is that performed on a nonselective channel in the rabbit corneal endothelium (Liebovitch et al., 1987). The data include 1,465 closed times and extend over three orders of magnitude in time. In Fig. 4 we plot the data, together with the results of Läuger's simulation and those of the calculation presented here. Läuger (1988) allowed the defect to perform a random walk on a cube containing 125 sites; he took $\gamma = 33$ and a jump rate of $(12/11)s^{-1}$. In Fig. 4 we chose $\gamma = 2$ and a jump rate of $32\ s^{-1}$.

We can see that, while the curve for $\gamma = 2$, $c = 0.5$ gives an excellent agreement with the experimental results, that for $\gamma = 2$, $c = 0$ yields a poorer representation. If $c < 0.5$, it is still possible to obtain a very good fit by increasing the value of γ. A relatively large c, however, permits a better adjustment, because it yields the long-term downward slant that is absent in the single-defect case. Note that we obtain a good fit with a value of γ smaller than that used by Läuger (1988). This may be due to the fact that in our one-dimensional version of the model the walker at site 1 can only jump into the limbo state or into site 2 (see Fig. 1). In the three-dimensional version, the walker in the site next to the channel has several paths available; this makes γ relatively less efficient. However, because our Γ is larger than Läuger's, the corresponding dimensional rates $\gamma\Gamma$ differ by a factor of <2.

Due to the finite experimental resolution, it is clear that some of the briefest closed events must have gone undetected. Therefore, the total number n of closed events must be taken as an adjustable parameter, which permits a vertical shift of the curve without changing its shape. We took $n = 1{,}560$. Liebovitch et al. (1987) obtained a good fit of their data using Kohlrausch's fractional expo-

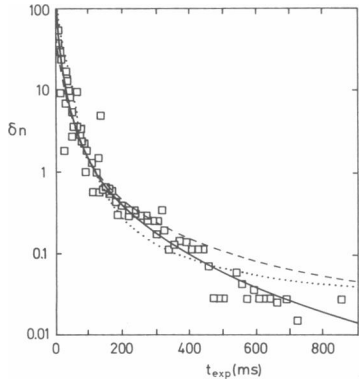

FIGURE 4 Closed-time distribution for a channel in the rabbit corneal endothelium. δn is the number of events per millisecond, $\delta n = n\Gamma f(t_{exp}) \cdot (10^{-3}s)$. The experimental data (*squares*) of Liebovitch et al. (1987) are plotted together with the results of Läuger's simulation (···) (Läuger, 1988) and our own results for $\gamma = 2$, $c = 0.5$ (—) and $\gamma = 2$, $c = 0$ (---). We took $\Gamma = 32\ s^{-1}$ and $n = 1{,}560$.

nential function (Kohlrausch, 1854) as an empirical ansatz (see their Fig. 13). The statistical significance of the fits was tested and compared with the multi-exponential fitting of the Markov model by Korn and Horn (1988). Although five fit parameters were used—compared with only two for the Kohlrausch function—the result of the Markov model was not significantly better for this type of channel. In other cases, however, in which a doubly logarithmic plot of the closed time distribution shows oscillatory behavior, the use of the Kohlrausch function was found to be unsatisfactory compared with the results of the Markov model (Mc Manus et al. 1988, Korn and Horn, 1988).

In the case of the rabbit corneal endothelium channel one could discriminate between Liebovitch's fractal model and our defect-diffusion model if data for shorter times were available. While in the model discussed here, as well as in the original version due to Läuger (1988) $f(t = 0)$ is finite, in the proposal of Liebovitch et al. (1987) $f(t)$ increases without limit as $t \rightarrow 0$ (although, of course, the model has a proper distribution, i.e., Eq. 10 is satisfied). In particular, we conclude that the number of undetected brief events in the experiment under consideration must have been of the order of 10^2. On the other hand, Liebovitch et al. (1987) had to assume that n = 11,000, which would imply that the number of undetected events exceeded by an order of magnitude the number of recorded ones.

Frog end-plate channel

Läuger (1988) has obtained a good fit for another experiment performed over a long time interval, that of Colquhoun and Sakmann (1985) on a suberyl-dicholine-activated frog end-plate channel. The experimental data (*squares*) as well as Läuger's fit (*continuous line*) are plotted on a log-log scale in the inset of Fig. 3. The horizontal axis in the inset corresponds to t_{exp} varying between 10^{-5} and 1 s. The vertical axis indicates the number of events per second. The scale runs from 10^2 to 10^8 (Läuger, 1988).

Although we can also obtain a good fit for all the points corresponding to closed times shorter than 10 ms (for example, taking $\gamma \sim 20$, $\Gamma \sim 10^3$ s^{-1} and $n \sim 3{,}000$), we cannot account for the two points corresponding to the longest intervals: a curve that describes reasonably well the rest of the data always passes well below these points. It is certainly possible that there are other channels, having relatively long activation times, in the patch considered. These extra channels could account for the long-time data. If there was really only a single channel present and we want to understand its behavior in terms of the defect-diffusion model, we have to conclude that either (*a*) the diffusion is effectively three-dimensional, or (*b*) it is one-dimensional but there are traps where the defect can dwell for times longer than it would in a normal site.

NG108-15 neuroblastoma x glioma channel

McGee et al. (1988) studied a voltage-sensitive K^+ channel in NG108-15 cells. One of the main goals of that work was to examine the effects of changes in membrane lipid composition on the properties of the channel. To this end they enriched the cell phospholipids with polyunsaturated fatty acids. In Fig. 5 we reproduce their results for the closed time probability density functions corresponding to cells enriched through the addition of arachidonic acid (20:4) (the enriched patches), as well as to cells that were not subjected to this treatment (the control patches).

Although Mc Gee et al. (1988) used a superposition of six exponentials to fit the resulting curves, we can see from Fig. 5 *a* that the simple form $f(t) = \alpha t^{-3/2}$ fits well the data from the control patches over almost four orders of magnitude in time. This is exactly the behavior corresponding to a Läuger defect in the long-time regime. At the longest times ($t_{exp} > 1$s) the data points lie below the $t^{-3/2}$ line. This would be consistent with the presence of a low concentration of Glarum defects, which become asymptotically dominant. Deviations from $t^{-3/2}$ are also observed at the shortest measured times. Because the curve turns upward, we estimate that γ is in the range $2 < \gamma \lesssim 10$. (For higher values of γ a shoulder is present. [See Fig. 3])

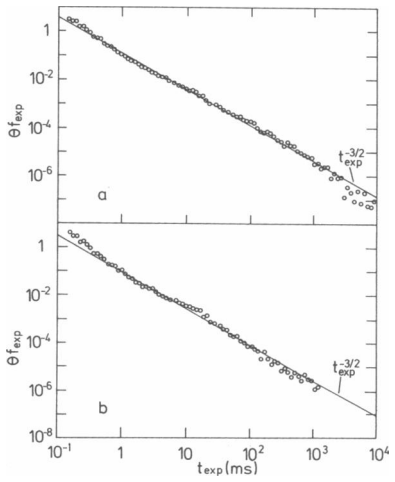

FIGURE 5 Closed-time distribution for the K^+ channel in NG 108-15 cells. The experimental data (*circles*) of McGee et al. (1988) are plotted for the control (*a*) and enriched (*b*) patches. The enrichment was due to the addition of arachidonic acid. The straight lines have a slope of $-3/2$. The constant Θ is proportional to the product $n\gamma\Gamma$.

Once the $t^{-3/2}$ form is accepted, a single fitting parameter (α) is left. Because α depends on Γ, the jump rate, and the total number of closed events, these three parameters cannot be separately identified when only the long-time data are available. On the other hand, the fact that the power law behavior becomes evident at times shorter than 1 ms suggests that the defect jump rate must be quite high.

For the enriched patches there is a gentle oscillation superimposed on the $t^{-3/2}$ line. These oscillations may be accounted for by a perturbation of the defect diffusion due to the enrichment. It is conceivable that the agonist causes a slight variation of the different transition rates (cf. Fig 1 *b*), which renders the defect diffusion along the chain of substates inhomogeneous and leads to the observed deviation from the ideal $t^{-3/2}$-behavior. While the Läuger defect diffusion still plays the main role, the enrichment is responsible for the onset of a second, much weaker, process that generates the observed fluctuations.

It is our opinion that the $t^{-3/2}$ behavior observed over a wide time range is a strong evidence in favor of the defect-diffusion model. This model does not require the parameter proliferation that ensues if one insists on making a fit with a superposition of exponentials.

Gramicidin A channel

Ring (1986) points out his gramicidin A channel measurements may be fit with a single process proportional to t^{-x}, with $1.5 < x < 2$. His multiple regression plot gives $x = 1.7$. (There is apparently a typographic error in the caption to his Fig. 7; the values $x = 1.7$ and $x = 1.9$ are transposed). This suggests that the channel may have been observed in the long-time regime of a Läuger defect diffusing essentially along a one-dimensional path.

Rat skeletal muscle channel

Blatz and Magleby (1986) investigated single fast Cl^- channels from rat skeletal muscle. They fit their data, which cover almost five orders of magnitude in time, with a superposition of five exponential components. We note, however, that the data seem to oscillate about the line t^{-x}, with $x \sim 1.65$. Although the oscillations (or humps) are more marked than in the case of the enriched neuroblastoma *x* glioma channel, the results suggest that a modified Läuger defect-diffusion process is also effective here.

Rat colonic channel

R. Reinhardt et al. (1987) studied the properties of an anion-selective channel from rat colonic enterocyte plasma membranes reconstituted into plane phospholipid bilayers. From an experimental record lasting 380 s they obtained a closed-time histogram that included 2,888 events. They used a bin-width of 1 ms and fit the data with a superposition of two exponentials. Taking a jump rate of the order of 670 s^{-1} and $n > 4{,}500$, it is possible to obtain a good fit with values of γ ranging from $\gamma \simeq 2.5$ up to $\gamma \simeq 6$, with $c \gtrsim 0.2$.

The data are clearly consistent with a defect-diffusion interpretation. Unfortunately, the histogram does not span a range of time wide enough to allow a precise determination of γ.

SENSITIVITY TO EXTERNAL PARAMETERS

The channel kinetics can be affected by different external factors e.g., the temperature, applied potential, agonist nature and concentration, frequency of applied field, etc. In the model discussed here, these external factors influence the measured closed-time distribution only through their effect on the three parameters γ, c, and Γ. Experimental variation of such factors therefore provides an opportunity for a stringent test of the applicability of the model to a given channel. In general, when the dependence of the model parameters upon externally controlled conditions is not known, the parameter values need to be determined by fitting for every independent measurement. However, some external factors, like the electrolyte agonist concentration or an applied electric field, are likely to influence mainly the jump rate γ of the molecular group blocking the channel, and to have little effect on the defect concentration and hopping rate in the interior of the protein molecule. In such cases, the variation of the closed-time distribution is essentially determined by its dependence on γ. Therefore it is useful to analyze in detail the dependence of our result for f on γ, neglecting the contribution of the Glarum defects. This is done by looking at the first derivative, f_γ, of f with respect to γ. We obtain,

$$f_\gamma(\gamma, 0; t) = \frac{2}{\pi}\int_0^\pi dq \frac{\sin^2 q[2(1 - \cos q) - \gamma^2]}{[1 + 2(\gamma - 1)\cos q + (\gamma - 1)^2]^2} \cdot \exp[-2t(1 - \cos q)] + \theta(\gamma - 2)\left[\gamma^3 - 3\gamma^2 + 4\gamma - \frac{t\gamma^3(\gamma - 2)^2}{\gamma - 1}\right] \frac{\exp[-\gamma^2 t/(\gamma - 1)]}{(\gamma - 1)^3}. \quad (13)$$

Here $\theta(\gamma - 2)$ is the step function, which indicates the contribution of the surface state for $\gamma \geq 2$. The short-time form of Eq. 13 is given by

$$f_\gamma = 1 - (2\gamma + 1)t + 0.5(3\gamma^2 + 4\gamma + 2)t^2 + \ldots, \quad (14)$$

while the long-time limit is (see Eq. 9),

$$f_\gamma \simeq -\frac{1}{2\pi^{1/2}\gamma^2 t^{3/2}}. \tag{15}$$

The function f_γ is plotted in Fig. 6 for several values of γ. In all cases the curves start from unity at $t = 0$ and go to zero asymptotically from below as $t \to \infty$. In the intermediate region they show a minimum, which becomes deeper and occurs at shorter times for large values of γ.

At short times (shaded areas in the inset to Fig. 6), f grows with an increase in γ. As t evolves, f grows less and less with an increment in γ; beyond the solid line in the inset, the response of f to a modification in γ changes sign. The minima of f_γ are reached along the dashed line.

When $\gamma \gg 2$, the vanishing of the response (i.e., $f_\gamma = 0$), occurs at a time $t \simeq \gamma^{-1}$. The reason for this is that the real solution (surface state) contribution vanishes at $t \simeq \gamma^{-1}$, a fact that may be easily verified. This contribution goes very fast from positive to negative values whose magnitude is much larger than that of the contribution of the complex solutions. Another general observation is that the minimum in f_γ always occurs at a time which is slightly below twice the time at which $f_\gamma = 0$.

The sensitivity of f to changes in γ is then well-determined in this defect-diffusion model. However, the dependence of γ (or c, or the jump rate) on the external parameters is not specified *a priori*. It can be determined only via experimental observations.

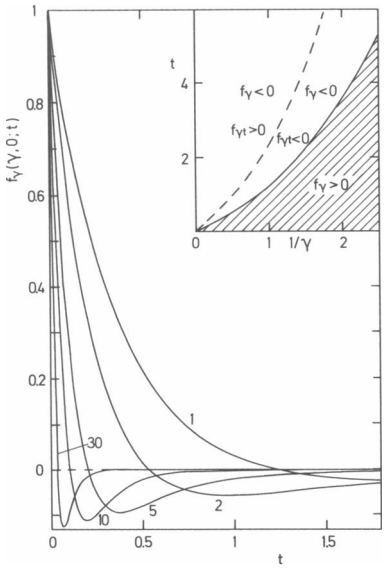

FIGURE 6 First derivative f_γ of the distribution of closed times with respect to γ, as a function of time. The numbers next to the curves are the corresponding values of γ. The solid line in the inset separates the regions where $f_\gamma > 0$ (*shaded*) and $f_\gamma < 0$. The dashed line gives the position of the minima of f_γ. The time derivative of f_γ is indicated as $f_{\gamma t}$.

Suppose a fast exponential is clearly identifiable in a set of experimental data for a given channel. By clearly identifiable we mean that, in a fit using several exponentials, the time constant τ_B corresponding to the briefest closures must be much smaller than all the other time constants. The existence of a fast exponential suggests a high value of γ, the fast exponential itself corresponding to the surface state contribution. We can obtain a relation between γ and Γ by equating $\Gamma\tau_B$ to the time constant in the last term of Eq. 12:

$$\frac{\gamma^2}{\gamma - 1} = \frac{1}{\Gamma\tau_B}. \tag{16}$$

Colquhoun and Sakmann (1985) studied the effects of different agonists on the end-plate channel currents. Because the briefest time constant is clearly identifiable in each case, we can apply Eq. 16. (In a few of their experiments, they observed signs of the possible presence of a superfast exponential, which we disregard in this analysis.) Under the assumption that the internal jump rate Γ is not substantially affected by the agonist, we estimate the following values of γ for the various cases: Acetylcholine, $\gamma \simeq 54$; suberyldicholine, $\gamma \simeq 21$; carbachol, $\gamma \simeq 70$; decan-1, 10-dicarboxylic acid dicholine ester, $\gamma \simeq 15.6$. Here we used the value of Γ obtained from our fit to the suberyldicholine data. Although the jump rate may be expected to depend on temperature, the differences between the temperatures at which the various runs were performed were probably too small to generate detectable changes. Variation in the agonist concentration did not modify the observed value of τ_B, so γ and Γ seem to be independent of the concentration. From the data for this channel it is also apparent that the dependence of these rates on the applied potential must be quite weak.

SUMMARY AND OUTLOOK

An analytical solution to Läuger's defect-diffusion model in one dimension has been worked out. The influence of pre-existent defects in the protein has been investigated. Their contribution, although relatively small (except at very long times) was shown to be important in the cases of the corneal endothelium and the rat colonic channels. It accounts also for the $t_{exp} > 1$ s downturn in the curves for the NG108-15 channel.

It is opportune to remark here that, the protein being a finite structure, only a limited (although probably large) number of sites is available for the walk. For the M-site lattice our predictions break down for times $t \gtrsim M^2$. The experiments of Liebovitch et al. (1987) and Reinhardt et

al. (1987) extended up to $t \sim 32$ and $t \sim 20$, respectively. Hence, the number of sites that effectively took part in the diffusion process is of the order of 10. The experiment of Colquhoun and Sakmann (1985), on the other hand, extended up to $t \sim 500$, so ~50 sites must have participated.

Some qualitative aspects of the contribution of the Glarum defects may remind us of a finite-size system. In both cases the value of $f(t)$ at intermediate times is higher than that corresponding to a single Läuger defect in the semi-infinite chain. From Eq. 10 we see then that in both cases the value of $f(t)$ at long times must drop below the $t^{-3/2}$ prediction for the Läuger defect in the semi-infinite chain.

We note that a model described by a master equation analogous to Eq. 2, with $s = 1$, was analyzed independently in a just published work (Millhauser et al., 1988). This master equation was obtained by assuming that the ion-channel proteins have a very large number of states of similar energy. The authors considered explicitly the $\gamma = 1$ and $\gamma \ll 1$ cases and obtained the asymptotic behavior of $f(t)$, which is exactly the one that follows by setting $c = 0$ in our Eq. 9. They also pointed out the power-law-like behavior of $f(t)$ in the experiments of Ring (1986) and Blatz and Magleby (1986). A computer simulation for an M-site system was made, showing that at times $t \sim M^2$ the $t^{-3/2}$ behavior of $f(t)$ is cut off by finite-size effects, as it should be expected.

The diversity of the channels existing in nature is enormous. Therefore one cannot expect a model as simple as ours to account for all the data. E.g., there are cases where a kinetic model containing only a small number of substates (referred to as the Markov model, see Fig. 1 *a*) gives a better fit of the data. At this point it is of interest to note that our defect diffusion model (Fig. 1 *b*) could be generalized so as to include the Markov model as a limiting case. This could be achieved by allowing the (asymmetric) transition rates for a small number of sites to be different from the common value (denoted by Γ). If, in particular, the transition rates were modified in such a way that a small number of sites in the chain act as traps, the long-time behavior would be governed by these trap states. In this case the model would lead to a closed-time distribution similar to those described by the Markov model (cf. the discussion in McManus et al. 1988). In principle, a continuum of different versions of the defect-diffusion model could be constructed, with our version of a "democratic" participation of all states and a "two-class" version with many transition states and a small number of dominant traps as limiting cases. Needless to say, it would be of interest to work out (by computer simulation or analytically) the solution of such a generalized defect-diffusion model for the channel kinetics.

APPENDIX A:

Integrals for the evaluation of $f(t)$

In this appendix we express $f(\gamma, c; t)$ in terms of integrals, which are readily evaluated numerically for arbitrary values of γ, c, and t.

Taking the derivative of Eq. 3 we arrive at

$$f(\gamma, c; t) = \{cP_1(t)[\dot{P}_1(t) + \gamma P_1(t)] - \dot{P}_1(t)\} \exp\{c[1 - P_1(t) - Q(t)]\}. \quad \text{(A1)}$$

The various functions appearing here may be calculated using the following forms:

$$P_1(t) = \int_0^\pi dq(1 + \cos q)\psi(q, t) + \left(\frac{\gamma - 2}{\gamma - 1}\right) g(t)\theta(\gamma - 2), \quad \text{(A2)}$$

$$\dot{P}_1(t) = -2\int_0^\pi dq \sin^2 q\psi(q, t) - \frac{\gamma^2(\gamma - 2)}{(\gamma - 1)^2} g(t)\theta(\gamma - 2), \quad \text{(A3)}$$

and

$$Q(t) = \frac{\gamma}{2}\int_0^\pi dq\{\exp[2t(1 - \cos q)] - 1\}h(q)\psi(q, t) - \frac{(\gamma - 2)}{\gamma}[g(t) - 1]\theta(\gamma - 2). \quad \text{(A4)}$$

Here $\theta(\gamma - 2)$ is the step function. We have also defined:

$$g(t) = \exp[-\gamma^2 t/(\gamma - 1)], \quad \text{(A5a)}$$

$$h(q) = (1 + \cos q)/(1 - \cos q), \quad \text{(A5b)}$$

and

$$\psi(q, t) = \frac{\gamma \exp[-2t(1 - \cos q)]}{\pi[\gamma^2 + 2(1 - \gamma)(1 - \cos q)]}. \quad \text{(A5c)}$$

The terms containing $g(t)$ have their origin in the surface state, active for $\gamma > 2$. This contribution is important only at short times.

APPENDIX B

The $\gamma \gg 2$ case

If $\gamma \gg 2$, all the quantities appearing in Eq. A1 can be evaluated in closed form. We obtain

$$f(\gamma, c; t) = (A_1 + cA_2) \exp(cB), \quad \text{(B1)}$$

where

$$A_1 = \frac{e^{-2t}}{\gamma t}\left\{-\frac{2I_0}{\gamma} + \left[1 + \frac{2}{\gamma}\left(1 + \frac{1}{t}\right)\right]I_1\right\} + \frac{\gamma^2(\gamma - 2)}{(\gamma - 1)^2} g(t), \quad \text{(B2)}$$

$$A_2 = \frac{e^{-4t}}{\gamma}\left(I_0 + I_1\right)\left(I_0 + I_1 + \frac{I_1}{\gamma t}\right) + \frac{\gamma(\gamma-2)e^{-2t}}{(\gamma-1)^2} \cdot \left[\left(1 - \frac{2}{\gamma}\right)I_0 + \left(1 - \frac{2}{\gamma} - \frac{2}{\gamma^2 t}\right)I_1\right] g(t) - \frac{\gamma(\gamma-2)^2}{(\gamma-1)^3} g^2(t), \quad \text{(B3)}$$

and

$$B = \frac{1}{2} + \frac{1}{\gamma} - e^{-2t}\left[\frac{I_0}{2} + 2t(I_0 + I_1) + \frac{1}{\gamma^2}(I_0 + I_1)\right] - \frac{(\gamma-2)}{\gamma(\gamma-1)} g(t). \quad \text{(B4)}$$

The function $g(t)$ was introduced in Eq. A5a. The argument of the Bessel functions is again $2t$ everywhere. The sources for the different terms are easy to identify. By explicit evaluation and comparison with the results of the numerical integration (Appendix A), we can verify that the large γ approximation embodied in Eq. B1 works well down to values of γ not very far from 2.

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