Reptation theory of ion channel gating

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ABSTRACT Reptation theory is a highly successful approach for describing polymer dynamics in entangled systems. In turn, this molecular process is the basis of viscoelasticity. We apply a modified version of reptation dynamics to develop an actual physical model of ion channel gating. We show that at times longer than microseconds these dynamics predict an α-helix-screw motion for the amphipathic protein segment that partially lines the channel pore. Such motion has been implicated in several molecular mechanics studies of both voltage-gated and transmitter-gated channels. The experimental probability density function (pdf) for this process follows $t^{-3/2}$ which has been observed in several experimental systems. Reptation theory predicts that channel gating will occur on the millisecond time scale and this is consistent with experimental results from single-channel recording. We examine the consequences of reptation over random barriers and we show that, to first order, the pdf remains unchanged. In the case of a charged helix undergoing reptation in the presence of a transmembrane potential we show that the tail of the pdf will be exponential. We provide a list of practical experimental predictions to test the validity of this physical theory.

I. INTRODUCTION

The physical basis of ion channel gating remains a mystery. In a number of recent and important studies the structure of several ion channel systems are becoming clear but the conformational change associated with the gating process is still open to conjecture (see for example, Grenningloh et al., 1987). Following the lead to allosteric models in enzymology and other branches of biochemistry, traditional gating models assume a small number of open and closed channel states connected by first-order rate constants. Solution of the master equation for such models predicts that the lifetime probability density function (pdf) for channel opening or channel closing will be distributed as a multiexponential function (Colquhoun and Hawkes, 1983). Multiexponential functions do fit the pdf's, however, the distinct underlying states have not been identified nor are they concretely suggested by recent structural models.

While addressing such issues, several researchers have noticed a striking pattern in the closed time distributions of a number of channel systems, most of which are transmitter-gated. In these particular channels it appears that the pdf follows an algebraic, or power law, decay of the form

$$f(t) \approx t^{-a}, \tag{1}$$

where $a \approx 3/2$ (Millhauser et al., 1988*a*; Condat and Jäckle, 1989). This law is obeyed over many decades in time. The rate-amplitude correlation provides evidence for this type of decay law in four different ion channel systems (Millhauser et al., 1988*b*). In past work we showed that a simple kinetic model for such a pdf is a one-dimensional (1D) diffusion process (Millhauser et al., 1988*a*).

Power law relaxation profiles are common in solid-state physics and low-temperature biological systems (Ansari et al., 1985), and several researchers have borrowed important models from these disciplines to explain gating kinetics. For instance, Liebovitch's fractal model of ion channel gating postulates that there are energy minima within energy minima on the ion channel protein potential energy surface and that the associated kinetics follow a fractal scaling scheme (Liebovitch et al., 1987). In the limit of high fractal dimension ($D \rightarrow 2$) this model gives the law expressed in Eq. 1 although the value of a is not specified. More recently Läuger (1988) used the concept of defect diffusion to explain the closed time behavior of the acetylcholine receptor (AChR). Such models were initially developed to describe how solvent and ligand molecules are able to diffuse into the interiors of large proteins. Condat and Jäckle (1989) solved Läuger's model exactly for the one-dimensional case by using a master equation identical to the one previously postulated by us. The essence of their model is that channel closing results in the formation of a small defect near the channel lumen. This defect is free to diffuse through the channel protein, and the channel remains closed until the defect returns to the area where it was formed. Rubinson (1982)

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and Levitt (1989) have both used the 1D diffusion scheme to develop continuum models of voltage-dependent channel gating. In Levitt's approach, the diffusion process is attributed to the rotation of an α-helix such as that in the helix-screw model of sodium channel gating (Catterall, 1988; Armstrong, 1981). In our own work we have suggested that the 1D diffusion process may be related to (*a*) a twisting motion of the channel protein such as that proposed for the gap junction or (*b*) an α-helix $\rightarrow$ coil transition which is a 1D process in the configuration space of hydrogen bonds.

Our goal here is to propose a new physical mechanism behind the apparent 1D diffusion process as suggested by Eq. 1. It is quite possible that a helix-screw process is responsible for channel gating and we describe this as originating from polymer reptation dynamics. If the idea of polymer reptation is accepted, then the helix-screw model arises as a natural consequence. Our ideas are developed in light of the following observations:

(*a*) Molecular biological studies indicate that putative transmembrane amphipathic sequences are often highly conserved in both ligand-gated (Boulter et al., 1987) and voltage-gated channels (Salkoff et al., 1987). Very recently, the amphipathic S4 sequence in the first homologous repeat of the sodium channel was modified and shown to be the probable carrier of gating charge (Stühmer et al., 1989). Most researchers agree that such transmembrane segments are probably α-helical in nature.

(*b*) The helix-screw model, as developed by Armstrong, provides an explanation of gating charge. Molecular mechanics studies have shown that such a model is plausible for sodium channels (Guy and Seetharamulu, 1986).

(*c*) Studies in molecular mechanics have recently demonstrated that gating in the AChR may also involve a type of helix-screw process (Vogelaar and Chan, 1989).

(*d*) Unwin (1986) has pointed out the structural similarities among a wide range of channel systems, including both voltage-activated and transmitter-activated, and has suggested that all channels may share a common gating mechanism.

(*e*) Reptation dynamics uses the concept of defect diffusion but gating arises in a way quite different than that proposed by Condat and Jäckle (1989).

(*f*) Reptation dynamics gives rise to the pdf of Eq. 1.

Below we outline the reptation process which was originally proposed by de Gennes (1971, 1983*b*) to explain polymer creep in highly entangled systems. We speculate on the expected time scale for such processes and demonstrate that there are several important consistencies. The effect of random potential barriers is then briefly discussed. Finally, we discuss the behavior of a charged helix in the presence of a transmembrane potential and we conclude with several experimental predictions that may be testable in the near future.

II. REPTATION THEORY OF CHANNEL GATING

In the helix-screw model of voltage-gated channels, helix translation arises from the driving force of the transmembrane potential acting upon the charged residues of the helix (Catterall, 1988). If we are going to apply a helix-screw description to transmitter-gated channels we must develop a mechanism for random helix translation in the absence of an applied potential. The highly successful reptation theory provides such a mechanism.

Consider a polymer chain entangled in a polymer network. Movement of this chain is highly restricted and mostly occurs within a 'tube' defined by the network (de Gennes, 1971; Doi and Edwards, 1986). In standard reptation theory the structure of the tube is random and is often described by a Gaussian distribution. After a predictable interval in time the polymer diffuses a distance similar to its curvilinear length and the tube is renewed. For our purpose here we must depart from a description that uses a random tube. Let's assume that the reptating helix of interest is one of the amphipathic helices lining the channel pore. Most of the helix surface area is in contact with adjacent helices and it has been proposed that the helix–helix packing follows a ridges-into-grooves motif (Guy and Seetharamulu, 1986). Thus the neighboring helices provide a rigid, tubelike environment in which the helix of interest translates. A two-dimensional projection of a helix within this helical tube is shown in Fig. 1. The end-to-end length of the tube is approximately equal to the thickness of the membrane. The transmembrane segment of channel protein that forms part of the channel pore is certainly helical when in the tube. The ends of the helix which connect to the intracellular and extracellular domains of the channel may be of either helical or random coil configuration. As the protein chain moves through the tube, new helical segments will be formed upon entering the tube and existing helical segments may likewise assume a random coil structure upon leaving the tube. To complete this picture we place a blocking region represented as a gray area along the helix backbone. This blocking region, which may be one or two charged residues or side-chains that introduce steric constraint, restricts ion flow when it is along the channel pore. Recognizing that closed-time distributions from single-channel recording usually exhibit power law decays we assume that the channel remains closed as long as any part of the blocking region is along the channel pore. Ultimately, there will be a limit to the extent of movement through the tube due to structural constraints

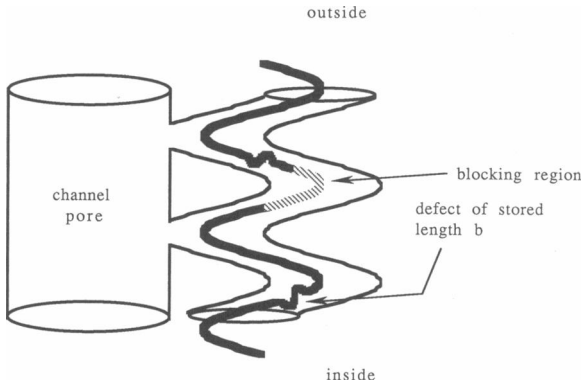

FIGURE 1 Schematic two-dimensional view of the transmembrane amphipathic α-helix backbone (*bold line*) which forms part of the lining of the ion channel. In a real transmembrane helix there would be approximately seven turns but we only show three for clarity. The tube around the helix is formed by the adjacent α-helices in the channel protein. These helices are in close contact except where the amphipathic helix lines the pore. The diffusing defects, which give rise to reptation of this amphipathic segment, are shown as small distortions of the backbone. Net movement of this segment appears as a α-helix-screw rotation in three dimensions. As the helix turns, the blocking region, represented as a gray section of the helix, is moved into position along the pore which stop the flow of ions.

from the remainder of the channel protein. For our model here we assume that there are always several residues in the loops between transmembrane segments and, therefore, each helix can move independently during the time scale of interest.

Reptation arises from the spontaneous formation and translation of defects along the α-helix structure. These defects, or kinks, are simply a small amount of stored length concentrated in a localized region. Because of the tight interhelical packing, the amount of stored length in a single defect will be small and probably much less than the backbone length of a single residue. This is discussed in more detail below. The defects migrate both up and down the helix with equal probability and net helix translation occurs when a defect deposits its stored length at the end of the tube. In Fig. 1 we indicate that the amount of stored length in a single defect is b (following the notation of de Gennes). Defect migration along the helix (i.e., along the curvilinear path) is governed by a diffusion equation and the methods for solution of this equation are well developed elsewhere (Doi and Edwards, 1986). The important result for our arguments here is that the displacement, $\sigma(t)$, of a general point on the helix, relative to the tube, from a starting point at $t = 0$ follows from the equation,

$$\langle \sigma^2(t) \rangle = \begin{cases} (2/\pi^{1/2}) \langle \rho \rangle b^2 \Delta^{1/2} t^{1/2} & t < T_R \quad (2a) \\ 2 D_c t & t > T_R, \quad (2b) \end{cases}$$

where $\langle \rho \rangle$ is the average number of defects per unit length along the helix backbone and Δ is the diffusion constant for a single defect (de Gennes, 1971). T_R, the defect equilibration time or Rouse relaxation time, is the time required for defects to spread along the helix and is given by

$$T_R = (Md)^2/\pi^2\Delta, \quad (3)$$

where d is the distance between the residues along the helix and M is the number of residues. Lastly, D_c is the diffusion constant for helix migration through the tube

$$D_c = \langle \rho \rangle b^2 \Delta / Md. \quad (4)$$

To understand these relations we begin with Eq. 2b which states that for times longer than T_R helix movement is governed by standard diffusion processes (where the square of the displacement is proportional to t). The associated diffusion constant is proportional to the diffusion constant for a single defect divided by the number of residues in the helix. Thus, the mobility of the entire helix decreases linearly with the number of residues that make up the helix. At times shorter than T_R the behavior is more complicated but can be understood through an argument provided by de Gennes (1982). Consider the movement of a single atom along the helix backbone. At extremely short times the atom diffuses as though it were not attached to its neighbors. As the atom moves over longer distances the motion will necessarily involve the neighboring backbone atoms and this results in a loss of local mobility. Because this is a diffusion process, the distance travelled will scale as $t^{1/2}$ and, likewise, the number of atoms involved in the movement will also scale as $t^{1/2}$. This results in a decrease in the local mobility and a corresponding decrease in the effective diffusion constant, D_{eff}, of $t^{-1/2}$. Hence, the square of the local displacement follows

$$\langle \sigma^2(t) \rangle \approx D_{eff} t \propto t^{-1/2} t \propto t^{1/2}, \quad (5)$$

and not t as in standard diffusion processes.

So far we have discussed the origin and the nature of helix displacement within the ion channel. We must now relate this process to channel gating and the structure of the closed-time distribution. From Fig. 1 we see that the channel closes and remains closed when the blocking region is along the channel pore. The closed-time pdf is then derived as the waiting time distribution for return of the helix so the point where the channel was open. (We can also wait until all of the blocking region has passed by the pore side of the helix but we will assume here that such motion would involve considerable protein distortion and is therefore restricted by the remainder of the channel

protein.) As we have shown in previous work, channel gating is then described as a diffusion model with an absorbing barrier (discussed below). We can use the methods of Chandrasekhar (1943) to recast the results in Eq. 2 for free diffusion to diffusion with an absorbing barrier and we find the pdf follows

$$f(t) \propto \begin{cases} t^{-5/4} & t < T_R \quad (6a) \\ t^{-3/2} & t > T_R \quad (6b) \end{cases}$$

This result follows from a standard derivation. The probability of the helix remaining in a particular configuration is $p(t) \propto 1/\sigma(t)$ (Alexander et al., 1981). [A specific case of this relation can be understood in terms of the probability distribution for a random walk, with a diffusion constant D, which follows

$$p(x, t) = (2\pi\sigma^2)^{-1/2} \exp(-x^2/2\sigma^2),$$

with $\sigma = (2Dt)^{1/2}$. This is simply a Gaussian function with a time-dependent variance. The probability of remaining at a particular location, say $x = 0$, then follows $p(0, t) \propto 1/\sigma(t)$.] If an absorbing barrier is included then $f(t) \propto -dp(t)/dt$ at long times. Thus, the physical process of reptation of an α-helix naturally gives the pdf of Eq. 1 at times longer than T_R.

In our previous work we argued that the kinetics of the closed time distribution for certain channels followed the kinetic scheme

$$0 \overset{\alpha}{\leftarrow} 1 \underset{\beta_1}{\overset{1}{\rightleftharpoons}} 2 \underset{\beta_2\gamma_2}{\overset{\gamma_2}{\rightleftharpoons}} 3 \underset{\beta_3\gamma_3}{\overset{\gamma_3}{\rightleftharpoons}} \cdots \underset{\beta_{N-1}\gamma_{N-1}}{\overset{\gamma_{N-1}}{\rightleftharpoons}} N, \quad (7)$$

where the states 1 through N represent the continuously connected closed states of the channel (Millhauser et al., 1988*a*). A closed interval ends when the system passes from state 1 to state 0. We showed that when $\alpha = 1$ and $\gamma_n = \beta_n = 1$ an analytic solution can be derived from the master equation. We further postulated that the hopping time (which is unity in our units of time here) between individual states was very short compared to the patch clamp time scale. With this assumption, only the long time behavior of the solution is important and we showed that the solution of Eq. 7 gives Eqs. 1 and 6b in the long time limit (and $t < N^2$ using unitless rate constants). Hence, we propose here that reptation is a highly plausible model for ion channel gating. The kinetic scheme in Eq. 7 is an approximate description of reptation at times longer than T_R. The continuously connected closed states represent different curvilinear positions of the α-helix within the helical tube when the blocking region is along the channel pore. The diffusion behavior observed by us and other researchers in the field arises from curvilinear displacement of the amphipathic segment within the helical tube and this displacement appears as an α-helix-screw rotation.

III. TIME SCALES FOR α-HELIX REPTATION

For reptation within an unchanging tube, as in our model here, curvilinear motion is distinguished by two different temporal regimes which are separated by T_R. We have shown above that the pdf will follow different power laws in these two regimes. It is estimated that $T_R \approx W^{-1}M^2$ in polymer melts (highly concentrated polymer solutions) and $W \approx 10^9$–10^{11} s^{-1} (de Gennes, 1982). In ion channels the transmembrane α-helix contains ~25 residues, so a rough estimate of T_R is 0.01–1.0 μs.

As discussed above, the pdf character expressed in Eqs. 1 and 6b is well established in the closed-time distribution of several channel systems through the rate-amplitude correlation (Millhauser et al., 1988*b*) and observation by other researchers in the field. To our knowledge, however, the early time behavior predicted by Eq. 6a has not been observed. (A pdf that follows Eq. 6a would give $p = 0.25$ in the rate-amplitude correlation and this is substantially smaller than the reported values.) This is not surprising given that the shortest observable events in the patch clamp experiment are on the order of 10 μs (Sakmann and Neher, 1983) which is longer than the predicted value for T_R extrapolated from studies of polymer melts.

Following this reasoning, we can use 10 μs as an upper bound for T_R. This, in turn, allows us to estimate a lower bound on Δ, the diffusion constant for a single kink along the α-helix. Using Eq. 3 and approximating $M \approx 25$ and $d \approx 5$ Å we find $\Delta \geq 10^{-8}$ cm^2/s. To put this quantity into perspective, the diffusion constant for glycine in water is ~10^{-6} cm^2/s. We expect that a kink will displace a certain volume in the ion channel protein and given the highly viscous environment within a protein interior our value for Δ seems quite reasonable.

We now turn to estimating the approximate timescale for gating events. Using our value for Δ and Eq. 4 we can approximate D_c the diffusion constant for α-helix creep within the helical tube. Before proceeding we must determine order-of-magnitude values for b and $\langle\rho\rangle$. Because helices are relatively rigid structures we believe that the stored length will be small compared to the distance between residues and so we estimabe $b \approx 0.1$ Å. As for the number of defects, we expect only a few per helix which gives $\langle\rho\rangle \approx 0.1$ Å^{-1}. Of course, these values are crude approximations but we are not aware of a more rigorous approach for calculating these quantities at this time. (However, these approximations are consistent with de Gennes' original description of reptation.) Combining

these values with Δ and again assuming $M \approx 25$ and $d \approx 5$ Å we find from Eq. 4 that $D_c \approx 10^3$ Å^2/s ($=10^{-13}$ cm^2/s) which is extremely slow again reflecting both the rigid environment within a ion channel protein and the general slowness of reptation processes. Note that if b is increased, so that a single defect involves more of the helical segment, then there will necessarily be fewer defects along the helix and $\langle \rho \rangle$ will decrease. D_c depends upon the produce of these two quantities and is, therefore, somewhat insensitive to such changes.

From molecular mechanics studies a single α-helix is believed to rotate about 60° during the gating process (Guy and Seetharamulu, 1986). This corresponds to a curvilinear displacement through the helical tube of ~3 Å. The time required for this excursion can serve as an estimate of the average time for gating. Rearranging Eq. 2b this time is calculated as

$$t_g = (3 \text{ Å})^2/2\, D_c \approx 5 \text{ ms}, \tag{8}$$

which is approximately correct for a typical channel closed period.

We conclude this section by determining the rotational diffusion constant, R, for the reptating α-helix because this quantity may be measurable by spectroscopic techniques such as electron spin resonance. R can be calculated by dividing D_c by the square of the curvilinear distance covered from a helix rotation of one radian. This distance is ~3 Å so that $R \approx 100$ s^{-1}. Again this is very slow but, nevertheless, this value is very close to R calculated by Levitt for helix rotation in the sodium channel (Levitt, 1989). It is also interesting to note that the rotational correlation time for the entire AChR complex in native membranes is $\approx 10^{-3}$ s, suggesting a rotational diffusion constant of <1,000 s^{-1} (Rousselet et al., 1982).

IV. DIFFUSION MODELS WITH RANDOM RATE CONSTANTS

As discussed above, we and other researchers have solved the kinetics scheme in Eq. 7 when the rate constants are all equal. For helix rotation, on time scales longer than T_R, it is reasonable to expect that the helix will experience greater motional restriction in certain regions of the tube and less restriction in other regions. Such a situation is represented by a variation of γ_n in Eq. 7. Without detailed knowledge of the structure of the tube, it is impossible to determine the nature of this variation so an unbiased approach is to let the rate constants vary in a random fashion.

The problem of unrestricted one-dimensional diffusion over random barriers has recently been solved (Alexander et al., 1981). In the language of this work, our present solution is for the ordered case ($\gamma_n = \beta_n = 1$). For the random case the barrier heights become independent random variables described by a continuous probability density $p(\gamma)$ with $\beta = 1$. An example of a distribution for γ, along with the ordered case, is shown in Fig. 2. The long time behavior for systems containing a random distribution of γ's mainly depends on the characteristics of $p(\gamma)$ as $\gamma \to 0$. There are three class types and qualitatively these classes are: class a, where $p(\gamma) \to 0$ as $\gamma \to 0$; class b, where $p(\gamma) \to$ finite as $\gamma \to 0$; and class c, where $p(\gamma) \to \infty$ as $\gamma \to 0$. Hence, the case shown in Fig. 2 is class a. Classes b and c correspond to situations where there is a probability of finding a rate constant equal to zero, i.e., an unpassable barrier. There is no reason to believe that unpassable barriers exist in the pathway of the rotating α-helix, so we restrict our attention to class a systems.

The long-time behavior of class a systems, where γ is random and β is unity, is described by the diffusion relations,

$$\langle \sigma^2(t) \rangle \approx 2 \langle \gamma \rangle t \tag{9a}$$

$$\langle \gamma \rangle^{-1} = \int p(\gamma) \gamma^{-1}\, d\gamma, \tag{9b}$$

and integration is over the domain $(0, \infty)$. It is important to note that Eq. 9 is correct regardless of the details of $p(\gamma)$ as long as the system fulfills the requirements of a class a system and, of course,

$$\int p(\gamma)\, d\gamma = 1. \tag{10}$$

This result shows that at sufficiently long times the behavior of diffusion is not affected by the presence of

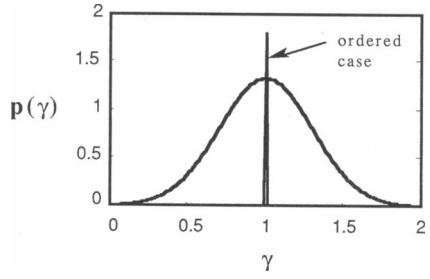

FIGURE 2 Example of a distribution of random rate constants for Eq. 7. The ordered distribution is when all of the rate constants are equal. The master equation for this configuration can be solved analytically giving a pdf of $t^{-3/2}$. The random distribution, which approximately follows a normal distribution here, is an example of a class a system. Remarkably, this system of random rate constants also gives a pdf of $t^{-3/2}$.

random barriers. The square of the displacement is still linear in time. It should be noted that Eq. 9 represents only the lowest order term for $\langle \sigma^2(t) \rangle$ so it is possible that at shorter times, where the higher order terms are important, there can be moderate deviations from this behavior.

Eq. 9 is proportional to Eq. 2b and so we are again led to pdf's that follow Eqs. 1 and 6b. There are certainly situations that can lead to the encounter of random barriers during the α-helix-screw process. Large side-chains can collide or interact through hydrogen bonding temporarily slowing the diffusion process. The results from the findings above indicate that such interactions will not be observable in the general shape of the pdf. This is important for any physical model that is kinetically described by Eq. 7. A pdf that follows Eq. 1 is not a special case for systems with uniform rate constants. On the contrary, $f(t) \approx t^{-3/2}$ is a general result for any one-dimensional scheme with or without a distribution of rate constants. From the higher order terms mentioned above, there may be some deviation from the algebraic behavior expressed in Eq. 1, however, the extent of these deviations are not predicted from the theory of 1D diffusion over random barriers. For ion channels such deviations may be very important because experimental pdf's following Eq. 1 are usually not smooth functions. The detailed nature of these deviations can only be studied with simulations, and we are presently pursuing this in our lab.

V. DISCUSSION

From the theory above we find that the pdf for ion channels should follow $t^{-5/4}$ at very short times (which are probably less than than the times observable on the patch clamp time scale). At longer times $f(t)$ follows $t^{-3/2}$, which has been observed in many systems. At even longer times the rotating helix will reach a limit beyond which further rotation will be restricted by intramolecular strains in the ion channel protein. This is equivalent to reaching the state N in the 1D kinetic scheme in Eq. 7 and the time required for this scales as N^2. Beyond this time the pdf will have an exponential tail and the details of this have been discussed previously.

There are several facts that make the reptation theory an attractive one for ion channel gating. First, reptation theory is a highly successful and accepted theory for the diffusion of polymers in melts which leads to the molecular basis viscoelasticity. The originator of reptation theory has hinted that it might be an appropriate approach for certain types of intramolecular protein dynamics (de Gennes, 1983*a*). Second, using the construct of a dynamic amphipathic helix, our approach predicts the corredt pdf and provides a physical basis for the $t^{-3/2}$ behavior. Third, reptation correctly predicts the approximate time scale for gating events. To our knowledge this is the first theory for channel gating which does this. Finally, our approach predicts dynamics which lead to the α-helix-screw model and this model has been suggested by several previous studies.

To this point we have been abstract in describing the defects. Defects do exist inpolymers and there is NMR evidence that backbone defects also exist in polypeptides (Nusser et al., 1988). In fact, analysis of these NMR experiments suggest that there is a local displacement of the peptide backbone which follows $\langle \sigma^2(t) \rangle \approx t^{1/2}$ for times $<10\ \mu s$ which may be consistent with the short time reptation behavior expressed in Eq. 2a. At this juncture we simply propose that the defects are localized strains that travel along the protein backbone. Because of the tight ridges-into-grooves packing, these strains result in amounts of stored length that are necessarily small compared to the distance between helix residues. The strain may arise from the dihedral angles (ϕ and/or Ψ) in an individual residue being displaced from the potential energy minimum. The only way to relieve this strain is to pass it on to the next residue.

We point out that our use of diffusing defects is fundamentally different than that in the model proposed by Condat and Jäckle. In their model the defects are directly responsible for the gating process through a recombination process with a group blocking the channel pore. In our approach, using reptation theory, the defects migrate along the protein backbone and this leads to a screwing motion of the amphipathic helix which in turn moves the blocking region into the channel pore.

We now turn to a brief consideration of the nature of the blocking region. We have shown that a helix rotation of ~60° requires 5 ms, on average, and this is consistent with closed-channel durations. A rotation of 60° is enough to move a single residue side-chain out of the channel pore and we believe, therefore, that the blocking region is only one, or possibly two, residues in length. Consistent with this is the proposal of a 'phenylalanine shutter' for the AChR where conserved phenylalanines rotate into the channel lumen (Vogelaar and Chan, 1989).

At this juncture is it worth mentioning how the pdf will be influenced if the helix has a net charge and there is a transmembrane potential. The amphipathic helix is oriented normal to the membrane so that the electric field, E, arising from the potential runs in the same direction as the helix. With this geometry and with reference to the kinetic scheme in Eq. 7, the effect of E is to add a net drift to the helix motion which is reflected by $\langle \beta \rangle \neq 1$. Introducing a new parameter $\xi = \langle \beta \rangle - 1$ to reflect a nonzero drift and assuming $|\xi| \ll 1$ it can be

shown that (when $\xi^2 > N^{-2}$),

$$f(t) \approx t^{-3/2} \exp(-\xi^2 t), \tag{11}$$

so that the pdf now has an exponential tail (Millhauser, G. L., E. E. Salpeter, and R. E. Oswald, manuscript in preparation). The terminal velocity of a charged species with diffusion constant D_c in a field E follows

$$s = zeED_c/kT, \tag{12}$$

where ze is the net charge of the species, k is Boltzmann's constant, and T is the absolute temperature. The drift parameter ξ will be proportional to s so combining the expressions above

$$f(t) \approx t^{-3/2} \exp[-\text{const.}\ (ED_c/T)^2 t], \tag{13}$$

and the constant contains factors that are not effected by the electric field. Hence, the location in time of the exponential tail in the pdf will scale with the square of the electric field. Eq. 13 also suggests that an algebraic decay for a voltage-sensitive channel will only persist at a single value of the transmembrane potential. At all other values the short time behavior will be algebraic followed by an exponential tail.

For neurotransmitter-gated channels, the pdf in Eqs. 1 and 6b applies only at saturating quantities of agonist. At lower concentrations a strict algebraic pdf is not correct and this is demonstrated in the rate-amplitude correlation. Experimental pdf's often exhibit exponential character at early times. At this juncture we do not have a specific reptation model for these unsaturated cases. However, reptation theory may provide a framework for the introduction of ligand effects. For instance, absence of a ligand may trap the amphipathic helix in a particular configuration inhibiting the reptation process. Physical characterization of ligand binding sites, in the AChR for example, will certainly help clarify these issues.

Because our application of reptation theory provides a physical model for channel gating we can list several experimental predictions to test its validity:

(*a*) Eq. 6a shows that the pdf will follow an algebraic form of $t^{-5/4}$ at early times and we have estimated these times to be on the order of microseconds. Such behavior is not observable in present experiments with a temporal resolution of $\sim 10\ \mu s$. However, slowing the gating kinetics by reducing the temperature, for example, may clearly reveal this early time characteristic of the pdf.

(*b*) Recently, synthetic 22-mers, mimicking the amphipathic segments of sodium channels, were incorporated into membranes yielding a cation-selective channel (Oiki et al., 1988). It was suggested that the channel structure was comprised of four α-helices in a bundle with the pore through the middle. Such a structure does not contain the additional framework of a second shell of α-helices around the amphipathic helices. Without this shell, and the interhelical ridges-into-grooves packing, the reptation tube does not exist and, therefore, reptation dynamics will not be observed. In fact these synthetic channels exhibit exponential, and not algebraic, pdf's, which is consistent with our expectations. With these channels as a starting point, we expect that more complicated assemblies containing a second shell of helices will follow the behavior of reptation dynamics. If these more complicated channels can be synthesized, then the M-dependence of D_c and T_R can be tested directly. By increasing the length of the amphipathic segment from 22 to 25 residues, for example, T_R will be increased possibly revealing the $t^{5/4}$ behavior in $f(t)$ mentioned above.

(*c*) We showed above that if the amphipathic segment in a channel contains a net charge, introducing a voltage sensitivity, then the tail of the pdf will be exponential. Hence, we expect that algebraic decays will only persist at a single transmembrane voltage in these channels. When algebraic decays are observed, however, the long time behavior will always follow $\sim t^{-3/2}$.

(*d*) Finally, we predict that molecular dynamics of a single helix packed in a bundle of helices will exhibit reptation modes at time scales longer than microseconds (if $M \approx 25$). Stochastic simulations of α-helix Brownian motion in solvent have been performed and these studies have revealed considerable backbone dynamics in the microsecond regime (McCammon et al., 1980). In fact, the simulation techniques were developed from the theories of polymer dynamics just as we have done here.

VI. CONCLUSION

We have developed a physical theory of channel gating by using the successful theory of polymer reptation dynamics. Our model predicts α-helix-screw dynamics of the amphipathic segments at times longer than microseconds. We showed that channel gating events will be on the time scale of milliseconds and that $f(t)$ will follow an algebraic decay of $t^{-3/2}$ under the appropriate conditions. This is one of the first times that reptation theory of polymer dynamics has been applied to describe the motional characteristics of polypeptides or proteins. If this reptation approach is correct for channel gating, then a new mode of protein dynamics is suggested along with new ideas that relate these dynamics to protein function. It will be very interesting to see whether reptation processes play a role in other areas of protein function of dynamics.

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