Model for the Binding of the Inactivation N-Terminal to the Ion Pore of Shaker Potassium Channel: Both Electrostatic Attraction and Covalent Linkage Are Required for Rapid Inactivation

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A model is presented for relating the binding of the inactivation N-terminal to the ion pore of the Shaker potassium channel (ShB) to the bimolecular binding of the N-terminal peptide with the deletion mutant ShBAΔ6–46. The binding site is modeled as a small patch on the surface of the channel protein, to which the N-terminal “inactivation ball” is tethered by a flexible linker. The potential energy due to electrostatic interactions between the channel and the N-terminal is \( U(r) = -Q \exp[-(r - a)/\lambda] \) for \( a \) is the closest approach distance and \( \lambda \) is the screening length determined by the ionic strength. The probability density for the end-to-end vector of the flexible linker (with \( L \) residues) is taken from a previous study [Zhou, J. Phys. Chem. B 2001, 105, 6763] as \( \rho(r) = (3/4\pi l_d^3 bL)^{3/2} \exp(-3r^2/4l_d^2 bL)(1 - 5r^4/4bL + ...) \). The intramolecular binding rate constant \( k_{on}^{in} \) of the intact ShB is related to the bimolecular binding rate constant \( k_{on}^{in} \) via \( k_{on}^{in} = k_{on}^b \rho(a)/(\int \exp(-\beta U(r))\rho(r)dV) \). The model rationalizes a number of important experimental observations. (1) The weaker ion strength dependence of \( k_{off}^{in} \) is quantitatively reproduced by the relation between \( k_{on}^{in} \) and \( k_{on}^b \). (2) The linker length dependence of \( k_{on}^{in} \) (observed when the linker length is reduced by deletion and extended by insertion) is qualitatively predicted by the \( L \) dependence of \( \rho(r) \). (3) The fact that \( k_{on}^s = k_{on}^b \) and both are insensitive to the change in ionic strength is due to the stereospecificity of the binding site. If the binding of the activation N-terminal were to occur in a bimolecular fashion, the millisecond inactivation time would have required the presence of the N-terminal at a concentration of 0.2 mM, even after considering the binding rate enhancement by the electrostatic attraction of the channel pore. The difficult task of maintaining such a high concentration underscores the importance of covalently linking the inactivation peptide to the ion channel.

Introduction

Voltage-gated ion channels open upon membrane depolarization and then close on a millisecond time scale. The rapid inactivation is crucial for the modulation of the firing frequency of the neurons. Inactivation occurs by the occlusion of the ion pore by an inactivation “ball” or “gate”. The ion channel is thus faced with the problem of having to affect rapid binding between the inactivation segment (IS) and the ion pore. If the binding were to occur in a bimolecular fashion and given that the stereospecificity of the binding between proteins limits the binding rate constant to \( 10^5 \) to \( 10^6 \) M\(^{-1}\)s\(^{-1}\), the millisecond inactivation time scale would require the maintenance of a population of the IS at a concentration of 1 to 10 mM. Long-range electrostatic interactions may enhance the binding rate constant, but given the high ionic strength of the cytosol, one may expect an rate enhancement of just 10-fold. This still leaves the required supply of the IS at the mM range. Of course, the channels solve the problem of rapid inactivation by covalently linking the IS to the transmembrane region. This paper deals with the relation between bimolecular and intramolecular binding kinetics of a peptide segment to a protein receptor.

The paper builds on and extends earlier work on the relation between the thermodynamics of dimeric and single-chain protein folding. We were able to relate the folding equilibrium constant \( K^d \) of a dimeric protein that has an interfaceal hydrophobic core and unfold upon dissociation to the equilibrium constant \( K^s \) of a single-chain form obtained by the covalent linking of the C terminal of one subunit and the N terminal of the other. The result is

\[
K^d / K^s = p(d) \tag{1}
\]

where \( p(r) \) is the probability density of the end-to-end vector of the flexible linker and \( d \) is the end-to-end distance of the linker in the folded protein. For a flexible linker consisting of \( L \) residues, we showed that

\[
p(r) = (3/4\pi l_d^3 bL)^{3/2} \exp(-3r^2/4l_d^2 bL)(1 - 5r^4/4bL + 2r^2l_d^2 - 33r^4/80l_d^4 + 37r^6/160l_d^6 - 329r^8/12l_d^8 + 6799r^{10}/160l_d^{10} - 3441r^{12}/2800l_d^{12} + 1089r^{14}/12800l_d^{12}) \tag{2}
\]

where \( l_d = bL \) with \( b = 3.8 \) Å and \( l_p = 3.2 \) Å. When the linker length \( l_d \) is much greater than both \( r \) and \( l_p \), eq 2 reduces to

\[
p(r) \approx (3/4\pi l_d^3 bL)^{3/2}, \text{ which is the classical result of Jacobson and Stockmayer.} \tag{6}
\]

Recently, we further showed that the DNA-binding of a single-chain protein consisting of two DNA-binding domains connected by a flexible linker can be related to the DNA-binding of the individual domains. If the dissociation constants of the two domains for their respective half sites are \( K_A \) and \( K_B \), then

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the dissociation constant \( K_{\text{A-B}} \) of the single-chain protein for the full binding site is

\[ K_{\text{A-B}} = K_A K_d / p(d_0) \] (3)

Here, we introduce a long-range interaction between the domains connected by the flexible linker. Our focus is to relate the intramolecular binding and unbinding rate constants \((k_{\text{on}})\) and \((k_{\text{off}})\) to the bimolecular binding and unbinding rate constants \((k_{\text{on,B}})\) and \((k_{\text{off,B}})\). In two situations, the relations are simple. The first consists of a spherical receptor and a spherical ligand interacting with a centrosymmetric potential. This simple model allows us to set up the framework of our approach. The second corresponds to the formation of a stereospecific ligand–receptor complex so that the binding site is small relative to the range of the interaction force. This situation is what is relevant for biological applications.

The theory developed is applied to the binding of the inactivation N-terminal to the ion pore of the Shaker potassium channel. We are able to rationalize a number of important experimental observations. (1) The weaker ionic strength dependence of \( k_{\text{on}} \) is quantitatively reproduced by the relation between \( k_{\text{on,B}} \) and \( k_{\text{off,B}} \) (observed when the linker length is reduced by deletion and extended by insertion) is qualitatively predicted by the L dependence of \( r(r) \). (2) The fact that \( k_{\text{off,B}} = k_{\text{off}} \) and both are insensitive to the change in ionic strength is due to the stereospecificity of the binding site.

Theory

Binding of Spherical Ligand to Spherical Receptor.

Consider the simple model shown in Figure 1a, where a spherical ligand interacts via a potential \( U(r) \) with a spherical receptor. The potential has a deep well when the ligand is near contact with the receptor and immediately switches to a smooth function that slowly approaches zero (see Figure 1b). The ligand is considered bound whenever it falls to the potential well. The binding constant for the bimolecular reaction is \( K_{\text{bi}} = \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \) (4) where \( \beta = (k_B T)^{-1} \). Unbinding occurs when the ligand escapes the potential well via diffusion. The rate constant can be calculated according to Kramers’ theory.\(^{11}\) The result is \(^{10} k_{\text{off}} = \)

\[ D\left[ \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \int_a^b 4\pi r^2 \exp[\beta U(r)]dr \right]^{-1} \] (5)

where \( D \) is the diffusion constant. The binding rate constant is thus

\[ k_{\text{on}} = k_{\text{on,B}} k_{\text{off}} = D\left[ \int_a^b 4\pi r^2 \exp[\beta U(r)]dr \right]^{-1} \] (6)

which is the same as a result derived by Debye.\(^{12}\)

We now consider the intramolecular binding shown in Figure 1c, where the ligand and receptor is connected by a flexible linker. If the end-to-end vector of the linker by itself has a probability density \( p(r) \), then under the influence of the interaction between the ligand and the receptor, the probability density of the end-to-end vector is \( p(r) \exp[-\beta U(r)] \). The equilibrium constant for the intramolecular binding is thus

\[ K_{\text{in}} = \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \] (7)

Note that \( p(r) \) is normalized such that \( \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr = 1 \). We are interested in cases where the width of the potential well is small. Then, between \( a \) and \( a_1 \), \( p(r) \) is nearly a constant and one has

\[ K_{\text{in}} = \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \] (8a)

\[ = K_{\text{in}} p(a) \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \] (8b)

If the long-range interaction potential is absent the integral in eq 8b reduces to \( \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \), which is nearly one because of the assumed closeness of \( a_1 \) to \( a \) and the normalization condition on \( p(r) \). Then, we have \( K_{\text{in}} = p(a_1) K_{\text{in}} \). This result is equivalent to eq 1. Hence, eq 8b may be considered a generalization of eq 1 when long-range interactions between the binding partners are present.

We now move on to the kinetics of intramolecular binding. A flexible linker is equally unlikely to have both extremely small and extremely large end-to-end distances because the number of chain configurations diminishes in both limits, \( 4\pi r^2 p(r) \) will have a maximum at an intermediate value. The location \( r = r_{\text{max}} \) of this maximum can be viewed as the equilibrium position of the unbound state. According to Kramers’ theory, the unbinding rate constant is

\[ k_{\text{off}} = D\left[ \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \int_a^b 4\pi r^2 \exp[\beta U(r)]dr \right]^{-1} \times \exp[\beta U(r)]dr \] (9)

Note that we have assumed that the linker does not change the diffusion constant of the ligand. The intramolecular binding rate constant is

\[ k_{\text{on}} = K_{\text{in}} k_{\text{off}} = D\left[ \int_a^b 4\pi r^2 \exp[-\beta U(r)]dr \int_a^b 4\pi r^2 \exp[\beta U(r)]dr \right]^{-1} \] (10)
Equilibrium and rate constants when the ligand is tethered to the receptor by a flexible linker, the equilibrium constant becomes

\[ K^\text{eq} = \int p(r) \exp[-\beta U(r)] dV/\int p(r) \exp[-\beta U(r)] dV \]  

(15)

where the prime signifies that the integration is over the whole space except for the region occupied by the binding site. Considering the small size of the binding site, we may write

\[ K^\text{eq} \approx p(d_0) \int \exp[-\beta U(r)] dV/\int p(r) \exp[-\beta U(r)] dV \]  

(16a)

where \( d_0 \) is the mean end-to-end distance of the tether when the ligand is bound. When the interaction potential is absent, eq 16b reduces to eq 1.

By the same argument that is used to justify eq 14, we assume that the tether will not significantly hinder the escape of the ligand from the binding site. Hence

\[ k_{\text{off}}^\text{in} \approx k_{\text{off}}^\text{bi0} \approx k_{\text{off}}^\text{bi} \]  

(17)

The intramolecular binding rate constant is thus

\[ k_{\text{on}}^\text{in} = K^\text{in} k_{\text{off}}^\text{in} \approx k_{\text{on}}^\text{bi} p(d_0) \int p(r) \exp[-\beta U(r)] dV \]  

(18)

Equations 16b, 17, and 18 are the main theoretical results of the paper. Note that when no interaction potential is present, we have

\[ k_{\text{on}}^\text{in} \approx k_{\text{on}}^\text{bi} p(d_0) \]  

(18a)

because of the normalization condition of \( p(r) \). This result was suggested in a previous work.\(^4\)

**Separation of Short and Long-Range Interactions.** It is important to recognize that, when a ligand approaches the binding site, it first encounters long-range electrostatic interactions. Once inside the binding site, it is stabilized by short-ranged van der Waals, hydrophobic, and hydrogen bonding interactions (long-range electrostatic interactions perhaps play a minor role here). The potential function depicted in Figure 1b is intended to illustrate the division of the short-range interactions inside the binding site (deep potential well between \( a \) and \( a_1 \)) and the long-range electrostatic interaction outside (smooth potential for \( r > a_1 \)). This division does not affect the main results for the present paper, eqs 16b, 17, and 18. We do need to note that the potential in eqs 16b and 18 refers to the long-range electrostatic interactions outside the binding site and \( k_{\text{off}}^\text{bi0} \) in eq 17 refers to the bimolecular unbinding rate when the potential outside the binding site is turned off.

As noted previously,\(^10\) eq 13 \( k_{\text{on}}^\text{bi} \) and \( k_{\text{on}}^\text{bi0} \) refer to the diffusion-controlled bimolecular binding rate constants in the presence and absence of the long-range electrostatic interactions, and the average of the Boltzmann factor is over the outer edge of the binding site (corresponding to \( r = a_1 \) in Figure 1b).

**Results**

We now use the theory developed in the previous section to analyze experimental data on the binding of the inactivation N-terminal to the ion pore of the Shaker potassium channel (ShB). Our focus is the relations of the rate constants for the intramolecular binding in the intact ShB and the rate constants for the bimolecular binding of the N-terminal peptide with the deletion mutant ShBA6–46. The N-terminal peptide consists
of the first 20 residues of ShB. The ShBΔ6–46 mutant is incapable of inactivation because of the disruption of the inactivation N-terminal. Of particular interest is the ionic strength dependence of the intramolecular unbinding rate constant (k_{\text{on}}^{\text{in}}) for the binding of the N-terminal peptide with the deletion mutant ShBΔ6–46, observed by Murrell-Lagnado and Aldrich. It is modeled well by eq 20 with a = 20 Å, \( Q = 240 \) and \( k_{\text{on}}^{\text{bd0}} = 1.5 \times 10^5 \text{ M}^{-1}\text{s}^{-1} \). Moreover, the ionic strength dependence of \( k_{\text{on}}^{\text{bi}} \) for a mutant N-terminal peptide (E12KD13K) is modeled equally well by increasing \( Q \) to 450.

In sharp contrast to the 20-fold and 60-fold decreases of \( k_{\text{on}}^{\text{bi}} \) for the binding of the N-terminal peptide with the deletion mutant ShBΔ6–46, \( k_{\text{off}}^{\text{bi}} \) has a nearly constant value of \( \sim 15 \text{ s}^{-1} \) for both peptides when the ionic strength is increased from 50 to 600 mM. This disparate ionic strength dependence between \( k_{\text{on}}^{\text{bi}} \) and \( k_{\text{off}}^{\text{bi}} \) is in line with results observed on a wide range of associating proteins, including the binding of the peptide toxin Lq2 to ShB. It is exactly what is predicted by eq 14 and indicates that the binding of the N-terminal peptide is limited by the electrostatic enhanced diffusional encounter with the ion pore of ShBΔ6–46.

**Discussion**

We have developed a theory relating the kinetics of the intramolecular binding of a ligand tethered to a receptor and the kinetics of the bimolecular binding of the ligand to the receptor. The theory accounts for both the long-range interactions between the ligand and the receptor and the effect of the linker (via the probability density \( p(r) \) of the end-to-end vector) on the ion pore of the Shaker potassium channel leads to the rationalization of a number of important experimental observations. (1) The weaker ionic strength dependence of the intramolecular binding rate constant \( k_{\text{on}}^{\text{in}} \) is quantitatively predicted by the relation between \( k_{\text{on}}^{\text{in}} \) and \( k_{\text{on}}^{\text{bi}} \). (2) The linker length \( L \) dependence of \( k_{\text{on}}^{\text{in}} \) is qualitatively predicted by the \( L \) depen-
rate constant to $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ (at $I = 150 \text{ mM}$) and lowers the required concentration of the N-terminal peptide to 0.2 mM. The difficult task of maintaining such a high concentration is simply solved by the covalent linking of the N-terminal to the ion channel.

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References and Notes

(21) However, the magnitude of $k_{off}$ predicted by eq 21 is uniformly higher by a factor of 10 than the experimental results in the ionic strength range of 50 to 600 mM. In addition, the experimental results may correspond to $4k_{off}$ because the channel is a homo-tetramer with 4 N-terminals that bind independently with the ion pore (Gomez-Lagunas, F.; Armstrong, C. M. Biophys. J. 1995, 68, 89; MacKinnon, R.; Aldrich, R. W.; Lee, A. W. Science 1993, 262, 757). The lower experimental values perhaps can be attributed to interference of the ligand binding by the flexible linker. In this context, we note that it is now known that the inactivation N-terminal binds deeply into the hydrophobic central cavity of the channel (Zhou, M.; Morais-Cabral, J. H.; Mann, S.; MacKinnon, R. Nature 2001, 411, 657), through one of four openings defined by a linking sequence between the T1 domain and the first transmembrane helix. It is likely that this “hanging gondola” architecture (Kobertz, W. R.; Williams, C.; Miller, C. Biochemistry 2000, 39, 10347) will interfere with the motion of the linker to which the N-terminal is attached.