Investigations of Ion Channels with Computational Simulations and Biochemical Experiments

Thesis by

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Abstract

Chapter one describes studies of the voltage-dependent hydration and conduction properties of the hydrophobic pore of the mechanosensitive channel of small conductance, MscS. A detailed picture of water and ion properties in small pores is important for understanding the behavior of biological ion channels. Several recent modeling studies have shown that small, hydrophobic pores exclude water and ions even if they are physically large enough to accommodate them, a mechanism called hydrophobic gating. This mechanism has been implicated in the gating of several channels, including MscS. Although the pore in the crystal structure of MscS is wide and was initially hypothesized to be open, it is lined by hydrophobic residues and may represent a nonconducting state. Molecular dynamics simulations were performed on MscS to determine whether or not the structure can conduct ions. Unlike previous simulations of hydrophobic nanopores, electric fields were applied to this system to model the transmembrane potential, which proved to be important. Although simulations without a potential resulted in a dehydrated, occluded pore, the application of a potential increased the hydration of the pore and resulted in current flow through the channel. The calculated channel conductance was in good agreement with experiment. Therefore, it is likely that the MscS crystal structure is closer to a conducting than to a nonconducting state.

Chapter two describes work toward a method using protein transduction domains (PTDs) to deliver tRNA to cultured mammalian cells. *In vivo* incorporation of unnatural amino acids using nonsense suppression is a powerful technique to study proteins. However, one challenge to the method is that the amount of unnatural protein that can be

produced is directly limited by the amount of unnatural aminoacyl-tRNA presented to the cellular translation machinery. Therefore, the success of this technique depends heavily on the ability to deliver aminoacyl-tRNA, which is produced *in vitro*, into cells. Currently, the most commonly used system involves injection of a *Xenopus* oocyte. It is desirable to transfer the technology to a mammalian expression system, but because mammalian cells are so much smaller than oocytes, injection is not a practical delivery method, so other techniques must be utilized. An intriguing possibility is the use of PTDs, small peptides that greatly enhance the internalization of extracellular material. Several PTD-based approaches for tRNA delivery were attempted: covalent ligation of tRNA to a PTD, noncovalent complexation of tRNA and PTDs, and production of a fusion protein containing a PTD and a tRNA-binding domain. However, none of these methods was useful in delivering tRNA into mammalian cells in culture.

Chapter three describes efforts to develop a high throughput assay for gating of the mechanosensitive channel of large conductance, MscL. The bacterial ion channel MscL is an ideal starting point for understanding the molecular basis of mechanosensation. However, current methods for the characterization of its mutants, patch clamp and bacterial growth analysis, are difficult and time consuming, so a higher throughput method for screening mutants is desired. We have attempted to develop a fluorescence assay for detecting MscL activity in synthetic vesicles. The assay involved the separation of two solutions—one inside and one outside the vesicles—that are separately nonfluorescent but fluorescent when mixed. It was hoped that MscL activity due to downshock of the vesicles would bring about mixing of the solutions, producing fluorescence. The development of the assay required the optimization of several

variables: the method for producing a uniform vesicle population containing MscL, the fluorescence system, and the lipid and protein composition of the vesicles. However, no MscL activity was ever detected even after optimization, so the assay was not fully developed. The probable cause of the failure was the inability of current techniques to produce a sufficiently uniform vesicle population.

Table of Contents

Chapter 3 (continued)

List of Figures

Chapter 1

Chapter 2

Chapter 2 (continued)

Chapter 3

List of Tables

Chapter 1

Chapter 2

Chapter 3