dase, thus indicating that it may act specifically on the enzymes. The other study comes from the research group of van Aalten, which investigated the inhibition of another bacterial chitinase by a cyclic dipeptide called CI-4 [12]. However, until now, the mechanism of interaction between the peptide-mimicking carbohydrates and the protein, and its binding site, was unknown; the structure of the complexes between chitinase and the cyclic peptide presented by Rao et al. [1] give several hints on how to increase the affinity of the peptides for the family 18 chitinase and how to modify, for example, the side chains to increase the efficiency of the inhibitor. Concurrent analysis of the data presented by Rao et al., and another prominent study of a peptide binding to the a mannopyranoside site of ConA, showed related types of hydrogen bonds and hydrophobic interactions during the replacement of a carbohydrate by a peptide [13].

In conclusion, a new development of carbohydrate minotopes is seen through this study. Screening of natural peptides or peptide libraries, chemically synthesized or obtained by phage display, could lead to the identification of new, simple inhibitors with known interactions. The manuscript of Rao et al. is a landmark in this area. It provides not only a way to analyze interactions and modify chemically the cyclopeptide inhibitors to obtain species-specific inhibitor but also opens new research avenues for nonsugar derivatives to inhibit glycosylhydrolases. The results also suggest a potential for peptides to inhibit other cell wall enzymes in addition to the chitinases. For example, echinocandins are cyclohexapeptides that inhibit fungal β1,3 glucan synthases. Based on the results of Rao et al., the use of peptides that mimic carbohydrates to inhibit glycosylhydrolases should become prominent in the near future.

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Chemistry & Biology, Vol. 12, January, 2005, DOI 10.1016/j.chembiol.2004.12.008

The End of Single-Molecule Envy

Molecular interactions can be detected using single engineered ion channels as stochastic sensors. This approach is used to examine events between a kinase and an inhibitor peptide tethered to an engineered channel [1] showing agreement with macroscopic biochemical data, as well as a tantalizing surprise.

Most of our knowledge of the behavior of biological molecules is derived from macroscopic studies which measure the activity of thousands or millions of molecules. The activity of single molecules (or single macromolecular complexes) can be measured—albeit for relatively few classes of biological molecules. Microscopic studies reveal that events that occur at the single-molecule level cannot be described as deterministic, but are stochastic. That is, the behavior of single molecules is probabilistic. Single behavior of ion channels was shown first three and a half decades ago using sensitive amplifiers to measure the activity of certain antibiotics that act as ion channels in lipid bilayers [2]. The development of patch clamp not long afterwards fueled explosive interest in single-molecule behavior, augmented further by the cloning and functional characterization of ion channels. These were heady days indeed, prompting one leading researcher in the field to quip that the enterprise was so heroic that it could be portrayed in the style of a Soviet-era social realist poster depicting an intrepid graduate student at work proclaiming in bold script "one investigator, one rig, one molecule!" Detailed kinetic behavior can be mined from the richness of single-molecule data. For example, complex models of ion channel

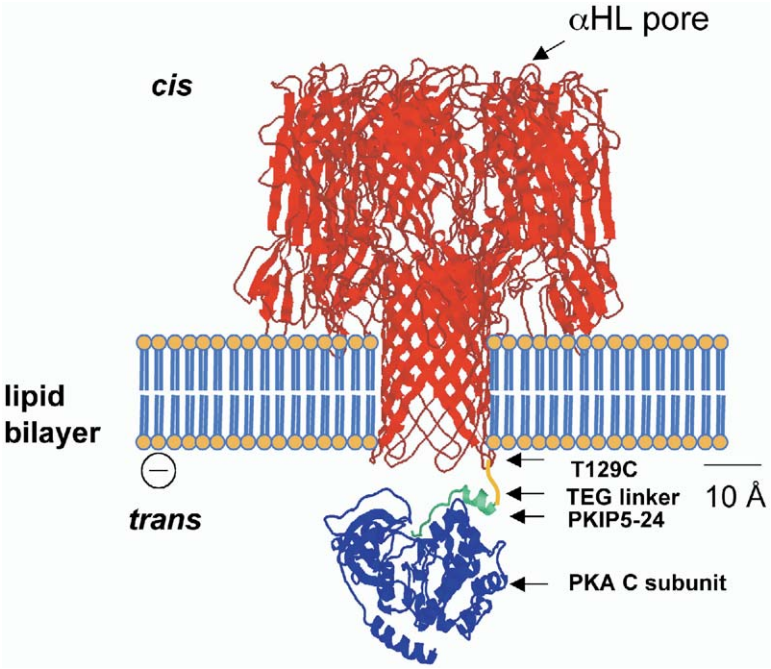

Figure 1. Molecular Model of the α-Hemolysin Channel with Tethered PKIP Bound to the Catalytic Subunit of cAMP-Dependent Protein Kinase

Single-channel conductance is altered when binding occurs; thus, the engineered channel acts as a reporter for kinase/inhibitor binding events [1].

functional substates have been developed in part from single-channel recordings.

So are investigators of other important biological molecules, including protein kinases, left to languish in single-molecule envy? Hagan Bayley's laboratory has established a highly innovative approach incorporating elements of chemistry and biology that permits the measurement of single (and bimolecular) behavior of a growing and varied class of molecules [3]. This is achieved by chemically coupling small molecules of interest (including ligands for much larger molecules) to genetically modified staphylococcal α-hemolysin ion channel pores that act as sensors. This scheme for the article herein is depicted in Figure 1. A high-resolution structure of the α-hemolysin channel was solved a number of years ago by a collaborative effort that included Eric Gouaux and Bayley [4]. The heptameric α-hemolysin channel has a distinct *cis* side mushroom-shaped cap with a β barrel stem that crosses the lipid bilayer with an average pore diameter of 20 Å through the stem. Following synthesis and preparative biochemical fractionation of subunits, α-hemolysin channels are incorporated into membranes with known stoichiometry and measured for single-channel electrophysiological recording using a standard bilayer apparatus. Stochastic sensing by modified α-hemolysin channels has been used by Bayley's group to measure a variety of analytes including proteins. Single binding events between large receptor molecules including proteins and ligands tethered to the modified α-hemolysis channels are reported as changes in the amplitude and ionic selectivity in single-channel recordings [5]. Within this issue, Xie, Bayley, and colleagues describe the implementation of their approach to measure single-molecule interactions between the catalytic subunit of cAMP-dependent protein kinase (PKAc) and a protein kinase inhibitor peptide (PKIP) that is tethered to the end of the stem *trans* side of the channel pore with a flexible chain of tetra ethylene glycol [1].

The stochastic sensing experiments with the modified α-hemolysin channel and the catalytic subunit of PKAc yielded results that validate the system—and some intriguing surprises as well. Current-voltage curves determined from single-channel current recordings of wild-type and PKIP-tethered α-hemolysin channels are similar, indicating that the PKIP peptide does not appear to interact with the transmembrane barrel pore by itself. Microscopic kinetic and binding constants of PKAc interaction with PKIP determined by stochastic sensing at a given intermediate membrane potential are similar to those values determined by macroscopic measurements made in bulk solution. These are exciting results, as protein kinases are critical regulators of many physiological processes and are important targets for potential drug therapies. High-throughput screening of protein kinase modulators could yield rapidly acquired and highly detailed data sets if stochastic sensing approaches could be scaled-up and made to be more robust. PKAc is relatively well understood in terms of high-resolution structure, molecular mechanism, and pharmacology. The general approach of single-channel stochastic sensing is broadly applicable to many proteins—including those that are much less understood.

Most unexpectedly, Xie and colleagues found that microscopic kinetic and binding constants derived from tethered PKIP and PKAc are influenced profoundly by applied membrane potential. In contrast to the binding events between the tethered PKIP and PKAc that are observed at the applied potential of –80 mV, curiously, no binding events are observed at positive potentials. This raises questions about the voltage dependence of

binding and the behavior of proteins at the membrane surface. Many signal transduction events occur at the cell membrane. For a few well-characterized examples such as Ras and Src, we know that a combination of electrostatic interactions and lipid modifications influence signaling at the membrane [6]. However, there is a growing body of evidence that suggests that the activity of other membrane-associated signaling proteins, including protein kinases, are influenced by voltage changes at the membrane [7, 8]. At first glance, the voltage-dependent differences in stochastic sensing of the interactions between tethered PKIP and PKAc appear to be an unexplained quirky feature of the measurement. Perhaps these unexpected results may lead to powerful new studies to examine the voltage-dependent behavior of proteins kinases and other important signaling molecules at the membrane interface.

The work described above signifies the latest progress in ion channel engineering, a new field which has two major branches. One is devoted to in vivo/therapeutic applications and the other to sensor technology. Recently, engineered channels have been applied to in vivo studies for manipulating electrical excitability in specific neural circuits in transgenic animals [9, 10]. Other work examining expressed engineered channels in cultured neurons in vitro shows that rapid temporal control over the activity of engineered ion channels can be achieved by small chemical ligands or light [11, 12]. These results point to future potential therapeutic applications for engineered ion channels. The use of engineered ion channels as sensors has been in existence for a few years longer. The earliest examples of ion channels as chemical sensors made use of naturally occurring channels. “Patch-cramming,” developed by Richard Kramer, was one notable early example of naturally occurring ion channels used as chemical sensors [13]. The work of Bayley’s group builds on this through the development of engineered channels as specific sensors. To date, virtually all engineered ion channels have been constructed using naturally occurring ion channels as scaffolds. Selection-based membrane protein evolution and de novo design of engineered ion channels are areas that will probably receive substantial attention in the near future. Specifically regarding chemical sensors, there are several challenges that must be solved in order to make channel-based sensors useful as an industrial technology [3]. First, they must be made more physically robust so that they are able to withstand the passage of large volumes of analytes over time. Second, channel-based sensors need to be scaled-up to true multiplex systems to allow for greater throughput. If these technical hurdles can be overcome, then engineered ion channels will likely be directed toward an enormous number of chemical sensing applications.

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