New and Notable

Activation of an Enzyme Simulated by Explicit Dynamics of an Active Site Lid

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In this issue of the *Biophysical Journal*, Peters et al. (1996) present a study in which the activation of a lipase enzyme is simulated as a hinge-type motion of a single 23-residue helix that acts as a lid over the active site. Much attention has been paid in recent years to the hypothetical gated control of access of substrates to enzyme active sites. Although much theoretical progress has been made in describing the rate effect of gating on a phenomenological level, very little is known about actual gate dynamics from an explicit all-atom structural viewpoint. Lipases have been studied as prototypical systems of the regulation of interfacial enzyme-catalyzed reactions. Through analysis of crystal structures of lipases, the hinge-type motions of helices covering the active site have been identified as the salient structural element of the gate. Still the regulation aspect has not been understood, that is, the relationship between gate motion and physicochemical details. What facilitates the gate opening and how does this contribute to regulation? The x-ray diffraction data provides only static information about lipase structures and their complexes, whereas the intermediate process of the dynamical response of the gate in its solution environment in the presence of the lipid substrate is where all the fun is, and is also very complicated. The present study provides a more comprehensive picture in that it links important known structural details to little-known dynamical aspects important to regulation.

The strength of this study is the implementation of two complementary simulation methods to piece together dynamical and thermodynamical information relating to the gate opening process. A constrained molecular dynamics simulation was first performed in which the 23-residue helical lid conformation was pried open in 20 steps into the active state by the application of a bias potential to enforce its movement. This was done in forward and reverse as a reliability check. The exciting discovery here was that it is energetically favorable for the enzyme to become active in a low dielectric (hydrophobic) environment, where strong electrostatic interactions lead to stabilization of the active conformer. This phase of study provided the thermodynamical framework for understanding the mechanism of hinge opening and its enhancement by a hydrophobic environment.

The fundamental shortcoming of the molecular dynamics method is that it is limited to time scales too short to observe hinge opening and closing without a biasing potential. Therefore, the interesting biological dynamics are not accessible to an explicit all-atom simulation method. This is where the Brownian dynamics method really begins to shine, with its ability to model dynamics all the way to the microsecond time scale with ease. The peptides of the lid region were modeled as soft spheres centered on the C_β atoms according to Levitt (1976) in his early protein folding simulations of bovine pancreatic trypsin inhibitor with modifications made by McCammon et al. (1980) in their studies of helix-coil transitions. The charged residues of the lid interact with the charge field of the remainder of the protein via a Poisson-Boltzmann electrostatic grid. Solvent is treated as a dielectric continuum with dielectric constant of 80 (for water) or 4 (for the hydrophobic environment). By observing microsecond trajectories, one readily sees the increased rate of lid opening in the hydrophobic environment. This is highly suggestive of a mechanism for the regulation of lipase activity by response to its environment.

The success and ease of implementing approximate Brownian models for subunit motions in complicated multisubunit systems has been exemplified here, and could conceivably carry over to a wide variety of biological processes.

REFERENCES

Levitt, M. 1976. A simplified representation of protein conformations for rapid simulation of protein folding. J. Mol. Biol. 104:59–107.

McCammon, J. A., S. H. Northrup, M. Karplus, and R. Levy. 1980. Helix-coil transitions in a simple polypeptide model. *Biopolymers.* 19: 2033–2045.

Peters, G. H., O. H. Olsen, A. Svendsen, and R. C. Wade. 1996. Theoretical investigation of the dynamics of the active site lid in Rhizomucor miehei lipase. *Biophys. J.* 71: 119–129.

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Coping with Cellular Stress: The Mechanical Resistance of Porous Protein Networks

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Most eukaryotic cells contain a system of filamentous protein polymers that collectively comprise the cytoskeleton, a three-dimensional polymer matrix that appears to be the primary determinant of the cell's viscoelastic character. Unlike rubberlike networks where the macroscopic viscoelasticity is directly interpretable in terms of the entropy of the constituent random coil polymers, the cytoskeleton is composed of rigid or semiflexible polymers for which no adequate viscoelastic theory is yet available. In a report in

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this issue, Satcher and Dewey (1996) take a step toward providing a conceptual framework for the viscoelasticity of these networks and show that it predicts quantities that are in agreement with experimental results.

Three chemically distinct linear polymers—microtubules, intermediate filaments, and microfilaments (F-actin)—are independently assembled in the cytoplasm, joined together by a host of associated proteins, and further linked at specific sites to the cell membrane and to the nucleus. The chemical complexity of this system of polymers makes a detailed viscoelastic characterization difficult, and the active response of the cytoskeleton to mechanical perturbations complicates efforts to measure the passive viscoelasticity of the cell. Indeed measurements of different types of cells that are similarly rich in cytoskeletal proteins yield rather different results. Resting neutrophils, measured by aspiration into micropipettes behave as viscoelastic liquids with viscosities around 1000 poise (Hochmuth and Needham, 1990), about as thick as honey. Platelets, on the other hand, when attached to a surface and probed by atomic force microscopy are viscoelastic solids with shear moduli on the order of 10 kPa (Radmacher et al., 1996), stiffer than a blood clot, and similar to typical polyacrylamide gels used for electrophoresis. Endothelial cells, which line the surface of blood vessels and are the subject of the current study, have shear moduli on the order of 1000 Pa. Presumably the differences among these cell types depend not only on the mass of the cytoskeleton but also on its architecture and the nature of its linkages to the cell membrane and other structures. Understanding the viscoelasticity of endothelial cells is biologically relevant because these cells respond directly to mechanical stresses caused by the fluid flow within blood vessels.

To begin to provide a molecular basis for the endothelial cell's viscoelasticity, Satcher and Dewey make several important assumptions. First, based on a number of biochemical and cell biological data, they assume that the viscoelasticity is primarily due to actin filaments, rather than to intermediate filaments or microtubules. Because the general appearance of these filaments in the electron microscope resembles the gently curving fibers comprising materials like paper and cotton wool (except that F-actin is 1000 times thinner), it is reasonable to expect a similar basis for their elasticity. Therefore a starting point is to model the network of actin filaments as a cubic lattice whose deformation is resisted elastically by the bending of its linear elements (actin filaments). Although many biochemical and geometric details are ignored by this model, it does provide quantitative predictions with experimental results and allows for additional contributions, such as the extra resistance to shear strain provided by bundles of actin filaments (stress fibers) that form in the direction of applied stress in vivo.

Given the assumptions of the model, the molecular parameter that most strongly determines the elastic modulus of the cell is the bending stiffness of the actin filament, and here the authors have a variety of experimental results to consider. Recent measurements of filament stiffness range between values corresponding to persistence lengths of 2 to 18 μm. A similar variation in results has been obtained for microtubules. Therefore, unless there are surprisingly large errors in some measurements, these filaments have different stiffness under different conditions. Satcher and Dewey choose an elastic constant on the high end of this range, corresponding to a persistence length greater than 10 μm. Therefore on the scale of the cytoskeletal mesh size, ~200 nm, these filaments are closely approximated as straight but bendable rods.

A striking result of Satcher and Dewey's calculations is that the predicted elastic moduli are very high: 10^4 Pa (10^5 dyne/cm^2) for a 1% volume fraction of polymer. This value is several orders of magnitude higher than the elastic modulus of similar concentrations of entropic networks, but is roughly consistent with experimental measurements of both purified F-actin and some types of cells. One reason that this large modulus is surprising is that the magnitude of deformation expected from the measured stresses that endothelial cells encounter in vivo is so small that their activation by shear stress cannot be explained by changes in cell structure, unless there are local regions of much lower elasticity.

How well the current model will account for the way in which changes in network structure alter elasticity remains to be seen. For example, the large elastic modulus appears to depend on the fact that the filaments are arranged orthogonally and cross-linked to each other at junction points. This is just the type of network observed in the cortex of some cell types (Stossel, 1994), and changes in viscoelasticity probably come about by changing the nature of these cross-links or the filament length, aspects that are not explicitly considered by this model. Another question is whether the elasticity of the cytoskeleton really derives primarily from the bending energy of filaments modeled as stiff rods. Actin filaments may be closer to the model of semiflexible polymers in which both entropy and the finite stiffness of the filaments contribute to elasticity, as shown recently by a different theoretical treatment (MacKintosh et al., 1995).

The validity of the Satcher and Dewey model for cytoskeletal mechanics will require further experimental verification, and it is a virtue of this model that a number of testable predictions are made. Whatever the eventual outcome, this study, and others like it, allow the characterization of cell mechanics to progress beyond being primarily phenomenological, and demonstrate how the study of biological polymers can motivate and test concepts of general interest in material science.

REFERENCES

Hochmuth, R. M., and D. Needham. 1990. The viscosity of neutrophils and their transit times through small pores. *Biorheol.* 27:817–828.

MacKintosh, F., J. Käs, and P. Janmey. 1995. Elasticity of semiflexible biopolymer networks. *Phys. Rev. Lett.* 75:4425–4428.

Radmacher, M., M. Fritz, C. M. Kacher, J. P. Cleveland, and P. K. Hansma. 1996. Measuring the viscoelastic properties of human platelets with the atomic force microscope. *Biophys. J.* 70:556–567.

Stossel, T. P. 1994. The machinery of cell crawling. *Sci. Am.* 271:54–5, 58–63.

Visualizing Proton Conductance in the Gramicidin Channel

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In this month's *Biophysical Journal*, Pomes and Roux (1996) provide a molecular dynamics simulation of protonic conductance along a single strand of hydrogen-bonded water in the gramicidin channel. This result complements an earlier report by Sagnella and Voth (1996) and is significant because protons do not depend on diffusion alone to move through aqueous media. Instead a proton can hop along chains of hydrogen-bonded water molecules, so that the measured ionic mobility of protons in water is several times higher than that of sodium or potassium ions. Proton conduction by hopping along water chains was first proposed by Hladky and Haydon (1972) to explain the anomalously high proton conductance through the gramicidin channel, which is approximately 15 times that of potassium ions.

Although proton conductance through the gramicidin channel is intrinsically interesting, it may also provide a useful model system for biological systems of more general interest. The term "proton wire" was coined by Nagle and Morowitz (1978) to describe possible proton conductance pathways in proteins in which hydrogen-bonded side chains of amino acids could provide pathways for protons. Although a proton wire composed purely of amino acid side chains has not yet been discovered, there are now a number of proposed protonic conductance processes in which water molecules are likely to be involved. For instance, proton conductance along water chains in proteins has been proposed for bacteriorhodopsin and bacterial reaction centers (see Pomes and Roux, this issue, for references) and the F_1Fo ATP synthase (Schulten and Schulten, 1985; Akeson and Deamer, 1992). It is here that the new molecular dynamics simulations will be most useful in guiding further research. Pomes and Roux show that protons in the channel are present as $O_2H_5^+$, rather than OH_3^+. Furthermore, a proton strongly orients the water in the channel and moves within the water by a semi-collective transfer mechanism, rather than by random diffusion or a highly coordinated process. Proton translocation is limited by the hydrogen bonds between water molecules and polar groups on the sides of the channels. If the hydrogen bonding forces are turned off in the simulation, proton mobility increases dramatically. Taken together, these results provide a new conceptual framework to investigate mechanisms by which proteins conduct protons as part of their function.

REFERENCES

Akeson, M., and D. W. Deamer. 1991. Proton conductance by the gramicidin water wire: a model for proton conductance in the F_1Fo ATPase? *Biophys. J.* 60:101–109.

Hladky, S. B., and D. A. Haydon. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. *Biochim. Biophys. Acta.* 274:294–312.

Marrink, S. J., F. Jahnig, and H. J. C. Berendsen. 1996. Proton transport across transient single-file water pores in a lipid membrane studied by molecular dynamics simulations. *Biophys. J.* In press.

Nagle, J., and H. J. Morowitz. 1978. Molecular mechanisms for proton transport in membranes. *Proc. Natl. Acad. Sci. USA.* 75: 298–302.

Sagnella, D. E., and G. A. Voth. 1996. Structure and dynamics of hydronium in the ion channel gramicidin A. *Biophys. J.* 70:2043–2051.

Schulten, Z., and K. Schulten. 1985. A model for the resistance of the proton channel formed by the proteolipid of ATPase. *Eur. Biophys. J.* 11:149–155.

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Slow Inactivation of Sodium Channels: More Than Just a Laboratory Curiosity

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Sodium currents are usually regarded as fast transients. In response to depolarization, channels open but then rapidly close within a millisecond to an inactive state. Repolarization of the membrane is necessary to reprime channels for subsequent depolarization-induced opening. The recovery from inactivation is voltage dependent and, after brief depolarizations, occurs within 10 ms or so at the resting potential of excitable cells (−70 to −90 mV). This orchestrated sequence of rapid voltage-dependent changes in sodium channel conformation produces many of the salient features of an action potential: fast upstroke of the depolarizing phase (activation), termination of depolarization (inactivation), and the refractory period (recovery from inactivation), as originally described so elegantly by Hodgkin and Huxley.

In addition to these rapid gating transitions, sodium channels undergo very slow voltage-dependent shifts in availability for opening, on a time scale of seconds to minutes. In this issue of the *Biophysical Journal*, Cummins and Sigworth (1996) show that slow inactivation is impaired in a mutant form of the skeletal muscle sodium channel that causes the hereditary muscle disorder hyperkalemic periodic paralysis (HyperPP). This result may be the key to a previously unanswered question about the mechanism of the prolonged episodes of weakness in HyperPP (Ruff, 1994) and provides the first direct evidence for a potential physiological role of slow inactivation.

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