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.

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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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