A commentary on gating of the active site of triose phosphate isomerase: Brownian dynamics simulations of flexible peptide loops in the enzyme, by R. C. Wade, M. E. Davis, B. A. Luty, J. D. Madura, and J. A. McCammon

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A detailed modeling analysis of the role of protein flexibility on the kinetics and mechanisms of enzyme substrate reactions is made difficult by the long timescales of the motions involved. Molecular dynamics, which is the obvious choice for a study of protein motion at an atomic level of resolution, has its limitations. Computational constraints place an upper limit of several hundred picoseconds (ps) on the overall simulation time of a protein or macromolecule in general. For triose phosphate isomerase (TIM), it takes, on the average, 16,000 ps for the enzyme and its substrate, GAP, to diffuse a distance of 40 Å relative to each other. Thus, for the long trajectory times needed to study enzyme substrate reactions, molecular dynamics is not feasible.

In the work of Wade et al. (1), a strategy is evolved which extends the overall simulation time to several hundred nanoseconds which is three orders of magnitude longer than the longest times achievable in molecular dynamics. It is applied to TIM whose structural and kinetic properties have been extensively studied. Kinetically, the rate limiting step of TIM with substrate GAP is diffusion controlled. It has been termed a "perfect" enzyme since any further acceleration of the catalytic steps that follow the binding of substrate would have no effect on the rate of the overall reaction (2). Structurally, TIM is a dimeric enzyme which contains disordered, probably mobile loops of nine amino acid residues near the active sites. However, these loops fold down and cover the sites once the substrate is bound, which suggests a kinetic and mechanistic importance of the loop flexibility. The question which this paper addresses is whether or not the flexible loops affect the rate of diffusional encounters by gating substrate access to the active sites.

In order to simulate protein motion on the nanosecond time scale, a number of simplifications in the model are needed. First, all atoms of the TIM dimer except those in the two flexible loops are held fixed in their crystallographically observed positions. Second, a simple polypeptide (SP) model is used to represent the loops (references 19–21 of Wade et al.). A polypeptide is modeled as a string of beads with each bead representing a single amino acid residue. Forces on individual residues of the loops arise from their interaction with other loop residues, the rigid region of the protein, and (for charged residues) mobile ions present in the surrounding fluid. Third, Brownian dynamics is used to generate the diffusional trajectory of the protein. In Brownian dynamics, the solvent is replaced by a bath of random noise. Exposed residues are subjected to random displacements which mimic stochastic collisions with solvent.

With these modifications it is possible to study protein motion on the nanosecond timescale. In the work of Wade et al., two simulations of duration 74 and 104 ns are carried out. They conclude that the loops prevent access to the active site about 50% of the time with an opening/closing period of about 1 ns. Because this is much shorter than the time TIM and GAP remain in close proximity once they diffuse together (about 16 ns), during which time the two species come into contact on numerous occasions, this gating does not reduce the reaction rate below that expected for an "always open" active site. Whatever the role the flexible loops play in catalysis of TIM, they do not reduce the catalytic rate even though they block the active sites a significant fraction of the time. It should be emphasized, however, that this is due to a gating time that is small compared to the time of diffusional encounter and will not be true in general.

In a previous simulation of the diffusional encounter between TIM and GAP, diffusion controlled rate constants between one and two orders of magnitude larger than experimental values were obtained (3). Detailed topographical and electrostatic models were employed, but TIM was treated as a purely rigid body and GAP as a structureless bead. A motivation for the present study was to see if loop flexibility could help reconcile the difference between predicted and observed rates, and the answer appears to be no. It is suggested that a more detailed model of the substrate and a more stringent geometrical definition of a "reactive encounter" between GAP and the active site of TIM may bring calculated and observed rates into better agreement. This will undoubtedly be investigated in the near future.

The work of Wade et al. should stimulate interest in a

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number of areas. As mentioned above, it can be expected to promote additional studies of TIM to determine what additional factors affect rate. It also opens the door to incorporating protein flexibility in other detailed studies of diffusional encounters (4). One in particular is the reaction between Mn superoxide dismutase (SOD) and superoxide, where the active site of SOD is blocked by a tyrosine residue (5). Wade et al. will undoubtedly spark renewed interest in the SP model of Levitt, Warshel, McCammon and co-workers, which has been largely neglected up to now. Future efforts can be expected to further refine and optimize its parameters. Since it is a general polypeptide model, we can expect to see the SP model applied to other important problems in Biophysics, such as the protein folding problem.

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