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Simulation of enzyme-substrate interactions: the diffusional encounter step

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Enzymes are targets for structure-based redesign and drug design. One potentially important design strategy is to create molecules — enzymes and ligands — with desired diffusional encounter properties. Rates of diffusive bimolecular encounter can be calculated by Brownian dynamics simulation. This methodology and its application to study the factors influencing the rates of diffusion-influenced enzymes are described here.

Enzymes are important chemotherapeutic tools and targets. As tools, they can be used to perform desired therapeutic tasks either in their native form or as engineered mutant proteins. As targets, they can be inhibited by drugs that bind to them with high affinity. A wide range of molecular modelling and simulation methods are being developed and applied to both the engineering of enzymes and the design of new ligands. Here, I focus on methods that utilize the three-dimensional atomic structures of enzymes and their complexes with ligands.

The binding of a substrate to an enzyme can be conceptualized as three consecutive events:

–1. Diffusion of the substrate towards its binding site on the enzyme.

–2. Formation of the bound substrate-enzyme complex with specific non-bonded interactions such as hydrogen-bonds.

–3. Catalysis of the substrate to form product.

All three events are targets for chemotherapeutic design. As they take place on different spatial and temporal scales, different computational methods are required to study them. Diffusional encounter can be studied using Brownian dynamics simulation with a simplified representation of the ligand and a continuum treatment of the solvent. Binding affinities can be estimated by methods that use an atomic description of the ligand and enzyme and range from simple rule-based approaches to computationally demanding calculations of free energies by molecular dynamics simulation. Catalysis generally requires a quantum mechanical treatment. Computational studies of the first event — enzyme-substrate diffusional encounter — will be the focus of this article.

The diffusion-dependence of enzymes is discussed in the next section. Then a brief outline of the Brownian dynamics simulation methodology is given, and this is followed by a description of the current status of its application to study enzyme-substrate diffusional encounter.

SIMULATION OF ENZYME-SUBSTRATE DIFFUSIONAL ENCOUNTER

The rate-limiting step of some enzymes is diffusional encounter with their substrate. Such diffusion-controlled enzymes have been referred to as "perfect" enzymes [1] as they typically have very high bimolecular rate constants

(approx. 10^8–10^9 $M^{-1}s^{-1}$). The second-order rate constant (k_{cat}/K_m) of these enzymes is viscosity dependent. Examples of enzymes whose rates are dependent on diffusional encounter with substrate include superoxide dismutase [2], triose phosphate isomerase [3], acetylcholinesterase [4], alkaline phosphatase [5], β-lactamase I [6, 7], glycoxalase II [8], phosphotriesterase [9], and adenosine deaminase [10]. The extent of diffusion-control varies among these enzymes and can also vary for different substrates of one enzyme. Although viscosity dependence is a characteristic of enzyme-substrate diffusion-control of the rate, it may also arise because of viscosity-dependent conformational changes of the enzyme (see e.g. [10]). Dependence on enzyme-substrate diffusional encounter is also often characterized by ionic strength dependence of the enzyme rate (see e.g. [2]).

It is possible to increase the rate of such "perfect" enzymes by using mutagenesis to construct "superperfect" enzymes [11]. This has been shown for Cu,Zn-superoxide dismutase by Getzoff and colleagues [12]. This enzyme protects against oxidative damage by dismuting the negatively charged superoxide radical $O_2^{\bullet-}$ to molecular oxygen and hydrogen peroxide. Simulations show that diffusion of the substrate towards the active site is guided by the electrostatic field of the protein [13–15]. Brownian dynamics simulations by Getzoff [12] and other workers [14, 15], indicated that mutation to glutamine of two specific glutamic acid residues (132 and 133) at the mouth of the substrate access channel in human Cu,Zn-superoxide dismutase would increase the rate of diffusion of superoxide to the active site. Subsequent site-directed mutagenesis experiments showed that the rate could be increased 2–3-fold (depending on the salt concentration). This demonstrates that enzymes can be engineered to have specific kinetic properties by designing their charge distribution. It also indicates that it may be possible to engineer enzymes (and ligands) of therapeutic or industrial value by designing their diffusional encounter properties. Of course, enzymes designed for practical purposes will also have to be designed to behave optimally with respect to other properties. Indeed, superoxide dismutase probably does not function at it maximum speed *in vivo* because of constraints present under physiological conditions, such as presence of phosphate ions. In the future, it may also be possible to engineer enzymes that are not normally diffusion-controlled to the extent that they become so efficient that they become diffusion-dependent.

In order to design new or modified diffusion-controlled enzymes and their ligands it is necessary to calculate the rate of diffusional encounter between the enzyme and its ligand and the impact of changes in the properties of the enzyme and the ligand on the rate. The diffusional encounter rate between highly symmetric particles can often be calculated analytically. However, enzymes and their substrates are usually far from symmetric: they are flexible and have irregular shapes and charge distributions. The consequences of these features for the rate of diffusional encounter can be evaluated by means of Brownian dynamics simulations, e.g. with the UHBD Program [16], as follows.

THE BROWNIAN DYNAMICS SIMULATION METHODOLOGY

The steady state bimolecular diffusion-controlled rate constant k_D for enzyme-substrate diffusional encounter may be written [17, 18] as $k_D = k(b)\beta$ where $k(b)$ is the rate at which a diffusing substrate first encounters a spherical surface of radius b centred on the enzyme, and β is the probability that, having reached this surface, the substrate goes on to reach an active site and react. When b is chosen large enough that the forces on the substrate due to the enzyme are centrosymmetric, $k(b)$ can be calculated analytically. For example, when the forces are negligible, $k(b)$ is given by the Smoluchowski equation [19, 20]: $k(b) = 4\pi Db$ where D is the relative diffusion constant. On the other hand, β cannot be calculated analytically but can be obtained by simulating many trajectories of the substrate that start with the substrate in a random position and orientation at the distance b from the centre of the enzyme. β Is the fraction of these trajectories during which the substrate reaches the active site and satisfies specified criteria for the occurrence of a reaction.

Because of the long distance and time-scales of diffusion, it is not feasible to simulate these trajectories with full atomic detail by classical

molecular mechanics. The Brownian dynamics method permits the elimination of the less important features of the system while allowing the properties that can have a crucial impact on diffusional motion to be taken into account. It entails treating the solvent as a viscous continuum that exerts frictional and random Brownian forces on the solute molecules [21], and employing a simplified model for simulating the motion of the solute molecules. In addition to stochastic and viscous forces from the solvent, a diffusing ligand is subject to systematic forces which can be modelled as follows:

- (i) Electrostatic forces may be derived from a precalculated electrostatic potential map of the enzyme. This may be computed taking account of the atomic charge distribution over the enzyme and the different dielectric permittivities of the enzyme and the surrounding solvent by numerical solution of the Poisson-Boltzmann equation [22, 23].
- (ii) Excluded volume forces that prevent the ligand from penetrating the enzyme are usually treated by disallowing molecular overlap during the simulations.
- (iii) Hydrodynamic interactions can influence bimolecular encounter [24, 25] and may be treated by using approximate hydrodynamic interaction tensors [26].
- (iv) If the internal flexibility of the ligand or the enzyme is simulated, then forces between the moving parts must be calculated. For example, the motion of a peptide may be simulated by treating it as a chain of spherical beads, each bead representing a residue that interacts with the other residues *via* bonded (pseudo-bond, angle and torsion) and non-bonded (excluded volume, electrostatic and solvent hydrophobic effect) forces [27–29]. This type of representation can be applied to multisubunit substrates and particularly flexible regions of the target enzyme.

APPLICATIONS OF BROWNIAN DYNAMICS SIMULATIONS OF ENZYME-SUBSTRATE ENCOUNTER

The Brownian dynamics simulation method has so far been used to study enzyme-substrate encounter for three enzymes which appear to be diffusion-controlled and whose crystal structures are known: superoxide dismutase, triose phosphate isomerase and acetylcholinesterase. These studies have provided the following insights into enzyme-substrate diffusional encounter.

The electrostatic fields of enzymes can translationally and orientationally steer their substrates into their active sites

In all three enzymes studied, electrostatic steering of the ligand towards the enzyme's active sites is observed in Brownian dynamics simulations [13, 14, 30, 31]. It generally increases the rate over that for an uncharged model system without electrostatic interactions by 1–2 orders of magnitude. The enzyme's electrostatic potential can even accelerate the rate at which a substrate reaches the active site when both enzyme and substrate have net charges of the same sign. This is the case for superoxide dismutase. The ionic strength dependence of its rate is opposite to that expected for two like charged objects and can be reproduced in Brownian dynamics simulations [13, 14]. Thus, the non-uniform charge distribution over an enzyme is important for its interaction with its substrate. Electrostatic steering is usually enhanced by the heterogeneity of the dielectric medium with the boundary between low-dielectric protein and high-dielectric solvent distorting the potential, particularly when it has high curvature. Other dielectric models, e.g., with distance-dependent or uniform (at approx. 80) relative dielectric constant throughout the system, usually lead to less electrostatic steering [14].

The effects of point mutations have been investigated by simulation for superoxide dismutase and acetylcholinesterase. For the former, increased rates were predicted from simulations for certain point mutants and the predictions were confirmed experimentally [12] as discussed above. For the latter, many charge-altering mutations have been observed experimentally to have small effects on the rate. Although this seems counterintuitive for electrostatically driven substrate binding, simulations reproduce the observed trends and show that this is consistent with electrostatic steering [32], for which local attractive fields tend to have a dominant effect.

Rotational steering of the ligand by the electrostatic field of the enzyme has been investigated in simulations of triose phosphate iso-

merase [33] and acetylcholinesterase [34] in which the substrate is represented as a dumbbell with a dipole moment. For triose phosphate isomerase, rotational steering enhances the probability that the substrate enters the active sites in the orientation required for reaction. It is a shorter range effect than the translational steering and is only detectable when the substrate is within approx. 5 Å of the position in the active site where it is assumed to react. For acetylcholinesterase, less good agreement with experimental rates is observed in simulations in which the ligand is modelled as a dumbbell [34], and this indicates that the dynamics of the protein influence the rate of diffusion of the substrate into the active site.

Enzyme flexibility can have varying effects on the rate of enzyme-substrate diffusional encounter

When the active site is rather buried in the protein and the ligand must travel down an access channel, fluctuations in the protein structure that should be modelled on the atomic scale can affect the rate at which the substrate reaches the active site. For such enzymes, the diffusional encounter rate may be evaluated by coupling Brownian dynamics simulations of the substrate diffusing to the mouth of the access channel to molecular dynamics simulations of the passage of the substrate through the channel to the active site using Markov chain models [35, 36]. When this approach was applied to superoxide dismutase, the rate constant was approximately halved compared to that from calculations with the rigid protein.

Motions of parts of the enzyme that take place on a much longer time-scale than is presently accessible by molecular dynamics may also affect the rate of enzyme-substrate diffusional encounter. These may be treated by simulating their motion by Brownian dynamics at the same time as diffusion of the substrate is simulated. This has been done for triose phosphate isomerase [29, 37] which is an enzyme with highly conserved flexible peptide loops that close over its active sites when the substrate binds. The motion of eleven consecutive residues in the loop was simulated by treating them as a chain of suitably parameterized beads. Figure 1 shows snapshots from one trajectory. Although the peptide loops diffused back and forth over the active sites during the simulations, they did not have a significant effect on the calculated rate of enzyme-sub-

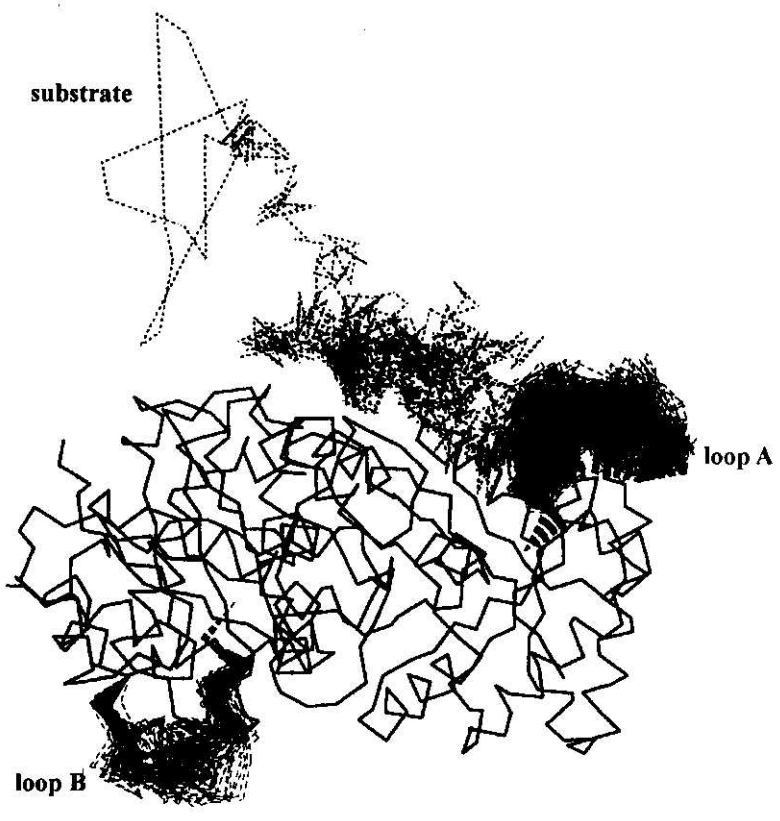

Fig. 1. Superimposed snapshots from one 11 ns long trajectory generated by Brownian dynamics simulation in which the substrate reached an active site of the dimeric enzyme, triose phosphate isomerase.

The substrate trajectory is shown by short dashed lines connecting consecutive positions. The motion of the flexible peptide loops at the active sites was simulated and is shown by long dashed lines. Loop A opens up during the simulation and allows the substrate to enter the active site next to it. Note that in most of the trajectories generated, the substrate does not reach an active site, and often if it does, it takes a much longer and less direct route towards the active site than shown here.

strate encounter. Simulations in the absence of substrate [29] showed that the loops could be considered as gates to the active sites that are closed sufficiently to prevent substrate access for about half of the simulation time and open and close with an average period of about 1 ns. This gating period is much shorter than the characteristic diffusion time of about 20 ns required for the substrate to explore the region around the protein [37]. This explains why the loops do not slow down the simulated rate of substrate access to the active site: the substrate can "wait around" long enough for a loop to open up. In other proteins, such active site loops might influence the rate of substrate access either by slowing it by acting as a gate or by increasing the rate of access by acting as a scoop.

Hydrodynamic interactions appear to have a modest effect on the rate of enzyme-substrate diffusional encounter

Hydrodynamic interactions arise because a moving solute displaces the solvent surrounding it which in turn displaces other solutes. They usually tend to decrease diffusional encounter rates. Hydrodynamic interactions have only been partially included in detailed studies of enzyme-substrate encounter; e.g. between the monomers of the dumbbell substrate models [33]. Brune & Kim [24] suggested that hydrodynamic interactions would favour encounters between an enzyme with a binding cleft and an elongated substrate when the long axes of the molecules were perpendicular. Studies of simple model systems indicate, however, that hydrodynamic interactions between enzymes and their substrates should have a weak effect on orientational steering compared to electrostatic interactions although they can be expected to reduce rates by about 20–30% compared to those calculated without hydrodynamic interactions [25].

CONCLUDING REMARKS

Diffusional encounters between enzymes and their ligands can be simulated by Brownian dynamics. Encounter rates that are in good agreement with experimental measurements can be calculated and their sensitivity to the steric, electrostatic, dynamic and hydrodynamic features of both enzymes and ligands investigated. Here, the application to the encounter of enzymes with small substrates has been discussed. The method can also be applied to examine protein-protein encounter, e.g. the association of cytochrome *c* and cytochrome *c* peroxidase prior to electron-transfer [38] and antibody-antigen complexation [39]. There are still many improvements to be made to the simulation methodology, particularly in the treatment of molecular flexibility. Nevertheless, the accuracy of the simulations is sufficient that, as has been shown for superoxide dismutase, they can be used to guide site-directed mutagenesis experiments to alter diffusional encounter rates. The simulation methodology should prove a useful tool for studies of diffusion-influenced enzymes of therapeutic and industrial value.

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