Communications to the Editor

A 240-Fold Electrostatic Rate-Enhancement for Acetylcholinesterase−Substrate Binding Can Be Predicted by the Potential within the Active Site

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An approximate relation that allows easy prediction of k/k^0, the enhancement of enzyme−substrate binding rates by an interaction potential U, was derived recently by one of us.[1] This is given by

$$\boldsymbol{k} = \boldsymbol{k}^0 \langle \exp(-\beta \boldsymbol{U}) \rangle \tag{1}$$

where the average of the Boltzmann factor is over the region in which the substrate can effectively bind to the enzyme. Equation 1 becomes exact when this binding region reduces to a point. In this paper, we present a test of this relation on the enzyme *Torpedo californica* acetylcholinesterase (TcACHE).

We focus on the rate enhancement by electrostatic interactions between the enzyme and a positively charged substrate, such as the natural substrate acetylcholine. The interaction potential was found by treating the substrate as a test charge in the presence of the electrostatic potential of TcACHE. The electrostatic potential was calculated using the crystal structure of TcACHE (PDB entry 1ACE)[2] and the UHBD program, as described previously.[3] In particular, nonionizable residues of the enzyme were assigned partial charges from the CHARMm 22.0 polar hydrogen parameter set[4] and ionizable residues were given charges representative of the distribution of ionization states at pH 7 and the desired ionic strength. A 140 × 140 × 140 cubic lattice with a 0.8 Å spacing was used in the calculation of the electrostatic potential.

Binding rate constants were calculated from Brownian dynamics (BD) simulations using an algorithm developed previously.[5] Briefly, substrates are started in the binding region (see below) with a Boltzmann distribution. If a substrate is inside the binding region, it can bind to the enzyme with an intrinsic rate constant k_{in}. When this happens, its trajectory is terminated. All trajectories are terminated if they reach a preset cutoff time. The rate constant is given by the long-time limit of the fraction of trajectories that are not terminated due to binding. By taking the limit $k_{in} \to \infty$, the diffusion-controlled rate constant k_{DC} is obtained.

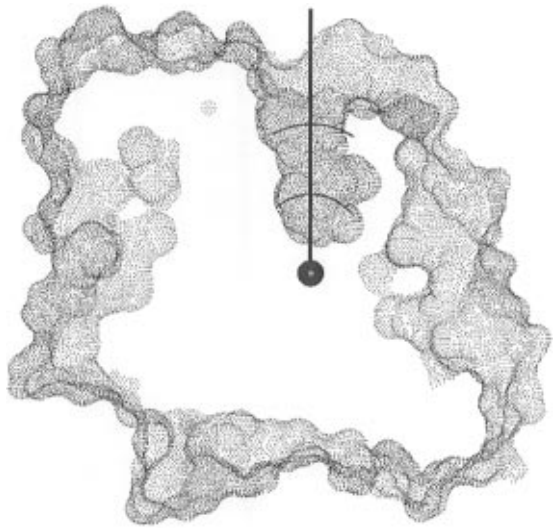

Figure 1. Cross section of a dot representation of the surface of TcACHE accessible to the center of a 2 Å spherical substrate. The gorge axis of the active site is shown as a line pointing upward from CD of Ile444. Arcs intersecting the gorge axis represent the upper boundaries of two definitions of the binding region. They are ∼9 and 17 Å away from the gorge base.

Substrate trajectories were propagated according to the Ermak−McCammon algorithm[6]

$$\mathbf{r} = \mathbf{r}_0 + \beta \mathbf{F} D \Delta t + \mathbf{s}\sqrt{2D\Delta t} \tag{2}$$

where **s** is a random vector with components that are normally distributed and D is the sum of the diffusion constants of the enzyme and the substrate. The diffusion constants were estimated by using hydrodynamic radii of 32.0 and 3.5 Å, respectively, for the enzyme and substrate,[3] resulting in D = 78.3 Å^2/ps at a temperature of 300 K. Overlap of the substrate with the enzyme was prevented by representing enzyme atoms as van der Waals spheres and the substrate as a 2 Å sphere.[3] The distance beyond which there is no chance of enzyme−substrate collision was r_{surf} = 44.8 Å, when measured from the geometric center of the enzyme. The time step Δt was 1 ps if the enzyme−substrate distance r was less than r_{surf} and 1 ps + $(r - r_{surf})^2/200D$ otherwise. Each component of the force **F** at a position inside an elementary cube was calculated by first finding its values at the eight corners of the cube by central difference and then interpolating using the formula

$$\begin{aligned} f = {} & (1-x)(1-y)(1-z)\, f_{i,j,k} + xyz f_{i+1,j+1,k+1} + \\ & x(1-y)(1-z)\, f_{i+1,j,k} + (1-x)\, yz f_{i,j+1,k+1} + \\ & (1-x)\, y(1-z)\, f_{i,j+1,k} + x(1-y)\, z f_{i+1,j,k+1} + \\ & (1-x)(1-y)\, z f_{i,j,k+1} + xy(1-z)\, f_{i+1,j+1,k} \end{aligned} \tag{3}$$

Here x, y, and z are the displacements, in the three directions, of the position under study from the lattice site with indices ijk, measured in units of the lattice spacing.

As shown in Figure 1, the active site of TcACHE is a narrow but deep gorge with a width ∼5 Å and a depth of ∼20 Å. Following previous studies,[3] a line that connects the CD atom of Ile444 and the geometric center of four atoms, CA of Glu73, CB of Asn280, CG of Ap285, and O of Leu333, was defined

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as the gorge axis. The binding region was defined as the part of a 6 Å sphere that was accessible to the 2 Å substrate. This sphere was centered at a point that was on the gorge axis and 3 Å away from the gorge base (i.e., the CD atom of Ile444). A uniformly distributed collection of positions inside the binding region were generated by first randomly selecting points in the sphere and then discarding those resulting in overlap between the substrate and atoms of the enzyme. The potentials at these positions were obtained by the interpolation formula given in eq 3 and were used to calculate the average Boltzmann factor. Each of the above positions was either repeated as initial positions for the substrate or discarded according to the Boltzmann weight at that position to ensure a Boltzmann distribution of initial positions.

From 4487 positions uniformly distributed in the binding region, the average Boltzmann factor $\langle \exp(-\beta U) \rangle$ at an ionic strength of 150 mM was calculated to be 1003.6. Equation 1 would predict that the electrostatic interactions between the enzyme and the substrate enhance the binding rate by this enormous factor! Using these positions as initial positions, the diffusion-controlled rate constant when the electrostatic interactions were turned off, k^0_{DC}, was found to be 7.4×10^6 M^{-1} s^{-1} by BD simulations. The diffusion-controlled rate constant in the presence of the electrostatic interactions, k_{DC}, is thus predicted to be 7.4×10^9 M^{-1} s^{-1}. When 4487 initial positions with a Boltzmann distribution were started and the electrostatic interactions were turned on, k_{DC} was found to be 1.7×10^9 M^{-1} s^{-1} by BD simulations.[7] This agrees with the prediction of eq 1 to within a factor of 4.1.[8]

An increase in ionic strength will reduce the electrostatic rate enhancement. At an ionic strength of 250 mM, the average Boltzmann factor became 296.2. The rate enhancement is predicted by eq 1 to decrease from that at a 150 mM ionic strength by 3.4-fold. In comparison, the decrease was found to be 2.6-fold by BD simulations. The potential within the binding region is thus very predictive of the electrostatic rate enhancement. One consequence of this result is that charge mutations far away from the binding region are expected to have little effect on the rate enhancement. This is precisely what was observed in recent experimental[9] and simulation[3b] studies.

While it is interesting that the potential within the binding region is predictive of the rate enhancement when the binding region is restricted to the 6 Å sphere near the base of the active site gorge, one has to ask whether such a binding region provides a good model for realistic enzyme−substrate binding. We address this question by comparing simulation results with experimental data.[10] Experimentally, the rate constant for the diffusional encounter of acetylcholine with TcACHE is $\sim(0.5-1.0) \times 10^9$ and $\sim(0.1-0.2) \times 10^9$ M^{-1} s^{-1}, respectively, at ionic strengths of 150 and 250 mM, and the rate constant for the diffusional encounter of a neutral analog, 3,3-dimethylbutyl acetate, with the enzyme is $\sim 7 \times 10^6$ M^{-1} s^{-1}. The electrostatic rate enhancements at ionic strengths of 150 and 250 mM are thus ∼100- and ∼30-fold, respectively. These are in rough agreement with the BD simulation results of 240- and 90-fold, respectively. Moreover, the experimental binding rate constant for the neutral substrate matches the BD simulation result in the absence of electrostatic interactions. The 6 Å spherical binding region near the base of the active site gorge thus appears to model TcACHE−acetylcholine binding reasonably well.

In conclusion, we have demonstrated that, for a molecular model that roughly reproduces experimental data on TcACHE−acetylcholine binding, the electrostatic potential within the active site can be used to predict the rate enhancement due to electrostatic interactions. The predictive power is particularly significant, since the rate enhancement in the present case is about 2 orders of magnitude.

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(7) Binding rate constants were also calculated by running the UHBD program with a quite different algorithm.[3] The result in the presence of the electrostatic interactions was 1.6×10^9 M^{-1} s^{-1}. Unfortunately, a statistically significant result could not be obtained for k^0_{DC} since its value was too small.

(8) The accuracy of eq 1 will deteriorate as the size of the binding region is increased. For example, when the binding region was based on a 9 Å sphere centered at a point on the gorge axis and 8 Å away from CD of Ile444, $k^0_{DC} = 23 \times 10^6$ M^{-1} s^{-1} and $k_{DC} = 2 \times 10^9$ M^{-1} s^{-1} at a 150 mM ionic strength by BD simulations. The electrostatic rate enhancement is thus 90-fold. This is smaller than $\langle \exp(-\beta U) \rangle$ by a factor of 17.

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