Acetylcholinesterase: Electrostatic Steering Increases the Rate of Ligand Binding†

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ABSTRACT: Brownian dynamics simulations have been used to calculate the diffusion-controlled rate constants for the binding of a positively charged ligand to several models of acetylcholinesterase (AChE). The crystal structure was used to define the detailed topography and the active sites of the dimeric enzyme. The electric field around AChE was then computed by solving the Poisson equation for different charge distributions in the enzyme at zero ionic strength. These fields were used in turn to calculate the forces on the diffusing ligand. Significant increases in the rate constant resulted in going from a model with no charges to one with the net charges concentrated at the centers of the monomers and then to a model with a realistic distribution of charges throughout the enzyme. The results show that electrostatic steering of ligands contributes to the high rate constants that are observed experimentally for AChE.

Acetylcholinesterase (AChE) is an enzyme which is bound to the postsynaptic membrane at neuromuscular and other junctions (Stryer, 1988). Its principal role is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) to yield acetic acid and choline (Barnard, 1974). *N*-Methylacridinium (NMA) is an inhibitor of AChE (Rosenberry, 1975). The reactions between AChE and ACh or NMA are diffusion controlled; i.e., the rate-limiting step is the diffusional encounter between the enzyme and its substrate or inhibitor (Bazelyansky et al., 1986; Hasinoff, 1982; Rosenberry, 1975). There is evidence that electrostatic forces may influence the rate of this encounter (Nolte et al., 1980). Here, we describe a theoretical study of NMA binding to AChE. We first calculate the electrostatic potential field around the AChE dimer. We then use this potential in Brownian dynamics simulations of the diffusional encounter between AChE and NMA. The results indicate that electrostatic steering of ligands makes a substantial contribution to the efficiency of this important enzyme.

In the next section we discuss the model used for electrostatic potential calculations and the results of those calculations. In the section that follows we describe the use of Brownian dynamics to calculate diffusional encounter rate constants. Results and a discussion of the calculations are presented in the final section.

ELECTROSTATICS

The enzyme–solvent system is modeled as a heterogeneous dielectric continuum in which point charges are embedded. More specifically, each atom of AChE is modeled as a point charge embedded in a low dielectric ($\epsilon = 2$) sphere. The solvent is modeled as a continuum with a high dielectric coefficient ($\epsilon = 80$). The inhibitor is considered to be a test charge which does not affect the electrostatic field of the enzyme–solvent system. The effect of added salt is not included in these initial studies.

To calculate the electrostatic potential of the enzyme–solvent system, we solved the Poisson equation

$$\nabla^2\Phi = \frac{4\pi}{\epsilon}\rho \qquad (1)$$

via a finite difference scheme developed in this laboratory (Davis & McCammon, 1991a). Equation 1 relates the electrostatic potential Φ to the charge density ρ in the enzyme and the dielectric coefficient ϵ; each of these is a function of position relative to a coordinate system fixed in the enzyme.

The 2.8-Å crystal structure of AChE from *Torpedo californica* was used to build our model of the AChE dimer (Sussman et al., 1991). Eleven residues were missing from the X-ray structure as were the side chains of several other residues. All of these residues were more than 25 Å from the active site. The missing heavy atoms were built in using Quanta (Molecular Simulations, Inc., Waltham, MA). Polar hydrogens were patched in using CHARMm (Brooks et al., 1983). Twenty-six water molecules (13 per AChE monomer) found in the X-ray structure were also included. The resulting structure was relaxed by 100 steps of steepest descent energy minimization using CHARMm.

The electrostatic potential around the enzyme was calculated with the UHBD program (Davis et al., 1991b). CHARMm parameters for charge and radii were used in the electrostatic calculations. The net charge of the AChE dimer was −16. For the finite difference calculation of the potential, the AChE dimer was placed on a 190^3 grid with a spacing of 2.0 Å. The potential has the general characteristics of a monopole plus quadrupole field, as shown in Figure 1. Near the enzyme active site the potential becomes negative. On the side opposite the active site the potential becomes positive. Thus, one might postulate a mechanism for steering a positively charged substrate (or inhibitor) toward the active site. If the substrate is far from the enzyme, it only sees the net negative charge. It is attracted to the enzyme from all directions. Near the enzyme, the substrate begins to feel the quadrupole field. If the substrate approaches the active site directly, it is attracted

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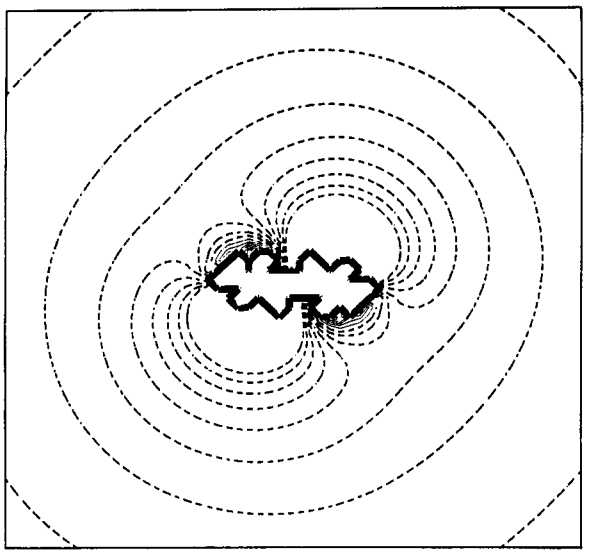

FIGURE 1: Contour plot of AChE electrostatic potential. The thick line denotes the AChE dimer. Dashed lines denote negative potential. Solid lines denote positive potential. Fifteen equally spaced contours were taken between −1.0 and +0.5 kcal/(mol·charge).

and reacts. If the substrate approaches the side opposite the active site, it is repelled and then steered toward the active site.

BROWNIAN DYNAMICS

The rate constants for detailed models of diffusion-controlled reactions can be calculated by Brownian dynamics simulations (Ermak & McCammon, 1978; Northrup et al., 1984; Davis et al., 1991c). In these, one computes and then analyzes many trajectories of one reactant diffusing toward its partner under the influence of any intermolecular forces. More formally, one begins by dividing the space around the target molecule (in this case the enzyme) into three regions based on the distance, r, from the center of the target: ($r < b$), ($b < r < q$), and ($r > q$). The parameter b is usually chosen so that interaction forces between the two molecules are centrosymmetric for $r > b$. The parameter q is chosen so that the flux of molecules is centrosymmetric for $r > q$ (Zhou, 1990).

All simulations are in the frame of reference of the target. A trajectory is started by randomly placing the diffusing molecule (in this case the inhibitor) on the surface $r = b$ (b surface). The molecule's trajectory is then calculated step by step by numerically integrating the Langevin equation that describes the diffusion of the inhibitor (Ermak & McCammon, 1978). A spherical model is used for NMA in this initial study, so the Ermak–McCammon equation takes on the simple form

$$\vec{r} = \vec{r}^0 + (k_B T)^{-1} D\vec{F}(\vec{r}^0)\Delta t + \vec{S} \qquad (2)$$

where $\vec{r}^0$ and $\vec{r}$ are the location of the NMA center before and after a step is taken, k_B is Boltzmann's constant, T is the temperature, D is the relative diffusion constant, $\vec{F}$ is the force on NMA at $\vec{r}^0$, Δt is the time step, and $\vec{S}$ is a random vector with statistical properties $\langle \vec{S} \rangle = \vec{0}$ and $\langle S_i S_j \rangle = 2D(\delta_{ij})\,\Delta t$. Equation 2 does not take hydrodynamic interactions into account, though they can be included. The force $\vec{F}$ is calculated from the electrostatic potential discussed above. The time step, Δt, is chosen so that the forces on the molecule do not change by more than 10% during a step. The trajectory is continued until the diffusing molecule reacts with the target or hits the q surface. A correction is applied to the encounter probability to allow for the possibility of the diffusing molecule recrossing the q surface and reacting with the target (Northrup et al., 1984). Several thousand trajectories are calculated to get an estimate of the rate constant.

The association rate constant is given by (Northrup et al., 1984)

$$k = k_D(b)\beta[1 - (1 - \beta)\Omega]^{-1} \qquad (3)$$

where $k_D(b)$ is the steady-state rate at which diffusing molecules with $r > b$ would first hit the b surface, β is the fraction of trajectories that lead to reaction rather than truncation at the q surface, and Ω is the correction factor for truncation effects. Assuming that intermolecular interactions are centrosymmetric and that hydrodynamic interactions are negligible for $r > b$, $k_D(b)$ is given by

$$k_D(b) = 4\pi\left(\int_b^\infty (e^{U/k_B T}/r^2 D)\, dr\right)^{-1} \qquad (4)$$

The quantity Ω is given by

$$\Omega = k_D(b)/k_D(q) \qquad (5)$$

These methods have been applied to the study of the diffusional encounter of superoxide with superoxide dismutase (Sines et al., 1990, 1992; Sharp et al., 1987; Head-Gordon & Brooks, 1987; Allison & McCammon, 1985), of cytochrome c with cytochrome peroxidase (Northrup et al., 1988), of D-glyceraldehyde phosphate with triosephosphate isomerase (Madura & McCammon, 1989; Luty et al., 1993), and of ligands with competing receptor sites (Northrup, 1988). Recently, Getzoff et al. have shown that such simulations can aid in the design of mutagenized enzymes with rate constants several times larger than those of the wild type (Getzoff et al., 1992; McCammon, 1992).

In the present study, the hydrodynamic radius of the AChE dimer was taken to be 60 Å while that of NMA was 5 Å. AChE had a net charge of −16.0 e and NMA had a charge of +1 e. The b and q surfaces had radii of 130 and 300 Å, respectively. A dummy atom was placed in the active site near the catalytic residues. The dummy atom was approximately 10.8 Å from the Cδ atom of Glu 327, 6.8 Å from the Oγ atom of Ser 200, and 5.4 Å from the Cδ2 of His 440. A reaction was considered to occur when the inhibitor passed within 13 Å of this atom. Because of excluded volume effects, the reaction surface was not a 13-Å sphere but rather a patch on a 13-Å sphere. In order for the inhibitor to react, it had to diffuse down a narrow gorge. Finally, a variable time step was used in this study (Davis et al., 1991c). The values were chosen so that forces did not change significantly at each step and so that the displacements are small compared to the distances to the b and q surfaces.

Calculations were performed under three different conditions: diffusion subject to full electrostatic forces, diffusion subject only to the monopole moments of the monomers, and diffusion without electrostatic forces. For the monopole calculations, a charge of −8 was placed at the center of each monomer. To calculate forces for Brownian dynamics, two descriptions of the electrostatic potential were used, a "coarse" description and a "fine" one. A coarse 190^3 grid with 2.0-Å spacing was calculated as described under Electrostatics. A fine 190^3 grid with 1.0-Å spacing was then calculated using the coarse grid above to set boundary conditions. When the inhibitor was far from the enzyme, the coarse grid was used to calculate forces. When the inhibitor was near the enzyme, the fine grid was used to calculate forces.

Table I: Rate Constants k for Differently Charged Models of AChE

charge model	k (10^9 M^{-1} s^{-1})[a]
all charges	43 ± 1
monopoles only	26 ± 1
no charges	0.5 ± 3

[a] Errors are estimated by propagation of the 90% confidence intervals calculated for β.

RESULTS AND DISCUSSION

The results of these initial calculations are summarized in Table I. The results suggest that electrostatic interactions between AChE and ACh or NMA play a major role in enzyme–substrate or enzyme–inhibitor encounter.

The diffusion-controlled rate constant increases by an order of magnitude when the charge on the enzyme is increased by 16 units upon including the monopole interactions. This change is reminiscent of the order of magnitude decrease in rate seen in experimental and simulation studies of superoxide dismutase when the charge on that enzyme was decreased by 15 units at low ionic strength by partial acylation of its lysine residues (Cudd & Fridovich, 1982; Allison et al., 1988).

The rate constant of AChE increases by another 65% when the detailed distribution of its charges is used. In addition to the attraction of substrate by the overall charge of AChE, there is therefore a significant steering of substrate toward the active sites by the quadrupole and higher moments of the electrostatic field of the enzyme. Substrate steering by quadrupole and higher moments is also now well established for superoxide dismutase (Davis et al., 1991b; Getzoff et al., 1992).

Because the monopole and quadrupole components of the electrostatic field both tend to increase the rate constant of AChE, this rate constant can be expected to decrease monotonically with increasing ionic strength. But previous results for superoxide dismutase suggest that the quadrupole component and even the longer range monopole component will increase the rate constant of AChE significantly at physiological ionic strengths (Allison et al., 1988).

The experimentally determined rate constants for positively charged ligands to bind to AChE at zero ionic strength range up to about 4×10^9 M^{-1} s^{-1} (Quinn, 1987). That the rate constant calculated here for the model with the full charge distribution is about 10 times larger is very likely due to the simplified reaction criterion used in this initial study of AChE. Simulation studies of diffusion-controlled reactions catalyzed by another enzyme, triosephosphate isomerase, showed that the calculated rate constants decreased by 1–2 orders of magnitude when simple spherical models of the substrate were replaced by structured models and orientational criteria for reaction were introduced (Luty et al., 1993). Future work will address these issues and also the possibility that orientational steering of ligands (Luty et al., 1993), or the aromatic guidance mechanism due to the large number of aromatic residues in the active site gorge as recently suggested by Sussman et al. (1991), may occur in addition to the translational steering demonstrated in the present study. Finally, electrostatic effects may also play an important role in product escape from AChE (D. Ripoll et al., private communication).

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