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Conformational Effects in Enzyme Catalysis: Reaction via a High Energy Conformation in Fatty Acid Amide Hydrolase

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ABSTRACT Quantum mechanics/molecular mechanics and molecular dynamics simulations of fatty acid amide hydrolase show that reaction (amide hydrolysis) occurs via a distinct, high energy conformation. This unusual finding has important implications for fatty acid amide hydrolase, a key enzyme in the endocannabinoid system. These results demonstrate the importance of structural fluctuations and the need to include them in the modeling of enzyme reactions. They also show that approaches based simply on studying enzyme-substrate complexes can be misleading for understanding biochemical reactivity.

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Under native conditions, proteins are subject to a variety of random thermal motions and conformational transitions that may be crucial for their function (1,2). Enzyme structure should therefore be considered as an ensemble of conformational states rather than as a single static structure. Molecular dynamics (MD) simulations have played a crucial role in developing this view (1). Such simulations of proteins typically apply empirical force fields that can provide a good description of protein structure and dynamics. These ''molecular mechanics'' methods are, however, generally not applicable for the modeling of chemical reactions, because of their simple functional forms and parameterization for stable molecules. Simulations of enzyme-substrate complexes can provide insight into some potentially important determinants of reactivity, such as the proximity of reacting groups and the conformational behavior of substrates: for example, it is known that some enzymes bind substrates in conformations that are similar to the transition state for reaction (3). However, we show here that predictions of reactivity based purely on the conformational preferences of enzyme-substrate complexes can be misleading in some cases.

Enzyme-catalyzed reactions can be modeled using combined quantum mechanics/molecular mechanics (QM/MM) methods (4). The use of a quantum chemical method allows the study of chemical bond-breaking and making while the effects of the protein environment are included by a MM force field. QM/MM calculations are relatively computationally demanding, particularly when high levels of QM theory are used. Due to these computational demands, an adiabatic mapping approach is often employed in QM/MM modeling, in which a potential energy surface (PES) is calculated by minimization along approximate reaction coordinates. This type of approach has been shown to perform well for several enzyme reactions (e.g., by correlation with experimental data (4)). Often a single, minimum-energy starting structure is employed, with the assumption that it is a reasonable representation of the important conformational space of the reacting enzyme-substrate complex. This approach has been useful in identifying probable mechanisms in many enzymes, including fatty acid amide hydrolase (FAAH) (5). It does not, however, account for structural fluctuations in the protein, which can lead to significant fluctuations in calculated barrier heights (6). More fundamentally, an inappropriate choice of starting structure could lead to an incorrect mechanism being modeled. Similar problems could arise when the definition of reaction coordinate is difficult, and simulation of a large set of reaction paths is not feasible.

FAAH is responsible for the deactivating hydrolysis of the endocannabinoid anandamide and other bioactive lipid amides (7). We have recently identified a likely mechanism for the first step of the acylation reaction of FAAH with oleamide, using QM/MM methods (5). The results indicated that (neutral) Lys-142 initiates catalysis by deprotonating the nucleophilic residue Ser-241 via Ser-217, which acts as a proton shuttle during the reaction. B3LYP/6-31+G(d)//PM3-CHARMM QM/MM calculations indicate that deprotonation of Ser-241 represents the rate-limiting event of the first step of the acylation reaction. The calculated barrier is consistent with experiment (5). Previously, only a single enzyme-substrate Michaelis complex was studied. We now report a detailed investigation of the effects of conformational fluctuations.

The first step of the FAAH acylation reaction with oleamide was explored here by using multiple conformations of the enzyme-substrate complex. We apply a well-tested QM/MM approach (8), at the B3LYP/6-31+G(d)//PM3-CHARMM level, for reliable calculation of reaction energetics (9).

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The results show that geometrical fluctuations of the active site can significantly affect the overall energetic barrier, and that significant conformational changes occur in the reaction. Conformational fluctuations do not affect the general shape of the PESs; however, consistency between experimental and calculated barriers was observed only with a specific arrangement of the reactants. These reactive configurations correspond to a conformation observed only rarely during MD simulations, indicating that the so-called ''NAC'' proposal, which tries to relate catalysis to an enzyme's ability to maintain high populations of reactive conformers (3), does not work in FAAH. The free-energy difference between the reactive (low populated) conformational state from the prevalent, unreactive conformations was calculated with two different methods (by applying the Boltzmann distribution law and by umbrella sampling techniques (10)). The energetic cost for the conformational transition was found to be relatively small (see below), compared to the barrier for the catalytic process, suggesting that the Curtin-Hammett principle (11) of chemical reactivity can be applied to enzyme catalysis.

The simulation system consisted of a molecular model of the functional subunit of the FAAH-oleamide complex, prepared following the procedure described previously (5). CHARMM (v. 27b2) was used for all calculations (12). An adiabatic mapping approach was used to calculate PESs, generating approximate models of the transition states and intermediates. Briefly, the PES was explored by selecting two reaction coordinates (R_x and R_y) to represent the key steps of the acylation process, i.e., deprotonation of Ser-217 by (neutral) Lys-142, proton transfer from Ser-241 to Ser-217, and nucleophilic attack on the carbonyl of oleamide by Ser-241, leading to the formation of the tetrahedral intermediate (Fig. 1). R_x was defined as $R_x = d[O_1, H_1] - d[O_2, H_1] - d[O_1, C]$), including proton abstraction from Ser-241 by Ser-217 and the nucleophilic attack by Ser-241. R_y, defined as $R_y = d[O_2, H_2] - d[N_1, H_2]$, describes the proton transfer between Ser-217 and Lys-142. R_x and R_y were increased in steps of 0.2 and 0.1 Å, respectively, with harmonic restraints of 5000 kcal/mol $Å^{-2}$. Geometry optimization of the structures to an energy gradient of 0.01 kcal/mol $Å^{-1}$ was performed at each point. The energy was then computed by a single point calculation, removing the energy contributions due to reaction coordinate restraints. The QM atoms of each structure were isolated and single point energy calculations (~300 points per surface, almost 1500 in total) were performed at PM3 and B3LYP/6-31G+(d) DFT levels (15). The corrected PESs were obtained by subtracting the PM3 energy of the isolated QM region from the total QM/MM energy, and adding the B3LYP energy (5,9). Four different representative starting structures from a MD simulation (5) were used to investigate the role of conformational effects. Comparison of the PESs indicated that all four substrate complexes follow similar reaction pathways. In three of the four cases, a small variation in the energy barrier was observed (barriers of 30 ± 3 kcal/mol). However, for the fourth structure (*D*), the PES showed a barrier of only 18 kcal/mol. It should be noted that the effective calculated barrier will be lowered by zero-point energy and tunneling effects (13). Even taking these into account, given the known properties of the B3LYP method, it appears that only the barrier calculated for structure *D* is consistent with experiment (apparent activation barrier of ~16 kcal/mol (14)). Crucially, this remains true when the free-energy difference between reactive and unreactive conformations (3.6–4.7 kcal/mol) is considered (see below), as the overall estimated barrier for the reaction is ~22 kcal/mol.

Geometrical parameters, such as distances between QM atoms (Table 1), those involved in substrate binding, the nucleophile attack angle, and dihedral angles of oleamide (Tables S1 and S2 in the Supplementary Material) indicate that the reactive conformation is significantly different from the unreactive ones. In particular, the distance between the nucleophilic oxygen of Ser-241 (O_1) and the nitrogen (N_1) of the neutral Lys-142 is significantly different between the reactive and unreactive structures.

The potential energy profiles for the chemical transformation of the system from configurations 2 to 4 are reported for conformations *B* and *D* in Fig. S1 in the Supplementary Material. As the second proton transfer proceeds and negative charge increases on the Ser-241 oxygen, the distance between O_1 and N_1 becomes smaller; at the transition state (TS), where the H_1 proton is nearly equidistant between the oxygens of Ser-217 and Ser241, it reaches its minimum value. Lys-142, protonated during the first part of the reaction, stabilizes the proton transfer between the two serine residues. In the reactive substrate complex (structure *D*), the [N_1, O_1] distance is only 4.09 Å, similar to the TS value of 3.91 Å, whereas in all the other structures (*A–C*), this distance is on average 4.59 ± 0.08 Å. This suggests that an active site conformation that brings Ser-241 O_1 and Lys-142 N_1 closer together reduces the barrier to reaction. The structure of the TS in the reactive conformation is different from those in the other structures, especially considering the distances between the reactant atoms (e.g., [H_1, O_1], [H_1, O_2] or [N_1, O_1]), showing that the effect observed here is not a simple consequence of reactants being closer to one another.

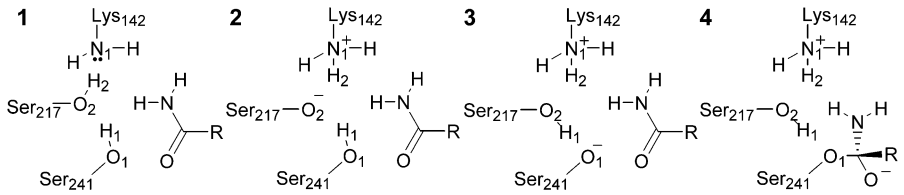

FIGURE 1 Configurations along the FAAH catalytic pathway.

TABLE 1 Reactant distances and energy barriers (kcal/mol)

Structure	$[O_1, C]$*	$[H_1, O_2]$*	$[N_1, H_2]$*	$[N_1, O_1]$*	B3LYP‡
A	2.77	1.82	1.60	4.60	28
B	2.70	1.81	1.79	4.66	33
C	2.61	1.81	1.68	4.50	29
D	2.79	1.76	1.61	4.09	18

*Distance in angstroms.
‡B3LYP/6-31+G(d)//PM3-CHARMM barrier.

To investigate this effect further, a PES was explored by forcing the $[N_1, O_1]$ distance to the value of 4.1 Å in the less reactive structure *B*. The new PES shows a catalytic pathway consistent with the mechanism proposed and a barrier of 26 kcal/mol, with a TS geometry resembling some features of the TS found for structure *D*. (Table S3 in the Supplementary Material). These findings indicate that the $[N_1, O_1]$ distance influences the rate of oleamide hydrolysis and that a full description of the reaction should consider this interaction.

MD simulations (see the Supplementary Material) were used to explore the conformational space of the enzyme-substrate complex; 4000 structural ''snapshots'' were collected over 1 ns, and clustered in different classes, based on the $[N_1, O_1]$ distance, ranging from 6.0 to 4.0 Å with a class width of 0.1 Å. The distribution of the $[N_1, O_1]$ distances was determined and the free-energy difference between different conformations was estimated by applying the Boltzmann law distribution (Fig. S2 in the Supplementary Material). The free-energy difference between the ground conformation (represented by a $[N_1, O_1]$ distance of 4.9 Å) and the reactive one (with a shorter distance of 4.1 Å), is estimated as 3.6 kcal/mol.

Standard MD simulations on accessible timescales typically may not adequately sample high energy regions of conformational space (3). Umbrella sampling MD was used here to obtain a potentially more accurate estimate of the free-energy profile for this conformational transition. The $[N_1, O_1]$ distance was chosen as a reaction coordinate, and nine independent MD simulations were run to move the system from 4.9 to 4.1 Å in the presence of a bias potential of 50 kcal/mol $Å^{-2}$. The weighted histogram analysis method gave the unbiased free-energy profile (10). The reactive conformation was found to be 4.7 kcal/mol higher in free energy, a slightly larger value than that estimated from relative populations above. Again, this result indicates that the energetic cost to reach the reactive conformation is low, compared to the difference in barrier heights of reactive and unreactive structures (18 and 33 kcal/mol, respectively). According to the Curtin-Hammett principle, the first step of FAAH acylation will be controlled by the difference in (free) energy between the transition states, and reaction via the high-energy (low population) conformation will be favored.

The results here identify crucial structural determinants of reactivity in FAAH. To our knowledge, this is the first example in which the Curtin-Hammett principle has been found to apply to an enzyme reaction. They also suggest that the substrate specificity of FAAH might be related to a differential ability of substrates to induce the catalytically relevant high energy conformation. These findings stress the need to model transition states, to understand the factors involved in enzyme reactivity and catalysis.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org

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