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Conformational effects in enzyme catalysis: QM/MM free energy calculation of the 'NAC' contribution in chorismate mutase

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The controversial 'near attack conformation' (NAC) effect in the important model enzyme chorismate mutase is calculated to be 3.8–4.6 kcal mol^{-1} by QM/MM free energy perturbation molecular dynamics methods, showing that the NAC effect by itself does not account for catalysis in this enzyme.

Chorismate mutase (CM) is at the centre of current debate about the fundamental nature of enzyme catalysis. CMs catalyse the Claisen rearrangement of chorismate to prephenate, a rare example of a biochemically-catalysed pericyclic reaction. Chorismate mutases have been isolated from several organisms including *Bacillus subtilis* (BsCM), *Escherichia coli* (EcCM) and yeast (YCM). BsCM, the smallest chorismate mutase, is the most widely studied. Catalysis does not involve covalent bond formation with the enzyme[1,2] and the rearrangement occurs *via* the same mechanism in solution and in the enzyme.[3] This makes it an ideal test case for studying the principles of enzyme catalysis. This enzyme has been the focus of many theoretical[4–6] and experimental studies,[1,3,7] but the nature of catalysis still remains a matter of debate. Until recently it has been generally accepted that preferential stabilization of the transition state (TS) through electrostatic interactions with the enzyme is central to catalysis in this enzyme.[4,6,8,9] Substrate conformational effects are also thought to contribute to catalysis.[4–6,10–13]

In contrast to these widely accepted proposals, Hur and Bruice[14] have recently proposed that catalysis in this enzyme (and others) is due almost entirely to the ability of the enzyme to bind 'near attack conformations' (NACs) of the substrate. This idea is based on the fact that for covalent bond formation to occur, the atoms must come together at a suitable distance and angle, regardless of the environment. As a result, catalysis is proposed to arise from the fact that the enzyme can maintain high populations of these reactive substrate conformations, whereas they have a low probability in solution. A schematic diagram of a reaction profile in solution and in an enzyme involving NACs is shown in Fig. 1(a). While it has been thought for many years that conformational effects play a role in catalysis by chorismate mutase,[1–6] the NAC proposal[14] in its strongest form suggests that other factors, such as TS stabilization, do not contribute. This conflicts with combined quantum mechanics/molecular mechanics (QM/MM) calculations which show significant TS stabilization by the enzyme.[4,6,8,9] NACs have been defined in several different ways:[14] *e.g.* as conformations of the substrate in which the bond-forming atoms are distances apart less than or equal to the sum of their van der Waals radii [*e.g.* ≤3.7 Å in chorismate mutase;[14b] see Fig. 1(b)]. Based on structures obtained from MM molecular dynamics (MD) simulations of the substrate in solution and bound to the enzyme, the mole fraction of NACs in the enzyme and in solution were calculated and used to estimate a free energy cost for NAC formation, initially in EcCM, but also more recently in BsCM, the catalytic antibody 1F7, and some mutant enzymes. Their results suggest that the observed catalytic effect of the enzyme is 90% due to the ability of the enzyme to support NACs, compared to the very low concentrations of NACs in solution, with transition state stabilization playing only a minor role.[14b] This proposal has been the subject of considerable debate and controversy. The calculation of potential catalytic contributions through unrestrained molecular dynamics simulations in this way has been criticized as being unreliable due to limited sampling and related statistical uncertainty.[15] Shurki *et al.*[15] have recently proposed a more reliable free energy perturbation (FEP) method for estimating the catalytic effect of NAC formation.

We have calculated the NAC contribution in CM catalysis (*i.e.* the free energy cost of forming the same NAC in solution as observed in BsCM) by QM/MM MD at the AM1/CHARMM level.[16] This method has been shown to treat this system well.[5,6a,12] We use a simple definition of a NAC proposed by Hur and Bruice [*i.e.* bond-forming C–C distance ≤ 3.7 Å; see Fig. 1(b)],[14b] and a FEP approach similar to that proposed by Shurki *et al.*[15] We examine here the (NAC) effect of conformational restriction for the substrate, not the TS. Differences in conformational restriction between the TS in the enzyme and solution are likely to be relatively small.[15] All calculations were performed using the CHARMM program (version 27b2).[17a] QM/MM MD simulations were carried out in the enzyme and in solution, with chorismate treated QM, and the enzyme and/or solvent described by the CHARMM22 MM force field.[17b] The models comprised chorismate surrounded by a 25 Å sphere either of solvent, or protein[2] and solvent. The set-up of the enzyme model is described in detail elsewhere[6a] (the same methodology was used for the solution model). The stochastic boundary MD approach was used.[18] More

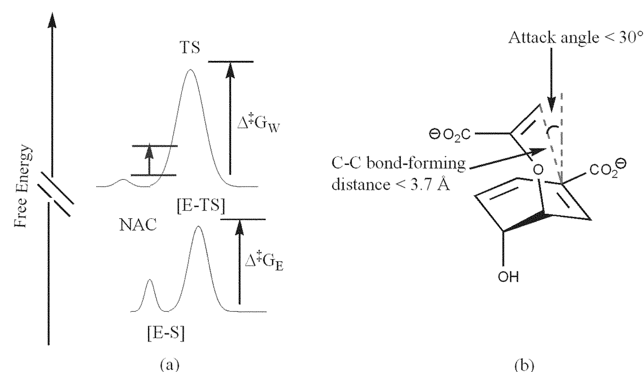

Fig. 1 (a) Schematic representation of a reaction profile for the same reaction in water and in an enzyme environment. (b) Chorismate showing one definition of a NAC, as used by Hur and Bruice in ref. 14*a*.

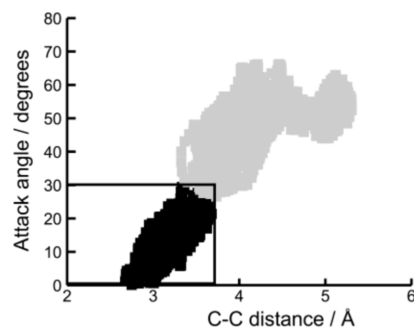

Fig. 2 Attack angle *vs.* C–C bond length for chorismate in solution (grey) and in the enzyme (black). The black box indicates the structures that are NACs using the definition used by Hur and Bruice in ref. 14*a*.

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than 1.4 ns of MD simulation was carried out (60 ps at each point), using a 1 fs time step.

Unrestrained QM/MM MD simulations were first carried out in both environments to establish the conformational distributions. Fig. 2 shows the resulting attack angle *vs*. C–C bond-length distributions in the enzyme and in solution. The distributions for the two environments are very different, with all of the conformations in the enzyme being NACs (using the bond-length criterion alone, or including the angle), but only a very small fraction of conformations in solution fitting the definition of a NAC. This difference in substrate conformation between the two environments is in agreement with previous QM/MM studies,[4–6,13,19] and is suggestive of a NAC effect.

To evaluate the energy cost of this difference in conformation between the two environments using a FEP approach, a suitable force constant is required to restrain the C–C distance distribution in solution to that in the enzyme. A harmonic force constant of 25 kcal $mol^{-1}Å^{-2}$ applied to the C–C distance was found to achieve the desired distribution. A series of simulations was then carried out, gradually reducing or increasing this restraint (48 in total). The C–C bond-forming distance was recorded at every step of the dynamics for use in evaluating ΔG_{NAC}. The value of V_{res} (eqn. 1) was also evaluated using the force constant above and below that used in the simulation, a technique known as double-wide sampling, in both directions. The difference between the values was turned into a weighted average (eqn. 2), and then summed to find the free energy associated with removing the restraint (eqn. 3).

$$V_{res,m} = \frac{1}{2}k(x_i - x_{i,0})^2 \quad (1)$$

$$\Delta g_{res} = \sum_{m=0}^{n} \Delta\Delta g_m \quad (2)$$

$$\Delta\Delta g_m = -\beta^{-1} \ln \left\langle e^{-\beta\left(V_{(res)m+1} - V_{(res)m}\right)} \right\rangle \quad (3)$$

Where: $\langle \ldots \rangle$ denotes an average of the trajectories propagated with the restraint *m*, and *n* is the number of simulations.

The FEP approach gives a NAC contribution of 3.8 or 4.6 (± 1.3) kcal mol^{-1} for applying or removing the restraint, respectively. This is significantly lower than the estimate of 8.4 kcal mol^{-1} (based on populations from MM MD simulations) given by Hur and Bruice.[14b] Very recently, Štrajbl *et al.*[9] published an estimate of the NAC effect, from calculations with an empirical valence bond (EVB) FEP method. Their finding of an apparent NAC effect of approximately 5 kcal mol^{-1} is in good agreement with our QM/MM results.

A proposal related to the recent NAC hypothesis is that distortion/strain of the substrate by the enzyme (compression of the C–C bond-forming distance) destabilizes the substrate, and so lowers the barrier to reaction.[4,6,10,13] This was first suggested by early QM/MM modelling.[4] This C–C distance is clearly shorter in the enzyme, even for similar substrate conformations (Fig. 2). This effect is included in the calculated free energy effect here, and so does not appear to be very large. This finding is in agreement with recent *ab initio* QM/MM results.[6b] AM1 appears to overestimate somewhat the compression of chorismate by CM.[6b] The use of the same method here for the enzyme and solvent simulations makes our approach consistent. It is also encouraging to note the good agreement with the recent findings of Štrajbl *et al.*, which used an entirely different (EVB) modelling method. The finding here of a relatively small (though not insignificant) conformational contribution to catalysis is consistent with the fact that TS stabilization by the enzyme has been shown by a number of QM/MM studies.[4,6,8,9,12] It appears that both conformational effects and TS stabilization contribute to catalysis by this enzyme; both lower the barrier to reaction.

When viewed on the basis of the geometric profile of attack angle *vs*. C–C distance (Fig. 2), the large difference in appearance between the solution and enzyme profiles might suggest a significant 'NAC effect'. However, when the free energy difference between the two situations is considered, calculated by reliable methods, it can be seen that the 'NAC effect' can play a role, but it is clearly not entirely responsible for catalysis in this enzyme. Our results of 3.8–4.6 kcal mol^{-1} suggest that the NAC effect (strain) may account for 40–50% of the observed $\Delta\Delta G^{\ddagger}$ of ~9.1 kcal mol^{-1} in chorismate mutase.[3] Transition state stabilization is important in catalysis, as shown by modelling[4,6,9] and experimental results.[7] NACs, by definition, are structures that closely resemble the TS, and as a result, the same interactions that stabilize the TS will stabilize NACs in the enzyme. The NAC effect therefore probably arises *because* of TS stabilization by the enzyme:[4,6,9] *e.g.* the same residues that stabilize the TS (*e.g.* Arg90) also stabilize the substrate in a reactive conformation or 'NAC'.[4–6,11,12,19] Formation of a 'NAC' is, however, clearly insufficient by itself to account for the observed catalytic power of chorismate mutase. Transition state stabilization is central to catalysis, *i.e.* the enzyme stabilizes the transition state relative to the bound (NAC) form of the substrate.

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