Insights into Chorismate Mutase Catalysis from a Combined QM/MM Simulation of the Enzyme Reaction

Paul D. Lyne,[†,‡] Adrian J. Mulholland,[‡] and W. Graham Richards*[,†]

Contribution from the Physical and Theoretical Chemistry Laboratory, Oxford Centre for Molecular Sciences, Oxford University, South Parks Road, Oxford OX1 3QZ, UK, and Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

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Abstract: The results from a combined QM/MM study of the reaction catalyzed by *Bacillus subtilis* Chorismate Mutase are presented. Chorismate Mutase catalyzes the skeletal rearrangement of chorismate to prephenate with a 2×10^6 rate acceleration over the solution phase reaction. Chorismate Mutase does not chemically catalyze the reaction but selectively chooses a destabilized conformer of chorismate. The simulation is consistent with previous structural studies insofar as the enzyme does not chemically catalyze the reaction. It is found that the minimum energy enzyme–substrate complex has chorismate in a distorted geometry relative to the ground state structure in the gas phase. The pyruvyl sidechain has rotated to maximize the interactions in the active site with the result that the distance between the reaction centers is shorter. In addition it is shown that the enzyme preferentially binds the transition state with the biggest interactions being due to Arg90 and Glu78. Thus the catalysis of chorismate may be rationalized in terms of a combination of substrate strain and transition state stabilization.

Chorismate Mutase, an important enzyme in the Shikimate pathway,[1,2] catalyzes the Claisen rearrangement of chorismic acid to prephenic acid, the latter being a precursor for aromatic amino acids. The enzyme represents the only known example of a biocatalyzed pericyclic reaction that also occurs readily in solution, and has therefore provided the impetus for a considerable amount of research.[3–9] *Bacillus subtilis* Chorismate Mutase catalyzes the skeletal rearrangement of chorismate to prephenate with a 2×10^6 rate acceleration over the solution phase reaction.[3] Despite the extensive study of the enzyme catalyzed reaction, the source of the enzymic rate acceleration is still unclear.[10] Sogo *et al.*[5] demonstrated that the enzymic reaction proceeds via a chair-like transition state (see Figure 1) and Copley *et al.*[6] confirmed that the non-enzymic reaction follows a similar reaction path. A comprehensive analysis of various possible mechanisms by Guildford *et al.*[7] eliminated many plausible explanations for the enzymic reaction and found the kinetic data to be consistent with a non-dissociative mechanism with the suggestion that the rate acceleration is due to general base catalysis. However, the recent publication of the structure of an enzyme–hapten complex showed the active site to be devoid of suitable candidates for participation in enzyme catalysis.[7,11,12] This view is reinforced by an NMR study[8] and an FTIR study[9] indicating that Chorismate Mutase does not use

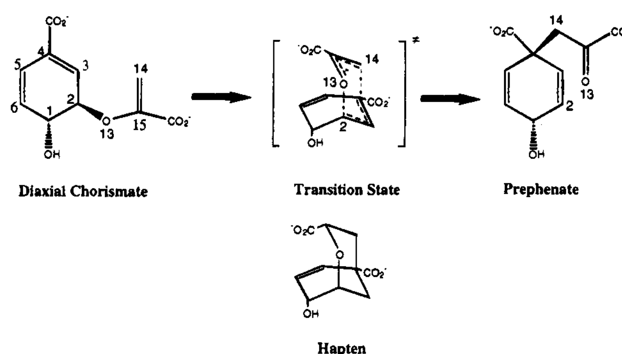

Figure 1. A schematic depiction of the Claisen rearrangement of chorismate to prephenate via a chair-like transition state. The endo-oxabicyclic hapten, which has been published bound to *Bacillus subtilis* Chorismate Mutase,[11] is also shown.

electrophilic catalysis to accelerate the rearrangement of chorismate. The absence of suitable active site residues to take part in chemical catalysis suggests that the enzyme does not react with its substrate.

Chorismate predominantly exists in a diequatorial conformation, but may also adopt a diaxial conformation. Experimentally the diequatorial conformer has been determined to be 0.9–1.4 kcal/mol more stable than the diaxial conformer.[6] Copley *et al.*[6] have shown that in solution 20% of chorismate exists as the diaxial conformer at physiological pH. Current consensus[9,13] suggests that Chorismate Mutase selects the reactive diaxial conformer which readily proceeds to product.

Several researchers have applied theoretical methods to study aspects of the interconversion of chorismate to prephenate. Wiest *et al.*[14] and Davidson *et al.*[15] have reported *ab initio* calculations

† Oxford Centre for Molecular Sciences.
‡ Harvard University.
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(1) Haslam, E. *The Shikimate Pathway*; Wiley: New York, 1974.
(2) Weiss, U.; Edwards, J. M. *The Biosynthesis of Aromatic Compounds*; Wiley: New York, 1980.
(3) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.
(4) Addadi, L.; Jaffe, E. K.; Knowles, J. R. *Biochemistry* **1983**, *22*, 4494.
(5) Sogo, S. G.; Widlanski, T. S.; Hoare, J. H.; Grimshaw, C. E.; Berchtold, G. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2701.
(6) Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 5008.
(7) Guildford, W. J.; D., C. S.; Knowles, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 5013.
(8) Gray, J. V.; Eren, D.; Knowles, J. R. *Biochemistry* **1990**, *29*, 8872.
(9) Gray, J. V.; Knowles, J. R. *Biochemistry* **1994**, *29*, 8872.
(10) Lee, A. Y.; Stewart, J. D.; Clardy, J.; Ganem, B. *Chem. Biol.* **1995**, *2*, 195.
(11) Chook, Y. M.; Ke, H.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8600.
(12) Chook, Y.-M.; Gray, J. V.; Ke, H.; Lipscomb, W. N. *J. Mol. Biol.* **1994**, *240*, 476.
(13) Lee, A. Y.; Karplus, P. A.; Ganem, B.; Clardy, J. *J. Am. Chem. Soc.* **1995**, *117*, 3627.
(14) Wiest, O.; Houk, K. N. *J. Org. Chem.* **1994**, *59*, 7582.

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on the transition state for the gas phase rearrangement of chorismate, while Bowlus[16] and Andrews *et al.*[17] have previously studied the gas phase reaction using semiempirical methods. Recently an analysis of the binding of the transition state in the active site has been reported.[18] Attempts to characterize Claisen rearrangements in solution by theoretical methods have been completed using a variety of methods and have focused primarily on the rearrangement of allyl vinyl ether.[19,20] However, to date, only the gas phase rearrangement of chorismate to prephenate has been studied. To obtain a more detailed impression of the biologically catalyzed reaction to supplement the available experimental data, the results of a computer simulation of the reaction in the enzyme are presented here. Hybrid quantum and molecular mechanical (QM/MM) methods have been developed to study chemical processes in solution and enzyme phases.[21-27] These methods counter the computational demands made by macromolecular processes by partitioning the system into a small quantum mechanical region (to study the chemical processes) and a larger molecular mechanical region. The QM/MM method developed by Field *et al.*[21] has been employed for this study. The method has previously been successfully employed to model the reactions catalyzed by triosephosphate isomerase[28] and citrate synthase[29] and for the study of nitrous acid induced cross-linking in DNA.[30]

Methods

The CHARMM23 QM/MM method is employed here to study the enzyme-catalyzed reaction. This method treats a subset of atoms quantum mechanically and the remaining atoms using the molecular mechanics force field of CHARMM.[31] Both semiempirical (AM1[32]) and *ab initio* (GAMESS[33]) molecular orbital methods have been incorporated into CHARMM. For consideration of such a large system as Chorismate Mutase the semiempirical method was adopted for the quantum mechanical region. Semiempirical methods have been extensively applied in the past to study gas phase pericyclic reactions with varying degrees of success.[34] Many of the doubts about the suitability of semiempirical methods applied to pericyclic reactions are concerned with the description of transition states. Indeed, semiempirical methods have been shown to perform poorly in their description of transition states relative to *ab initio* methods for some pericyclic reactions.[34] Thus, in order to ensure the suitability of the AM1 method for studying the Claisen rearrangement of chorismate, the gas phase reaction was studied using both semiempirical and *ab initio* methods. These simulations are a necessary precursor to the analysis and discussion of the enzyme-based calculations.

Gas Phase Calculations. Semiempirical calculations were performed using the AM1 Hamiltonian in MOPAC.[35] The transition state was optimized and characterized using the eigenvector following method and the reaction pathway was generated using the intrinsic reaction coordinate (IRC) method.[36] *Ab initio* calculations were performed using the GAUSSIAN92 program.[37] All geometry optimizations were performed at the restricted Hartree–Fock level using a 6-31G* basis set. Geometry optimizations, at semiempirical and *ab initio* levels, included all degrees of freedom and each stationary point was characterized by harmonic frequency analysis.

Enzyme Calculations. The calculations were performed using the QM/MM technique incorporated into CHARMM23.[33] This technique partitions the system into quantum and molecular mechanical regions. The Hamiltonian used for calculations in the quantum regions is given as:

$$\hat{H}_{eff} = \hat{H}^0 + \hat{H}_{MM} + \hat{H}_{QM/MM} \quad (1)$$

where $\hat{H}^0$ is the standard AM1 Hamiltonian, $\hat{H}_{MM}$ is the CHARMM force field, and $\hat{H}_{QM/MM}$ is given as:

$$\hat{H}_{QM/MM} = -\sum_{iM} \frac{q_M}{|r_{iM}|} + \sum_{A} \frac{Z_A q_M}{|R_{AM}|} + \sum_{AM} \left\{ \frac{A_{AM}}{R_{AM}^{12}} - \frac{B_{AM}}{R_{AM}^{6}} \right\} \quad (2)$$

The coordinates i and A correspond to the QM electrons and nuclei, respectively, and M refers to the MM atoms. The first two terms represent electrostatic interactions between the MM atoms and the QM electrons and nuclei. The final term accounts for van der Waals interactions between the MM and QM atoms with a Lennard-Jones 12/6 potential. This is the general expression for the QM/MM interaction Hamiltonian. The electrostatic form is modified slightly to incorporate the formalism used by AM1 (see Field *et al.*[21])

Extensive testing of the program has been performed.[21,29] The initial coordinates of the enzyme were obtained from the 2.2 Å refined X-ray crystal structure of *Bacillus subtilis* complexed to a transition state analogue.[11] The gas phase optimized structure (using AM1) of the diaxial conformer of chorismate (the starting structure from the gas phase AM1 IRC profile) was superimposed on the hapten of the crystal structure using a rigid body least-squares fit, and the hapten was subsequently deleted.

The choice of a partitioning scheme between quantum and molecular mechanical regions is important, and several options were considered. Due to the increasing computational demands associated with increasing the size of the quantum region a balance was sought between a reasonably (from a computational perspective) sized quantum region and a region large enough to describe realistically the electronic structures of any reacting species (bond making and bond breaking). From the crystal structure it may be seen that there are many close contacts between the enzyme and the substrate. The closest residues to the pyruvyl sidechain of chorismate, or the analogous groups of the transition state hapten, are Arg7, Arg90, and Tyr108. Chook *et al.*[12] have suggested that the π-electrons of the Phe57 sidechain could stabilize the positive charge that develops on the cyclohexenyl ring as the rearrangement proceeds. Various combinations of these four residues were considered for the quantum region in addition to chorismate: (i) nothing; (ii) Arg90 and Arg70; (iii) Arg90, Arg7, and Tyr108; and (iv) Arg90, Arg7, and Phe57. It was found that the various partition schemes gave essentially the same results.

In order to reduce the computational time required only residues within a sphere of radius 16 Å centered on the C4 atom of chorismate

(15) Davidson, M. M.; Hillier, I. H. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1415.
(16) Bowlus, S. R. 1994; we thank Dr. Bowlus for a copy of his research on the gas phase rearrangement of chorismate.
(17) Andrews, P. R.; Haddon, R. C. *Aust. J. Chem.* **1979**, *32*, 1921.
(18) Davidson, M. M.; Gould, I. R.; Hillier, I. H. *J. Chem. Soc., Chem. Commun.* **1995**, 63.
(19) Severance, D. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1992**, *114*, 10966.
(20) Gao, J. *J. Am. Chem. Soc.* **1994**, *116*, 1563.
(21) Field, M. J.; Bash, P. A.; Karplus, M. *J. Comp. Chem.* **1990**, *11*, 700.
(22) Gerber, R. B.; Alimi, R. *Isr. J. Chem.* **1991**, *31*, 383.
(23) Gao, J.; Xia, X. *Science* **1992**, *258*, 631.
(24) Cramer, C. J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1992**, *114*, 8794.
(25) Stanton, R. V.; Hartsough, D. S.; Merz, K. M., Jr. *J. Phys. Chem.* **1993**, *97*, 11868.
(26) Waszkowycz, B.; Hillier, I. H.; Gensmantel, N.; Payling, D. W. *J. Chem. Soc., Perkin Trans. 2* **1991**, 225.
(27) Wei, D.; Salahub, D. R. *Chem. Phys. Lett.* **1994**, *224*, 291.
(28) Bash, P. A.; Field, M. J.; Davenport, R. C.; Petsko, G. A.; Ringe, D.; Karplus, M. *Biochemistry* **1991**, *30*, 5826.
(29) Mulholland, A. J. D. Phil. Thesis; Oxford University, 1995.
(30) Elcock, A. H. E.; Lyne, P. D.; Mulholland, A. J.; Nandra, A.; Richards, W. G. *J. Am. Chem. Soc.* **1995**, *117*, 4706.
(31) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comp. Chem.* **1983**, *4*, 187.
(32) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. A.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(33) Karplus, M. *CHARMm 24 (Developmental Version)*; Department of Chemistry, 12 Oxford St., Cambridge, MA 02138.
(34) Houk, K. N.; Gonzalez, J.; Li, Y. *Acc. Chem. Res.* **1995**, *28*, 81.
(35) Stewart, J. J. P. *MOPAC 93.00 Manual*; Fujitsu Ltd.: Tokyo, 1993.
(36) Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363.
(37) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. W.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomberts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzales, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *GAUSSIAN92*; Gaussian Inc.: Pittsburgh, PA, 1992.

Table 1. Selected Distances (Å) of the Optimized Geometries of Diaxial Chorismate and the Transition State for the Claisen Rearrangement, Calculated at the AM1 and RHF/6-31G* Levels in the Gas Phase

diaxial chorismate			transition state		
bond	AM1	RHF/6-31G*	bond	AM1	RHF/6-31G*
C2−O13	1.433	1.417	C2−O13	1.824	1.987
C15−O13	1.395	1.374	C15−O13	1.304	1.252
C14−C15	1.339	1.320	C14−C15	1.406	1.385
C2−C3	1.492	1.512	C2−C3	1.402	1.381
C3−C4	1.345	1.328	C3−C4	1.404	1.386
C4−C5	1.452	1.480	C4−C5	1.463	1.479
C5−C6	1.340	1.326	C5−C6	1.342	1.324
			C4−C14	2.119	2.389

(see Figure 1 for numbering) were included. This is a procedure which has been applied previously.[28] A 16-Å region is expected to incorporate sufficiently long range effects on the active site. A sphere of preequilibrated TIP3P water molecules was then placed at the center of the system and was incorporated into the calculations by using stochastic boundary methods.[38,39] Any waters within 2.7 Å of a heavy atom or beyond 16 Å from the center of the system were deleted. The region between 12 and 16 Å from the center of the system was defined as the buffer region.[39] Atoms within this region were subjected to harmonic constraints determined from averaged X-ray temperature factors, $B_i = 8\pi^2/3\langle\Delta r_i^2\rangle$. To simplify the treatment, values of B = 14, 15, and 16 Å^2 were assigned respectively to the main chain atoms, the sidechain heavy atoms in the β position, and the sidechain heavy atoms in the γ position. Atoms beyond 16 Å from the center of the system were held fixed. The total system consisted of 1780 protein atoms and 85 solvent molecules. Initially, all heavy atoms were held fixed and the system was minimized using steepest descent methods for 250 steps. Subsequently only water molecules were minimized for 250 steps by steepest descent methods before finally minimizing the whole system using conjugate gradient methods. This minimized structure was used as a starting point for calculating the reaction profile. The reaction path was followed (using a modified version of the umbrella sampling module of CHARMM 23)[29] by varying the ratio of C2−O13 bond distance to C4−C14 bond distance (see Figure 1 for numbering scheme) in 20 intervals from reactant to product. The method applies a penalty function to restrain the particular coordinates to the desired values. The values of the ratio used were taken from the IRC profile calculated for the gas phase reaction using AM1. A harmonic constraint (k = 5000 kcal mol^{-1}) was used to fix the reaction coordinate at each point, ensuring that the system is held very close to the defined reaction coordinate and preventing the system from drifting away from the reaction path. The system was minimized for 250 steps at each point. The QM/MM calculations were performed without a nonbonded cutoff.

Results

Gas Phase Reaction. Selected geometric parameters for the optimized geometries of the diaxial conformation of chorismate and the transition states for the interconversion of chorismate to prephenate, calculated at the semiempirical and *ab initio* levels of theory, are presented in Table 1. The atomic labeling employed is the same as for Figure 1. In general AM1 compares favorably with Hartree−Fock methods at determining ground state structures of organic molecules[35] and this is borne out by the calculations on the ground state geometry of chorismate. Previously, semiempirical methods have been shown to be particularly weak at characterizing the geometries of transition states for some pericyclic reactions,[34] and in the original test calculations of the QM/MM method some discrepancies were noted in the quality of AM1.[21] The transition state calculated here by the AM1 method is very similar to the transition state

(38) Brunger, A. T.; Brooks, C. L., III; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8458.
(39) Brooks, C. L., III; Karplus, M. *J. Chem. Phys.* **1983**, *79*, 6312.

Table 2. Energy Barriers for the Claisen Rearrangement of Chorismate to Prephenate in the Gas Phase Calculated at the AM1, RHF/6-31G*, and MP2/6-31G*//RHF/6-31G* Levels

	ΔE (kcal/mol)
AM1	42.30
HF/6-31G*	48.44
MP2/6-31G*//RHF/6-31G*	19.42

obtained at the RHF/6-31G* level (Table 1). The root-mean-square standard deviation between the transition states at both levels of theory is 0.135 Å. The main differences in the structures of the transition states are the lengths of the bonds being made and broken. The AM1 method predicts a smaller extent of bond breakage and a larger extent of bond formation. Nonetheless, the energy barriers for the reactions at the AM1 and RHF 6-31G* level are quite similar.

Both methods overestimate the barrier to rearrangement compared to the experimentally determined value for the solution phase reaction ($\Delta G^{\ddagger}$ = 24.2 kcal mol^{-1}).[3] This is consistent with previous theoretical calculations on the rearrangement of chorismate[14,15] and the related allyl vinyl ether (AVE).[40,41] The overestimation of the energy barrier in the gas phase has been attributed to the increased intercarboxylate interaction in the transition state relative to the ground state.[14] Explicit inclusion of correlation effects for the calculation of the Claisen rearrangement of AVE[41] has been shown to yield an energy barrier similar to the experimentally determined value. In this work, single point MP2/6-31G*//RHF-631G* calculations for chorismate rearrangement (see Table 1) result in a barrier that is similar to the experimental value in solution.

In view of the similarities between the AM1 and *ab initio* treatments of the gas phase rearrangement of chorismate and the successful application of AM1 based hybrid methods to modeling enzyme reactions[28,29] and the Claisen rearrangement of AVE in solution,[20] it appears that this AM1/MM method may be applied with a reasonable degree of confidence to modeling the rearrangement of chorismate in the enzyme.

Enzyme Reaction. The energy profile for the interconversion of chorismate to prephenate in the enzyme is plotted in Figure 2b. There are a number of interesting features of the reaction profile that warrant discussion and selected points on the reaction profile have been labeled to facilitate this discussion. In Table 3 selected geometrical parameters of structures A to C are presented. The first point, A, corresponds to the structure of the system after minimization with the reaction coordinate fixed at the gas phase value and corresponds to the starting point for adiabatically mapping the rearrangement of chorismate. The geometry of chorismate has deviated little from its gas phase geometry. As the reaction coordinate is increased the energy of the system decreases passing through a minimum corresponding to B. At B the geometry of the substrate has been considerably distorted from its starting structure. The C2−O13 distance has changed little but the C4−C14 distance has decreased by 0.449 Å, the torsion angle about the C2−O13 bond has increased by 5.21°, and the H−C2−O13 angle has opened out by 10.8°. There have also been some changes to the structure of the protein around the active site. This is depicted in Figure 2c. There are several close contacts between the substrate and sidechains of the enzyme. In particular, Glu78 forms a hydrogen bond to the hydroxyl group of chorismate. The positively charged sidechains of Arg7 and Arg90 are in close proximity to the carboxylate and ether oxygen of choris-

(40) Dewar, M. J. S.; Jie, C. *J. Am. Chem. Soc.* **1989**, *111*, 511.
(41) Vance, R. L.; Rondan, N. G.; Houk, K. N.; Jensen, F.; Borden, W. T.; Komornicki, A.; Wimmer, E. *J. Am. Chem. Soc.* **1988**, *110*, 2314.

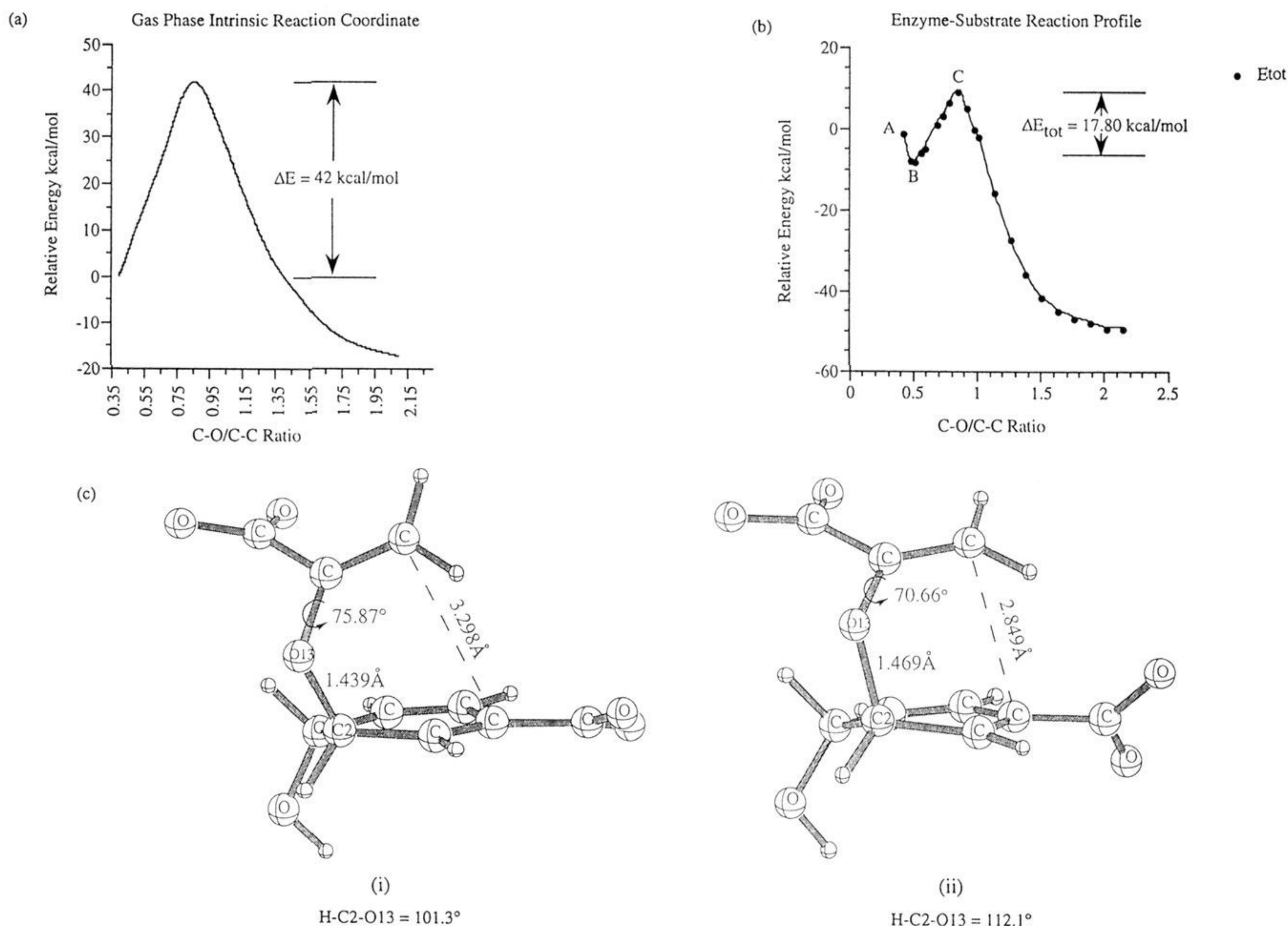

Figure 2. (a) Intrinsic reaction coordinate for the rearrangement of chorismate to prephenate in the gas phase, calculated by the AM1 method. (b) Reaction coordinate for the rearrangement of chorismate to prephenate in the active site of the enzyme, calculated using the QM/MM method. (c) (i) The geometry of chorismate at point A along the reaction profile shown in (b). (ii) The geometry of chorismate at point B along the reaction profile shown in (b).

Table 3. Bond Distances and Energies for Chorismate A, B, and C[a]

substrate	d(C4−C14) (Å)	d(C2−O13) (Å)	E_{tot}	$E_{QM/MM}$	E_{gas}
Chorismate A	1.439	3.298	0.00	0.00	0.00
Chorismate B	1.469	2.849	−8.23	3.91	13.02
Chorismate C	1.816	2.119	9.23	28.37	42.30

[a] All energies are relative to point A on the reaction profile shown in Figure 2b and are given in kcal/mol. The substrates are referenced by their respective positions on the reaction profile shown in Figure 3b. E_{tot} corresponds to the total energy of the enzyme complex. $E_{QM/MM}$[42] corresponds to the energy of the quantum region including the effects of the molecular mechanics region (see Methods). E_{gas} refers to the energy of the substrate in the gas phase. The E_{gas} of Chorismate B is a single point calculation since this does not correspond to a stationary point on the gas phase potential energy surface.

mate and the methylene group is only 2.43 Å away from the sidechain group of Leu47. Following the reaction coordinate from point B the energy goes through a maximum at point C before proceeding to product. The structure of the system at point C is partially recorded in Table 3. This point is an approximation to the transition state of the reaction. The structure of the substrate is almost identical to the AM1 gas phase transition state. The root-mean-square deviation between the substrate in C and the gas phase transition state (AM1) is only 0.08 Å. The interactions between the enzyme and the substrate at point C are similar to those at A and B, but there are differences which will be addressed below.

Residue Contributions. A quantitative assessment of the contributions made by individual residues and water molecules to the energetics of the reaction may be obtained by successively deleting residues, starting fom the residue furthest from C4, and then calculating the energy difference between the ground state and transition state. This method is similar to the perturbation method employed by Bash *et al.*[28] The calculations were performed for the lowest energy enzyme substrate complex (point B in Figure 2) and the highest energy complex, approximating the transition state of the reaction (point C in Figure 2). The differences in energy are plotted as a function of the distance of the residues from the active site in Figure 3. This method gives an indication of the relative effect of each residue on the lowest energy and highest energy structure along the reaction coordinate. For the purposes of the analysis of $E_{QM/MM}$[42] term of the total energy is employed since this should give the clearest indication of the effect of molecular mechanics atoms on the quantum mechanical region (refer to eq 2).

Considering that the substrate has a net negative charge of −2 it is to be expected that charged residues will have the strongest influence on the quantum region. The largest energy difference is associated with the Arg90 residue. This residue, on the basis of structural studies, may be identified as making ionic interactions with the pyruvyl sidechain ether. The perturbative method suggests that there is a strong interaction between the substrate and the enzyme of approximately 5 kcal mol^{-1} when the reaction has reached the point of maximum

(42) The actual values used correspond to the sum of E_{QM} and $E_{QM/MM}$ in eq 2. For Figure 3, since the relative differences in energy are ploted, the values effectively correspond to the contribution of the MM groups on the quantum region, i.e. $E_{QM/MM}$.

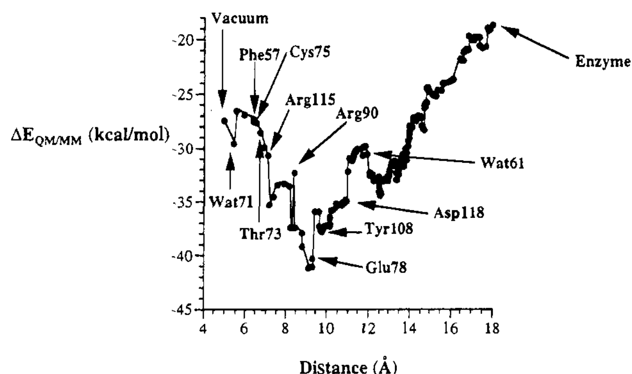

Figure 3. Estimate of the residue contributions to the energy along the reaction pathway. The abcissa represents an ordering of the amino acid residues by distance of their center of mass from the reaction center which has been defined as C4 of chorismate (refer to Figure 1 for numbering). The ordinate corresponds to the difference in $E_{QM/MM}$[42] of the minimum structure (point B in Figure 2) and the maximum structure (point C in Figure 3). The profile was constructed by sequentially deleting amino acid residues in decreasing order of their distance from the reaction center and recalculating the energy after each deletion. Protein corresponds to the energy difference of the entire simulation system; "vacuum" refers to the energy difference of the system after all the MM atoms have been deleted; specific points have been labeled and correspond to the energy difference after the residue indicated has been deleted.

energy (point C in Figure 2) along the reaction coordinate compared to the minimum energy structure (point B in Figure 2). This is to be expected since there is an increase in electron density on the ether oxygen as its bond to the cyclohexenyl ring begins to break. Another residue in the active site that has been previously postulated to have a role to play in enzyme catalysis is Glu78. The residue is proposed to contribute to rate enhancement by stabilization of the positive charge that is developing on the cyclohexenyl ring during the early stages of the rearrangement. From Figure 3 it may be seen that the Glu78 residue stabilizes the highest energy point relative to the ground state by approximately 3.5 kcal mol^{-1}. Other charged residues are seen to have influences on the quantum region and it is noted that the interaction between charged residues and the substrate is still relatively strong at large distances. For example, Lys110 is seen to preferentially stabilize the maximum energy system by approximately 1.0 kcal mol^{-1}. The charged sidechain of this group is located at the surface of the enzyme and its influence on the active site is likely to be much smaller in reality. This highlights the inability of the stochastic method to reproduce effectively dielectric screening in the system, as found in previous studies.[28,29]

In addition to the ionic residues in the active site some neutral residues are in close proximity to the substrate. The largest effect is seen to be from Thr73 whose sidechain comes into close contact with the carboxylate group of the pyruvyl sidechain. During the rearrangement the hydrogen bond between the threonine sidechain and the carboxylate diminishes as the bond between the pyruvyl group and the cyclohexenyl ring breaks. This leads to a destabilization of the "transition state" by approximately 1.5 kcal mol^{-1}. Other residues that were believed previously to be possible influences in the reaction rate include Tyr108 and Cys75. It is found that there is very little difference between the interaction energy of the ground state and highest energy state of the substrate with these residues.

Apart from the major changes identified above there are many small energy changes due to the differential interaction between the enzyme and the substrate in the minimum energy (point B) and maximum energy (point C) systems. The cumulative effect of these changes together with the larger changes mentioned above means that the energy difference between chorismate in structures B and C is reduced by 13 kcal mol^{-1} compared to the gas phase.

Discussion

Several mechanistic possibilities have been proposed to account for the rate acceleration achieved by Chorismate Mutases for the rearrangement of chorismate to prephenate.[7,10,43] Models based on either electrophilic or nucleophilic catalysis by the enzyme have been discounted in the light of recent experimental evidence. For the reaction catalyzed by the *Bacillus subtilis* Chorismate Mutases, a D_2O solvent isotope effect is not observed and the reaction rate is almost invariant over the pH 5−9 range.[7] Additionally, spectroscopic evidence reinforces the view that the enzyme does not covalently interact with the substrate.[8,9] The results from mapping the reaction pathway of Chorismate Mutase in this study reinforce previous assertions that the enzyme does not "chemically" catalyze the reaction. There is no significant movement in the positions of the active site residues along the rearrangement pathway and covalent interactions between the substrate and the enzyme are not found to occur.

In the absence of "chemical" catalysis by the enzyme, the source of the rate acceleration remains unresolved. Currently, the common explanation is that the enzyme selectively binds the diaxial form of chorismate in a conformation that is optimal for rearrangement. The presence of a positively charged wall in the active site provided by Arg7 are Arg90 has been proposed to ionically interact with the pyruvyl carboxylate of chorismate[43] and subsequent hydrogen bonding interactions exert a torque on the allyl vinyl ether system, ensuring the close proximity of C2 and C14.[11,12] Lee *et al.*[10] have expanded on this hypothesis by suggesting that diaxial chorismate enters the active site in an extended form, making ionic interactions between the carboxylate and a positively charged residue. Subsequently, rotation about the C2−O13 bond increases binding between the substrate and the enzyme. Experimentally, some attempts have been made to observe the nature of the ES complex by NMR, but by their nature enzyme−substrate complexes are extremely difficult to observe.[8,44]

Transition state stabilization is generally held to be the key factor involved in enzymic rate acceleration.[45−49] An equally valid method to reduce the activation barrier of a reaction is to destabilize the reactant. This classical strain mechanism, as proposed by Haldane,[50,51] is not often evoked. Lysozyme was proposed to bind sugar substrates in a destabilized sofa conformation.[52] Both theoretical calculations[53,54] and structural studies[55] ruled out substrate destabilization as a major contributor to catalytic function, but recently there have been some examples of a sugar intermediate that is substantially distorted bound to

(43) Gorisch, H. *Biochemistry* **1978**, *17*, 3700.
(44) Rajagopolan, J. S.; Taylor, K. M.; Jaffe, E. K. *Biochemistry* **1993**, *32*, 3965.
(45) Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219.
(46) Knowles, J. R. *Nature* **1991**, *350*, 121.
(47) Kraut, J. *Science* **1988**, *242*, 533.
(48) Menger, F. M. *Biochemistry* **1992**, *31*, 5369.
(49) Page, M. I. *Phil. Trans. R. Soc. London B* **1991**, *332*, 149.
(50) Haldane, J. B. S. *Enzymes*; Longman, Green & Co.: London, 1930.
(51) Haldane, J. B. S. *Enzymes*; M.I.T. Press: Cambridge, MA, 1965.
(52) Phillips, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 484.
(53) Warshel, A.; Levitt, M. *J. Mol. Biol.* **1976**, *103*, 227.
(54) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; Wiley: New York, 1991.
(55) Kelly, J. A.; Sielecki, A. R.; Sykes, B. D.; James, M. N. G. *Nature* **1979**, *282*, 875.

mutant forms of the enzyme.[56] Triosephosphate isomerase is believed to destabilize its substrate but the strain is manifested as polarization of a carbonyl group rather than geometric distortion. Jia *et al.*[57] have studied the induced fit conformational change of histidine-containing phosphoenolpyruvate and have reported that torsional strain in the active site of histidine-containing phosphoenolpyruvate amounts to approximately 4 kcal mol^{-1} and facilitates lowering of the activation barrier for phosphate transfer.

The computer simulations performed in this study suggest that in the minimum enzyme–substrate complex, chorismate has a considerably different structure compared to the diaxial minimum calculated in the gas phase at the AM1 and RHF/6-31G* levels. The distance between C4 and C14 has shortened by 0.449 Å and the torsion angle about the C2–O13 bond has changed by 5.21° (see Figure 2c). This structure is consistent with the model for substrate binding proposed by others.[10] The transition state complementarity of the active site has induced a change in the geometry of chorismate when it binds to the enzyme to form the Michaelis complex. There have been several attempts to explain the rates of intramolecular reactions (see Page[49] and references therein). Recently Menger[58] has stressed the importance of the relationship between reaction rate and distance in his so-called spatiotemporal theory. Here the enzyme binds the substrate in a geometry that has a C4–C14 distance that is shorter than the unbound form of the substrate.

Clearly, transition state stabilization will also have a major role to play in lowering the energy barrier to Claisen rearrangement. Severance *et al.*,[19] using a Monte Carlo simulation, and Gao,[20] using a QM/MM method (with the AM1 Hamiltonian), have studied the Claisen rearrangement of allyl vinyl ether in solution. Both studies find that the transition state is better solvated than the ground state. The enhanced hydrogen bonding of the transition state corresponds to a large contribution to the rate acceleration of the solvent phase reaction compared to the gas phase. The perturbative technique used to quantify the residue contributions to catalysis identifies several hydrogen bonding interactions that are enhanced in the transition state. It is clear that an interplay of numerous subtle interactions between the enzyme and substrate takes place, yet it is possible to indentify key residues for preferential binding of the transition state. In particular, the Arg90 and Glu78 are found to have the largest effect. Interestingly, Thr73 was found to have an adverse effect on preferential transition state binding, although the magnitude of its effect was much smaller than for Arg90 and Glu78.

Concluding Remarks

In this paper the QM/MM method developed by Field *et al.*[21] has been used to study the interconversion of chorismate to prephenate in *Bacillus subtilis* Chorismate Mutase. A modified AM1 Hamiltonian was used for the quantum region of the simulation. Preliminary gas phase studies show that results obtained by the AM1 method are on a par with RHF/6-31G* calculations for this system. One of the strengths of the AM1 method is that it has been parametrized for ground state organic molecules and therefore performs very well at representing ground state geometries of organic molecules. The minimum energy structure of the enzyme–chorismate complex is found to have chorismate bound in a distorted geometry compared to the ground state structure of chorismate in the gas phase. This may be rationalized by the increased hydrogen bonding in the complex as the chorismate distorts toward the structure of the transition state. There have been several attempts to determine the structure of the ground state for this reaction. These results provide a clear example of ground state strain/transition state complementarity in the enzyme–substrate complex.

It has been shown previously, and in this work, that electron correlation methods such as MP2 are a prerequisite for an accurate description of the energetics in this and related systems. Although the energetics determined here may only be viewed as approximate, by comparing relative energies, insight can be gained into the interactions between the enzyme and substrate during the reaction. The enzyme-catalyzed reaction is predicted to have an energy barrier of 17.8 kcal mol^{-1} compared to the gas phase barrier of 42.0 kcal mol^{-1}. Key residues in the active site may also be identified by an energy perturbation technique similar to that used by Bash *et al.*[28] In particular, Arg90 and Glu78 are seen to make large contributions to stabilizing the transition state relative to the ground state. The importance of preferential hydrogen bonding of the transition state in solution phase Claisen rearrangements has been recently established,[19,20] and it is expected that these residues make a catalytic contribution to the reaction compared to the gas phase. Thus, the energy barrier relative to the gas phase has been lowered in the enzyme by a combination of substrate strain and preferential hydrogen bonding of the transition state.

The recent publication of the crystal structure of an antibody, 1F7,[59] that catalyzes the Claisen rearrangement of chorismate, and the crystal structure of the *E. coli* Chorismate Mutase[10] offer the possibility of comparison with the *Bacillus subtilis* Chorismate Mutase mechanism. In addition, an activated dynamics study[60] and free energy calculations of the enzyme-catalyzed reaction is planned which should further our understanding of the thermodynamics and catalytic processes of Chorismate Mutases.

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(56) Kuroki, R.; Weaver, L. H.; Matthews, B. H. *Science* **1993**, *262*, 2030.

(57) Jia, Z.; Vandonselaar, M.; Quail, J. W.; Delbaere, L. T. J. *Nature* **1993**, *361*, 94.

(58) Menger, F. M. *Acc. Chem. Res.* **1993**, *26*, 206.

(59) Haynes, M. R.; Stura, E. A.; Hilvert, D.; Wilson, I. A. *Science* **1994**, *263*, 646.

(60) Brooks, C. L., III; Karplus, M.; Pettitt, B. M. *Proteins: A Theoretical Perspective of Dynamics, Structure and Thermodynamics*; Wiley: New York, 1988.