Substrate conformational transitions in the active site of chorismate mutase: Their role in the catalytic mechanism

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Chorismate mutase acts at the first branch-point of aromatic amino acid biosynthesis and catalyzes the conversion of chorismate to prephenate. The results of molecular dynamics simulations of the substrate in solution and in the active site of chorismate mutase are reported. Two nonreactive conformers of chorismate are found to be more stable than the reactive pseudodiaxial chair conformer in solution. It is shown by QM/MM molecular dynamics simulations, which take into account the motions of the enzyme, that when these inactive conformers are bound to the active site, they are rapidly converted to the reactive chair conformer. This result suggests that one contribution of the enzyme is to bind the more prevalent nonreactive conformers and transform them into the active form in a step before the chemical reaction. The motion of the reactive chair conformer in the active site calculated by using the QM/MM potential generates transient structures that are closer to the transition state than is the stable CHAIR conformer.

Chorismate occupies a central position in the biosynthesis of aromatic amino acids in microorganisms and plants. The isomerization of chorismate to prephenate, the first committed step in the synthesis of tyrosine and phenylalanine (Fig. 1), is formally a Claisen rearrangement and is catalyzed by chorismate mutase (CM) (chorismatepyruvate mutase, EC 5.4.99.5) with a rate enhancement of $2 \times 10^6$. The biological importance of this conversion and the synthetic value of the Claisen rearrangement have led to extensive experimental (1–23) and theoretical (24–33) investigations. In particular, the crystal structures of Bacillus subtilis, Escherichia coli (P-protein), and Saccharomyces cerevisiae (yeast) CMs complexed with a transition-state analog (TSA) inhibitor (4) are available (8–11), as well as that of a less active catalytic antibody 1F7 (12). Extensive electrostatic and hydrogen-bonding interactions between the TSA and these enzymes (11) have been examined by site-directed mutagenesis studies (13–17). Nevertheless, many questions remain concerning the details of catalytic processes, including substrate selection, rate enhancement, and roles of active site residues.

Knowles and coworkers demonstrated that the rearrangement of chorismate to prephenate proceeds through a “chair-like” transition state for the atoms of the [3,3]-pericyclic region (Fig. 1), both in solution and in the enzyme-catalyzed reaction (18–19). The bond breaking and making process is presumed to start from a pseudodiaxial (chair) conformer (CHAIR in Fig. 2) that is capable of reaching the transition state directly. One way for CM to speed up the reaction is, therefore, to bind this chair conformer preferentially from solution and to catalyze its chemical transformation at the active site (20). Many discussions of CM catalysis have been based on this mechanism (21, 25, 28–29, 32). However, quantum mechanical calculations (24–25, 27–28, 31) have either failed to identify this reactive conformer in the gas phase or in solution or have found that it is much less stable than some other conformers. For instance, the structure of the “chair-like” transition state was determined by Wiest and Houk (24) from ab initio and density functional calculations, but no energy minimum for CHAIR was located in their investigation.

Instead, they obtained a nonreactive extended pseudodiaxial conformer that was used later by Carlson and Jorgensen (27) to study the conformational equilibrium of chorismate in solution. The CHAIR conformer determined by Martí et al. (31) from the MP2/6–31G* calculations is 16 kcal/mol less stable than the lowest energy conformer (DIEQ2 in Fig. 2) in the gas phase. Moreover, in a recent transferred nuclear Overhauser effect study of chorismate in solution (23), the CHAIR conformer was not detected. Thus, an alternative to the proposed preferential binding of the CHAIR conformation by the enzyme appears to be required. One possibility is that the enzyme is able to bind the more abundant conformers and convert them to CHAIR in the active site (1, 21–23). In the present paper, this alternative is explored, and the focus is on the dynamics of the substrate conformational transitions in the active site of yeast CM (11).

A recently developed potential energy function (34) based on a semiempirical implementation of density functional theory (35) is used. This allows quantum mechanical/molecular mechanical (QM/MM) molecular dynamics simulations of the substrate in the active site.

Methods

The quantum mechanical calculations for the chorismate substrate conformers in the absence of the enzyme were performed by using the GAUSSIAN09 program (36) with the density functional B3LYP/6–31G* method; the PCM method formulation (37) as implemented in GAUSSIAN09 was used to estimate the effect of aqueous solvation on the energy of the gas-phase conformers. A fast semiempirical density functional approach [self-consistent charge density functional tight-binding (SCC-DFTB) method] (35), recently implemented in the CHARMM

Fig. 1. The Claisen rearrangement of chorismate to prephenate. The atoms involved in the [3,3]-pericyclic reaction are arranged in a chair configuration.

Abbreviations: CM, chorismate mutase; YCM, yeast CM; TSA, transition-state analogue; CHAIR, chair pseudodiaxial conformer; DIAx, a pseudodiaxial conformer; DIEQ2, the lowest energy conformer of chorismate in the gas phase; ex-DIAx, an extended pseudodiaxial conformer; SCC-DFTB, the self-consistent charge density functional tight-binding method; QM/MM, quantum-mechanical/molecular mechanical.

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program (34), was used for calculations on the same conformers and for comparison with the B3LYP/6-31G* results. Because the values for the stable conformers of chorismate from the two approaches were similar (see Results), the SCC-DFTB method was used for QM/MM molecular dynamics simulations in solution and in the enzyme active site; SCC-DFTB calculations are several thousand times faster than those with B3LYP/6-31G*.

The chorismate substrate was treated by QM and the rest of the system (explicit aqueous solvent or the enzyme active site) by MM. The quantum mechanical description of the substrate is advantageous because it does not require specific MM parameters to be determined and provides a more realistic treatment of the fluctuations of the covalent bond distances that cleave or form during the reaction (here the ether bond that is broken and the C1=C6 bond that is formed during the Claissen rearrangement). The QM/MM molecular dynamics simulations were performed by using the CHARMM program (38); the all-hydrogen potential function (PARAM22) (39) was used for MM atoms. A modified TIP3P water model (40, 41) was used for the solvent. The stochastic boundary molecular dynamics method (42) was used for the QM/MM calculations in the enzyme and in solution.

The initial coordinates for the simulations were obtained from the crystal structure of yeast CM (Protein Data Bank ID code 3cm) in the wild-type “super R” state, which has the TSA plus Trp (ITRP in ref. 11) bound to the active and regulatory sites, respectively. For the simulations, the inhibitor was replaced by the substrate in the various conformations that were examined. In addition to the reactive CHAIR conformer, DIEQ1 and DIAx (see Fig. 2) were studied, because they seemed most likely to be able to change to CHAIR in the active site and were relatively stable in solution; other conformations (e.g., DIEQ2) would have required more complicated motions. Solution simulations were done with and without two unconstrained guanidinium cations. The effects of the latter were examined to mimic the large positive counterions [bis(tetra-n-butylammonium)] present in the solution studies of Copley and Knowles (22).

Details of the methods, including the placement of the various conformers in the active site, are published as supplemental data on the PNAS web site (www.pnas.org).

Results

Substrate Conformations from Quantum Mechanical Calculations and from Solution Simulations. To select a set of conformers for introduction into the enzyme active site, gas-phase density functional calculations were made with and without PCM solvation correction and solution QM/MM simulations; the former served also to test the semiempirical SCC-DFTB method. The optimized structures for some of the chorismate conformers (CHAIR, DIEQ1, DIEQ2, and ex-DIAx) from gas-phase calculations are shown in Fig. 2, and their relative stabilities and structural parameters are listed in Table 1. Previous QM calculations (24–25, 27–28, 31) and the present results show that DIEQ1 is the lowest energy conformer in the gas phase and in solution, including PCM solvation correction. Consistent with previous studies (24–25, 27–28, 31), DIEQ1 and DIEQ2 are found to be considerably more stable than CHAIR. Both DIEQ1 and DIEQ2 are stabilized by a strong hydrogen bond between the side-chain carboxylate and the ring OH of C4 (31). The energy difference (\(\Delta E + \Delta G_{\text{soln}}\)) between CHAIR and DIEQ2 (DIEQ2) is 11 kcal/mol (8 kcal/mol) by using B3LYP/6-31G*. The gas-phase B3LYP/6-31G* values are close to those obtained by Martí et al. (31) from MP2/6-31G* calculations. The results from the SCC-DFTB calculations are in a reasonably good agreement with those from B3LYP/6-31G*, although the energy differences between CHAIR and DIEQ2 or DIEQ1 are somewhat smaller; see Table 1. The large energy difference between CHAIR and DIEQ2 (11.3 kcal/mol with PCM correction) suggests that the population of CHAIR is very small in solution.

Table 1. Energies and structural parameters from calculations

<table>
<thead>
<tr>
<th>Conf *</th>
<th>(\Delta E)</th>
<th>(\Delta G_{\text{soln}})</th>
<th>(R_1)</th>
<th>(t_1)</th>
<th>(t_2)</th>
<th>(t_3)</th>
<th>(\delta)</th>
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<td>CHAIR</td>
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<td>78.8</td>
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<tr>
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<td>161.0</td>
<td>-105.8</td>
<td>-43.1</td>
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</tr>
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<td>DIAx</td>
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<td>70.9</td>
<td>-57.6</td>
<td>44.7</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ex-DIAx</td>
<td>12.3</td>
<td>-6.4</td>
<td>5.2</td>
<td>-161.0</td>
<td>70.5</td>
<td>-120.5</td>
<td>44.8</td>
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</table>

\(\Delta E\) and \(\Delta G_{\text{soln}}\) values are in kcal/mol. The energies of DIEQ2 are taken as the zero. \(R_1 = R(C_1...C_6) (\AA), t_1 = r(O_2-C_5-C_6-O_1), t_2 = r(H-C_4-C_5-H), t_3 = r(C_4-O_2-C_5-O_1),\) and \(\delta = 1/2(t_1^2 - t_2^2).\) Except where otherwise noted, \(\Delta E\) values and the structural parameters were obtained from the gas-phase calculations. The values of \(R_1, t_1, t_2, t_3,\) and \(\delta\) for the experimental transition-state analogue structure are about 1.53 Å, -149°, 87°, 57°, and 31°, respectively.

\(\Delta G_{\text{soln}}\) values were obtained using the PCM approach. The total gas-phase energies are -837.1571, -837.1810, -837.1857, and -837.1661 a.u. for CHAIR, DIEQ1, DIEQ2, and ex-DIAx, respectively. The solvation free energies are -179.8, -172.5, -173.2, and -178.8 kcal/mol for CHAIR, DIEQ1, DIEQ2, and ex-DIAx, respectively.

The structural parameters in parentheses were based on the minimization of chorismate (CHAIR, DIEQ1, and DIEQ2) within a sphere of water molecules or based on molecular dynamics simulations (DIAx).

*See Fig. 2 for definitions.

**HF/6-31G*; with eight bridging waters (see Fig. 2).
solution, much smaller than the 12% estimated from the NMR measurements of Copley and Knowles (22). As shown in Table 1, one structural difference between CHAIR and DIEQs is that $\tau(\text{O}1\text{C}3\text{C}4\text{O}2)$ (17) and $\tau(\text{H}1\text{C}2\text{C}3\text{H})$ (22) are about $-135^\circ$ and $90^\circ$, respectively, in CHAIR, whereas they are about $0^\circ$ and $90^\circ$, respectively, in DIEQs. The parameter $\delta$ is a linear combination of $\tau_1$ and $\tau_2$ (i.e., $\delta = 1/2(\tau_1 - \tau_2)$), is useful for studying conformational transitions; i.e., $\delta$ is positive for the pseudodiaxial conformers (e.g., CHAIR and DIAX) and negative for the pseudoequatorial conformers (e.g., DIEQ1 and DIEQ2).

To explore the possible solution conformers, molecular dynamics simulations were performed with the QM/MM method (with or without two guanidinium cations interacting with the two carboxylates) starting from the CHAIR conformer. Although CHAIR is a local minimum in the gas phase, it is not stable in solution. Instead, it is rapidly (within 10–20 ps) converted to another conformer, called DIAX (see Fig. 2), and it spends most of the time in the DIAX conformation during the remainder of the simulations (several hundred picoseconds). Efforts to stabilize CHAIR in solution by the addition of harmonic constraints to the two guanidinium cations at their optimum positions for interaction with the two carboxylates in CHAIR were unsuccessful; CHAIR converted to DIAX by breaking one or more salt-bridge interactions with guanidinium cations. DIAX is not stable by itself in the gas phase, because the two carboxylates are near each other. A stable DIAX conformer was obtained from ab initio calculations when water molecules were introduced to bridge the two carboxylates; an optimized DIAX structure from the HF/6–31G* calculations with eight bridging waters is shown in Fig. 2. As can be seen from Fig. 2, there exist structures of the form: CO$_2$...HOH...O$_2$-C and -CO$_2$...HOH...O$_2$-C, where $n = 1$ or 2. Similar structures were found during the QM/MM simulations to stabilize the DIAX conformer in solution; the structural data for the solution conformation are given in Table 1. Such water-bridged structures stabilizing two charged ionic groups have been observed in an earlier simulation of the active site of ribonuclease A (44). Of particular interest about DIAX (see Table 1) is that the dihedral angles describing the ring ($\tau_1$ and $\tau_2$) are similar to those found in CHAIR; DIAX is distinguished from the latter by $\tau_1(\text{C}2\text{O}1\text{C}3\text{C}4)$; i.e., $\tau_1$ is about $70^\circ$ in CHAIR, whereas it is about $-30^\circ$ to $-70^\circ$ in DIAX, corresponding to the fact that DIAX has the side-chain carboxyl group, rather than the side-chain methylene group, over the C3 atom. Thus, DIAX is an inactive conformation, but it may well be the one observed by Copley and Knowles (22) in their solution NMR studies; its structure is consistent with the NMR measurement of Hilvert and coworkers (23).

A nonreactive extended pseudodiaxial conformer (ex-DIAX) in Fig. 2), which is 5 kcal/mol more stable than CHAIR in the gas phase, was obtained in earlier studies (24–25, 27–28, 31–32). This conformer was identified previously (24, 27), with the pseudodiaxial conformer observed in the NMR studies of Copley and Knowles (22). The structural data for ex-DIAX are also listed in Table 1; most of the parameter values of ex-DIAX are similar to those of DIAX except for $\tau_1$, which is more negative (i.e., $-120^\circ$ instead of $-30^\circ$ to $-70^\circ$ for DIAX), as expected for an extended conformation. Fixed HF/6–31G* structures and Electrostatic Potential Surface charge distributions of ex-DIAX and DIEQ2 were used by Carlson and Jorgensen (27) to study the conformational equilibrium of chorismate in solution. However, ex-DIAX is unstable in solution, and the two carboxylates moved closer to each other and were bridged by water molecules.

The results obtained in this section, in agreement with the experiments of Hilvert and coworkers (23), indicate that the original proposal in which the enzyme preferentially binds the CHAIR conformer is not tenable, because its concentration in solution is too small. Instead, a likely possibility is that DIAX, which could have been mistaken for CHAIR in the original NMR work and is relatively stable in solution, is one of the conformers bound by the enzyme.

Conformation Dynamics of the Active Site of Yeast CM (YCM). The dynamics of CHAIR, DIEQ1, and DIAX were studied in the active site of YCM; DIEQ2 and ex-DIAX were not considered further, because the significant conformational changes required for them to change into CHAIR are unlikely in the active site (10, 11), and the relative positions of the functional groups (i.e., the two carboxylates, the ether oxygen, and the C4 hydroxyl group) in these (extended) conformations prevent interactions with the corresponding active site residues. As described in Methods, energy minimizations were performed first on the complexes with the three different substrate conformers docked in the active site (see Fig. 3). Fig. 3A shows that the interactions of CHAIR with the active site residues remain the same as those observed in the X-ray structure (10, 11). As a result of the minimization, the interaction distances are improved significantly from the values in the docked structure (see Fig. 3 legend). The $R_1$ distance is 2.9 Å, about 0.5 Å smaller than that obtained in the gas phase (3.5 Å) and in solution (3.3 Å). Fig. 3B shows that DIEQ1 retains its conformation after the minimization (i.e., it has essentially the same structure as that shown in Fig. 2). Almost all of the electrostatic and hydrogen-bonding interactions between YCM and the CHAIR–YCM complex discussed above are found in the YCM–DIEQ1 complex, including the one involving the backbone amide group of Asn-194 and the C4 hydroxyl oxygen missing in the initial docked structure (see Methods); the only exception is that the hydroxyl hydrogen of C4 still makes the internal hydrogen bond with the side-chain carboxylate. Fig. 3C shows that, whereas energy minimization moves DIAX somewhat closer to CHAIR (i.e., $R_1 = 3.6$ Å and $\tau_1 = 2^\circ$ compared with $R_1 = 4.5$ Å and $\tau_1 = 30^\circ$ to $-70^\circ$ of DIAX in solution; in CHAIR, the values are $R_1 = 3.3$ Å and $\tau_1 = 65^\circ$), the environment of the ring carboxylate is different from that observed in the TSA X-ray structure (10–11). Specifically, Arg-157 interacts only with one of the ring carboxylate oxygens in DIAX (it interacts with both ring carboxylate oxygens in CHAIR), and the hydroxyl proton of Thr-242 is 6.7 Å away from $O_b$ (the distance for CHAIR is 1.7 Å). Moreover, the C4 hydroxyl of DIAX cannot hydrogen bond to Glu-198 and the backbone amide group of Asn-194 at the same time, in contrast to the CHAIR. These differences result from the incorrect orientation of the side chain with respect to the ring in DIAX, even after minimization. The minimized structures shown in Fig. 3B and C for DIEQ1 and DIAX, respectively, are not appropriate for the Claisen rearrangement, and catalysis starting with them would require large structural changes during the reaction.

To determine whether the reorientation of DIAX and DIEQ1 is likely to occur spontaneously and rapidly before reaction, we did QM/MM molecular dynamics simulations in the active site. The motions of CHAIR, DIEQ1, and DIAX in the enzyme active site are monitored in Fig. 4 by using $\tau_1$ and/or the angle $\delta$. Fig. 4A (Top) shows that, in contrast to the motion of CHAIR in solution (see above), no conformational transition occurs in the active site; the substrate remains in the neighborhood of CHAIR, with $\delta$ about $30^\circ$ and $\tau_1$ about $60^\circ$; the values for the minimized CHAIR are $25^\circ$ and $59^\circ$, respectively (see caption to Fig. 3A). Moreover, the important interactions with the active site residues shown in Fig. 3A are retained. The conformational changes of DIEQ1 at 100 and 200 K are monitored by $\delta$ in Fig. 4B (Middle); the behavior at 300 K is similar to that at 200 K. The $\delta$ value for DIEQ1 is about $-45^\circ$ for the substrate within the enzyme after energy minimization ($\sim 55^\circ$ in the gas phase or minimized in solution), whereas it is about $12^\circ$–$23^\circ$ for CHAIR.
hydrogen bond with the side-chain carboxylate rather than with Glu-198 (8 above). (G) DIAK. The initial orientation of DIAK in the active site is such that the side-chain groups (the carboxylate and ether oxygen) form the observed interactions with the active residues (i.e., 1, 2, 4, and 5). The substrate undergoes a rotation about the C₆-O₆ bond (R₆) toward CHAIR during the minimization, so the conformation is between DIAK and CHAIR. R₆ = 3.6°, γ₁ = −145°, γ₂ = 84°, γ₃ = 2°, and δ = 31°. Certain interactions involving the ring carboxylate and the C₆ hydroxyl proton cannot be formed, e.g., Arg-157 interacts only with one of the ring carboxylate oxygens, and the hydroxyl group of Thr-242 is 6 Å away from the oxygen (O₆) of the ring carboxylate.

The simulation of DIAK at 300 K (Fig. 4C, Bottom) shows that τ₃, the torsional angle reflecting the relative orientation of the side chain and ring, initially moves from about 0° (the value after the minimization) to 30° in about 1 ps and then gradually increases to 50–60° by 50 ps, when the transition to CHAIR is essentially complete; the δ value is not sensitive to the transition, as it is similar in the pseudodiaxial conformers DIAK and CHAIR (i.e., 10–40°; see Table 1 and the discussions above). Fig. 5B shows the time dependence for the YCM–DIAK complex of the interactions that exist in the YCM–CHAIR complex (see Table 1). Fig. 4B (Middle) shows that the substrate changes to CHAIR with δ in the range of 5–40° in about 50 ps at 100 K and 5–10 ps at 200 K (or 300 K). It then fluctuates around the CHAIR conformation during the remainder of the simulation time (300 ps). The fluctuations after the transition are larger than those before, in part because of the absence of the internal hydrogen bond between the C₆ hydroxyl proton and the side-chain carboxylate. Fig. 5A examines the internal hydrogen bond between the C₆ hydroxyl proton and side-chain carboxylate in DIAK at 100 and 200 K as a function of time. It is evident that this hydrogen bond is broken at the same time as the substrate changes from DIAK to CHAIR (see Fig. 4B). As mentioned earlier, the only interaction between the substrate and active site residues that is lacking in the YCM–DIAK complex, relative to those in the YCM–CHAIR complex, is the hydrogen bond between the C₆ hydroxyl proton and Glu-198, as long as the hydroxyl proton is involved in the internal hydrogen bond.

The simulation of DIAK at 300 K (Fig. 4C, Bottom) shows that τ₃ increases from 0° to 50–60° (CHAIR) as a result of the rotation of the ring carboxylate.

Fig. 4. Motions of CHAIR, DIAK, and DIAK in the active site of YCM as a function of time. (A) CHAIR at 300 K. The motion is monitored by δ (magenta, dashed line) and τ₃ (red, dotted line). No conformational transition occurs, and the substrate remains in the neighborhood of CHAIR (i.e., δ = 30° and τ₃ = 60°). (B) DIAK at 100 K (blue, dashed line) and 200 K (red, dotted line). The motion is monitored by δ. The substrate changes to CHAIR in about 50 ps at 100 K and 5–10 ps at 200 K, as indicated by the change of δ from negative to positive values. (C) DIAK at 300 K. The motion is monitored by τ₃ (red, dotted line). τ₃ increases from 0° to 50–60° (CHAIR) as a result of the rotation of the ring carboxylate.

Fig. 3. Active site structures after energy minimization and before dynamics. (A) CHAIR. The interactions between the substrate and the active site residues are the same as those observed in the X-ray structures (10–11). The interactions and distances are (with the values in the initial docked structure given in parentheses): 1) salt bridge between Arg-16 and the side-chain carboxylate with the corresponding distances between the protons of Arg-16 and the oxygens equal to 1.6 Å (2.0–2.3 Å); 2) salt bridge between Lys-168 and the side-chain carboxylate with a distance between the proton of lys-168 and the oxygen of the carboxylate equal to 1.5 Å (2.5 Å); 3) salt bridge between Arg-157 (H) and the ring carboxylate (O) with distances equal to 1.6 Å (1.9–2.5 Å); 4) hydrogen bond between Glu-246 (H) and the ether oxygen with a distance equal to 2.4 Å (2.6 Å); 5) interaction between Glu-198 (H) and the C₄ hydroxyl proton (with the values in the initial docked structure given in parentheses): 1) salt bridge between Arg-16 and the side-chain carboxylate with the corresponding distances between the protons of Arg-16 and the oxygens equal to 1.6 Å (2.0–2.3 Å); 2) salt bridge between Lys-168 and the side-chain carboxylate with a distance between the proton of lys-168 and the oxygen of the carboxylate equal to 1.5 Å (2.5 Å); 3) salt bridge between Arg-157 (H) and the ring carboxylate (O) with distances equal to 1.6 Å (1.9–2.5 Å); 4) hydrogen bond between Glu-246 (H) and the ether oxygen with a distance equal to 2.4 Å (2.3 Å); 5) hydrogen bond between Lys-168 (H) and the ether oxygen with a distance equal to 2.4 Å (2.6 Å); 6) interaction between Thr-242 (H) and the ring carboxylate with a distance equal to 1.7 Å (2.9 Å); 7) interaction between the backbone amide group (H) of Asn-194 and the hydroxyl oxygen with a distance equal to 1.9 Å (2.7 Å); and 8) interaction between Glu-198 and the C₆ hydroxyl proton with a distance equal to 2.0 Å (3.5 Å). The energy minimization of the YCM–CHAIR complex does not lead to a different conformation, although there are some modifications of the structural parameters (see above). R₁ = 2.9 Å, δ = 137°, τ₁ = 87°, τ₂ = 59°, and δ = 25°. (D) DIEQ₁. The substrate is still in DIEQ₁ after the energy minimization. R₁ = 3.7 Å, τ₁ = −70°, τ₂ = 160°, τ₃ = 68°, and δ = −45°. All of the interactions in A exist here, except that the hydroxyl proton is involved in the internal
the internal hydrogen bond between the C4 hydroxyl proton and the side-chain carboxylate oxygen in the YCM–DIEQ1 complex monitored as a function of time; the trajectories shown are the ones used to produce Fig. 4B. This figure shows that the hydrogen bond is broken at the same time as the conformation transition from DIELQ to CHAIR occurs at each temperature (compare Fig. 4B). Certain interactions of the ring carboxylate and C4 hydroxyl group with the active site residues (Arg-157, Thr-242, Glu-198, and Asn-194) in the YCM–DIEQ complex as functions of time. The trajectory is the one used to produce Fig. 4C. There are no interactions initially between H of Arg-157 and O2 of the ring carboxylate, between Thr-242 and O3, and between O3 of Glu-198 and the C4 hydroxyl proton in the YCM–DIEQ complex (see Fig. 3B). (c) shows that these interactions are all formed after 50-ps simulations as the substrate changes from DIAx to CHAIR.

The fluctuations of the C1...C9 distance (R1) and the C1—O1 distance (R2) during 2 ps (from 172 to 174 ps) in the active site at 300 K, starting from the minimized structure in Fig. 3A. R1, blue-solid line; R2, red-dashed line.

Fig. 5. (A) The internal hydrogen bond between the C4 hydroxyl proton and the side-chain carboxylate oxygen in the YCM–DIEQ1 complex. The fluctuations of the C1...C9 distance (R1) and the C1—O1 distance (R2) during 2 ps (from 172 to 174 ps) in the active site at 300 K, starting from the minimized structure in Fig. 3A. R1, blue-solid line; R2, red-dashed line.

Fig. 6. The fluctuations of the C1...C9 distance (R1) and the C1—O1 distance (R2) during 2 ps (from 172 to 174 ps) in the active site at 300 K, starting from the minimized structure in Fig. 3A. R1, blue-solid line; R2, red-dashed line.

and in the enzyme. This result has led to the suggestion that the reaction starts from the chair, pseudodiaxial conformer (CHAIR) where C1 and C9 are positioned to form a carbon—carbon bond. Thus, one way for CM to catalyze the reaction is to bind the CHAIR conformer preferentially from solution and to catalyze its chemical transformation at the active site. A requirement for this mechanism is a sufficiently large population of CHAIR in solution.

Copley and Knowles (22) measured the temperature variation of the 1H coupling constants for the protons in the ring of chorismate and showed that, whereas the pseudodiaxial conformer(s) is dominant, a pseudodiaxial conformer(s) exists at reasonable levels (∼12%) in water. They assumed it was the CHAIR conformer, but the results of the present solution simulations suggest that the NMR data are likely to correspond to a nonchair, pseudodiaxial conformer (DIAx), which has the side-chain carboxylate group instead of the methylene group over the C1 atom (the C1...C9 distance is 5 Å) and so is in an inactive conformation. Indeed, the CHAIR conformer is unstable in solution, and the solution simulations starting from CHAIR lead to the stable DIAx in 10–20 ps. DIAx may not be distinguishable from CHAIR, on the basis of the coupling constants of the ring protons. Moreover, the study of the transferred nuclear Overhauser effects for chorismate in solution (23) showed no evidence for the existence of the CHAIR conformer.

Thus, the enzyme could bind the more abundant nonchair conformations from solution. To determine whether the conversion of such conformers to CHAIR is possible in the enzyme–substrate complex, we have explored the dynamics of DIAx, which is abundant in solution, and a second conformer, DIELQ, after they are bound to the enzyme in the inactive (solution) conformations. In the active site, both DIAx and DIELQ are rapidly converted (within 50 ps at 300 K) to CHAIR in a molecular dynamics simulation using a QM/MM potential for the enzyme–substrate complexes. This result suggests that the selection of the reactive CHAIR conformer is not necessary for the CM-catalyzed reaction. Instead, more abundant conformers can be bound and converted to the active CHAIR form in a fast step that is not rate limiting.

A recent transferred nuclear Overhauser effect study of the catalytic antibody 1F7 (23) indicates that, whereas the CHAIR conformer is not observed in solution, it is detectable in the
antibody 1F7: the preorganization apparently takes place at the antibody active site. The present study suggests that this may well be true for the CM from yeast and perhaps for CM from other species. Because the conformational transformations from non-reactive conformers to the reactive CHAIR are very efficient in structures that approach the transition state for the length of the bond to be formed and the orientation of the methylene group. This result is in part a consequence of the use of a QM/MM potential, which would lead to reaction in the simulations if the barrier were low enough. The relation of this observation to catalysis is of interest (45).

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Details of the simulation system and how different conformers were placed in the active site.

For the substrate in the CHAIR conformer, the position within the active site was chosen to mimic that of the transition-state analogue (TSA) with the corresponding interactions (1) (see Fig. 3A legend). The initial orientations of DIEQ$_1$ and DIAX within the active site were such that the side-chain carboxylate formed salt bridges with Arg-16 and Lys-168, observed in the TSA x-ray structure (1). The relative positions of the two carboxylates and ether oxygen in DIEQ$_1$ allow it to form most of the interactions of the yeast chorismate mutase (YCM)–TSA complex (i.e., 1-6 listed in Fig. 3A legend); the orientation of this conformer is similar to that of TSA. However, the C$_4$ hydroxyl group, which is in an equatorial position and makes an internal hydrogen bond with the side-chain carboxylate of DIEQ$_1$, cannot interact with the backbone amide group of Asn-194 and Glu-198 (i.e., interactions 7 and 8 in Fig. 3A legend are absent). For DIAX, the relative position of the side-chain carboxylate and the ether oxygen allows interactions with ether oxygen to be formed (i.e., interactions 4 and 5 in Fig. 3A legend). However, the ring carboxylate is too far from Arg-157 and Thr-242 to interact with them (i.e., interactions 3 and 6 in Fig. 3A legend are absent); the C$_4$ hydroxyl group is also unable to form the interactions observed in TSA. Energy minimizations performed for the YCM–CHAIR, YCM–DIEQ$_1$ and YCM–DIAX
complexes in the active site led to small adjustments of the various substrate models, as well as the active residues; some of the missing interactions are formed (e.g., between the backbone amide group of Asn-194 and Glu-198 in YCM–DIEQ₅) or partially formed (e.g., between the ring carboxylate and Arg-157 in YCM–DIAX) after the minimization (see em Results).

The stochastic boundary molecular dynamics method (2) was used in the quantum mechanical/molecular mechanical (QM/MM) calculations. The system was separated into a reaction zone and a reservoir region, which was deleted (3); the reaction zone was further divided into the reaction region and the buffer region. The reference point for partitioning the system was chosen as the bridge ether oxygen of the substrate (see Fig. 1). The reaction region was a sphere with radius $R$ of 16 Å, the buffer region had $R$ equal to 16 Å $\leq R \leq 18$ Å. The simulation systems consisted of 115 protein residues (out of 256), the substrate, and 400 waters; a corresponding model system is shown in Fig. 4 of Ref. 3. Inside the reaction region, the atoms were propagated by molecular dynamics, whereas atoms in the buffer region were propagated by Langevin dynamics. Protein atoms in the buffer region were restrained by a harmonic potential to their x-ray positions, with force constants derived from the temperature factors in the crystal structure (3). The friction constants for the Langevin dynamics (2) were 250 ps⁻¹ for the protein atoms (3) and 62 ps⁻¹ for the water molecules (3). Water molecules were confined to the active site region by a deformable boundary potential (2). For the simulations of the substrate in solution, spheres with radii equal to 16 Å (550 water molecules) and 25 Å (2,200 water molecules), including a 2 Å boundary
region, were used for the systems without and with two unconstrained guanidinium cations, respectively. The effects of the latter were examined to mimic the large positive counterions \[\text{bis(tetra-n-butylammonium)}\] present in the solution studies of Copley and Knowles (4). The initial positions of guanidinium ions were chosen so that each one forms a salt bridge with one of the carboxylates. As in the enzyme case, a stochastic deformable boundary was used. During the simulation, all of the covalent bonds involving hydrogen atoms were fixed by the SHAKE algorithm (5). A 1-fs time step was used for integration of the equations of motion. In initiating the runs, 500 steps of minimization using the steepest descent method were performed for the protein and solvent atoms. Then 4,000 steps of minimization were performed for the entire stochastic boundary system (including the substrate) with the Adapted Basis Newton Raphson method (5). The temperature of the system was gradually increased from 50 K to 300 K and equilibrated at 300 K (30 ps). The simulations were performed on the resulting enzyme–substrate complexes and the substrate in solution for several hundred picoseconds. Because the conformational transformations occur rather fast (i.e., before the temperature reaches 300 K), simulations were also performed at lower temperatures (100 and 200 K) to determine the effects of temperature on the rate of the conformational transformation, the motions of the substrate, and certain side chains in the enzyme active site. Dynamics of three conformers of chorismate (DIEQ₁, DIAAX, and CHAIR) were studied. The computer time required was about 1 day for a 100-ps simulation on a single processor of an Origin 2000.


