Molecular Modeling of Enzymes

Understanding the Role of Active-Site Residues in Chorismate Mutase Catalysis from Molecular-Dynamics Simulations**

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Understanding the role of active-site residues in enzymatic catalysis is of fundamental importance for a microscopic description of the catalytic mechanism, but also for the design of effective enzyme mimics or improvement of existing enzymes (or abzymes) for catalyzing chemical reactions. Site-directed mutagenesis and kinetic studies are able to identify key residues involved in the catalysis, but determination of their exact role from experimental information alone can be difficult. This is the case for the chorismate mutase of *Bacillus subtilis* (BsCM), which catalyzes the Claisen rearrangement of chorismate to prephenate (Figure 1 a and b) in the biosynthetic pathway that forms Tyr and Phe.[1] Mutation experiments[1–5] have identified several residues that are important for the catalysis. For instance, it was shown that replacement of Arg 90 by Gly or Ala leads to a significant reduction in k_{cat} by a factor of more than 10^5, while k_{cat}/K_m is decreased by a factor of 10^5–10^7. Interestingly, the double mutants Cys 88 Lys/Arg 90 Ser and Cys 88 Ser/Arg 90 Lys restore a factor of more than 10^3 in k_{cat}.[5] Arg 90 interacts with the ether oxygen atom of the transition-state analogue (TSA) in the X-ray structure of the wildtype BsCM complex,[6–7] and this observation led to the suggestion that Arg 90 stabilizes developing negative charge on the enolic oxygen atom in the transition state[1–3,6] as well as the transition-state conformation.[2,6] Nevertheless, the exact role of Arg 90 and other active-site residues is not clear.

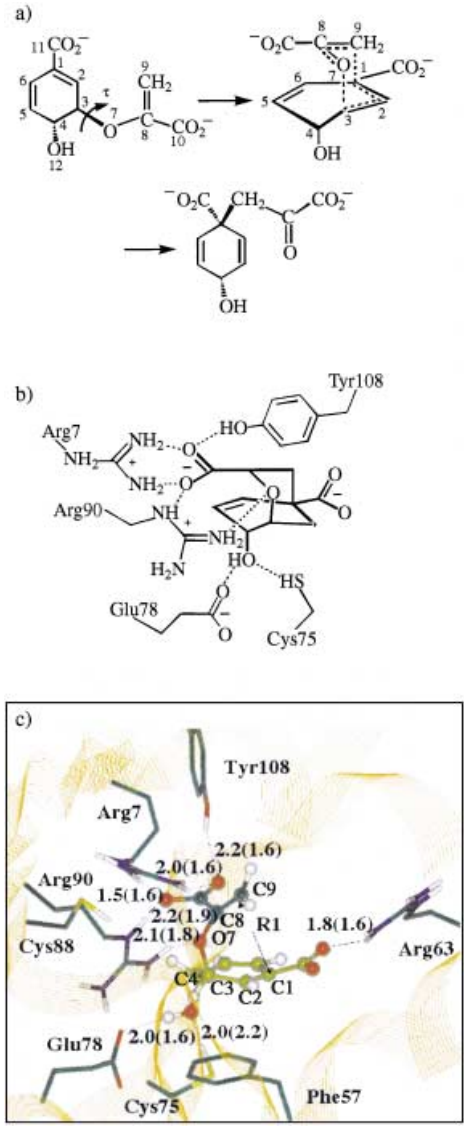

Figure 1. a) Claisen rearrangement of chorismate to prephenate via the chair-like transition state. b) Interactions between the TSA and important active site residues in BsCM.[6] c) The initial docking configuration of CHAIR in the wild-type BsCM as well as certain structural data after energy minimization (in parentheses). Distances are in Å. R_1(C1···C9) and τ(C2–C3-O7–C8) are used in monitoring the motion of the substrate during dynamics. Insight II of Molecular Modeling System from Accelrys, San Diego, was used to make the figure.

Herein we apply quantum-mechanical/molecular-mechanical (QM/MM) molecular-dynamics simulations to study the role of Arg 90 in the BsCM catalysis. It is demonstrated that the existence of this residue is essential for stabilizing the reactive-substrate conformation (CHAIR) in the active site, as both the Arg 90→Ala and Arg 90→Gly mutations destroy the ability of the wildtype enzyme to stabilize CHAIR.[8] Free-energy simulations suggest that the loss of this ability may contribute significantly to the reduction of k_{cat} for Arg 90 Ala and Arg 90 Gly observed

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experimentally.[2–5] The restoration of the catalytic efficiency by the double mutant Cys88Lys/Arg90Ser can be explained by a corresponding argument; that is, the CHAIR conformer is found to be more stable in this double mutant than in Arg90Ala. Thus, the simulations suggest that the removal of the single key residue Arg90 destroys the balance of the interactions in the active site required for stabilizing the reactive conformation of the substrate,[8] leading to a dramatic reduction in the catalytic efficiency of BsCM.

The reactive conformation (CHAIR) obtained from gas-phase ab initio calculations[8] was manually docked into the active sites of the wildtype enzyme, Arg90Ala, Arg90Gly, and Cys88Lys/Arg90Ser based on the BsCM TSA structures.[5–6] The initial docking and minimized configurations in the wildtype enzyme are given in Figure 1c, and those of the Arg90Ala, Arg90Gly, and Cys88Lys/Arg90Ser complexes are given in the Supporting Information. Figure 1c shows that CHAIR has interactions with the active-site residues that are similar to those of TSA (Figure 1b). The motion of CHAIR in BsCM and in different mutants during the QM/MM molecular-dynamics simulations is monitored by τ(C2–C3-O7–C8) and R_1(C1···C9) (See Figure 1c for definition) in Figure 2a and Figure 2b, respectively. The substrate remains in CHAIR in the wildtype enzyme ($\tau = 60$–$70°$ and $R_1 = 3$–3.5 Å), but it undergoes a conformational transition to a nonreactive conformation in Arg90Gly and Arg90Ala in 50–100 ps, as indicated by the decrease in τ (to about $\sim -40°$) and the increase of R_1 (to 4.5–5 Å). Strikingly, for Cys88Lys/Arg90Ser, the substrate remains in the neighborhood of the reactive conformation, although it is distorted and there are large fluctuations of R_1 (i.e., $\tau \sim 40°$ and $R_1 = 3.5$–5 Å). Figure 3 shows active-site structures for the wildtype enzyme and the mutants from the MD simulations. Figure 3a shows the substrate in the reactive CHAIR conformation at 1 ns with essentially the same interactions as those in the minimized structure (Figure 1c) and in the BsCM TSA complex structure (Figure 1b). For instance, Arg90 interacts with both the side-chain carboxylate group and the oxygen atom of the ether function, whereas Arg7 interacts with the side-chain carboxylate group only. Detailed discussions of the different interactions and a comparison with the available X-ray structures[5–7] will be given in a full paper. In the Arg90Gly and Arg90Ala complex structures (Figure 3b and c, respectively) the substrate has moved away from CHAIR, consistent with Figure 2, and it is now in an inactive (DIAX) conformation in which the side-chain carboxylate group, rather than the side-chain methylene group, is over C1. The DIAX conformer was identified earlier[8] as a stable conformation in solution simulations. In the DIAX conformation, it is Arg7 that interacts with the ether oxygen atom and the side-chain carboxylate group; these interactions replace those involving Arg90 in the wildtype enzyme complex (see Figure 3a). Figure 3d shows that the substrate is near the reactive CHAIR conformation in the double mutant Cys88Lys/Arg90Ser: the side chain is rotated toward DIAX by about 20°. The substrate is stabilized by a hydrogen-bonding network (side-chain carboxylate···S90···Lys88···Glu78···C4 hydroxy), which along with Arg7, appears to play a similar role in stabilizing CHAIR to that of the Arg90,

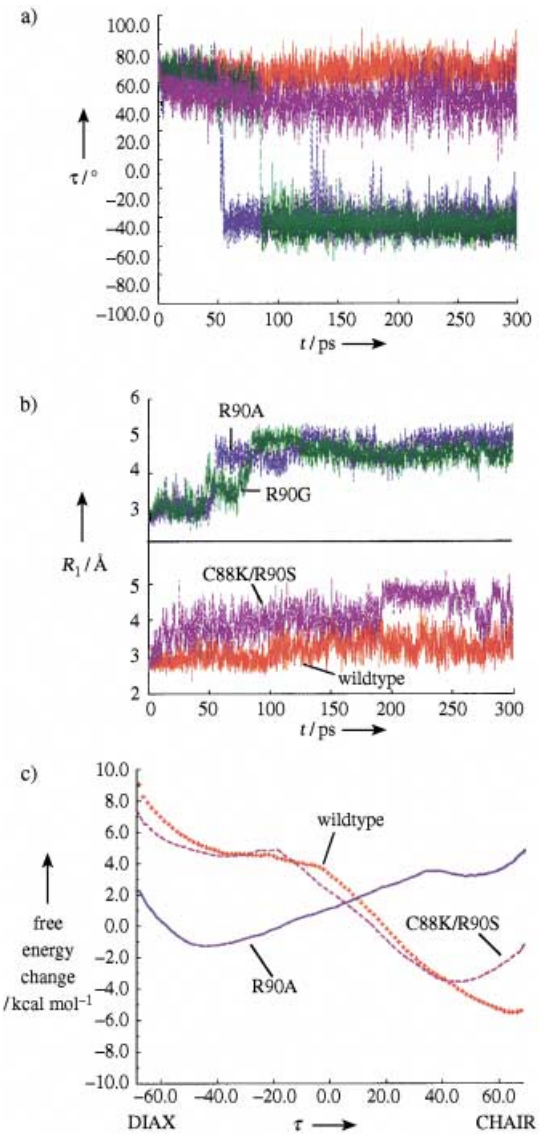

Figure 2. The motion of the substrate during the QM/MM MD simulations and the free energy (potential of mean force) changes. a) τ; b) R_1; c) free-energy changes. The initial substrate conformation is CHAIR. Red: wildtype; blue: Arg90Ala; green: Arg90Gly; purple: Cys88Lys/Arg90Ser.

Glu78 diad in the wildtype (see above). Consistent with the X-ray structure of Cys88Lys/Arg90Ser without TSA,[5] Lys88 forms a salt bridge with Glu78. No hydrogen bond is observed between Lys88 and the ether oxygen atom during the MD simulations; this hydrogen bond formed in the initial docking configuration, but broke during energy minimization and was absent during the dynamics simulations (see Supporting Information).

The removal of the Arg90 side chain through the Arg90→Ala or Arg90→Gly mutation leads to a reduction in k_{cat} by at least 5 orders of magnitude,[2–5] corresponding to an increase in the activation barrier by more than 8 kcal mol^{-1}. The X-ray crystal structure[5] shows that the integrity of the three-dimensional structure is well-maintained after the mutations. One widely accepted role for

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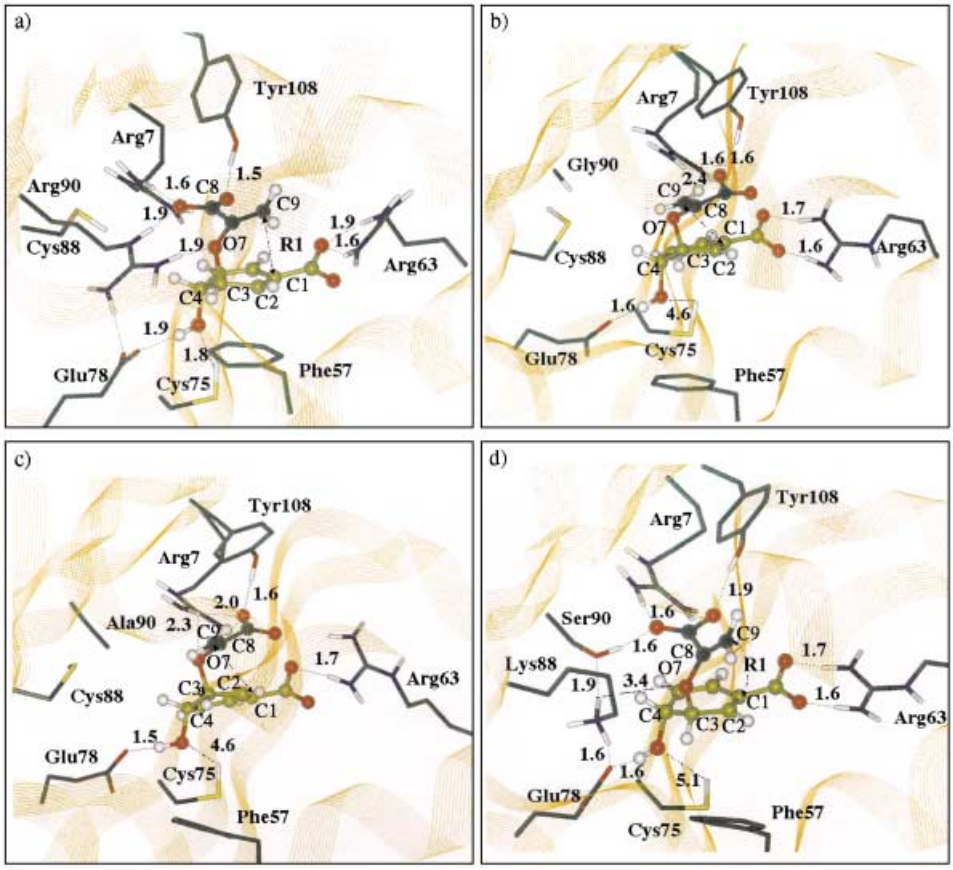

Figure 3. Typical active-site structures observed during the MD simulations; a single structure is shown. a) Wildtype at 1 ns: $R_1 = 3.0$ Å and $\tau = 53°$; b) Arg90Gly at 400 ps: $R_1 = 4.9$ Å and $\tau = -49°$; c) Arg90Ala at 400 ps: $R_1 = 4.9$ Å and $\tau = -45°$; d) Cys88Lys/Arg90Ser at 400 ps: $R_1 = 4.0$ Å and $\tau = 37°$.

Arg90 in the catalysis is to stabilize developing negative charge on the enolic oxygen atom in the transition state.[1–6] This suggestion is supported by a QM/MM study[10] of the BsCM catalysis by using a perturbation approach[11] based on fixed BsCM CHAIR and BsCM transition-state (TS) structures, in which it was shown that the deletion of Arg90 increases the activation barrier by 5 kcal mol^{-1} in going from CHAIR to TS. This increase is significantly smaller than the effect observed experimentally (> 8 kcal mol^{-1}).

To examine the free energy involved in the stabilization of the CHAIR form in the active site, umbrella sampling simulations[9] were performed for the wildtype and mutant enzymes. Figure 2c shows that whereas CHAIR is about 10 kcal mol^{-1} more stable than DIAX in the wildtype BsCM, it becomes 5 kcal mol^{-1} less stable in Arg90Ala. CHAIR is also found to be considerably less stable than DIAX in solution; the calculated free-energy difference is about 10 kcal mol^{-1}. CHAIR is stable in the mutant Cys88Lys/Arg90Ser, but the free-energy minimum is shifted somewhat ($\tau = 40°$). Thus, the results of the MD simulations point to an additional role for Arg90. Its existence appears to be essential for stabilizing the reactive substrate conformation (CHAIR) in the active site. The extra energy cost ~5 kcal mol^{-1} necessary for generating CHAIR in Arg90Ala and Arg90Gly would contribute to the loss of activity of the mutants; presumably, this would appear in the observed reduction in k_{cat}.[5] Correspondingly, the activity of the double mutant Cys88Lys/Arg90Ser (i.e., k_{cat} is 10^3-fold greater than that of Arg90Ala and Arg90Gly) involves its ability to stabilize a substrate conformation close to CHAIR. In this mutant, the substrate is distorted, and additional energy (~2 kcal mol^{-1}, see Figure 2c) is necessary to generate the optimal reactive CHAIR conformation (i.e., from $\tau = 40°$ to 60°). This factor, as well as the lack of a stable hydrogen-bonding interaction with the ether oxygen atom, would make the double mutant less efficient than the wildtype enzyme (k_{cat} is 10^2-fold less than that of the wildtype).

BsCM has been the subject of a large number of experimental[1–7,12–13] and theoretical investigations.[10,15–17] It has been shown experimentally that the transformation of chorismate into prephenate in solution has a free energy of activation of 24.5 kcal mol^{-1}[12] and that the activation barrier is reduced to 15–16 kcal mol^{-1} in the BsCM-catalyzed reaction.[13] Our earlier study of yeast CM[8] suggested that the catalysis may arise from the ability of the enzyme to lower the free energy of the transition state by stabilizing the reactive CHAIR conformation, in addition to the lowering of the chemical reaction barrier once the CHAIR conformation is present; the latter had been analyzed in many QM/MM studies.[10,15,17] The role of the stabilization of the CHAIR

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conformation is supported by the present free-energy simulations for BsCM, which demonstrate that Arg90 stabilizes the reactive conformation relative to the solution conformation.[18] Free-energy calculations for the much less active Arg90Ala and Arg90Gly mutants show that they destroy the ability of the enzyme to stabilize the CHAIR conformation. Moreover, the Cys88Lys/Arg90Ser mutant, which increases k_{cat} by a factor of more than 10^3 relative to Arg90Ala is found to be able to stabilize the CHAIR conformation in the active site. The present results provide strong evidence for the hypothesis that chorismate mutase uses conformational optimization of the substrate to lower the transition-state barrier. Efforts to design enzyme mimics or to improve existing enzymes (or abzymes) for transforming chorismate into prephenate should thus consider the conformational change required to achieve the transition-state geometry.

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