Computational Study of the Ground State of Thermophilic Indole Glycerol Phosphate Synthase: Structural Alterations at the Active Site with Temperature

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The reduced flexibility of hyperthermophilic enzymes is responsible for their stability at high temperature.[1,2] What is open to investigation is the following question: What structural changes in the active site of a hyperthermophilic enzyme occur with temperature, and can these explain the temperature dependence of the activity?

The enzyme chosen for the present study is a monofunctional hyperthermophilic indole-3-glycerol phosphate synthase from the archaeon *Sulfobus solfataricus* (sIGPS) and is a member of the family of enzyme having a $(\beta\alpha)_8$ fold barrel, commonly known as the TIM barrel.[3] The sIGPS is the terminal enzyme in the tryptophan biosynthesis pathway. The enzyme IGPS catalyzes the ring closure of the substrate 1-(o-carboxyphenylamino) 1-deoxyribulose 5-phosphate (CdRP) to form the product indole-3-glycerol phosphate (IGP) as shown in Scheme 1.[4] On the basis of the available crystal structures and by modeling the intermediates in the enzyme active site, Kirschner and co-workers provided insights into the catalytic mechanism of IGPS.[5] However, in the X-ray crystal structures with the substrate (CdRP)- and reduced substrate analogue (rCdRP)-bound sIGPS, both ligands are bound in an extended, unproductive conformation, such that the two reactive carbon centers C1 and C2′ are separated by a distance that is too large to initiate bond formation.[5]

In covalent bond formation, regardless of the environment, nucleophilic and electrophilic atoms must come together at a van der Waals distance and at an angle approximating that in the transition state. We term such members of the Michaelis conformers as near attack conformers, or NACs.[6] The transition state can only be reached through these reactive Michaelis conformers. The NACs for the CdRP → IGP reaction are defined as those ground-state conformers that have the two reacting atoms (C1 and C2′) within van der Waals contact distance (≤3.5 Å) at an attack angle of 120° ± 20°[7] (Chart 1).

We have performed molecular dynamics (MD) simulations of the Enzyme·CdRP complex at both room temperature (298 K) and at high temperature (385 K) for 2 ns duration each.[8] They will be referred to as E·S298 and E·S385, respectively. The average distance between the two bond-forming centers (C1 and C2′) in E·S298 is 4.0 ± 0.2 Å.[8] Only 1.6% of the conformers have the bond-forming distance that satisfies the NAC criteria, that is, $R_{C1···C2'} \leq 3.5$ Å. After applying the NAC angle definition in conjuncture with the distance criteria, only 1 out of 2760 conformers is identified as a NAC in E·S298 (Figure 1A). In the E·S385 system, the average distance between C1 and C2′ is 3.5 ± 0.2 Å, which is shorter as compared to low temperature. The percent conformers having a bond-forming distance less than 3.5 Å is 47. The mole percentage of the Michaelis complex present as NACs at 385 K is 40 (Figure 1B).

The experimentally determined increase in enzyme activity in going from 298 to 385 K is ~4200-fold (Table 1).[9] The increase

Scheme 1
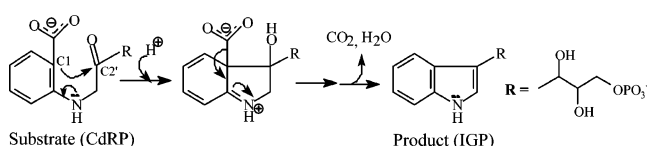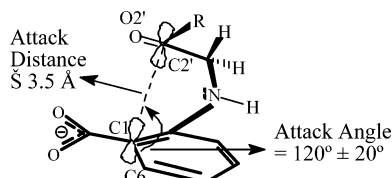
Substrate (CdRP)   Product (IGP)

Chart 1. Definition of NAC

in the mole percent of NACs from 0.036 at 298 K to 40 at 385 K (~1100-fold increase) correlates well with the experimentally determined increase in enzyme activity. Thus, the increase in time that the Michaelis complex exists as a NAC is correlated to the change in activity of this enzyme with an increase in temperature.

Taking a closer look at the active site of sIGPS, the higher population of NACs at elevated temperature may be attributed to the interplay between the substrate and the residues Lys53 and Lys110 (Chart 2). Lys110 functions as a general acid. The extent of electrostatic interactions between the positively charged Lys53
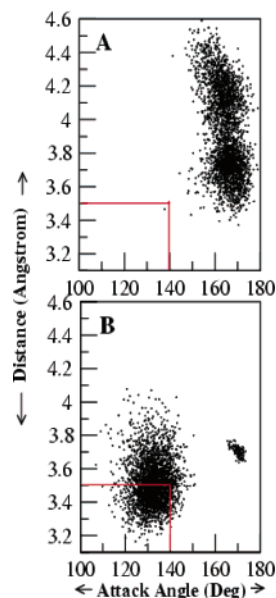
Figure 1. Plot displaying observed distances and attack angles in the MD simulation of (A) E·S298 and (B) E·S385. The conformers within the red box correspond to NAC population. There is only 1 NAC at 298 K, whereas there are 1103 NACs at 385 K.

Table 1. Relative Specific Activities[9]

T	relative specific activities
298 K (25 °C)	1[a]
310 K (37 °C)	4
328 K (55 °C)	30
353 K (80 °C)	320
385 K (110 °C)	4200[a]

[a] Extrapolated from the Arrhenius plot using the equation: $\log k = -4797.7(1/T) + 14.79$, correlation coefficient $= 0.99$.

Chart 2. Structural Alterations in E·S298 and E·S385[a]
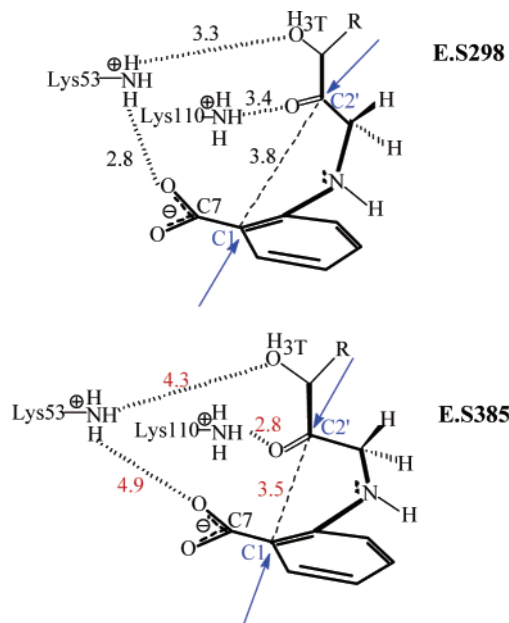
[a] Distances in angstroms from the average structure generated from the last 200 ps of MD.

and the negatively charged carboxylate (O71 and O72), and the O3T hydroxyl of CdRP is crucial in determining the attack angle (Chart 1) and bringing the reactive centers C1 and C2′ in proximity. The Lys53 interacts strongly with the carboxylate and O3T of the CdRP simultaneously, fixing them in the same plane at 298 K (Chart 2). This conformation disfavors the NAC formation at 298 K because the angle at which C2′ approaches C1 is inappropriate. It is worth pointing out that even though the reacting centers C1 and C2′ are at van der Waals distance, the reaction is only favorable when they approach at the correct orientation, that is, the π-orbital of C2′ pointing at C1. This is another[10] example that shows the importance of the angle dependence for the NAC formation. At 298 K, the general acid Lys110 hydrogen bonds with the O2′ of the substrate with a distance of 3.4 ± 0.3 Å (averaged from the final 200 ps of the MD trajectory). Lys53 exhibits more flexibility at 385 K. Chart 2 shows that Lys53 is further away from both the carboxylate and the O3T of CdRP at 385 K. This results in the substrate adopting the NAC conformation. However, it should be pointed out that Lys53 is needed for guiding the substrate to bind in a correct orientation at the active site. If this interaction is too strong, the substrate becomes trapped in nonproductive conformations as it happens in the case of E·S298. In the case of E·S385, interaction with Lys53 is moderate. This allows enough room for the substrate to form the reactive conformer. At the same time, the

general acid Lys110 migrates closer to the O2′ of the substrate maintaining an average distance of 2.8 ± 0.1 Å (averaged from the final 200 ps of MD trajectory). The observed changes in the active site structure with temperature are large enough that they could be, at least in principle, detected by sensitive experimental techniques.

In conclusion, we find that the population of reactive Enzyme·Substrate conformers (NACs) at 385 K is significantly higher (~1100-fold) than that at 298 K. This increased population of NAC conformers in the Michaelis complex correlates well with the increase in rate in going from 298 to 385 K. The positioning of the two active site residues Lys53 and Lys110 controls the binding of the substrate in the favorable orientation for the general acid-catalyzed intramolecular ring formation reaction.

This is a continuation of studies of a series of single-substrate intramolecular reactions, which do not involve covalent enzyme—substrate intermediates.[11−13]

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