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Diels−Alder Ribozyme Catalysis: A Computational Approach

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Abstract: A computational comparison of the Diels−Alder reaction of a maleimide and an anthracene in water and the active site of the ribozyme Diels-Alderase is reported. During the course of the catalyzed reaction, the maleimide is held in the hydrophobic pocket while the anthracene approaches to the maleimide through the back passage of the active site. The active site is so narrow that the anthracene has to adopt a tilted approach angle toward maleimide. The conformation of the active site changes marginally at different states of the reaction. Active site dynamics contribution to catalysis has been ruled out. The active site stabilizes the product more than the transition state (TS). The reaction coordinates of the ribozyme reaction in TS, R_{C1-CD1} and R_{C4-CD2}, are 2.35 and 2.33 Å, respectively, compared to 2.37 and 2.36 Å in water. The approach angle of anthracene toward maleimide is twisted by 18° in the TS structure of ribozyme reaction while no twisted angle is found in TS of the reaction in water. The free energy barriers for reactions in both ribozyme and water were obtained by umbrella sampling combined with SCCDFTB/MM. The calculated free energy barriers for the ribozyme and water reactions are in good agreement with the experimental values. As expected, Mulliken charges of the atoms involved in the ribozyme reaction change in a similar manner as that of the reaction in water. The proficiency of the Diels−Alder ribozyme reaction originates from the active site holding the two reactants in reactive conformations, in which the reacting atoms are brought together in van der Waals distances and reactants approach to each other at an appropriate angle.

Introduction

To date, naturally occurring ribozymes are known to catalyze only a narrow reaction spectrum, such as, the hydrolysis and transesterification of phosphodiester bonds.[1] The introduction of an *in vitro* selection technique (SELEX) has provided a way to produce non-natural ribozymes for many biological important chemical reactions.[2] The Diels−Alder ribozyme is a 49-nucleotide RNA molecule obtained by use of the SELEX method.[3−6] It catalyzes a [4 + 2] cycloaddition reaction between the anthracene and maleimide (Charts 1 and 2). The ribozyme reaction has been estimated to be about 20 000-fold faster than the reaction in water.[4,7]

The Diels−Alder reaction is not only a quintessential pericyclic reaction in organic synthesis but also postulated to account for biosynthesis of a number of natural products.[8] The rate of the Diels−Alder reaction in water has been proved to be accelerated by hydrophobic terms.[9−14] The finding of naturally occurring Diels−Alderases,[15−18] catalytic antibody Diels−Alderases,[2,19] Diels−Alder ribozymes,[4,20] and a DNA-based Diels−Alderase[21] has drawn attention. It has been demonstrated that the reactions in gas and water proceed *via* a concerted mechanism. On the other hand, both concerted and stepwise mechanisms have been proposed for some biocatalytic system.[16,22−25]

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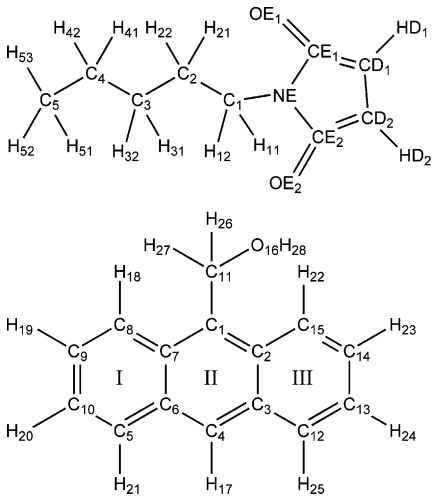
Chart 1



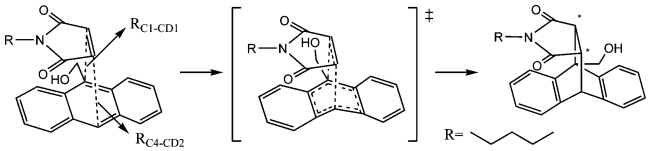
The X-ray structure of the Diels−Alder ribozyme (PDB entry: 1ykv) complexed with the reaction product has been determined.[26] The Diels−Alder ribozyme adopts a double-pseudoknot architecture,[27] with two λ-shaped hydrophobic pockets nested in the two neighboring centers of the three-helical conjunctions.[26] Each hydrophobic pocket is basically bracketed by the A3-U45 base pair, U23·A43 noncanonical base pair, and U42·(G2-C25) base triple to form a wedge shape, which is precisely complementary to the reaction product (Figure 1). The active site has two opened edges as passages to the solvent, which have been named the front and back. Eight Mg^{2+} cations, found in the ribozyme X-ray structure, are not directly involved in the catalysis reaction.[26] Chemical interaction between the active site and substrate does not play a major role in the Diels−Alder ribozyme reaction.[28]

We have reported previously both MD simulations of ground state conformers and QM/MM calculations of the reaction coordinate of the hammerhead ribozyme.[29,30] In the present study, the Diels−Alder ribozyme reaction has been simulated by the QM(SCCDFTB)/MM method[31−33] combined with Umbrella sampling.[34,35] The SCCDFTB/MM method has been applied to a number of biosystems,[36−38] demonstrating its reliability.[39,40]

Methods

The QM/MM simulations were carried out employing the self-consistent-charge density functional tight binding (SCCDFTB) module by the use of CHARMM version 31b1.[31−33] Initial coordinates for the ribozyme simulations were modified from the X-ray structure of the

Chart 2



Diels−Alder ribozyme (PDB entry: 1ykv, resolution: 3.3 Å).[26] The products in the two neighboring active sites were replaced with the maleimide and anthracene reactants. Only one pair of the reactants in one of the active sites were handled by quantum mechanics (QM = SCCDFTB). A total of 53 atoms was included in the QM portion. The CHARMM force fields of the maleimide and anthracene were also obtained for the preliminary molecular dynamics (MD) simulations.

The ribozyme−substrate complex was solvated using a water sphere with radius of 25 Å, centered at the QM region. Water molecules within 2.8 Å of any non-hydrogen ribozyme−substrate complex atoms were removed. Magnesium and sodium ions were added to neutralize the charge of system. Energy-minimization was carried out to remove the unfavorable interactions between the solute and solvent. The voids generated after briefly Langevin dynamics were filled with water molecules so that the solvent density is approximately correct. The resulting system contains 75 RNA nucleotides and 1350 TIP3P water molecules.[41] For the simulations of the Diels−Alder reaction in water, the initial coordinate was prepared in a similar way as that of ribozyme but involved only one pair of reactants and a water sphere of 2312 TIP3P water molecules. Stochastic boundary conditions were applied to the system.[42] The setup procedure of stochastic boundary conditions can be found in the previous study.[43] Preliminary MD simulation was performed at 298 K. The SHAKE algorithm was applied to fix the covalent bonds involving hydrogen.[44] Time step of 1 fs was used for the integration of the equations of motion.

Initial structures for the potential energy surface (PES) calculation were randomly extracted from the stable trajectories obtained from the MD simulations. The two distances, R_{C1-CD1} and R_{C4-CD2} as labeled in Chart 2, were selected as reaction coordinate. The two-dimensional PES was created by the energy minimization of the structure associated with variation of reaction coordinates. The stationary points (minima and saddle points) were confirmed by normal-mode analysis (NMA).[45,46]

Both one- and two- dimensional umbrella sampling were employed to obtained one- and two- dimensional potential of mean force (PMF), respectively. For the one-dimensional umbrella sampling, the average of R_{C1-CD1} and R_{C4-CD2} was selected as reaction coordinate. The choice of average of R_{C1-CD1} and R_{C4-CD2} as reaction coordinate ensures that the synchronous region of the PES was sampled effectively, which is crucial to obtain accurate free energy profiles. The reaction coordinate was divided into 45 windows with 0.05 Å intervals from 1.5 to 3.7 Å in the simulations of ribozyme. The force constants of the umbrella were 200−400 kcal·mol^{-1}·Å^{-2}, in which the larger force constants were assigned to the windows around the TS in order to improve the degrees of overlap in the phase space between the adjacent windows. Good overlaps in the phase space are essential for obtaining reliable PMF

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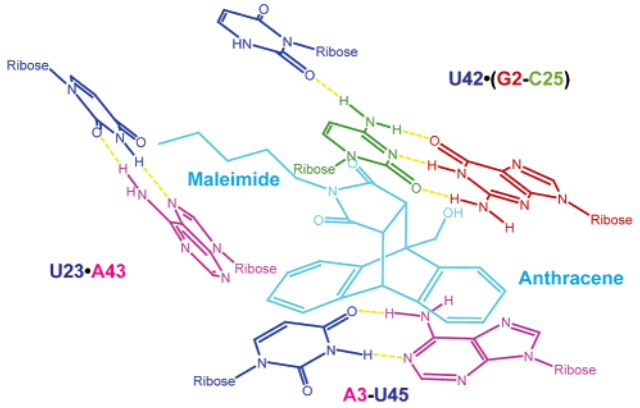

Figure 1. Active site of the Diels−Alder ribozyme. The A3-U45 and U23•A43 base pairs and U42•(G2-C25) base triple constitute a λ-shaped hydrophobic pocket, which is precisely complementary to the reaction product of maleimide and anthracene. The structures are colored the same as their labels.

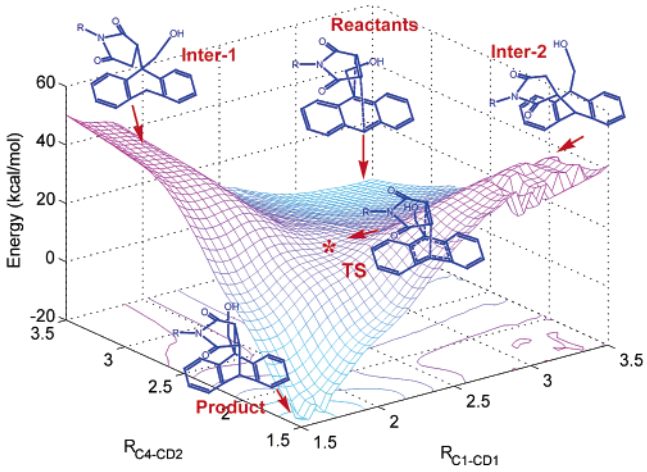

Figure 2. Three-dimensional plot of ribozyme reaction potential energy surface. The assumed intermediates (Inter-1 and Inter-2) are the structures with only one bond formation as depicted in the figure. TS is at the saddle point of the surface. Surface is offset by values of ground state energy minima.

profiles.[47] The same procedure was applied for the simulations in water but with 80 umbrella sampling windows with force constants of the umbrella of 200−500 kcal•mol^{-1}•Å^{-2}. For the two-dimensional umbrella sampling, the R_{C1-CD1} and R_{C4-CD2} were selected as reaction coordinates. Due to intensive computational resource requirement, only the region around TS (both R_{C1-CD1} and R_{C4-CD2}) ranging from 2.0 to 2.5 Å was sampled. The region was divided into $10 \times 10 = 100$ umbrella windows with 0.05 Å intervals for each reaction coordinate. In each umbrella sampling window, the system was energy minimized using Adopted Basis Newton−Raphson (ABNR) algorithm and equilibrated at 298 K for 50 ps. Data was collected at every 100 time steps during the production run for 200 ps after the equilibration. Both one- and two-dimensional PMFs were obtain *via* the Weighted Histogram Analysis Method (WHAM).[34,35]

Results and Discussion

Potential Energy Surfaces. To compare the mechanism of the [4 + 2] cycloaddition reaction in ribozyme and water, the potential energy surfaces (PES) at SCCDFTB/MM level were determined (Figure 2 and 3). The contour plot of the ribozyme PES is a typical double-well potential surface (Figure 2). The reactant energy minimum, TS, and product energy minimum locate on the synchronous region of the surface. Two energy barriers, marking stepwise reaction, juxtapose at either side of the transition state.

The transition state for the ribozyme reaction at the saddle point is characterized by R_{C1-CD1} of 2.31 Å and R_{C4-CD2} of 2.30 Å. In order to obtain more extensive TS ensemble of ribozyme reaction, the two-dimensional umbrella sampling combined with SCCDFTB/MM method was carried out in the vicinity of TS. The two-dimensional potential of mean force (PMF) was obtained by the WHAM method. About the saddle point, two-dimensional PMF is approximately symmetric along the reverse diagonal with TS at R_{C1-CD1} of 2.35 Å and R_{C4-CD2} of 2.33 Å for the ribozyme (Figure 4). The TS on PMF surface was found to be similar to that on the PES. The surface of two-dimensional PMF resembles that of PES around the TS region.

Reaction Mechanism. Along the minimum energy path on the PES from the reactant to the product, R_{C1-CD1} and R_{C4-CD2} change synchronously. The two assumed intermediates of stepwise mechanisms, shown in the PES of Figure 2, are both more than 25 kcal/mol higher than the TS of concerted [4 + 2] cycloaddition reaction. Thus, the concerted mechanism is the energy-favored reaction pathway. Judged from the potential energy surfaces, the reactions in water and ribozyme proceed *via* the same mechanism. The TS of reaction in water is at the saddle point with R_{C1-CD1} of 2.37 Å and R_{C4-CD2} of 2.36 Å, which are slightly larger than that of ribozyme reaction (R_{C1-CD1} of 2.31 Å and R_{C4-CD2} of 2.30 Å). This may be due to the active site of the ribozyme confining the reactants, which is felt at TS.

Free Energy Profiles. For the reason that full-scale two-dimensional umbrella sampling is difficult to achieve at SCCDFTB/MM level, we chose the average of R_{C1-CD1} and R_{C4-CD2} (RC) as reaction coordinate and carried out one-dimensional umbrella sampling for reactions in ribozyme and water in the synchronous region of PES. As can be seen in Figure 5, the calculated free energy barrier ($\Delta G^{\ddagger}_{calc}$) for the ribozyme reaction is 17.2 kcal/mol, which is consistent with the experimental value ($\Delta G^{\ddagger}_{exp} = 18.1$ kcal/ mol).[4] The free energy barrier in water is larger than that of the ribozyme by 5.3 kcal/mol ($\Delta\Delta G^{\ddagger}_{calc}$). Estimated by the experimental value,[4] the ribozyme reaction is about 20,000-fold more rapid ($\Delta\Delta G^{\ddagger}_{exp} = \sim 5.8$ kcal/mol less) than the uncatalyzed reaction in water. Thus, the theoretical and experimental values are in good agreement. The product in the active site of the ribozyme is more stable than that in water by 7.5 kcal/mol. An AM1/MM study of various Diels−Alder reactions in water by Chandrasekhar et al.[48] obtained a result similar to that of our simulation in water.

The reaction coordinate (RC) of the free energy barrier for the reaction in water is slightly larger than that of ribozyme (Table 1). This is consistent with the results from the PES that both R_{C1-CD1} and R_{C4-CD2} of TS determined by PES of water are slightly larger than that of ribozyme. The reaction coordinates of the product free energy minima of water and ribozyme are identical, which is equal to the carbon−carbon bond length

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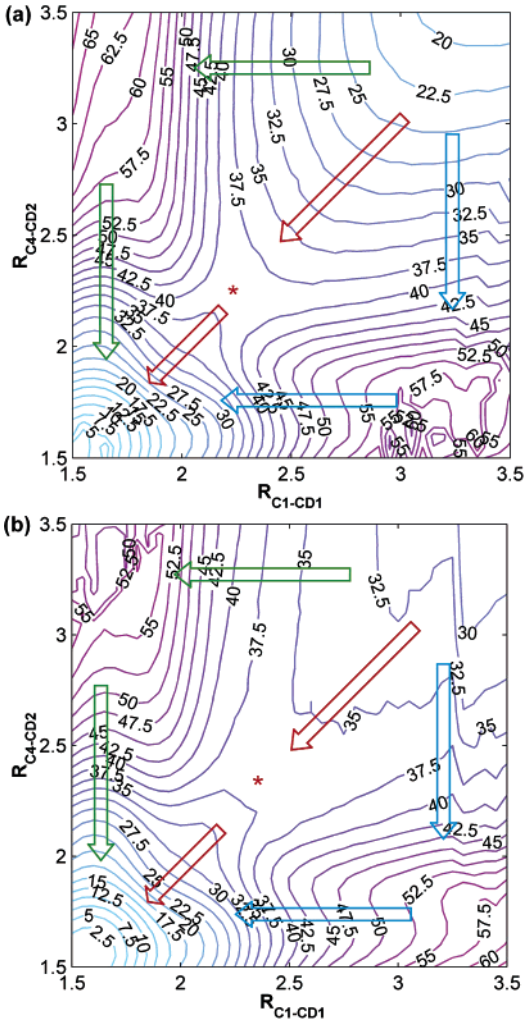

Figure 3. Contour plots of the reaction potential energy surface in the active site of Diels−Alder ribozyme (a) and water (b) at SCCDFTB/MM level. The two distances (Å), R_{C1-CD1} and R_{C4-CD2}, are chosen as reaction coordinates. Both R_{C1-CD1} and R_{C4-CD2} range from 1.5 to 3.5 Å. Surfaces are offset by values of the product energy. The TS are labeled by red asterisks. The red arrows denote the route of concerted mechanism. The green and blue arrows represent fictional routes of stepwise mechanism, respectively.

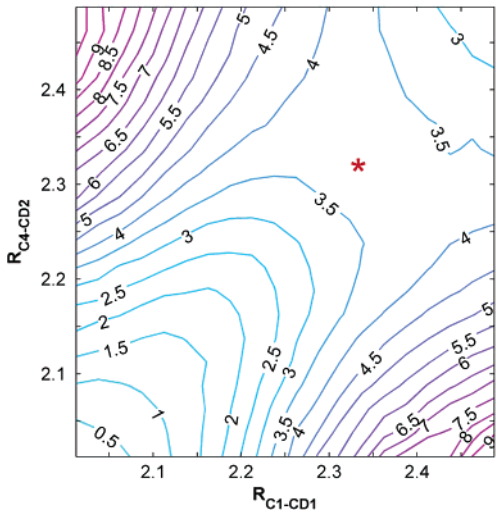

Figure 4. The two-dimensional potential of mean force (PMF) of Diels−Alder ribozyme reaction near TS. R_{C1-CD1} and R_{C4-CD2} (Å) are chosen as the reaction coordinates. Both R_{C1-CD1} and R_{C4-CD2} range from 2.0 to 2.5 Å. The surface is offset by the value of the minimum in the surface. TS is labeled by a red asterisk.

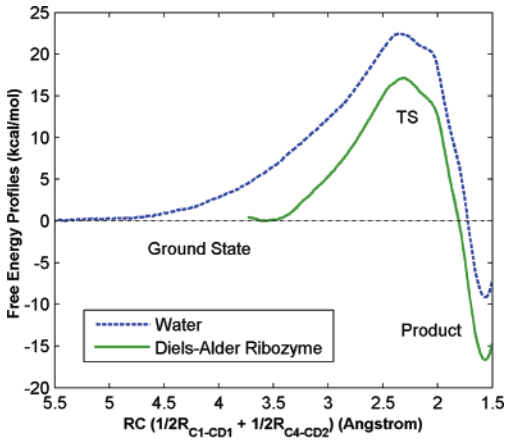

Figure 5. The one-dimensional free energy profiles of Diels−Alder reaction in water (blue dashed line) and ribozyme (green line). The average (Å) of R_{C1-CD1} and R_{C4-CD2} is chosen as the reaction coordinate (RC). Each free energy profile is offset by the values of their ground state free energy minima. The arrow points the direction of the reaction.

(1.57 Å). It seems that the locations of both TS and product are not significantly affected by their reaction environments. However, the locations of the ground state free energy minima between water and ribozyme are of remarkable difference (water: 5.46 Å; ribozyme: 3.61 Å). The functionality of the active site is to constrain the two reactants within van der Waals contacts, by which both R_{C1-CD1} and R_{C4-CD2} are limited to be within 3.7 Å. By doing so, the free energy barrier of the ribozyme reaction is lowered. Thus, the proficiency of the catalyzed reaction is achieved by stabilization of the reactive ground state conformation in the ribozyme active site.

For reactions in both ribozyme and water, the positions of TS are closer to products than to the reactants, although the free energy minima of products are much lower than the ground state free energy minima. This is not an expectation of the Hammond postulate.[49]

Mulliken Partial Charges. The Mulliken partial charges of reacting atoms, CD1, CD2, C1, and C4, have been determined for the ground state, TS, and product in both ribozyme and water reactions (Table 1). For both reactions, the negative partial charges of CD1 and CD2 atoms accumulate in going from ground state to TS while there is a decrease as TS goes to product. However, the partial charges of C1 and C4 atoms change marginally during the reaction course.

The similarities of reaction in ribozyme and water include both geometric and electronic structures in the different states. The reaction coordinates, R_{C1-CD1} and R_{C4-CD2}, change simultaneously during the course of reaction. The changes in partial charges of the reacting atoms are much the same for the reactions in both ribozyme and water. Thus, the ribozyme active site does not contribute to the stabilization of the accumulated charges of the reacting atoms in TS as compared to water. The reaction route can only follow the synchronous region of the PES.

Active Site Analysis. The active site of the ribozyme is a λ-shaped hydrophobic pocket surrounded by the A3-U45 base pair, U23·A43 noncanonical base pair, and U42·(G2-C25) base

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Table 1. Reaction Coordinate, Free Energies, and Partial Charges for the Diels−Alder Reactions in Both Ribozyme and Water

	Diels–Al der ribozyme			water		
	GS[a]	TS	product	GS	TS	product
reaction coordinate (Å)	3.61	2.31	1.57	5.46	2.35	1.57
free energy (kcal/mol)	0.0	17.2	−16.6	0.0	22.5	−9.1
Mulliken partial charge:						
C1	0.01	0.02	0.00	0.02	0.01	0.00
C4	−0.09	−0.08	−0.09	−0.08	−0.09	−0.10
CD1	−0.17	−0.19	−0.13	−0.14	−0.21	−0.14
CD2	−0.15	−0.17	−0.12	−0.16	−0.19	−0.12

[a] GS stands for ground state.

Table 2. The Geometry of Diels−Alder Ribozyme Active Site and Structures of Anthracene in Both Active Site and Water at Different States

	GS[a]	TS	product
Diels−Alder Ribozyme			
A3(C1)−U45(C1) (Å)	10.6 ± 0.3	10.7 ± 0.2	10.6 ± 0.2
U23(C1)−A43(C1) (Å)	9.5 ± 0.2	9.6 ± 0.2	9.6 ± 0.2
G2(C1)−C25(C1) (Å)	12.1 ± 0.4	12.3 ± 0.3	12.2 ± 0.3
U42(C1)−C25(C1) (Å)	9.4 ± 0.3	9.5 ± 0.3	9.3 ± 0.3
anthracene width (Å)[b]	9.2 ± 0.1	9.1 ± 0.1	8.6 ± 0.1
caret angle[b]	172°	159°	132°
Water			
anthracene width (Å)	9.3 ± 0.2	9.1 ± 0.1	8.3 ± 0.1
caret angle	179°	156°	122°

[a] GS stands for ground state. [b] Width of anthracene is measured by distance of H20 and H24, and the caret angle of anthracene is measured by the dihedral angle H20−C4−C1−H23.

triple (Figure 1). The distances between the C1′ atoms of A3-U45, U42−C25, G2-C25, and U23-A43 pairs has been average for the different states of the ribozyme reaction (Table 2). It can be seen that the average distance between the C1′ atoms of A3 and U45 changes marginally during the different reaction states, which indicates the A3-U45 base pair changes barely during the reaction. The same can also be found in the U42−C25, G2-C25, and U23-A43 base pairs. Thus, the shape of the active site changes slightly as the reaction proceeds. This is consistent with the experimental observation that active site structure of free Diels−Alder ribozyme determined by the X-ray crystallography is virtually identical to the one bound with product.[26] The active site acts merely as a reaction container to host the two reacting species. Evidence of preorganized active site indicates that dynamics of the active site is of no importance to the catalysis.

Conformations of Ribozyme in Different Reaction Stages. The structures of the ribozyme−substrates, TS, and product complexes are shown in Figure 6. The most convenient way for anthracene to enter the active site is through the back passage (Figure 6a). In the ground state of the ribozyme•reactants complex, ring III of anthracene is partially stacked between the interstice of the G2 and A3 bases. A pair of hydrogen bonds is formed instantly between the hydroxyl oxygen (O16) of anthracene with NH_2 group in the A3 base and the hydroxyl hydrogen (H28) with carbonyl group in U45 base as the anthracene enters the active site. These hydrogen bonds assist the binding of the anthracene into the active site. Nevertheless, the possibility of anthracene entering from the front passage of active site cannot be ruled out. These results are consistent with the observation by Wombacher et al.[6] After reaction, both hydrogen bonds are disrupted. Ring III of anthracene is then fully sandwiched by G2 and A3 bases. The product is stabilized by closer interactions between the aromatic rings of anthracene

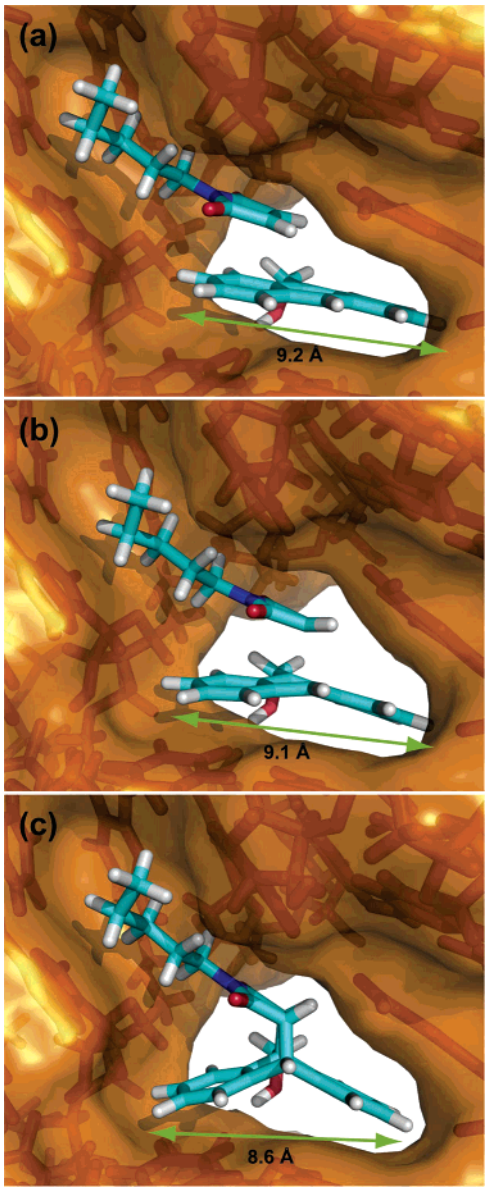

Figure 6. The structures of the ribozyme (a) −reactant, (b) −TS, and (c) −product complexes. The reacting species are represented by sticks. The ribozymes are denoted by sticks surrounded by surface. The width of anthracene is labeled by a green arrow.

and A3-U45 base pair which compensates for the loss of the hydrogen bonds. The product is too big to pass through the back passage and can be only released from the front passage.

Anthracene in Ribozyme. The conformation of anthracene undergoes significant change during the reaction. While the structure of ground state anthracene is relatively flat with a width

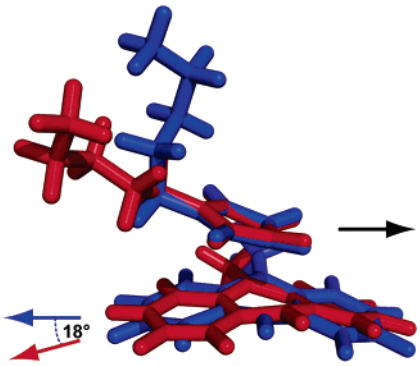

Figure 7. Overlapping of TS structures of ribozyme (red) and water (blue) reactions. The rings of maleimides from TS structures in ribozyme and water were aligned. Arrows are the directions that maleimide and anthracene approach each other. The angle between the directions of anthracenes in the two TS structures is 18°.

of anthracene (distance between H20 and H24) of 9.2 Å and the caret angle (dihedral angle H20−C4−C1−H23) of 172° (Table 2). As can be seen from Figure 6a, the space above the caret-shaped A3-U45 base pair is too narrow to bind the flat anthracene in the ground state. The anthracene has to adopt a position with its ring III (Chart 1) sticking out into the back passage. The anthracene must approach maleimide at a tilted angle as it is bound into the active site. During the reaction, ring II of anthracene bends while ring I and ring III remain flat. On formation of the product, the caret angle has changed from 172° to 132°. This leads to a decrease in anthracene width by 0.6 Å from ground state to product. In this way, the product perfectly fits in the bottom of the active site (Figure 6c). This is the result of SELEX method in that the HEG-linked Diels−Alder product[4] was used to derive the ribozyme. The degree of resemblance of TS to product provides some TS stabilization.

Comparison of Reacting Species in Ribozyme and Water. As one might expect, the ground state of the anthracene (widths and caret angles) in the active site and water are very similar. The same is true for the TS. Figure 7 shows an overlapping of the TS structures of ribozyme and water reactions. The reaction coordinate R_{C1-CD1} is roughly equal to R_{C4-CD2} in TS structures in both active site and water. The approach angle of anthracene to the maleimide is about 0° in water. However, it is about 18° for the ribozyme−TS complex. Thus, the TS of the ribozyme reaction is synchronous in distances but twisted in approach angle. The shape of anthracene in the product is less planar in water compared to that in active site. The difference is due to the stabilization of the product in the active site, in which the shape of anthracene in product is forged by the caret-shaped bottom of active site.

Maleimide. The maleimide is stacked onto the anthracene and oriented through hydrogen bonds formed by its carbonyl oxygen (OE2) with the NH2 group of G24 and the 2′-hydroxyl group of U42.[26] Its alkyl group is buried in the hydrophobic pocket of ribozyme. The conformation of its alkyl group changes only slightly at different states of reaction. Maleimide is in face to face contact with C25. Thus, the base of C25 interacting with maleimide is the major driving force for binding of maleimide in the active site.

It has been shown by Breslow and co-workers that an increase of hydrophobic effect in water facilitates the Diels−Alder reaction of the maleimide and anthracene by bringing the two reactants together.[9−13] The water molecules surrounding nonpolar anthracene and maleimide are highly structured. When the two reactants come together, face to face, the surface area in contact with water decreases and there is an increase in entropy accompanied by a decrease in free energy. The hydrophobic bringing together of reactants does not necessarily favor the formation of reactive conformers. The structure of the ribozyme active site does create reactive conformers, and the creation of such reactive conformers explains the kinetic advantage of the Diels−Alder ribozyme. Reactive conformers, conceptually named near attack conformers (NACs), are defined by the van der Waals distances of reacting atoms and correct approach angles.[50]

Conclusion

The computer simulations of Diels−Alder reactions in both the active site of a Diels−Alder ribozyme and water have been carried out by the use of SCCDFTB/ MM method combined with umbrella sampling. The potential energy surfaces support a concerted mechanism in both ribozyme and water reactions. The partial charges of reacting atoms during the reaction in both the active site and in water are much the same. The free energy profiles were determined by the one-dimensional PMF along the reaction coordinate of $1/2(R_{C1-CD1} + R_{C4-CD2})$. The calculated free energy barriers are in good agreement with the experimental values. TS of the reaction in the active site possesses a configuration with equal reacting distances but a twisted angle.

The proficiency of the ribozyme reaction is achieved by the active site bringing the reactants within van der Waals distances and in an appropriate direction. Stability is provided for the product rather than TS. The ribozyme catalysis of the Diels−Alder reaction resembles the chorismate mutase catalysis of the Claisen rearrangement of chorismate → prephenate.[43,51−55] In both ribozyme and mutase enzyme, the kinetic advantage over the reaction in water is due to the creation of a reactive conformer at the active site. TS stabilization is of less importance. In the ribozyme reaction, the active site is constructed to stabilize the ribozyme·product complex. Thus, the degree of resemblance of TS to product provides some TS stabilization. The concept that TS stabilization is the most important feature in all enzyme reactions is fading. The importance of reactive ground state conformations (NACs) may be greater for more complex systems. Thus, Wolfenden and co-workers have determined the proficiency of peptide bond formation in the ribosome is 2×10^7 fold.[56] This rate enhancement is due mainly to correct positioning of the reactants within the active site, rather than due to conventional chemical catalysis.

Abbreviations

TS, transition state; QM/MM, quantum mechanics and molecular mechanics; SCCDFTB, self-consistent-charge density-

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functional tight-binding; MD, molecular dynamics; WHAM, weighted histogram analysis method; PES, potential energy surface; PMF, potential of mean force.

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