The ONIOM molecular dynamics method for biochemical applications: cytidine deaminase.

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Abstract

We developed and implemented the ONIOM-molecular dynamics (MD) method for biochemical applications. The implementation allows the characterization of the functions of the real enzymes taking account of their thermal motion. In this method, the direct MD is performed by calculating the ONIOM energy and gradients of the system on the fly. We describe the first application of this ONOM-MD method to cytidine deaminase. The environmental effects on the substrate in the active site are examined. The ONIOM-MD simulations show that the product uridine is strongly perturbed by the thermal motion of the environment and dissociates easily from the active site.

1. Introduction

The ONIOM hybrid method, which combines a quantum mechanical (QM) method with the molecular mechanical (MM) method, is one of the powerful methods that allow to calculate large molecular systems [1-6] with the accuracy afforded for smaller molecular systems. The notable feature of this method is to include the environmental effects into the high level QM calculation through a simple extrapolation procedure [5]. Since the definition of the layer is simple and the layer is easily extended to the multiple-layers, the ONIOM method is more flexible and versatile than the traditional QM/MM method. The reliability of the ONIOM method has been well established by the Morokuma group and is increasingly adopted as an efficient approach beneficial to many areas of chemistry [7]. For example, Kerdcharoen et al. developed the ONIOM-XS method for the purpose of simulating ions in solution [8]. This is the only molecular dynamics method based on the ONIOM method that has been reported so far. Other groups have applied the ONIOM method to biological systems, because they represent excellent targets for this type of methods. However, the ONIOM energy/gradient/optimization method is not satisfactory in itself when the target is a biomolecule, such as an enzyme in which the property of the entire system is strongly affected by its dynamical behavior. The coupling of the ONIOM method with the molecular dynamics (MD) method is necessary in this case to account for the thermal fluctuations of the environment. In the present study, we aimed to characterize the function of an enzyme in a realistic simulation of the thermal motion while retaining the concept embodied in the ONIOM method. Thus, we coupled the ONIOM method with the MD method in an ONION-MD implementation in the HONDO program [9]. As a first application of the ONIOM-MD method, we studied the cytidine deaminase that combines with the substrate

uridine (product).

Cytidine deaminase is known to play an important role on the activation of the anticancer drug capesitabine in the human body. Some theoretical studies, including ours, have indicated the importance of the amino acid residue in the active site as a mediator of the reaction [10-13]. However, the effect of other amino acid residues inside the pocket of the active site has not been clearly investigated and established yet. This is the driving force for this work. To this end, we examined the environmental effects of the pocket of the active site on the substrate, using the ab intio ONIOM-MD method to take into account the thermal motion of the environment.

2. Methods

The integration of the ab initio ONIOM (QM+MM) method with the molecular dynamics (MD) method was performed by combining the HONDO-2001 program [9] with the TINKER Ver. 4.2 program [14-19]. In the present study, the 2-layered ONIOM methodology was used, where the entire system is divided into two layers, i.e., the core (active) part and the outer part. The integrated ONIOM energy and gradient of the entire system is expressed as follows:

$$E_{ONIOM,Real}(\boldsymbol{R}_{core},\boldsymbol{R}_{outer}) = E_{QM,Model}(\boldsymbol{R}_{core},\boldsymbol{R}_{link}) + E_{MM,Real}(\boldsymbol{R}_{core},\boldsymbol{R}_{outer}) - E_{MM,Model}(\boldsymbol{R}_{core},\boldsymbol{R}_{link}) \quad (1)$$

$$\partial E_{ONIOM,Real}/\partial R_{Real} = (\partial E_{QM,Model}/\partial R_{Model})*\boldsymbol{J}(\boldsymbol{R}_{link};\boldsymbol{R}_{core},\boldsymbol{R}_{outer}) + \partial E_{MM,Real}/\partial R_{Real} - (\partial E_{MM,Model}/\partial R_{Model})*\boldsymbol{J}(\boldsymbol{R}_{link};\boldsymbol{R}_{core},\boldsymbol{R}_{outer}) \quad (2)$$

Here, $\boldsymbol{J}$, the Jacobian matrix, projects the forces on the link atoms ($\boldsymbol{R}_{link}$) onto the atoms that belong to the core ($\boldsymbol{R}_{core}$) and outer ($\boldsymbol{R}_{outer}$) parts. The time evolution of the atomic nuclei is

performed according to the conventional MD procedure. The Newton's equations of motion is solved by calculating the ONIOM gradients on the fly:

$$F(GradE_{ONIOM,Real}) = ma \quad (3)$$

The flowchart of the ONIOM-MD calculation in the HONDO program combined with the TINKER program is presented in Figure 1.

---------- Figure 1----------

The MD simulations were performed using the Beeman algorithm [20] with a time step of 1 fs. The QM calculations were performed at the Hartree-Fock level of theory with the basis set BSI, which consists of a double-ζ (14s,8p,5d)/[5s,2p,2d] set for the Zn atom [21] and 3-21G for the others. For the MM calculation, the AMBER99 [22-26] and TIP3P [27] force field parameters were used for the enzyme and water solvent, respectively. The simulations were run under the constant temperature (298.15 K) through the use of Berendsen's velocity scaling algorithm [28].

We used the crystal structure of cytidine deaminase that binds with the substrate uridine (product) as an initial geometry, which is registered in the protein data bank (PDB) with the ID code 1AF2 (Figure 2) [29]. To mimic the water solvent, about 2500 water molecules were randomly placed around the cytidine deaminase. The periodic or spherical boundary conditions were not used. We confirmed that the entire system is stable in energy and there is no significant conformational change during the simulation and that the evaporation of the solvent water molecules does not occur. The substituent of Glu104 that mediates the reaction and the active site were cut out and the dangling bonds were capped with the H atoms to form the model system, CH_3COO^- + (uracil)$Zn(SH)_2(H_2O)$. For the substrate, the uridine is replaced by uracil without the ribose ring in the model system. To

mimic the lone electron-pair of the histidine that coordinates to the Zn atom we used a H_2O molecule. The model system was treated at the QM level of theory and the other part of the enzyme along with the solvent water molecules were treated at the MM level of theory. One water molecule from the solvent was included in the QM part, because the water molecule was located in the vicinity of the glutamic acid in the active site in the original structure registered in PDB. In the ONIOM-MD simulations, the motion of the $Zn(S\text{-})_2(H_2O)$ of the QM part was fixed. The QM-MD simulations were also performed for the model systems, CH_3COO^- + H_2O + (uracil)$Zn(SH)_2(H_2O)$ (model 1) and (uracil)$Zn(SH)_2(H_2O)$ (model 2), similarly fixing the motion of the Zn and S atoms and the H_2O coordinated to the Zn atom.

The average and standard deviation of the geometric parameters and the MM gradients of the substrate in the MD simulations were calculated from the data collected every 10 fs. For the dihedral angle, the absolute value was used (ignoring its sign) in order to assess the deviation of the selected atom from the plane of the 6-membered ring of the substrate uridine. When the absolute value $|\theta|$ was larger than 90°, the value was converted as follows: $\theta' = 180 - |\theta|$.

---------- Figure 2----------

3. Results and discussion

The crystal structure of cytidine deaminase with the substrate uridine (product) was optimized in the water solvent at the ONIOM(HF/BSI:AMBER99+TIP3P) level of theory before performing the ONIOM-MD simulation. In the optimized structure, the uridine binds with the Zn atom of the active site with the Zn1-O5 distance of 1.985 Å (see Figure 2 for the structure). The O5 atom is pulled into the Zn1 atom since the entire uridine is fixed by some

H-bonds with the neighboring amino acid residues. Therefore, the O5 atom largely deviates from the plane of the 6-membered ring with the dihedral angle ∠O5-C6-C8-C9 of 26.7°, which indicates that the potential energy of the uridine is increased by the environmental effects. The ONIOM-MD simulations were started from the optimized geometry.

The fluctuation of the energy of the QM part is presented in Figure 3(a). The potential energy of the QM subsystem is seen to display a sudden increase in magnitude in the initial stage of the simulation, due to the dissociation of the Zn1-O5 bond. The thermal motion of the amino acid residues easily breaks the Zn1-O5 bond even at room temperature. To examine the environmental effects on the substrate, the QM-MD simulation for the model 1 without the amino acid residues, which corresponds to the QM part of the real system, were performed. The substrate (product) never dissociated from the Zn atom during the simulation without the neighboring amino acid residues. As shown in Figure 3(b), the substrate is much more stabilized in energy. The fluctuation of the potential energy is also smaller. This suggests that the substrate is strongly perturbed by the neighboring amino acid residues. Even if plot (b) is shifted up by 38.3 kcal/mol (plot (c)) assuming the dissociation of the Zn1-O5 bond, it is still much lower in energy than plot (a). Thus, the substrate uridine is destabilized in energy in the cleft of the active site by the environmental effects of the amino acid residues. The thermal motion of the neighboring amino acid residues deforms the geometry of the uridine and increases its potential energy.

---------- Figure 3 ----------

We note in particular that the dihedral angles of the 6-membered ring of the uridine molecule are significantly affected by the neighboring amino acid residues, as presented in Table 1. The left-hand side of the 6-membered ring of the uridine, which is deeply inserted

into the cleft, is strongly perturbed as shown by the large dihedral angles, ∠N10-C11-N7-C6, ∠C11-N7-C6-C8, and ∠N7-C6-C8-C9. This suggests that His102, Tyr126, and Phe71, which sandwich the uridine molecule (see Figure 2), affect its dihedral angles through steric contact due to the thermal motion. In fact, these large values in the dihedral angles are reduced in the model 1 without the neighboring amino acid residues. The dihedral angles for the model 1 are quite similar to those for the model 2, indicating that the interactions of the substrate with the glutamic acid and one water molecule does not affect the dihedral angles of the substrate.

---------- Table 1 ----------

The forces acting on the 6-membered ring of the substrate uridine were examined on the basis of the MM gradients. The MM gradients calculated according to the eq. (4) correspond to the forces from the environment.

$$GradE_{MM} = GradE_{MM,Real} - GradE_{MM,Model} \qquad (4)$$

As presented in Table 2, the MM gradients are clearly large in the -N10(R)-C11(=O12)- part. His102 and Tyr126, which sandwich this part (see Figure 2), are likely responsible for the large forces to this part.

---------- Table 2 ----------

Three H-bonds between the substrate uridine and the amino acid residues, Ala103, Asn89, and Glu91, presented in Figure 2 were maintained during the ONIOM-MD simulation. These H-bonds prevent the uridine distorted by the steric contact with His102, Tyr126, and Phe71 from relaxing, which would increase the potential energy of uridine. It is therefore reasonable that the distortion is large in the left-hand side of the uridine that is deeply inserted into the cleft and has the H-bonds with the amino acid residues.

It was found that Glu104 extracts the H13 atom and shuttles between the N7 and O5

atoms after the Zn1-O5 bond dissociation as shown in Figure 4. This dynamical behavior of Glu104 supports the experimental proposal that Glu104 carries the hydrogen to complete the deamination reaction [30]. The H_2O molecule placed in the vicinity of Glu104 connects the carbonyl oxygen of the main chain of cytidine deaminase with one of the oxygens of Glu104 or the O5 atom of the uridine without going out from the cleft of the active site.

---------- Figure 4 ----------

4. Conclusion

We integrated the ONIOM method with the molecular dynamics (MD) method and implemented it into the HONDO program for the purpose of characterizing functions of realistic models of enzymes. In this first application of the ONIOM-MD method to cytidine deaminase, we examined the environmental effects of the pocket of the active site on the substrate uridine (product). The ab inito ONIOM-MD simulations showed that the uridine trapped in the pocket of the active site is fixed by H-bonds with some amino acid residues and is strongly perturbed by other amino acid residues, which sandwich the uridine, through the steric contact due to the thermal motion. As a result, the uridine easily dissociates from the Zn atom of the active site.

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Figure captions

Fig. 1. Flowchart of the ONIOM-MD calculation in the HONDO program combined with the TINKER program.

Fig. 2. Structures and illustration of the real system of cytidine deaminase combined with the substrate uridine (product) and its active site.

Fig. 3. Fluctuations of the QM energies of the active site of cytidine deaminase with the substrate uridine in the MD simulations at 298.15 K. (a) ONIOM-MD simulation for the real system. (b) QM-MD simulation for the model 1 (see section 2 for the model 1). (c) This plot corresponds to plot (b) shifted up by 38.3 kcal/mol (see the text for the details). All the energies are relative to that of the initial geometry of (a).

Fig. 4. Fluctuations of the distances of the H-bonds between the substrate uridine and Glu104 of cytidine deaminase in the ONIOM-MD simulation at 298.15 K.

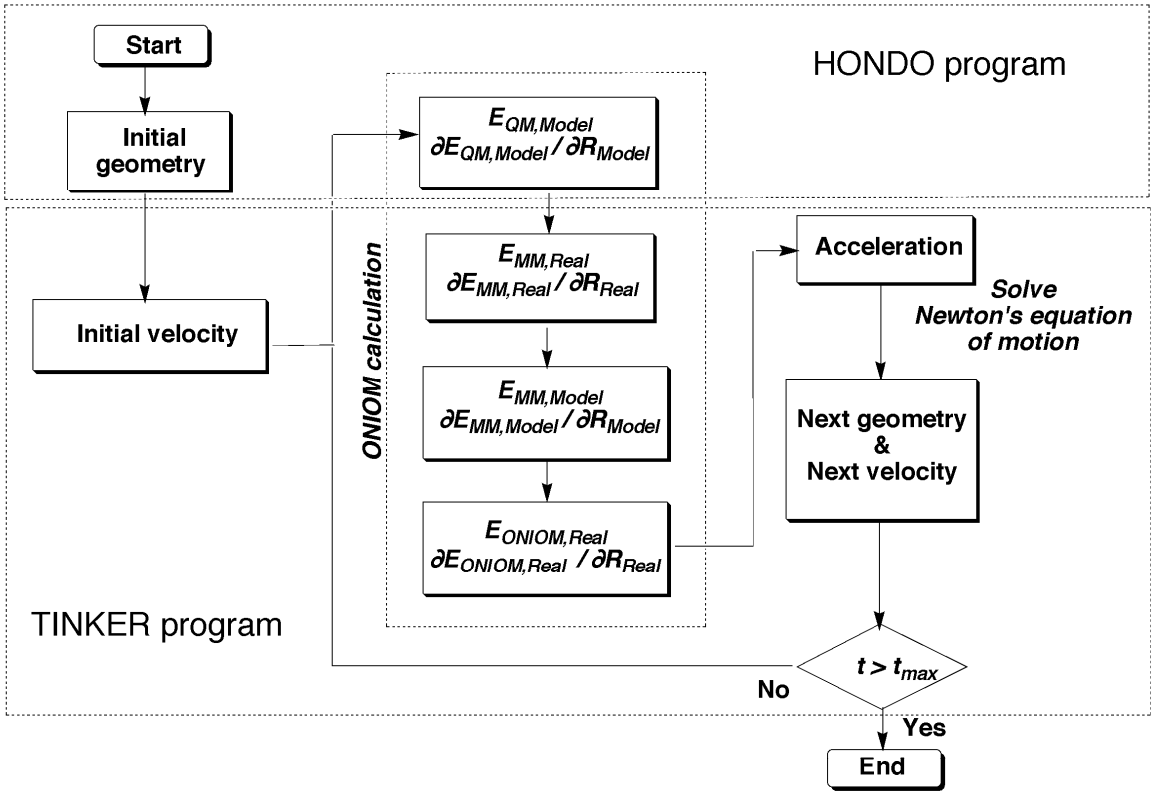

Figure 1. T. Matsubara

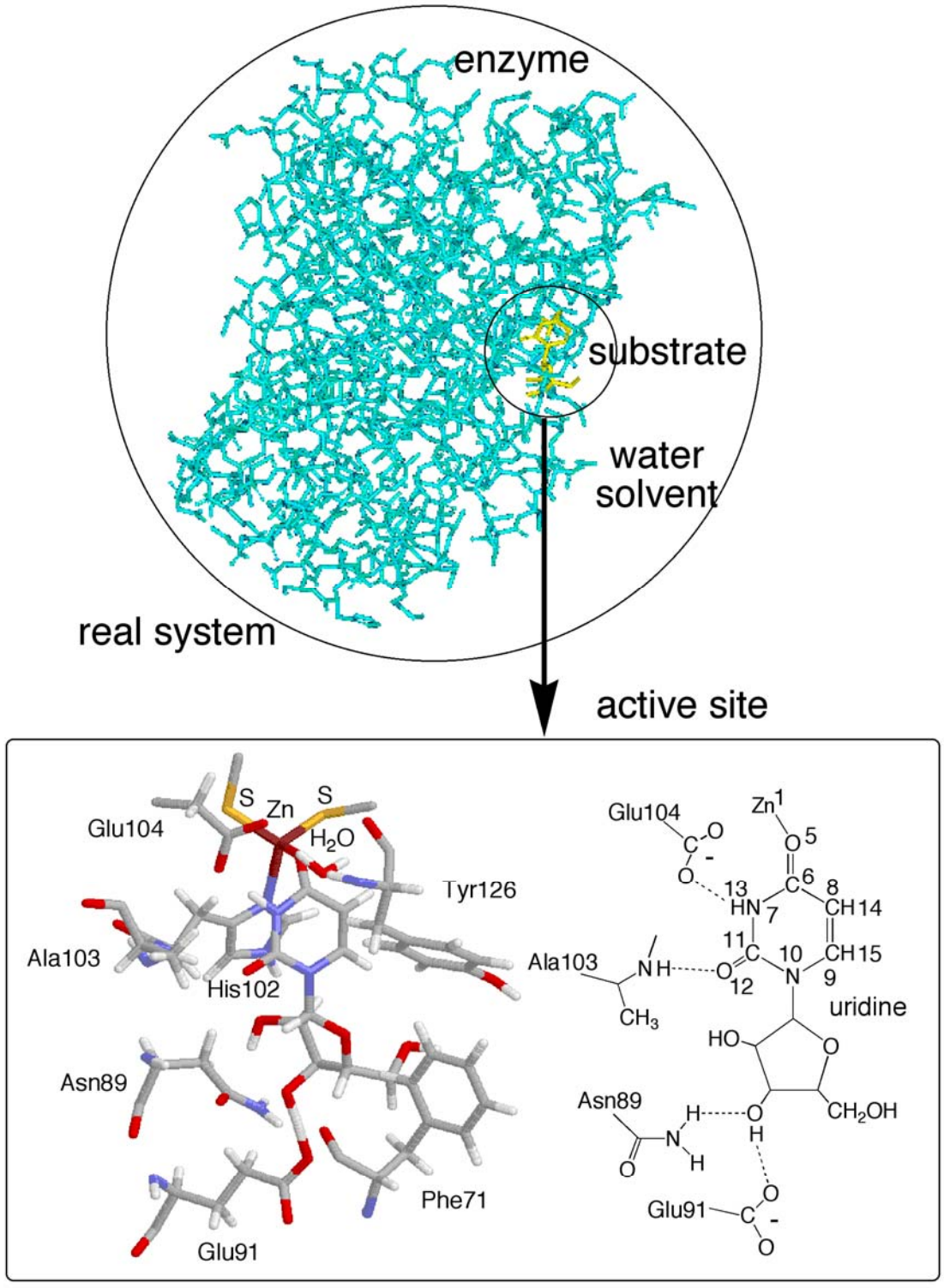

Figure 2. T. Matsubara

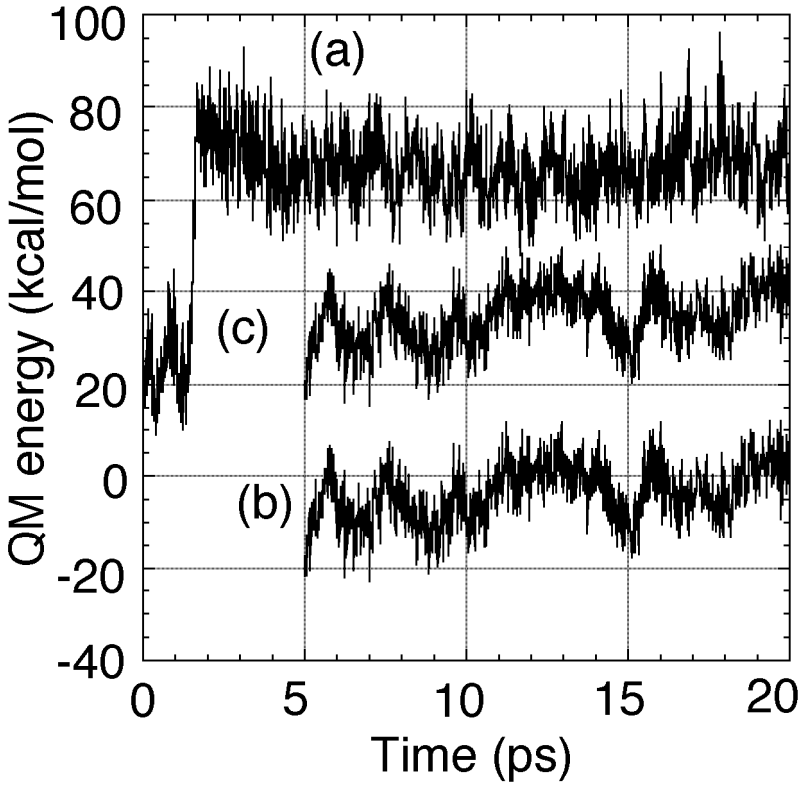

Figure 3. T. Matsubara

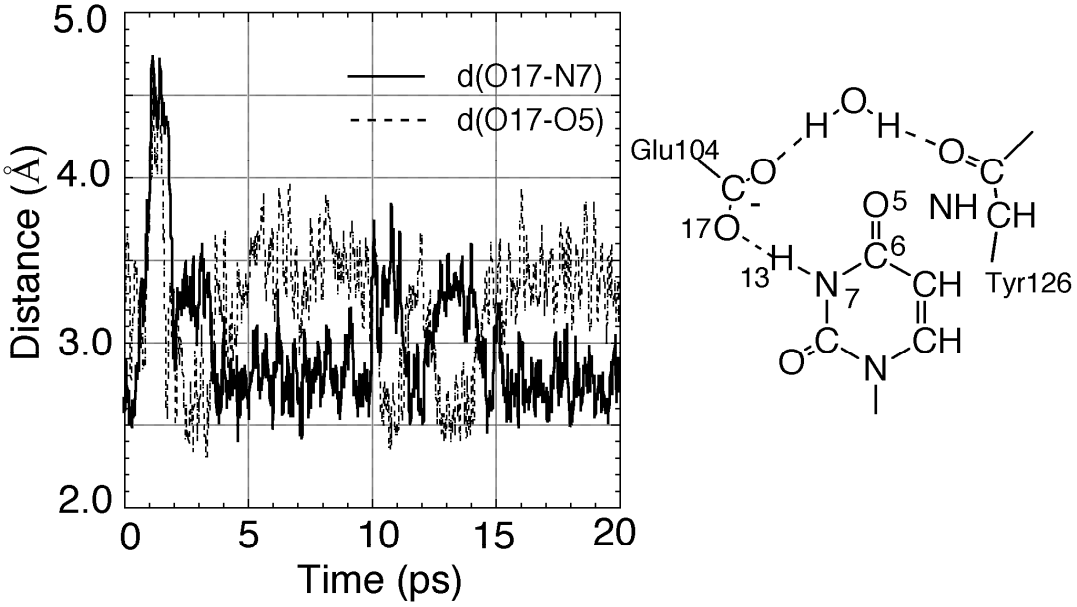

Figure 4. T. Matsubara

Table 1

Average dihedral angles (in degree) of the substrate uridine trapped in the active site of cytidine deaminase and in its model active sites, models 1 and 2, in the ONIOM- and QM-MD simulations at 298.15 K. [a]

dihedral angle[b]	real system	model 1	model 2
∠O5-C6-C8-C9	12.4	7.3	6.8
∠C6-C8-C9-N10	7.3	5.6	5.5
∠N7-C6-C8-C9	11.4	6.9	6.1
∠C8-C9-N10-C11	9.8	5.9	6.3
∠C9-N10-C11-N7	7.0	7.0	7.3
∠N10-C11-N7-C6	18.4	7.2	7.1
∠C11-N7-C6-C8	21.8	7.3	6.7
∠O12-C11-N10-C9	7.8	7.1	7.5

[a]These values are calculated using the dihedral angles from 5 to 20 ps. See section 2 for the models 1 and 2. The QM-MD simulations were performed for the models 1 and 2. [b]See Figure 2 for the numbers attached to the atoms.

Table 2

Average and standard deviation of the MM gradients (in kcal/mol·Å) of the selected atoms of the substrate uridine trapped in the active site of cytidine deaminase in the ONIOM-MD simulation at 298.15 K.[a]

atom[b]	MM gradient	
	average	standard deviation
O5	10.5	2.6
C6	12.1	1.6
N7	8.3	1.4
C8	8.2	2.2
C9	6.4	3.0
N10	24.5	13.4
C11	11.0	1.4
O12	17.1	4.9

[a]The MM gradients are calculated according to the eq. (4). The average and standard deviation are calculated using the data from 5 to 20 ps. [b]See Figure 2 for the numbers attached to the atoms.