Quantum chemical study of conformational preferences of intermediates and transition states in the alkaline hydrolysis of dimethyl phosphate

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Abstract. The phosphate diester is a basic structure in DNA and RNA. The mechanism of phosphate diester hydrolysis is important for understanding the decomposition reactions of nucleic acids. In this study, we have explored the reaction pathway of alkaline hydrolysis of dimethyl phosphate, which is the simplest phosphate diester, with a hydroxide ion. Since the conformations of the intermediates and transition states reportedly influence the reaction mechanism of transacylation of methyl acetate with methoxide, we considered the conformational preferences on the alkaline hydrolysis of dimethyl phosphate, by using the most stable conformer as a reactant. Upon the reaction with hydroxide, a concerted reaction pathway was obtained in the gas phase, whereas a stepwise reaction pathway was obtained in water. As compared to the earlier study, our computation shows more stable conformations in the hydrolysis reactions than the previous study.

1. Introduction

DNA and RNA play essential roles in life, such as the storage and decoding of genomic information [1]. In this series of processes, called the central dogma, the decomposition reactions of DNA and RNA; namely, the hydrolysis of the phosphate ester bonds in DNA and RNA strands, is important. For example, the production and decomposition of RNA primers in DNA replication and the modification of mRNA in transcription are accomplished by enzymes that catalyze the decomposition of nucleic acids [1, 2]. Besides the central dogma, some restriction enzymes in bacteria distinguish and decompose foreign DNA, and prevent their host from being infected with a virus. In addition, the hydrolysis of a phosphate ester bond is involved in the removal of damaged DNA strands. Decomposition reactions of DNA and RNA are essential to maintain life. Therefore, it is important to fully understand the mechanism of this reaction in biology.

Recently, the hydrolysis reaction of phosphate diester has been investigated, as a model of the decomposition reactions of DNA and RNA. In addition, the hydrolysis of the phosphate diester bond is also involved in the energy utilization from ATP and the mechanism of intracellular signal transfer by GTP [2]. However, the mechanism is still being debated. Iche-Tarrat [3] and Ribeiro [4] calculated the hydrolysis reaction pathway of dimethyl phosphate with a hydroxide ion as a nucleophile, by using B3LYP [5–8] with a double-ξ plus polarization valence basis set [9] and B3LYP/6-311++G(2d,2p), respectively, and showed that the reaction is concerted. However, Imhof [10] explored it by using the B3LYP/6-31+G(d,p) method, and showed that the reaction is stepwise. These results suggest that the computed hydrolysis reaction pathway depends on the basis set. Dejaegere [11] calculated the
hydrolysis reaction pathway under the same conditions as Iche-Tarrat, Ribeiro, and Imhof, by using three types of basis sets, HF/STO-3G, HF/3-21G(d), and HF/3-21+G(d). In all three calculations, one intermediate was observed in the pathway. However, the greater the accuracy of the basis set, the higher the energy of that intermediate.

In our previous study [12], we performed a conformational analysis for the transition states and the tetrahedral intermediates of the transacylation of methyl acetate, and compared the conformations involved in the transacylation to those of the phosphate inhibitors found in lipases and esterases. We concluded that the three-dimensional shapes of the active sites of lipases and esterases accommodate the conformational preferences of the tetrahedral intermediates and inhibitors.

In the present study, we have explored the alkaline hydrolysis pathway of phosphate diester at the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) level of theory, considering the conformational preferences of the stationary points. We used dimethyl phosphate, the simplest phosphate diester, as the reactant and examined the hydrolysis pathway with a hydroxide ion in vacuum and water.

2. Computational procedure

2.1. QM calculation

All calculations were done using the Gaussian09 program packages [13]. We optimized all possible conformations of dimethyl phosphate, and investigated the most stable conformation. Then, we explored the hydrolysis pathway of dimethyl phosphate in the most stable conformation with a hydroxide ion in the gas phase and the aqueous environment at the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) level of theory, because it is time-consuming to perform geometry optimizations, frequency calculations, and IRC calculations at the coupled cluster (CC) level [14–16]. In addition, Gaussian09 does not implement the geometry optimization in the implicit water model such as the polarizable continuum model (PCM) [17, 18] at the CC level of theory, and it is known that geometrical parameters converged very quickly with improving theoretical methods. However, since it is shown that B3LYP is not adequate functional to study the energetics of phosphodiester systems [4], single point energy calculations were performed by using the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) method [5–8].

All stationary points were characterized by frequency calculations. The reaction pathway was determined by following the IRC procedure [19–21]. Zero-point energies and thermal corrections at 298K were also included, to obtain the Gibbs free energies. Aqueous solvation energies were computed with the IEF-PCM [14, 15], and we also included the non-electrostatic term to estimate the aqueous solvation free energies. To remove the excessive estimation of entropies attendant upon the formation of complexes, we did not include the translational and rotational entropies in the thermal corrections, in the hydrolysis pathway in water [4], because the complex water–dimethylphosphate should always exist in solution and the nucleophile that attacks dimethylphosphate will substitute that water molecule.

2.2. Definition of conformations

Dimethyl phosphate has two methoxy groups, and they adopt various conformations depending on the orientation of these methoxy groups. The definitions of these conformations are shown in figure 1 [12]. The conformations of dimethyl phosphate are decided by the two dihedral angles of the methoxy groups, O1-C1 and O2-C2, with respect to the P-C2 and P-C1 bonds, respectively. We defined three notations, gauche+ (G+), gauche− (G−), and anti (A), to represent dihedral angles of 0˚ to 120˚, −120˚ to 0˚, and 120˚ to 180˚ or −180˚ to −120˚, respectively.

For an unsymmetrical case, there are nine conformers at the P atom of a phosphate, while the nine possible conformers can be reduced for a symmetrical case. Dimethyl phosphate has four distinct conformers (AA, AG−, G+G−, and G−G−), because the AG− conformer is the same as the G+A, AG+, and G−A ones, the G+G− conformation is the same as the G−G+ one, and the G−G− and G+G+ conformers have the same structures, respectively.
3. Results and discussion

3.1. Comparison of possible conformations of dimethyl phosphate

We optimized all four conformers of dimethyl phosphate, and calculated the Gibbs free energies. The bond lengths and dihedral angles of the optimized structures are shown in table 1. The relative Gibbs free energies of the optimized structures are shown in table 2.

3.1.1. Comparison of theoretical methods

We first assessed the validity of the theoretical methods with regard to the optimized geometrical parameters and the relative Gibbs free energies of the conformers of dimethyl phosphate. Table 1 indicates that the bond lengths computed by the B3LYP method are agreement with those by the MP2 method within 0.01 Å, and that the dihedral angles are close to each other within 15°, implying that geometry optimization of dimethyl phosphate does not depend on the theoretical methods. As shown in table 2, the B3LYP method provides smaller relative Gibbs free energies than the CCSD and CCSD(T) methods by 0.3–0.7 kcal mol\(^{-1}\) for the AA conformers and by about 0.3 kcal mol\(^{-1}\) for the AG– conformers, while the MP2 method shows the equivalent relative free energies to the CCSD and CCSD(T) methods with the error within 0.1 kcal mol\(^{-1}\). The difference in the relative Gibbs free energies between the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) and CCSD/6-311++G(2d,2p)//MP2/6-311++G(2d,2p) were evaluated to be within 0.1 kcal mol\(^{-1}\) in the gas phase and at most 0.25 kcal mol\(^{-1}\) in water, implying that the theoretical methods for the geometry optimization are insensitive to the relative Gibbs free energies. These findings indicate that our procedure (CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p)) is appropriate for the exploration of the alkaline hydrolysis pathway of phosphate diester. Hereafter, we use the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) method for the investigation.

3.1.2. Conformational preference of dimethyl phosphate

As shown in table 1, after optimization, conformational changes were observed in both vacuum (G+G– to AG–) and water (G+G– to AG–). The AA, AG–, and G–G– conformers were found as the energy minima in both the gas phase and water. The two close methoxy groups of the G+G–

![Figure 1. Definitions of the conformations of dimethyl phosphate (A), and the geometry of each conformation (B).](image-url)
conformer show a large steric hindrance, resulting in the conformational change to AG–. Table 2 shows that the G–G– conformer is the most stable conformation, in both vacuum and water. Since the two methoxy groups of the G–G– conformer point in opposite directions, the steric hindrance in the G–G– conformation is smaller than that in the others. The AG– and AA conformers are less stable than the G–G– conformer by about 1.2 and 2.2 kcal mol–1.

**Table 1.** Bond lengths (P–O1, P–O3, and P–O4)\(^a\) and dihedral angles (C2–O4–P–O3 and C1–O3–P–O4)\(^b\) of the optimized structures of dimethyl phosphate in the gas phase and water at the B3LYP/6-311++G(2d,2p) level of theory\(^c\)

| Conformer | Initial | Optimized | \(\Delta G\) |
|-----------|---------|-----------|-------------|
| P–O1 | P–O3 | P–O4 | C2–O4–P–O3 | C1–O3–P–O4 |
| **gas phase** | | | | |
| AA | 1.495 (1.499) | 1.658 (1.654) | 1.658 (1.654) | +153.7 (+166.5) | +153.7 (+166.5) |
| AG– | 1.497 (1.648) | 1.673 (1.671) | +171.0 (-73.23) |
| G+G– | 1.497 (1.648) | 1.673 (1.671) | +171.0 (-73.23) |
| G–G– | 1.489 (1.665) | 1.665 (1.664) | -73.26 (-73.26) |
| **water** | | | | |
| AA | 1.502 (1.507) | 1.636 (1.629) | 1.636 (1.629) | -180.0 (-180.0) |
| AG– | 1.500 (1.629) | 1.644 (1.636) | -172.9 (-71.36) |
| G+G– | 1.500 (1.629) | 1.644 (1.636) | -172.9 (-71.78) |
| G–G– | 1.497 (1.632) | 1.639 (1.632) | -67.04 (-67.04) |

\(^a\) Bond lengths are shown in angstroms.  
\(^b\) Dihedral angles are shown in degrees.  
\(^c\) The geometrical parameters computed at the MP2/6-311++G(2d,2p) level of theory are shown in parentheses.

**Table 2.** Relative Gibbs free energies of the optimized structures in the gas phase and water\(^e\)

| Conformer | \(\Delta G\) |
|-----------|-------------|
| B3LYP/6-311++G(2d,2p) | CCSD(T) | CCSD | B3LYP | MP2 |
| **Gas phase** | AA | 2.276 | 2.207 | 1.557 | 2.395 | 2.288 | 2.364 |
| AG– | 1.271 | 1.224 | 0.932 | 1.203 | 1.203 | 1.277 |
| G–G– | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Water** | AA | -b | 2.505 | 2.187 | -b | 2.251 | 2.252 |
| AG– | -b | 1.419 | 1.105 | -b | 1.278 | 1.195 |
| G–G– | -b | 0.000 | 0.000 | -b | 0.000 | 0.000 |

\(^a\) Free energies are with respect to the G–G– conformer, and shown in kcal mol–1.  
\(^b\) Relative Gibbs free energies are not shown, because Gaussian09 does not implement the CCSD(T) in the PCM.

### 3.2. Hydrolysis of phosphate diester

Since the G–G– conformer is the most stable conformation of dimethyl phosphate in both vacuum and water, we explored the hydrolysis reaction pathway of the G–G– conformer of dimethyl phosphate with hydroxide ion.

#### 3.2.1. Gas phase

Figure 2 shows the lowest energy pathway for the gas-phase reaction at the B3LYP/6-311++G(2d,2p) level of theory, and the bond lengths (cleaved bond: P–O3 and formed bond: P–O5) and the dihedral...
angles (C2-O4-P-O3 and C1-O3-P-O4) are listed in table 3. As illustrated in figure 2, the reaction proceeds in a concerted manner. The hydrogen atom of the hydroxide ion approaches the phosphorus atom of the G–G– dimethyl phosphate, forming a transition state (TS) with an activation barrier of 99.1 kcal mol⁻¹. In this process, the O4C2 methoxy group slightly rotates to the leaving group, resulting in the G–G– transition state. The transition state decomposes to the isolated products, methoxide and methyl hydrogen phosphate. The overall reaction is exothermic, \( \Delta G = -7.55 \) kcal mol⁻¹.

![Figure 2. Hydrolysis reaction pathway of dimethyl phosphate with a hydroxide ion in the gas phase and the optimized structures of stationary points (reactants, transition states, and products) at the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) level of theory. Gibbs free energies of the stationary points are plotted, relative to the reactants. The relative Gibbs free energies are given in units of kcal mol⁻¹.]

**Table 3.** The lengths of the cleaved (P-O3) and formed bonds (P-O5)a, and the dihedral angles for the definition of the conformation (C2-O4-P-O3 and C1-O3-P-O4)b.

| Structure                  | P-O3 | P-O5 | C2-O4-P-O3 | C1-O3-P-O4 |
|----------------------------|------|------|------------|------------|
| Dimethyl phosphate (Reactant) | 1.63 | –    | –73.26     | –73.26     |
| TS                         | 1.87 | 2.18 | –109.2     | –67.95     |
| Methyl hydrogen phosphate (Product) | –    | 1.65 | –          | –          |

a Bond lengths are shown in Å.
b Dihedral angles are shown in degrees.

However, when the CCSD/6-31+G(d)//B3LYP/6-31+G(d) method was used for the investigation of the gas-phase hydrolysis pathway, we found a stepwise reaction pathway with one intermediate, as shown in figure 3. Since the energy differences between TS1, TS2 and INT in these pathways are 1.9 kcal mol⁻¹ at most, we concluded that the greater the accuracy of the basis set, the higher the energy of the INT. This is consistent with the previous studies [10, 11].
Figure 3. Hydrolysis reaction pathway of dimethyl phosphate with a hydroxide ion in the gas phase and the optimized structures of stationary points (reactants, transition states, and products) at the CCSD/6-311++G(2d,2p)//B3LYP/6-31+G(d) level of theory. Gibbs free energies of the stationary points are plotted, relative to the reactants. The relative Gibbs free energies are given in units of kcal mol$^{-1}$.

3.2.2. Aqueous phase

The reaction pathway and the geometrical parameters (the bond lengths of P-O3 and P-O5 and the dihedral angles of C2-O4-P-O3 and C1-O3-P-O4) in water are shown in figure 4 and table 4, respectively. As illustrated in figure 4, stepwise hydrolysis proceeds in water at the CCSD/6-311++G(2d,2p)//B3LYP/6-31++G(2d,2p) level of theory. The B3LYP/6-31+G(d) computation also provides the stepwise reaction pathway of the hydrolysis of dimethyl phosphate in water. In this pathway, the hydroxide ion attacks the phosphorus atom of dimethyl phosphate, resulting in the formation of an intermediate (INT) via a transition state (TS1). Then, the dissociation of the methoxide occurs to form the isolated products, methoxide and methyl hydrogen phosphate, through another transition state (TS2). The aqueous environment favors the stepwise pathway and remarkably reduces the activation barriers, by about 74 kcal mol$^{-1}$, due to the reduction in the electrostatic repulsion between the hydroxide and dimethyl phosphate.

The difference of the dipole moments between the transition states (TS1 and TS2) and the intermediate (INT) is considered to be responsible for the stepwise mechanism in water. In general, a molecule with a larger dipole moment becomes more stable in water. In this reaction, the large dipole moment of INT contributes to the stepwise reaction pathway, because INT has the largest dipole moment (TS1: 8.23 D, INT: 10.8 D, and TS2: 8.55 D).

3.3. Comparison with the previous study

Ribeiro and colleagues [4] tested many DFT exchange–correlation functionals by considering the hydrolysis reaction of dimethyl phosphate. They explored the hydrolysis reaction pathway of dimethyl phosphate by the B3LYP/6-311++G(2d,2p) calculations, and then calculated the energy of each structure in the pathway by using various DFT exchange–correlation functionals. We compared the reaction pathway in this study to that in the Ribeiro's study.

In the Ribeiro's study, the reactant has the G–G– geometry. In vacuum, the computed pathway in the Ribeiro's study is also a concerted one, with an activation energy of 98.4 kcal mol$^{-1}$, equivalent to our results. However, the transition state was not G–G–, but AA. The differences in the conformations along the reaction pathway hardly influence the number of the intermediates and transition states.
Figure 4. Hydrolysis reaction pathway of dimethyl phosphate with a hydroxide ion in water and the optimized structures of stationary points (reactants, transition states, and products) at the CCSD/6-311++G(2d,2p)//B3LYP/6-311++G(2d,2p) level of theory. Gibbs free energies of the stationary points are plotted, relative to the reactants. The relative Gibbs free energies are given in units of kcal mol$^{-1}$.

Table 4. The lengths of the cleaved (P-O3) and formed bonds (P-O5)$^a$, and the dihedral angles for the definition of the conformation (C2-O4-P-O3 and C1-O3-P-O4)$^b$.

| Structure                  | P-O3 | P-O5 | C2-O4-P-O3 | C1-O3-P-O4 |
|----------------------------|------|------|------------|------------|
| Dimethyl phosphate (Reactant) | 1.65 | –    | –67.40     | –67.40     |
| TS1                        | 1.81 | 2.07 | –67.48     | –58.97     |
| INT                        | 1.91 | 1.85 | –63.21     | –57.82     |
| TS2                        | 2.02 | 1.79 | –65.28     | –54.51     |
| Methyl hydrogen phosphate (Product) | –    | 1.64 | –          | –          |

$^a$ Bond lengths are shown in Å.

$^b$ Dihedral angles are shown in degrees.

In water, the components of the reaction pathway, such as the Gibbs free energies and the conformation of each structure, are remarkably different from those in the Ribeiro's study. Although the stepwise pathway was obtained in the present study, the concerted pathway was shown in the Ribeiro's study. A clear conformational change was not observed in this study (figure 4), but in the Ribeiro's study, the conformation of the reactant (G–G–) is quite different from that of the transition state (AA). Since both the reaction pathway (stepwise or concerted) and the conformation of each structure are different between this study and the Ribeiro's study, it is possible that the differences in the conformations along the reaction pathway influence the number of the intermediates and transition states. Moreover, the first activation energy of the hydrolysis reaction in this study is lower than that in the Ribeiro's study, by 1.8 kcal mol$^{-1}$. Therefore, if the conformations do not largely affect the reaction pathway, the current stepwise reaction pathway is considered to be more favorable than the concerted reaction pathway in water.

Since we determined the hydrolysis pathways by following the IRC procedure, the pathways obtained in this study are more reliable than the Ribeiro's study.
4. Concluding remarks

In this study, we have investigated the hydrolysis reaction mechanism of the most stable conformation (G–G–) of dimethyl phosphate. We examined the hydrolysis pathway with a hydroxide ion in vacuum and in water.

In the reaction with hydroxide, a concerted reaction pathway was obtained in vacuum, whereas a stepwise reaction pathway was obtained in water conditions. Regardless of the conformational difference of the transition state, the reaction pathway shown in the previous study is concerted as well as our study, in the gas phase. On the other hand, the reaction mechanism is quite different from those of the previous study in water, suggesting that the conformations in the reaction pathway affect the number of the intermediates and transition states.

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