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The Role of Magnesium in Hydrolysis of Triphosphates in Water: Quantum Mechanical/Molecular Mechanical Modeling

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Abstract—The mechanism of hydrolysis of deprotonated methyl triphosphate (MTP) to methyl diphosphate (MDP) and inorganic phosphate (Pi) in water clusters in the presence and absence of magnesium cations has been modeled. Modeling has been performed by the effective fragment potential–based quantum mechanical/molecular mechanical method. The energies and energy derivatives in the quantum subsystem including MTP, reacting water molecules, and Mg^{2+} has been calculated at the density functional theory (B3LYP) level, whereas water–water interactions have been described by the TIP3P model potential. The minimum-energy path for the reaction MTP + $H_2O \longrightarrow$ MDP + Pi is consistent with a two-stage dissociative process in the absence of Mg^{2+} and with a one-stage mechanism in the presence of Mg^{2+}.

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The mechanisms of hydrolysis of triphosphates have received much attention, first of all, due to the importance of enzymatic transformations of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) in biological systems [1]. Analysis of the hydrolysis reaction of methyl triphosphate (MTP) in aqueous solutions provides necessary reference data for elucidating the catalytic effect of protein matrices in enzymatic transformations of ATP and GTP [2, 3]. Measurements of the hydrolysis rate constants of methyl monophosphate in water at various pH values [4] made it possible to estimate the activation barriers on the free energy surface at 27–31 kcal/mol [5, 6]; however, details of the reaction mechanism remained unclear. Theoretical approaches to the modeling of the mechanism of hydrolysis of completely deprotonated MTP in aqueous solutions were described in [7, 8] with the use of the Car–Parinello molecular dynamics method [7] (in this case, this strategy led to considerably overestimated activation barriers) and quantum mechanical/molecular mechanical (QM/MM) method [8]. Our previous results of calculation of the minimum-energy path of the reaction

$$MTP^{4-} + H_2O \longrightarrow MDP^{3-} + H_2PO_4^-, \qquad (1)$$

where MTP and MDP are, respectively, methyl triphosphate and methyl diphosphate surrounded by water molecules [8], point to a predominantly dissociative reaction mechanism. However, these results were obtained in the Hartree–Fock approximation, i.e., without the inclusion of correlation effects in the quantum subsystem, as well as without the participation of Mg^{2+} in the model molecular system, although it always exists in enzymes catalyzing the hydrolysis of AFP and GTP. In this work, we report the results of calculations of the minimum-energy paths of reaction (1) and discuss the reaction mechanism with and without the participation of the Mg^{2+} cation in the system on the bases of QM/MM calculations with the use of the density functional theory (DFT) approximation in the quantum subsystem.

COMPUTATION DETAILS

Energies and energy gradients were calculated by the QM/MM method based on the effective fragment potential theory [9]. In this approach, the molecular groups attributed to the molecular mechanical (MM) subsystem are taken to be effective fragments that make electrostatic, polarization, and exchange contributions to the Hamiltonian of the quantum subsystem. As distinct from the implementation of this method in the GAMESS(US) quantum-chemical program [10], in our computational scheme adapted for the PC GAMESS program [11], the interaction between water molecules assigned to the MM subsystem is described by the known empirical potential TIP3P [12].

In the present work, we considered two model molecular systems differing, first of all, by the presence or absence of the magnesium cation. In model I, the quantum subsystem comprises the quadruply charged MTP anion and five water molecules; another 75 water molecules described by effective fragments are assigned to the MM subsystem. In model II, the QM subsystem comprises the MTP anion, the Mg^{2+} cation, and seven water molecules; another 73 molecules

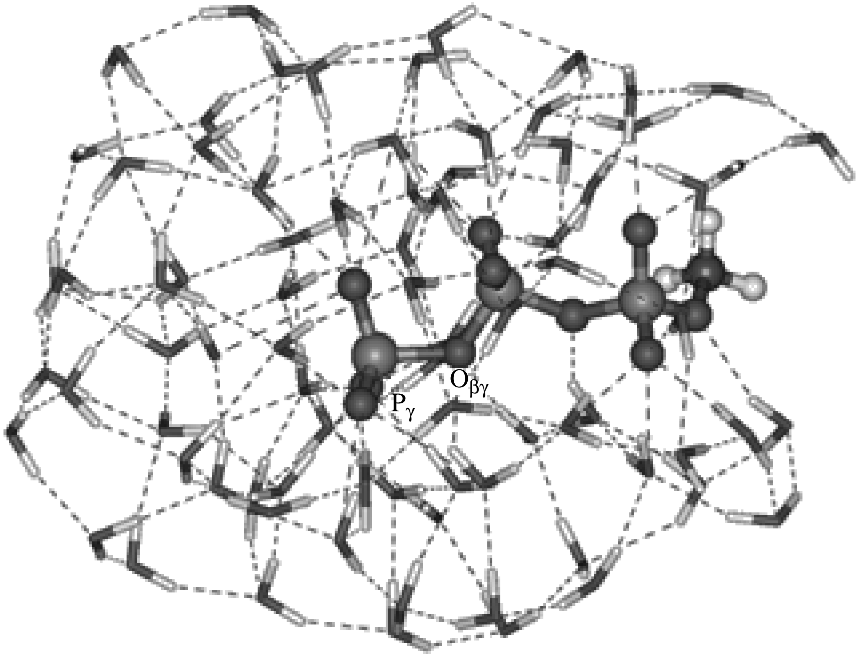

Fig. 1. General view of the model cluster (without the magnesium cation).

(effective fragments) constitute the MM subsystem. These molecular clusters were constructed by successively adding water molecules to the reacting particles ($MTP^{4-} + H_2O$), the geometric parameters of the entire system being optimized at each step in such a way that the hydrogen bond network persists. The selected number of water molecules (altogether 80 molecules) is sufficient for them to completely surround the reactive part (Fig. 1).

The inclusion of five (model I) or seven (model II) water molecules in the QM subsystem was caused by the necessity to ensure several variants of the choice of the proton transfer route to form inorganic phosphate. For the ultimate conclusion, the routes with minimal activation energies were selected.

In calculations of the energy in the QM/MM approximation, regions of multidimensional potential energy surfaces were scanned in which chemical or hydrogen bonds are broken and formed. Then, the positions of the stationary points on the potential energy surfaces were determined more precisely by geometry optimization. The geometric configurations of the saddle points (or transition states) were found based on the following criterion: at this point, the gradient along the internal coordinate related to the reaction coordinate of the stage under consideration should change the sign.

The energies and energy gradients in the QM subsystem were calculated by the DFT method with the known B3LYP potential using the PC GAMESS program package [11]. The LANL2DZdp_ECP basis set [13] with the corresponding pseudopotential for phosphorus was used. The orbitals of the magnesium cation were described with the use of the standard basis set 6-31G.

RESULTS AND DISCUSSION

The computation results for model I (without magnesium cation) are qualitatively consistent with the Hartree–Fock calculations in the QM subsystem [8]. The potential energy surface contains the stationary points corresponding to the reagents ($MTP^{4-} + H_2O$), the products ($MDP^{3-} + H_2PO_4^-$), the reaction intermediate (see Figs. 1–3 in [8]), and two transition states TS1 and TS2. The reaction proceeds by the dissociative mechanism: the P_γ–$O_{\beta\gamma}$ bond is broken and the γ-phosphate group $P_\gamma O_3$ is eliminated from methyl diphosphate followed by proton transfer along hydrogen bonds of water molecules and $P_\gamma O_3$ to form inorganic phosphate $H_2PO_4^-$. The table compares the relative energies of the stationary points on the reaction path calculated at the Hartree–Fock and DTF levels. The experience of calculations of the energy profiles of chemical reactions shows that the Hartree–Fock activation barriers are, most likely, overestimated, whereas the DFT barriers are underestimated. The calculation results for model II (with the magnesium cation) are considerably different. Figure 2 shows the equilibrium reagent configuration obtained by unconstrained optimization of geometric parameters. The magnesium cat-

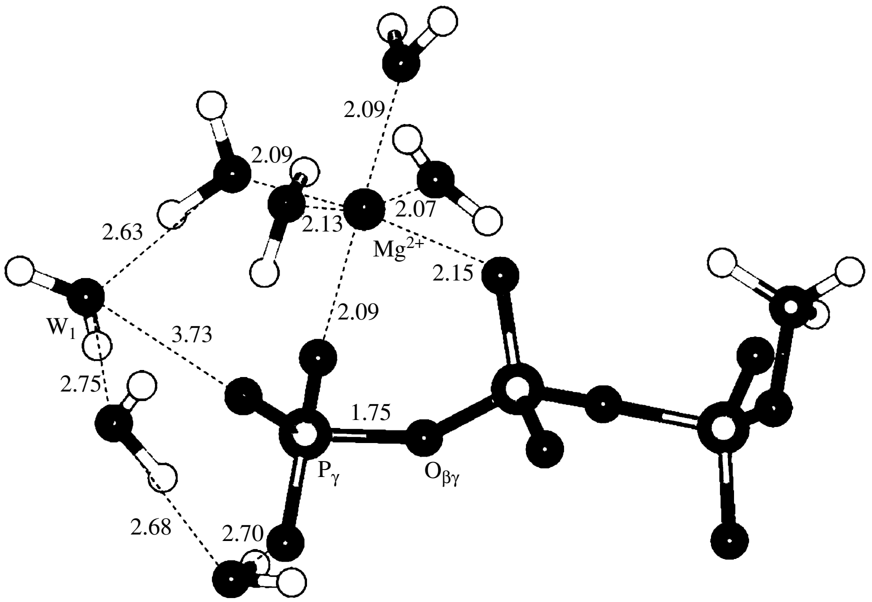

Fig. 2. Geometric configuration of the quantum subsystem of the reagents for model II (distances are given in angstroms).

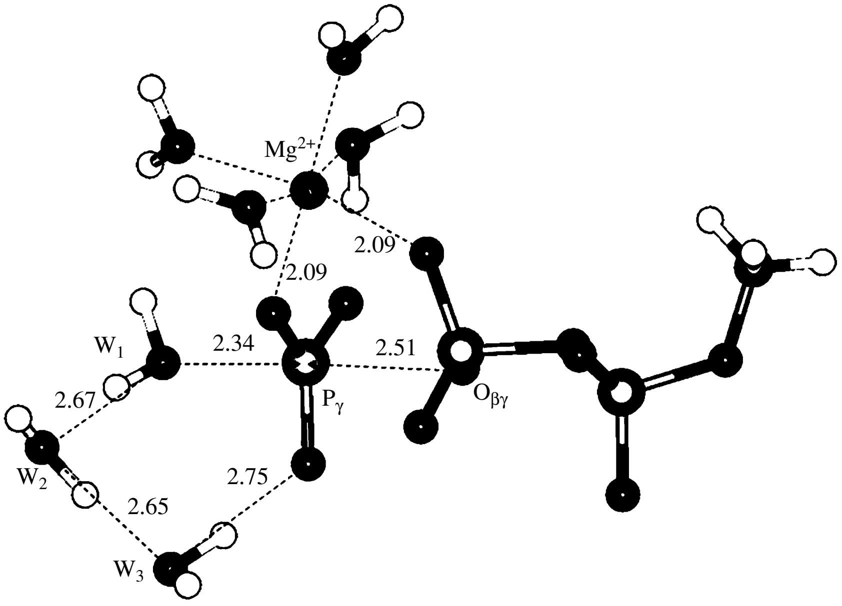

Fig. 3. Geometric configuration of the quantum subsystem for the only transition state in model II (distances are in angstroms).

ion is coordinated to four water molecules and the oxygen atoms of the γ- and β-phosphate groups of MTP. The reacting water molecule W_1 is located at a distance of 3.73 Å from P_γ.

As for model I, the transition state was sought by scanning the potential energy surfaces along two coordinates: the distance from the oxygen atom of the reacting water molecule (W_1) to the P_γ atom (the initial distance in the reagent configuration, 3.73 Å) and the P_γ–$O_{\beta\gamma}$ distances (the initial distance, 1.75 Å). After a series of constrained optimizations of geometric parameters, we obtained the transition state shown in Fig. 3. As in all hydrolysis reactions of triphosphates, the P_γ–$O_{\beta\gamma}$ bond surrounded by water molecules or a protein matrix is broken through the stereochemical inversion about the P_γ atom. As distinct from model I, Relative energies (kcal/mol) of the stationary points on the path of reaction (1) of hydrolysis of MTP in the model system without magnesium cation (model I) calculated by the Hartree–Fock method in [8] and by the DFT (B3LYP) method in this work. In both cases, the LANL2DZdp_ECP basis set was used in the QM subsystem and the TIP3P potential was used for water–water interactions in the MM subsystem

Structure	Hartree–Fock calculations [8]	DFT/B3LYP calculations
Reagents	0.0	0.0
TS1	20.0	12.9
Intermediate	7.0	3.0
TS2	14.1	4.5
Products	–20.7	–16.4

beyond the transition state point in model II, the minimum-energy path leads from the saddle point to the reaction products (MDP^{3-} + $H_2PO_4^-$) after a series of proton transfer events involving the W_1, W_2, and W_3 water molecules. The equilibrium configuration of the products, obtained by unconstrained optimization of geometric parameters, is shown in Fig. 4. A similar single-stage mechanism is characteristic of hydrolysis of ATP by the protein myosin.

With respect to the energy level of the reagents, the only transition state is 11.1 kcal/mol higher, and the energy effect of the reaction is –8.6 kcal/mol.

CONCLUSIONS

Molecular modeling by the QM/MM method of reaction (1) in water clusters in the presence and absence of the Mg^{2+} cation in a model molecular system allows us to draw the following conclusions. Hydrolysis of completely deprotonated MTP in water proceeds by the dissociative mechanism: the P_γ–$O_{\beta\gamma}$ bond is broken so that the γ-phosphate group $P_\gamma O_3$ is eliminated and, due to successive proton transfers along hydrogen bonds of water molecules and $P_\gamma O_3$, is transformed to the inorganic phosphate $H_2PO_4^-$. The activation barriers calculated in the B3LYP/LANL2DZdp_ECP approximation in the quantum subsystem are estimated at 13 and 11 kcal/mol in the absence and presence of the Mg^{2+} cation in the system, respectively. Taking into account that DFT calculations provide, as a rule, the lower estimate for free energy barriers and that the estimates of the free energy barriers (27–31 kcal/mol) [5, 6] based on experimental data [4] refer to hydrolysis of methyl monophosphate and obviously overestimate the barrier for completely deprotonated MTP, the agreement between the theoretical and experimental results is quite satisfactory. The calculated energy effect of reaction (1) in the presence of the magnesium cation in the model system (–8.6 kcal/mol) is also consistent with the values expected from the experimental data [14]. According to the modeling results, hydrolysis by reaction (1) in the presence of the magnesium cation somewhat differs from that in the absence of this ion. In the latter case, the cleavage of the P_γ–$O_{\beta\gamma}$ bond leads to the formation of the intermediate and its transformation into inorganic phosphate requires the second transition state. Hydrolysis of deprotonated

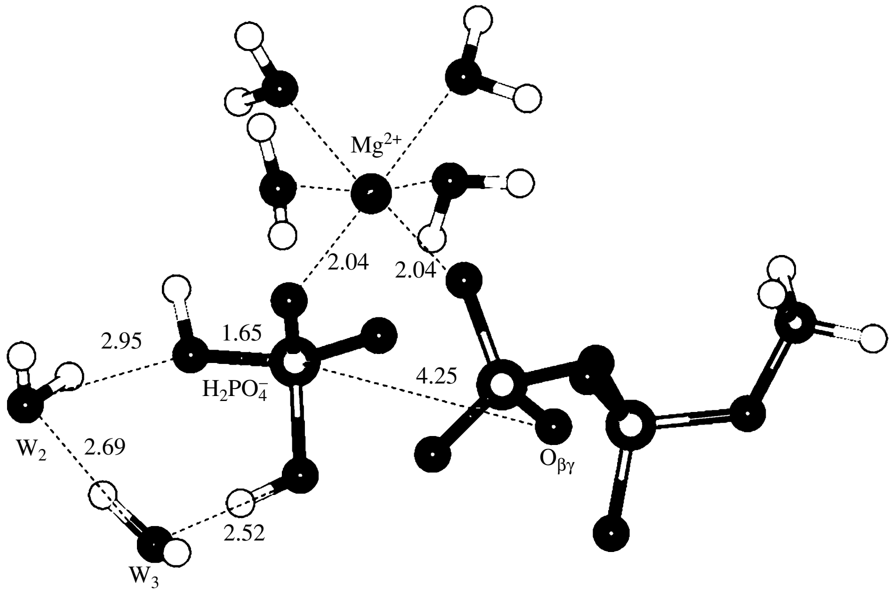

Fig. 4. Geometric configuration of the quantum subsystem of the reaction products in model II (distances are in angstroms).

MTP in water in the presence of the magnesium cation proceeds in one stage.

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