First Computational Evidence for a Catalytic Bridging Hydroxide Ion in a Phosphodiesterase Active Site

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Abstract: Phosphodiesterases are clinical targets for a variety of biological disorders, because this superfamily of enzymes regulates the intracellular concentration of cyclic nucleotides that serve as the second messengers playing a critical role in a variety of physiological processes. Understanding the structure and mechanism of a phosphodiesterase will provide a solid basis for rational design of the more efficient therapeutics. Although a three-dimensional X-ray crystal structure of the catalytic domain of human phosphodiesterase 4B2B was recently reported, it is uncertain whether a critical bridging ligand in the active site is a water molecule or a hydroxide ion. The identity of this bridging ligand is theoretically determined by performing first-principles quantum chemical calculations on models of the active site. All the results obtained indicate that this critical bridging ligand in the active site of the reported X-ray crystal structure is a hydroxide ion, rather than a water molecule, expected to serve as the nucleophile to initialize the catalytic degradation of the intracellular second messengers.

Introduction

The phosphodiesterase (PDE) superfamily catalyzes the hydrolytic degradation of 3′,5′-cyclic nucleotides (such as cAMP and cGMP) to the corresponding 5′-nucleotide metabolites (such as AMP and GMP).[1] These cyclic nucleotides are intracellular second messengers that are essential in vision, muscle contraction, neurotransmission, exocytosis, cell growth, and differentiation. The intracellular concentration of the second messengers is tightly regulated through both the synthesis by receptor-linked enzymes (such as adenylyl and guanylyl cyclase) and the degradation by PDEs. Hence, PDEs are clinical targets for such biological disorders as retinal degeneration, congestive heart failure, depression, asthma, erectile dysfunction, and inflammation.[2−4] Selective PDE inhibitors have already been shown or expected to exert beneficial effects in a number of therapeutic areas, including stimulation of myocardial contractility, inhibition of mediator release, inhibition of platelet aggregation, cancer chemotherapy, analgesia, the treatment of depression, Parkinson's disease, and learning and memory disorders.[5] Understanding the structure, particularly the active site structure, and the catalytic mechanism of a PDE will provide a solid basis for rational design of the more efficient therapeutics.

Xu et al. recently described a three-dimensional X-ray crystal structure of the catalytic domain of human phosphodiesterase 4B2B (PDE4).[6] In the reported X-ray crystal structure, the active site contains a cluster of two divalent metal ions, denoted by Me1 and Me2. Me1 is likely a Zn^{2+} ion based on the observed geometry of the metal-coordinating ligands, the anomalous X-ray diffraction behavior, the existing biochemical evidence,[7−10] and the known high affinity of PDE4 for zinc.[11] Me2 may be Mg^{2+}, Mn^{2+}, or Zn^{2+}. However, it is likely that either Mg^{2+} or Mn^{2+} is the relevant physiological ion. As depicted in Figure 1, in the PDE4 active site the Asp-392 residue coordinates to Me1 through an O_δ atom, His-238 and His-274 residues coordinate to Me1 through the N_ϵ atoms, and four solvent water molecules coordinate to Me2 through the O atoms. Besides, there are two bridging ligands. One bridging ligand is clearly Asp-275, whose two O_δ atoms respectively coordinate to Me1 and Me2.[6] However, it is uncertain whether the other bridging ligand is a water molecule or a hydroxide ion, since hydrogen atoms cannot be determined by X-ray diffraction techniques. Xu et al. described the second bridging ligand as a water molecule, although it had been clear that the second bridging ligand in the X-ray crystal structure of Zn^{2+}-substituted phosphotriesterase (PTE) should be a hydroxide ion.[12] They further

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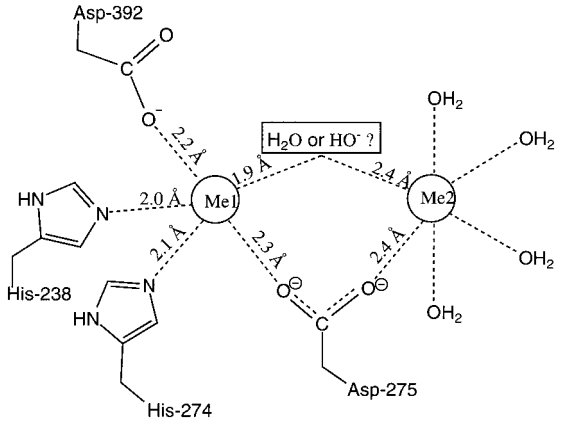

Figure 1. Schematic representation of the binuclear metal center of phosphodiesterase.

considered the possible position of the substrate in the PDE active site to discuss the catalytic mechanism based on the PDE structure in which Me1 and Me2 were considered as Zn^{2+} and Mg^{2+}, respectively. They concluded from a model of cAMP docked in the PDE4 active site that a water molecule coordinated to one or both metal ions could act as the nucleophile in the catalytic hydrolysis reaction, since they considered the second bridging ligand as a water molecule.[6] Obviously, compared to a water molecule coordinated to one metal ion, the postulated bridging water molecule should be a worse nucleophile whereas a possible bridging hydroxide ion should be a better nucleophile. For this reason, if the second bridging ligand is a water molecule the nucleophile is likely a water molecule coordinated to one metal ion (Me2). If the second bridging ligand is a hydroxide ion, the nucleophile is likely the bridging hydroxide ion. Thus, it is a key step for determining the nucleophile in the catalytic hydrolysis and understanding the catalytic mechanism to identify the structural form of the second bridging ligand. Here we theoretically determine the identity of this critical bridging ligand by performing a series of quantum chemical calculations on models of the Me1/Me2-PDE active site.

Calculation Methods

We considered all of the three possible combinations of metal ions mentioned by Xu et al.:[6] Me1/Me2 = Zn^{2+}/Mg^{2+}, Zn^{2+}/Mn^{2+}, and Zn^{2+}/Zn^{2+}. Construction of the initial structures for the geometry optimizations was based on the described X-ray crystal structure.[6] For each combination of the metal ions, we first fully optimized geometries of four different active site models by use of two first-principles methods implemented in the Gaussian98 program:[13] the Hartree−Fock (HF) method with the 3-21G basis set and density functional theory (DFT) using Becke's three-parameter hybrid exchange functional and the Lee−Yang−Parr correlation functional (B3LYP) with the 6-31G* basis set. Vibrational frequencies were evaluated at the optimized geometries to verify their true stability and to evaluate the zero-point vibration energies. The B3LYP/6-31G* geometry optimizations were followed by single-point B3LYP/6-31+G* energy calculations. Besides, geometries of the active site models with Me1/Me2 = Zn^{2+}/Mg^{2+} were also optimized at the B3LYP/6-31+G* level to further examine basis set dependence of the geometry optimizations.

Finally, the geometries determined by the DFT calculations were employed to perform self-consistent reaction field (SCRF)[14] calculations at the HF/6-31+G* level, for calculating free energies of solvation in aqueous solution. The calculated free energy in solution was taken as the energy calculated at the B3LYP/6-31+G* level in the gas phase (including zero-point vibration corrections) plus the solvent shift calculated at the HF/6-31+G* level. The SCRF method employed was developed and implemented recently in the GAMESS program[15] by one of us, and is called the surface and volume polarization for electrostatic interaction (SVPE).[16] The SVPE model was sometimes also called the fully polarizable continuum model (FPCM)[12b,17] because it fully accounts for both surface and volume polarization effects in the SCRF calculation. Since the solute cavity surface is defined as a solute electron charge isodensity contour determined self-consistently during the SVPE iteration process, the SVPE results, converged to the exact solution of Poisson's equation with a given numerical tolerance, depend only on the contour value at a given dielectric constant and a certain quantum chemical calculation level. This single parameter value has been determined as 0.001 au on the basis of an extensive calibration study.[16b]

Unless otherwise indicated, the Gaussian98 program was used to obtain the present results. All the calculations in this work were performed on Silicon Graphics, Inc. Origin 200 multiprocessor computers.

Results and Discussion

The optimized geometries were depicted in Figure 2. The optimized geometric parameters indicate that the geometries optimized at three different levels of theory are all consistent with each other; the geometric parameters optimized at the B3LYP/6-31G* level are all very close to those optimized at the HF/3-21G level and to those optimized at the B3LYP/6-31+G* level.

As depicted in Figure 2, 1S and 2S are two small models in which His-238 and His-274 residues are all simplified as ammonia molecules (NH_3) and Asp-275 and Asp-392 residues are all simplified as formate anions (HCO_2^-). The difference between 1S and 2S exists only in the structural form of the second bridging ligand, which is considered as a hydroxide ion (HO^-) in 1S and a water molecule in 2S. The internuclear distances optimized for Zn^{2+}/Mg^{2+}-1S and Zn^{2+}/Mn^{2+}-1S are all reasonably close to the available experimental values reported for the X-ray crystal structure of PDE4 determined to 1.77 Å resolution. The optimized geometry of Zn^{2+}/Zn^{2+}-1S is also consistent with the X-ray crystal structure[6] as far as the second ligand bridging the two metal ions is concerned, although the coordination numbers of the second metal ion are different. The optimized geometries of Zn^{2+}/Mg^{2+}-2S, Zn^{2+}/Mn^{2+}-2S, and Zn^{2+}/Zn^{2+}-2S are all qualitatively different from those of Zn^{2+}/Mg^{2+}-1S, Zn^{2+}/Mn^{2+}-1S, and Zn^{2+}/Zn^{2+}-1S. The hydroxide ion in 1S coordinates to the two metal ions simultaneously, whereas

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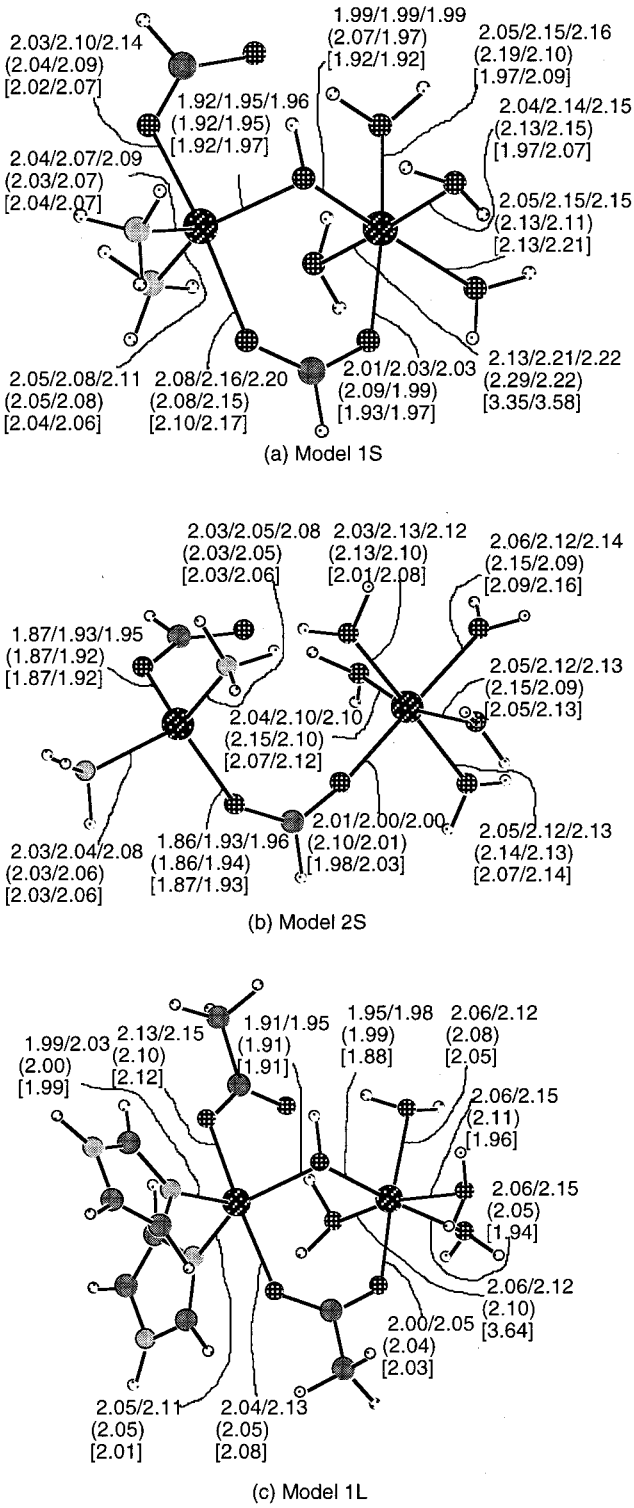

Figure 2. Geometries of the active site models of Zn^{2+}/Mg^{2+}-phosphodiesterase optimized with the B3LYP method. Indicated in the figure are geometrical parameters optimized at three different levels of theory: {HF/3-21G}/{B3LYP/6-31G*}/{B3LYP/6-31+G*} for all the models. The values in parentheses and brackets are the corresponding geometrical parameters optimized for Zn^{2+}/Mn^{2+}- and Zn^{2+}/Zn^{2+}-phosphodiesterases, respectively.

the water molecule in 2S coordinates only to one metal ion. These results indicate that the second bridging ligand in the X-ray crystal structure of PDE4 is a hydroxide ion rather than a water molecule.

Concerning the identity of the second metal ion (Me2), a notable difference between the optimized geometry of Zn^{2+}/Zn^{2+}-1S and the X-ray crystal structure exists in the coordination number of the second metal ion: 5 in model Zn^{2+}/Zn^{2+}-1S and 6 in the X-ray crystal structure. One of the four water molecules in Zn^{2+}/Zn^{2+}-1S gradually left the second zinc ion during the geometry optimizations. This difference further suggests that the second metal ion (Me2) in the PDE X-ray crystal structure[6] is unlikely to be Zn^{2+}. This conclusion supports the expectation of Xu et al. that either Mg^{2+} or Mn^{2+} is the relevant physiological ion.[6]

The qualitative results obtained from the quantum chemical studies of the small active site models can be examined through comparison with the corresponding calculations on larger active site models. Model 1L depicted in Figure 2 is a larger model with a hydroxide ion employed to theoretically examine the suitability of model 1S. In model 1L, each of the two ammonia molecules and each of the two formate anions in model 1S are replaced by imidazole and acetate anion, respectively. As one can see from Figure 2, the optimized internuclear distances involving the two metal ions do not change significantly from 1S to 1L for all three combinations of metal ions. We also tested geometry optimizations on an active site model, denoted by 2L, in which the bridging hydroxide ion in 1L is replaced by a water molecule. It turned out that during the optimizations the distances between the first metal ion and the "bridging water" oxygen became longer and longer for Zn^{2+}/Mg^{2+}-2L, Zn^{2+}/Mn^{2+}-2L, or Zn^{2+}/Zn^{2+}-2L. For each case, the optimization was stopped after the distance became considerably longer than the expected $Zn^{2+}-O$ bonding distance ($\sim$2 Å). The results obtained from the calculations on models 1L and 2L are qualitatively coincident with those from the calculations on models 1S and 2S, thus indicating that models 1S and 2S are sufficient for qualitatively determining the structural form of the second bridging ligand.

It should be mentioned that the quantum chemical calculations on the active site models have not completely accounted for the protein environment. Previous quantum chemical calculations and molecular dynamics simulations on other enzyme systems involving a binuclear divalent metal center indicate that the protein environmental effects do not qualitatively change the coordination of the divalent metal ions.[12b]

Although our calculated results indicate that the second bridging ligand in the reported X-ray crystal structure of PDE should be a hydroxide ion, the other PDE structure corresponding to model 2S still could exist in solution under a certain pH. The point is that the PDE structure corresponding to model 2S does not have a second bridging ligand and differs from the X-ray crystal structure.[6] The thermodynamic equilibration between the two structural forms of PDE corresponding to models 1S and 2S is characterized by pK_a associated with the bridging hydroxide ion. 2S is the protonated form of 1S. This pK_a may be estimated by evaluating the free energy change ΔG_a for the model reaction 2S (solvated) $\rightarrow$ 1S (solvated) + H^+ (solvated) in water. By employing the latest experimental data for a proton (the solvation free energy of the proton is -264.1 kcal/mol;[18] $T\Delta S = 7.8$ kcal/mol[19] for proton in the gas phase) and the free energies calculated for 2S and 1S with the geometries determined at the B3LYP/6-31G* level we obtained p$K_a = \Delta G_a/(2.303RT) = 5.3$ for Zn^{2+}/Mg^{2+}-PDE, -11.9 for Zn^{2+}/Mn^{2+}-PDE, and -0.2 for Zn^{2+}/Zn^{2+}-PDE when $T = 298$ K. By using the geometries optimized at the B3LYP/6-31+G* level, the change of the calculated pK_a value for

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Zn^{2+}/Mg^{2+}-PDE is smaller than 0.3. Note that these pK_a values were evaluated by using a dielectric constant of 78.5, which assumed a fully solvent-exposed binding site. Considering the fact that the binding site is partially buried, the actual pK_a values should be slightly smaller than what is estimated here.[20] So, the pK_a values estimated here are just the up limits of the actual pK_a values. These limited results suggest that the PDE structure with a bridging hydroxide ion should be dominant in neutral aqueous solution (pH 7).

Conclusion

All the calculations strongly support the conclusion that the second bridging ligand in the active site of the reported PDE X-ray crystal structure is a hydroxide ion rather than a water molecule. On the basis of this conclusion and the previously described position of substrate in the PDE active site, it is likely that the nucleophile during the catalytic hydrolysis should be this bridging hydroxide ion rather than other water molecules coordinated only to the second metal ion. The catalytic hydrolysis is likely to be initialized by the attack of the bridging hydroxide oxygen at the phosphorus atom of the substrate. Further computational studies on the detailed enzymatic reaction pathway are currently in progress in our laboratory.

(20) Because the net charges of models 1S and 2S are +1 and +2, respectively, solvation favors model 2S more than model 1S. So, the SVPE calculations using a larger dielectric constant (78.5) overestimated the stability of model 2S relative to model 1S. With the same experimental data for proton, the SVPE calculations using a smaller dielectric constant would lead to the smaller pK_a values.

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