J. Am. Chem. Soc. **1998,** *120,* 6113−6120 6113

An *Ab Initio* Study of the Isomerization of Mg− and Ca−Pyrophosphates

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Received August 5, 1997. Revised Manuscript Received March 27, 1998

Abstract: *Ab initio* calculations were performed on gas-phase calcium and magnesium dications chelated with various anionic pyrophosphate species: $H_2P_2O_7{}^{2-}$, $HP_2O_7{}^{3-}$, and $P_2O_7{}^{4-}$. The cleavage of the pyrophosphate into a metaphosphate and an orthophosphate complexed to either calcium or magnesium was also investigated. The studied isomerization reaction of the metal−pyrophosphate complexes can be written as $[M{\cdot}H_NP_2O_7]^{N-2} \rightarrow [PO_3{\cdot}M{\cdot}H_NPO_4]^{N-2}$ where M = Mg, Ca, and N = 0, 1, 2. Geometries for the complexes were optimized with the self-consistent-field (SCF) level of theory, and the total energy for each system was subsequently calculated with the second-order Møller−Plesset perturbation (MP2) method using 6-31+G** basis functions for the H, O, and P atoms and valence double-ζ basis functions polarization augmented with a diffuse function (pVDZ+) for the Mg and Ca atoms. Zero-point energies (ZPE) and entropies were calculated with the SCF harmonic frequencies from which enthalpies and Gibbs free energies were also estimated. The *ab initio* isomerization energies of all of the calcium-containing complexes were positive and had a large contribution of correlation, while those of the magnesium-containing complexes were negative and had a significantly lower contribution of correlation. These calculated gas-phase isomerization energies may provide an explanation to the observation that pyrophosphatases utilize magnesium complexes as substrates for the hydrolysis of pyrophosphates but do not utilize calcium complexes.

1. Introduction

The primary molecular structures which store and transmit energy in living biological systems contain the $\rangle P{-}O{-}P\langle$ linkage. The most important bioenergetic molecule, adenosine triphosphate (ATP), contains this linkage as does the inorganic molecule pyrophosphate (or diphosphate). Pyrophosphate (PP_i) can be a significant energy donor in procaryotes and eucaryotes, either being formed by decomposition of ATP to adenosine monophosphate (AMP) or, in some cases, even replacing ATP as the main bioenergetic molecule. Though there are differences between PP_i and ATP, there are enough similarities for pyrophosphate to be regarded as a prototype for modeling ATP chemistry.[1−5] However, the size of pyrophosphate and the environment in which its hydrolysis naturally occurs make it more amenable to experimental and theoretical evaluation.

The release of energy by pyrophosphates occurs during hydrolysis which is accompanied by the breaking of the $\rangle P{-}O{-}P\langle$ linkage. It is now clear that earlier theories[6] attesting to the strength of the phosphorous−oxygen bond itself as the source of bioenergy were misleading. *Ab initio* calculated harmonic frequencies[7,8] reveal that the force constants between the phosphorous atoms and the bridging oxygen in pyrophosphates are relatively low. Rather, it has been proposed that the difference in hydration energies of the reactants and products of the hydrolysis accounts for the available free energy. Early experimental results obtained calorimetrically[9] for the hydrolysis of various pyrophosphate species led to the above proposal, and there is also qualitative evidence from a recent numerical simulation[10] that indicates significant differences in the hydration layers of pyrophosphate and its ultimate hydrolysis product, orthophosphate (*i.e.*, phosphoric acid).

A general feature of the enzymes involved in the hydrolysis fo the $\rangle P{-}O{-}P\langle$ linkage is their requirement for divalent metal ions for activation, and their recognition of the metal ions complexed with PP_i, or ATP, as substrates, being magnesium the essential activator of membrane-bound enzymes[11,12] as well as regulator of their catalytic properties.[13−15] There is also ample evidence suggesting that a magnesium−metaphosphate

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intermediate ($Mg{\cdot}PO_3^-$) is formed at some point during pyrophosphate hydrolysis,[16] and proposed reaction mechanisms must account for this.

Recent extensive *ab initio* studies have examined the gas-phase hydrolysis of pyrophosphate and its anions[7,17] and the effect magnesium imparts.[8] These studies have led to a proposed reaction mechanism for the magnesium catalyzed hydrolysis of gas-phase dianionic pyrophosphate, consisting of the following four steps:[7]

$$H_2O + Mg^{2+} + H_2P_2O_7^{2-} \rightarrow H_2O + Mg{\cdot}[H_2P_2O_7] \quad (1)$$

$$H_2O + [Mg{\cdot}H_2P_2O_7] \rightarrow H_2O + [PO_3{\cdot}Mg{\cdot}H_2PO_4] \quad (2)$$

$$H_2O + [PO_3{\cdot}Mg{\cdot}H_2PO_4] \rightarrow [H_2PO_4{\cdot}Mg{\cdot}H_2PO_4] \quad (3)$$

$$[H_2PO_4{\cdot}Mg{\cdot}H_2PO_4] \rightarrow Mg^{2+} + 2H_2PO_4^- \quad (4)$$

It is interesting to note that the calculated orbital energies of the magnesium−pyrophosphates indicate that the metal creates stability; without the complexation to magnesium, the $HP_2O_7^{3-}$ and $P_2O_7^{4-}$ anions are predicted to be unstable in the gas-phase.[8]

The complexation of the pyrophosphates to magnesium produces, or enhances, asymmetry of the P−O bond lengths forming the $\rangle P{-}O{-}P\langle$ linkage and some bending of it. Using the elongated P−O bond as the reaction coordinate leading to the magnesium−metaphosphate isomer in reaction 2, it was found[7] that its calculated activation barrier is $\approx$3 times larger in the absence of Mg^{2+}. It was therefore proposed that the Mg^{2+} induces a strain that lowers the activation barrier, and the elongated P−O bond was termed "activated". Isomerizations as reaction 2 were predicted[8] to be spontaneous in the gas-phase for the neutral and monoanionic species, $Mg{\cdot}H_2P_2O_7$ and $[Mg{\cdot}HP_2O_7]^-$, and essentially thermoneutral for the dianion, $[Mg{\cdot}P_2O_7]^{2-}$. The magnesium dication has a relatively small ionic radius[18] (0.65 Å), and *ab initio* calculations have shown[7] that Mg^{2+} exhibits "tight" coordination throughout the isomerizations. The "intrinsic coordination requirements" of Mg^{2+} have been attributed as the force driving the isomerization of Mg−pyrophosphate.[7]

The pyrophosphatase that has been the most extensively characterized by *in vitro* experimental methods is the membrane-bound *Rhodospirillum rubrum* chromatophore. The chromatophores of this photosynthesizing bacteria exhibit high specificity in their requirement of magnesium for enzyme activation,[12] and, as in other pyrophosphatases, their use of magnesium−pyrophosphate complexes as substrate, whereas other dications with a similar ability to be bonded to pyrophosphates do not promote hydrolysis.[12−15] For example, Ca^{2+} readily forms complexes with pyrophosphate at concentrations comparable to those of Mg^{2+}. (In fact, the ability of polyphosphates to chelate either Mg^{2+}, or Ca^{2+}, was exploited in earlier decades as a detergent additive to prevent "soap-scum" formation.[19]) However, Ca^{2+} does not produce appreciable hydrolysis of PP_i with membrane-bound *Rhodospirillum rubrum* chromatophores at any tested molarity.[11] Even in the presence of the enzyme activated by Mg^{2+}, the complex $Ca{\cdot}PP_i^{2-}$ does not act as a substrate,[11] and supports very limited exchange of isotopically marked orthophosphate into pyrophosphate.[20] The presence of Ca^{2+} has even been observed to inhibit several types of pyrophosphatases.[21,22]

The calcium dication has a larger ionic radius[18] (0.99 Å) and greater polarizability than the magnesium dication (which is reflected in their computed values for hardness:[23] $\chi_{Ca^{2+}} = 31.4$ eV $< \chi_{Mg^{2+}} = 47.6$ eV). The coordination of the hydrated ions is also different; magnesium coordinates six water molecules in a regular octahedron, whereas calcium can coordinate up to seven water molecules in a more spherical array.[24] Though the resultant pyrophosphatase activities affected by Ca^{2+} and Mg^{2+} can be in part attriuted to both their differing atomic characteristics and coordination properties, an understanding of the structural and energy differences of calcium− and magnesium−pyrophosphates and their isomerization products is needed to elucidate pyrophosphatase selectivity.

The study presented in this paper is the *ab initio* investigation of the isomerization of three metal−pyrophosphates to their metaphosphate (PO_3^-) containing counterparts:

$$M{\cdot}H_2P_2O_7 \rightarrow PO_3{\cdot}M{\cdot}H_2PO_4 \quad (5)$$

$$[M{\cdot}HP_2O_7]^- \rightarrow [PO_3{\cdot}M{\cdot}HPO_4]^- \quad (6)$$

$$[M{\cdot}P_2O_7]^{2-} \rightarrow [PO_3{\cdot}M{\cdot}PO_4]^{2-} \quad (7)$$

where M = Mg, Ca.

The hypothesis is that the gas-phase reactions should reflect aspects of enzymatic catalysis behavior, and therefore noticeable differences between the *ab initio* calculated molecular energies and structures of the magnesium and calcium complexes should appear.

2. Theoretical Methodology

All electronic energies and harmonic vibrational frequencies were determined with the use of the GAUSSIAN94[25] suite of programs. Because the standard basis set[26] 6-31+G** used in previous calculations does not support Ca, the basis set utilized in this work was composed of a combination of functions from the 6-31+G** basis for elements H, O, and P and functions from the valence double-ζ quality with polarization basis[27] (pVDZ) for Mg and Ca. The functions centered on Mg and Ca were further enhanced with the diffuse functions for Mg from the 6-31+G** basis, which will be denoted with a "+", to yield a basis of similar design as those utilized in earlier *ab*

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initio studies.[7,8,10,17] The pVDZ basis was obtained from the Extensible Computational Chemistry Environment Basis Set Database.[28]

The *d*-type functions were composed of six primitive Gaussians (and therefore contain an extra *s* function). The *f*-type functions were comprised of linear combinations of primitive Gaussians to yield seven functions per atom.

Geometry optimizations were performed with the above described basis set with the SCF method. The order of these stationary-point structures was subsequently classified with calculated SCF harmonic frequencies from an analytically determined hessian. All reported equilibrium geometries correspond to structures in which the hessian eigenvalues are positive indicating a potential energy surface that is positively curved in all directions. The coordinates of each complex were referred to their respective centers of mass, with the axes aligned to affect diagonalization of the moment-of-inertia tensor (*i.e.*, the Principal Moments of Inertia frame: PMI). The structural variations between the complexes were quantified with the root-mean-square differences of their coordinates in their respective PMI frames.

Single point energy calculations using Møller−Plesset second-order perturbation theory (MBPT(2) = MP2) were then performed on the SCF-optimized geometries to evaluate electron correlation contributions to the electronic energy. The following compact notation will be used to denote the total molecular energy and the geometry optimization method used with the above described basis set: SCF//SCF and MP2//SCF.

Further calculations were made for the calcium−pyrophosphate complexes, up to the fourth-order perturbation theory, including single, double, and quadruple excitations (MP4SDQ).

Other basis sets were used to check the reliability of the MP2 results for the calcium−pyrophosphate complexes: pVDZ without diffuse functions on all atoms; pVDZ augmented with the diffuse functions of 6-31+G**, on all atoms, and a basis set without diffuse functions, composed of 6-31G** on H, O, and P, and pVDZ on Ca.

Estimates of the zero-point energy (ZPE) and the entropy were computed with the harmonic approximation with SCF//SCF frequencies. A correction for thermal energy was made to get an estimate of the enthalpy at room temperature. The free energies were then determined as the difference, $\Delta G = \Delta H - T\Delta S$.

3. Results

Summaries of the *ab initio* determined equilibrium geometries and energies for the isomerization of the neutral, monoanionic, and dianionic calcium− and magnesium−pyrophosphates follow in the next three subsections.

3.1. Neutral Complexes. The equilibrium geometries of the $Mg\cdot H_2P_2O_7$ and $Ca\cdot H_2P_2O_7$ complexes determined with the SCF level of theory are structurally similar. The equilibrium geometries are depicted in Figure 1 and correspond to structures in which

- the two hydrogens are located on the same moiety;
- the bridging P−O bond of the proton-free moiety is longer than the other (1.7 Å vs 1.5 Å), and the $\rangle$P−O−P$\langle$ linkage is bent (≈121°);
- the six nonbridging oxygens are in a staggered arrangement as viewed along an axis connecting the phosphorous atoms;
- the metal is coordinated to three oxygens, one of which is located within the moiety containing the two hydrogens and lies in the plane containing the bent $\rangle$P−O−P$\langle$ linkage.

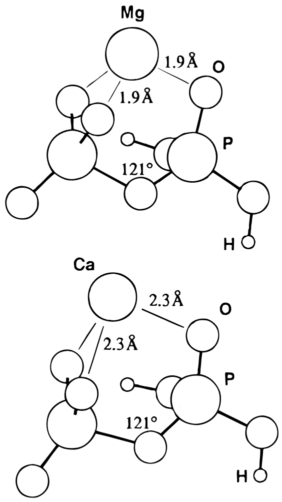

Figure 1. $Mg\cdot H_2P_2O_7$ and $Ca\cdot H_2P_2O_7$ complexes. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate, whereas the pyrophosphate geometry does not change appreciably.

The $Mg\cdot H_2P_2O_7$ complex has previously been studied with *ab initio* methodology,[7,8] and the structures determined in this present study match them well.

The most notable differences between the $Mg\cdot H_2P_2O_7$ and $Ca\cdot H_2P_2O_7$ equilibrium geometries are the oxygen−metal bond distances. The Ca·O bond distances are consistently longer than the Mg·O bonds by ≈0.4 Å. This value corresponds to the difference in the mean radii of the first hydration shells of calcium and magnesium ions.[24] The overall structure of the $Ca\cdot H_2P_2O_7$ complex can be viewed as a version of the $Mg\cdot H_2P_2O_7$ complex in which the metal is further distanced from the PP_i.

The RMS difference between the $Mg\cdot H_2P_2O_7$ and the $Ca\cdot H_2P_2O_7$ complexes is 0.45 Å. The displacement of the heavier calcium from the PP_i relative to magnesium's position in its complex contributes the most to this difference: When only the PP_i portions of the two complexes are considered, the RMS difference is just 0.12 Å.

The isomerization products of the calcium− and magnesium−pyrophosphates, $PO_3\cdot Mg\cdot H_2PO_4$ and $PO_3\cdot Ca\cdot H_2PO_4$, are also structurally similar (RMS = 0.38 Å). Their equilibrium geometries are depicted in Figure 2. The two hydrogens remain attached to the moiety that becomes the orthophosphate coordinated to the central metal by the two proton-free oxygens. The metaphosphate is also coordinated to the central metal by two oxygens so that the metal has four total ligands in a tetrahedral configuration. As in the metal−pyrophophate complexes, the Ca·O bond distances are longer in the isomerization products than are the Mg·O bonds (≈0.4 Å); this corresponds again to the difference in the mean radii of their first hydration shells.

The most significant differences between the neutral calcium− and magnesium−pyrophosphates are evidenced by their calculated isomerization energies. Due to a very large contribution of the correlation effects, the isomerization of the $Ca\cdot H_2P_2O_7$

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[28] The Extensible Computational Chemistry Environment Basis Set Database, Version 1.0, has been developed and distributed by the Molecular Science Computing Facility, Environmental and Molecular Sciences Laboratory which is part of the Pacific Northwest Laboratory, P.O. Box 999, Richland, WA 99352, and is funded by the U.S. Department of Energy. The Pacific Northwest Laboratory is a multiprogram laboratory operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC06-76RLO 1830. Contact David Feller, Karen Schuchardt, or Don Jones for further information.

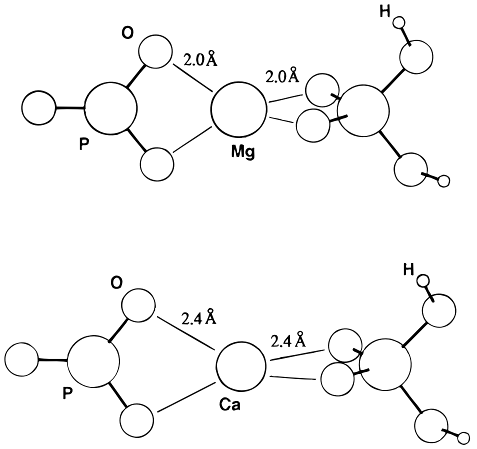

Figure 2. Products of the isomerizations of the neutral complexes. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

Table 1. Calculated Isomerization Reaction Enthalpies, Entropies, and Free Energies at T =298.15 K for the Neutral Complexes[a]

reaction	ΔE_e^{SCF}	ΔE_e^{MP2}	ΔE_{ZPE}^{SCF}	$\Delta H°$	$\Delta S°$	$\Delta G°$
$Ca{\cdot}H_2P_2O_7 \rightarrow PO_3{\cdot}Ca{\cdot}H_2PO_4$	−11.6	28.9	−1.8	16.9	15.4	12.3
$Mg{\cdot}H_2P_2O_7 \rightarrow PO_3{\cdot}Mg{\cdot}H_2PO_4$	−10.5	0.2	−1.4	−10.6	11.4	−14.0

[a] All energies and enthalpies are in kcal/mol, and entropies are in cal/(mol K). The ΔE_e^{SCF} was computed at the SCF//SCF level of theory. The ΔE_e^{MP2} is the contribution to correlation energy calculated at the MP2//SCF level of theory. The ΔE_{ZPE}^{SCF} and ΔS were computed within the harmonic approximation. The enthalpies $\Delta H°$ include a thermal correction for $T = 298.15$ K.

complex is endothermic, whereas the isomerization of the $Mg{\cdot}H_2P_2O_7$ complex is exothermic. Moreover, the gas-phase isomerization of $Mg{\cdot}H_2P_2O_7$ is spontaneous, but the isomerization of the $Ca{\cdot}H_2P_2O_7$ complex is not. The isomerization reaction enthalpies, entropies, and free energies are summarized in Table 1.

3.2. Monoanionic Complexes. The equilibrium geometries of the $[Mg{\cdot}HP_2O_7]^-$ and $[Ca{\cdot}HP_2O_7]^-$ complexes determined with the SCF level of theory are structurally alike; they are also similar to the geometries of the neutral complexes:

- the bridging P−O bond of the proton-free moiety is longer than the other (1.7 Å *vs* 1.6 Å) and the $\rangle P{-}O{-}P\langle$ linkage is bent (133° for $[Mg{\cdot}HP_2O_7]^-$ and 138° for $[Ca{\cdot}HP_2O_7]^-$);
- the six nonbridging oxygens are in a staggered arrangement as viewed along an axis connecting the phosphorous atoms;
- the metal is coordinated to three oxygens, one of which is located within the moiety containing the hydrogen, and lies in the plane containing the bent $\rangle P{-}O{-}P\langle$ linkage.

The equilibrium geometries are depicted in Figure 3.

The calculated RMS difference between the equilibrium geometries of the $[Mg{\cdot}HP_2O_7]^-$ and $[Ca{\cdot}HP_2O_7]^-$ complexes is 0.35 Å, the most notable structural differences being the shift of the center of mass due to the heavier calcium and consistently longer Ca•O bond distances relative to the Mg•O bonds by ≈0.3 Å. These differences constitute the main contribution to the value of the RMS difference. The calculated RMS difference between the PP_i portions of the complexes is 0.15 Å.

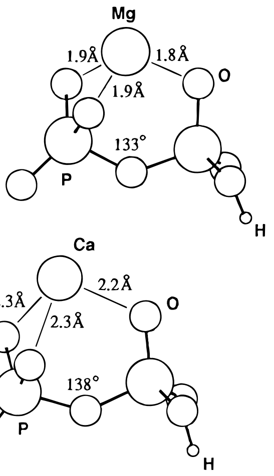

Figure 3. $[Mg{\cdot}HP_2O_7]^-$ and $[Ca{\cdot}HP_2O_7]^-$ complexes. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate. The $\rangle P{-}O{-}P\langle$ angle is consequently larger for the complex with calcium.

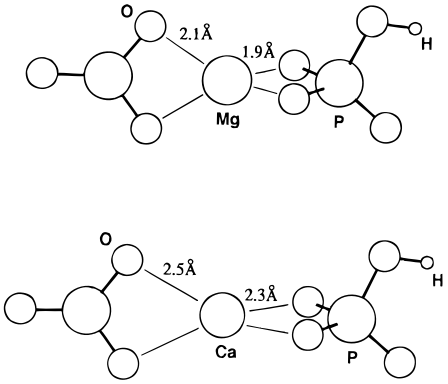

Figure 4. Products of the isomerizations of the monoanionic complexes. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

The isomerization products are also structurally similar (RMS = 0.37 Å). The equilibrium geometries are depicted in Figure 4. The hydrogen remains attached to the moiety that becomes the orthophosphate coordinated to the central metal by the two proton-free oxygens. The central metal also coordinates the metaphosphate by two oxygens, to attain a total of four ligands in a tetrahedral configuration. The Ca•O bond distances in the isomerization products are ≈0.3 Å longer than the analogous Mg•O bonds. Once again, the behavior of the cation−oxygen distances is the same as that found for the hydration shells of the metal cation, though the difference is somewhat shorter.

As for the neutral complexes, the calculated isomerization energies resulted significantly differerent: The isomerization of the $[Ca{\cdot}HP_2O_7]^-$ complex is endothermic and nonspontaneous, whereas the isomerization of the $[Mg{\cdot}HP_2O_7]^-$ complex is exothermic and spontaneous. The corresponding enthalpies, entropies, and free energies are summarized in Table 2.

3.3. Dianionic Complexes. Several equilibrium geometries for the $[Mg{\cdot}P_2O_7]^{2-}$ and $[Ca{\cdot}P_2O_7]^{2-}$ complexes were determined with the SCF level of theory, indicating conformation isomerization. In addition to the conformers in which the six nonbridging oxygens are in a staggerred arrangement as viewed along an axis connecting the phosphorous atoms (similar to the equilibrium geometries of the neutral and monoanionic complexes), other equilibrium geometries were found in which the six nonbridging oxygens are in an eclipsed arrangement. The staggered and eclipsed conformers of $[Mg{\cdot}P_2O_7]^{2-}$ and $[Ca{\cdot}P_2O_7]^{2-}$ are depicted on Figures 5 and 6, respectively.

The staggered dianionic complexes of the calcium− and magnesium−pyrophosphates share several common structural features:

Table 2. Calculated Isomerization Reaction Enthalpies, Entropies, and Free Energies at $T = 298.15$ K for the Monoanionic Complexes[a]

reaction	ΔE_e^{SCF}	ΔE_e^{MP2}	ΔE_{ZPE}^{SCF}	$\Delta H°$	$\Delta S°$	$\Delta G°$
$[Ca \cdot H_2P_2O_7]^- \rightarrow [PO_3 \cdot Ca \cdot H_2PO_4]^-$	−12.6	41.4	−1.2	28.7	10.7	25.5
$[Mg \cdot H_2P_2O_7]^- \rightarrow [PO_3 \cdot Mg \cdot H_2PO_4]^-$	−5.5	−2.3	−1.1	−7.9	8.6	−10.5

[a] All energies and enthalpies are in kcal/mol, and entropies are in cal/(mol K). The ΔE_e^{SCF} was computed at the SCF//SCF level of theory. The ΔE_e^{MP2} is the contribution to correlation energy calculated at the MP2//SCF level of theory. The ΔE_{ZPE}^{SCF} and ΔS were computed within the harmonic approximation. The enthalpies $\Delta H°$ include a thermal correction for $T = 298.15$ K.

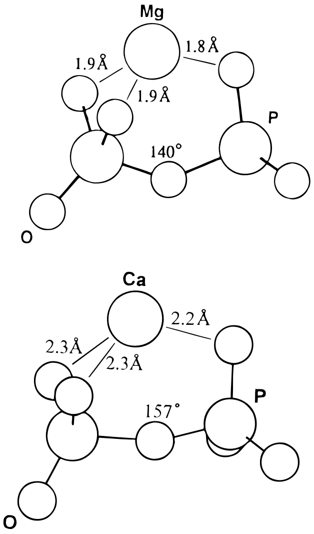

Figure 5. Staggered equilibrium geometries of the $[Mg \cdot P_2O_7]^{2-}$ and $[Ca \cdot P_2O_7]^{2-}$ complexes. The absence of intramolecular hydrogen-bonding allows for larger ⟩P−O−P⟨ angles. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

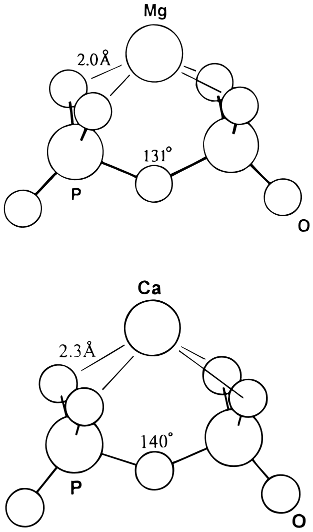

Figure 6. Eclipsed equilibrium geometries of the $[Mg \cdot P_2O_7]^{2-}$ and $[Ca \cdot P_2O_7]^{2-}$ complexes. The tighter coordination induces a bent on the ⟩P−O−P⟨ angles, as compared to the staggered configurations. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

- the six nonbridging oxygens are in a staggered arrangement as viewed along an axis connecting the phosphorous atoms;
- one of the bridging P−O bonds is longer than the other (1.7 Å *vs* 1.6 Å), and the ⟩P−O−P⟨ linkage is bent (140° for $[Mg \cdot P_2O_7]^{2-}$ and 157° for $[Ca \cdot P_2O_7]^{2-}$);
- the metal-ion is coordinated to three oxygens.

Table 3. Calculated Conformer Interconversion Reaction Enthalpies, Entropies, and Free Energies at $T = 298.15$ K[a]

complex	ΔE_e^{SCF}	ΔE_e^{MP2}	ΔE_{ZPE}^{SCF}	$\Delta H°$	$\Delta S°$	$\Delta G°$
$Ca \cdot P_2O_7^{2-}$ staggered → eclipsed	−11.7	4.8	0.5	−7.3	−4.7	−5.9
$Mg \cdot P_2O_7^{2-}$ staggered → eclipsed	3.0	−2.9	0.1	0.0	−3.1	0.9
$PO_3 \cdot Ca \cdot PO_4^{2-}$ 2 × 2 → 1 × 3	−12.3	−0.8	−0.1	−13.1	6.3	−15.0
$PO_3 \cdot Ca \cdot PO_4^{2-}$ 2 × 3 → 1 × 3	−2.8	−0.4	0.0	−3.2	4.9	−4.7
$PO_3 \cdot Mg \cdot PO_4^{2-}$ 2 × 2 → 1 × 3	−6.3	1.3	−0.2	−5.0	6.7	−7.0

[a] All energies and enthalpies are in kcal/mol, and entropies are in cal/(mol K). The ΔE_e^{SCF} was computed at the SCF//SCF level of theory. The ΔE_e^{MP2} is the contribution to correlation energy calculated at the MP2//SCF level of theory. The ΔE_{ZPE}^{SCF} and ΔS were computed within the harmonic approximation. The enthalpies $\Delta H°$ include a thermal correction for $T = 298.15$ K.

It is worthwhile to note that, in the $[Mg \cdot P_2O_7]^{2-}$ complex, the metal lies in the plane containing the ⟩P−O−P⟨ linkage. However, the equilibrium geometry of the staggered $[Ca \cdot P_2O_7]^{2-}$ complex has a structure where the metal does not lie in the plane containing the ⟩P−O−P⟨ linkage.

The calculated RMS differrence between the staggered conformers of $[Mg \cdot P_2O_7]^{2-}$ and $[Ca \cdot P_2O_7]^{2-}$ is 0.34 Å. The most notable structural differences occur in the angles of the ⟩P−O−P⟨ linkage, 17° larger for the latter, and in the metal−oxygen bond distances, ≈0.4 Å longer also for the latter. In this case, exclusion of the metal atoms does not reduce the RMS difference; it is even slightly larger for the PP_i portions of the complexes: 0.35 Å.

The $[Mg \cdot P_2O_7]^{2-}$ and $[Ca \cdot P_2O_7]^{2-}$ complexes also exhibit some basic structural differences from their monoanionic and neutral counterparts. In the neutral and monoanionic magnesium−pyrophosphates, the elongated P−O bond occurs opposite the moiety coordinated by two oxygens, whereas in the dianionic complexes it occurs opposite the moiety coordinated by one oxygen. The unique structure of the $[Mg \cdot P_2O_7]^{2-}$ complex compared to its neutral and monoanionic counterparts was also noted in an earlier study.[8]

The staggered dianionic $Ca \cdot PP_i$ and $Mg \cdot PP_i$ complexes were found to isomerize to their eclipsed conformers by rotation of one PO_3 group relative to the other PO_3 group. The conformational isomerization reaction enthalpies, entropies, and free energies are summarized in Table 3. The interconversion of the staggered and eclipsed conformers of $[Mg \cdot P_2O_7]^{2-}$ is almost thermoneutral, with the staggered conformer being slightly more favorable energetically. However, the $[Ca \cdot P_2O_7]^{2-}$ complex favors the eclipsed conformer by about 5 kcal/mol.

The eclipsed calcium− and magnesium−pyrophosphate dianionic complexes also share several common structural features with each other:

- the six nonbridging oxygens are in an eclipsed arrangement as viewed along an axis connecting the phosphorous atoms;
- the P−O bonds of the ⟩P−O−P⟨ linkage are essentially equal in length (≈1.7 Å), and the linkage itself is bent (131° for $[Mg \cdot P_2O_7]^{2-}$ and 140° for $[Ca \cdot P_2O_7]^{2-}$);
- the metal is coordinated to four oxygens, and the metal lies in the plane formed by the bent ⟩P−O−P⟨ linkage.

The RMS difference between the structure of the eclipsed $[Mg \cdot P_2O_7]^{2-}$ and $[Ca \cdot P_2O_7]^{2-}$ conformers is 0.25 Å the primary

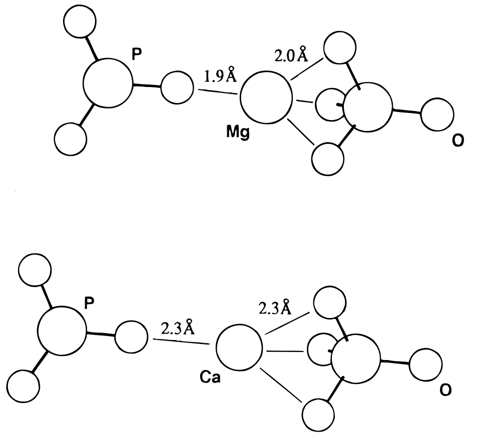

Figure 7. The global minima found for the products of the isomerizations of the dianionic complexes: the 1 × 3 configurations. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

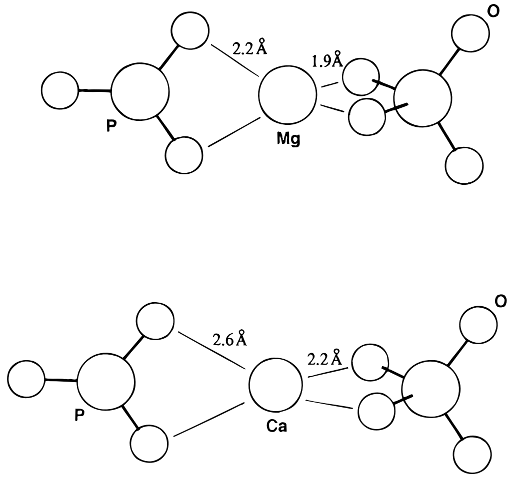

Figure 8. Products of the isomerizations of the di-anionic complexes, in the 2 × 2 configurations. The structural differences stem from the distinct coordination properties of the cations, reflected in the distances to the oxygens they coordinate.

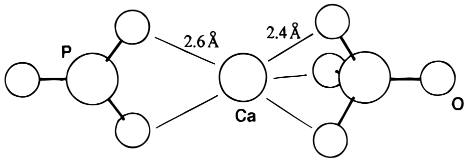

Figure 9. The 2 × 3 equilibrium configuration is unique to the complex with calcium, reflecting the "looser" coordination of Ca^{2+}, compared to that of Mg^{2+}.

Table 4. Calculated Isomerization Reaction Enthalpies, Entropies, and Free Energies at $T = 298.15$ K for the Dianionic Complexes[a]

reaction	ΔE_e^{SCF}	ΔE_e^{MP2}	ΔE_{ZPE}^{SCF}	$\Delta H°$	$\Delta S°$	$\Delta G°$
$[Ca{\cdot}P_2O_7]^{2-}_{st} \rightarrow [PO_3{\cdot}Ca{\cdot}PO_4]^{2-}_{1\times3}$	−7.8	81.9	−0.9	73.4	16.7	68.4
$[Mg{\cdot}P_2O_7]^{2-}_{st} \rightarrow [PO_3{\cdot}Mg{\cdot}PO_4]^{2-}_{1\times3}$	5.9	−2.0	−1.3	3.7	15.6	−1.0
$[Ca{\cdot}P_2O_7]^{2-}_{st} \rightarrow [PO_3{\cdot}Ca{\cdot}PO_4]^{2-}_{2\times2}$	4.5	82.7	−1.3	86.6	15.4	82.0
$[Mg{\cdot}P_2O_7]^{2-}_{st} \rightarrow [PO_3{\cdot}Ca{\cdot}PO_4]^{2-}_{2\times2}$	12.2	−3.4	−1.2	86	12.1	5.0
$[Ca{\cdot}P_2O_7]^{2-}_{ec} \rightarrow [PO_3{\cdot}Ca{\cdot}PO_4]^{2-}_{2\times3}$	6.7	77.5	−1.4	84.4	17.0	79.3

[a] All energies and enthalpies are in kcal/mol, and entropies are in cal/(mol K). The ΔE_e^{SCF} was computed at the SCF//SCF level of theory. The ΔE_e^{MP2} is the contribution to correlation energy calculated at the MP2//SCF level of theory. The ΔE_{ZPE}^{SCF} and ΔS were computed within the harmonic approximation. The enthalpies $\Delta H°$ include a thermal correction for $T = 298.15$ K.

differences being the ⟩P−O−P⟨ linkage angles, 9° larger for the latter, and the longer Ca·O bonds (by ≈0.3 Å). The RMS difference of the pyrophosphate portions alone has a much lower value of 0.12 Å.

Several conformers were also found in the isomerization products of the dianionic complexes. Two conformers for $[PO_4{\cdot}Mg{\cdot}PO_3]^{2-}$ and three conformers for $[PO_4{\cdot}Ca{\cdot}PO_3]^{2-}$ were located. The two conformers for the magnesium complex are structurally analogous to two of the calcium-complex conformers. These two shared structures involve the central metal in coordination to four oxygens but with varying contributions from the metaphosphate and orthophosphate moieties (see Figures 7 and 8). The first structure, denoted by "1 × 3", is coordinated by one oxygen from the metaphosphate moiety and three oxygens from the orthophosphate. It is the global minimum for both cations, and in the case of magnesium it corresponds to the geometry found previously.[8] The second structure, denoted by "2 × 2", is coordinated by two oxygens from both of the metaphosphate and orthophosphate moieties. The calcium containing complexes consistently have metal−oxygen bonds ≈0.3 Å longer than those of the magnesium complexes, again resembling the difference between the hydrated ions mean-radii. The calculated RMS differences between the calcium and magnesium complexes for both the 1 × 3 and 2 × 2 conformers are 0.37 Å. The unique third conformer of $[PO_4{\cdot}Ca{\cdot}PO_3]^{2-}$ has five coordinating oxygens: two from the metaphosphate moiety and three from the orthophosphate moiety (see Figure 9). It is denoted by "2 × 3". The relative energies are presented in Table 3.

The multitude of conformers found for the dianionic metal−pyrophosphates and their isomerization products slightly complicates determination of reaction energies. However, both the 1 × 3 and 2 × 2 isomerization product conformers were assumed to arise from staggered calcium− and magnesium−pyrophosphates reactants. All isomerizations are predicted to be endothermic. The only one predicted to be spontaneous is the reaction from staggered $[Mg{\cdot}P_2O_7]^{2-}$ to the 1 × 3 conformer, $\Delta G° = -1.0$ kcal/mol, in disagreement with a previous value of[8] 1.8 kcal/mol. However, both numerical values are in the limits of accuracy of the method, so the theoretical prediction is still consistent: the reaction should be reversible. The most significant differences found between the dianionic calcium− and magnesium−pyrophosphates are their calculated isomerization energies: Though at the SCF//SCF level of the theory they have similar values, the very large difference in correlation energy at the MP2//SCF level predicts a very unfavorable isomerization of $[Ca{\cdot}P_2O_7]^{2-}$. The isomerization reaction enthalpies, entropies, and free energies are summarized in Table 4.

4. Discussion

Although the hydrolysis of pyrophosphates in living cells is a complicated process mediated by enzymes under very controlled conditions, an attempt to model this reaction with the use of theoretical chemistry tools can provide revealing insight into the molecular mechanism of the destruction and formation of the diphosphate bond. The *ab initio* theoretical approach is particularly useful in characterizing the effects of interactions between distinct molecular systems and functional groups involved in the hydrolysis process because it permits isolation of the reactants from other reaction participants. The isomerization reactions (eqs 5−7) for the neutral, anionic, and dianionic complexes were chosen to evaluate differences between the effect of complexation of pyrophosphate with magnesium and calcium. These isomerizations probably represent the rate-determining step for pyrophosphate hydrolysis because metaphosphate readily reacts with aqueous media to yield orthophosphate.[32]

Theoretical studies of *in vacuo* anionic species should be carefully reviewed because anions can have low ionization potentials and can be unstable with respect to the lower charged, or neutral, forms. In an *ab initio* calculation, the use of a localized basis set (*e.g.*, LCAOs) runs the risk of confining excess electron density within the molecular frame when it would otherwise occupy diffuse orbitals indicating an ejected electron(s). Previously published *ab initio* calculations[8] that included diffuse functions in the basis set have predicted that otherwise unstable pyrophosphate anions in the gas-phase are stabilized by complexation to magnesium. This prediction was based on the value of the HOMO eigenvalues. In this current round of *ab initio* calculations on magnesium− and calcium−pyrophosphate isomerizations, the gas-phase stability of the dianions $Mg{\cdot}P_2O_7^{2-}$ and $Ca{\cdot}P_2O_7^{2-}$ was confirmed by performing UHF//UHF/6-31+G**(H,O,P),pVDZ+(Mg,Ca) calculations on the lower charged species. For all cases, the dianion was lower in energy than both the monoanion and neutral forms, with the following sequences (the numbers in parentheses are the relative energies, in kcal/mol):

$$\text{(staggered) } Mg{\cdot}P_2O_7^{2-}\ (0.0) < Mg{\cdot}P_2O_7^{-}\ (29.4) < Mg{\cdot}P_2O_7\ (228.6)$$

$$\text{(eclipsed) } Mg{\cdot}P_2O_7^{2-}\ (0.0) < Mg{\cdot}P_2O_7^{-}\ (13.4) < Mg{\cdot}P_2O_7\ (251.3)$$

$$\text{(staggered) } Ca{\cdot}P_2O_7^{2-}\ (0.0) < Ca{\cdot}P_2O_7^{-}\ (3.3) < Ca{\cdot}P_2O_7\ (218.0)$$

$$\text{(eclipsed) } Ca{\cdot}P_2O_7^{2-}\ (0.0) < Ca{\cdot}P_2O_7^{-}\ (35.7) < Ca{\cdot}P_2O_7\ (218.0)$$

It is therefore physically meaningful to study the isomerization of metal-pyrophosphate anions *in vacuo* to gain insight into the reaction mechanism of hydrolysis.

Some further validation of the use of *in vacuo* results for insight into biological system behavior is provided by evidence of a relatively anhydrous microenvironment within the active site of the enzyme.[29,30] However, it should be kept in mind that the active site creates an electrostatic field and that thermal and electronic fluctuations can account for the lowering of the activation energy of hydrolysis, or synthesis, of pyrophosphate. Since no structure for the membrane-bound *Rhodospirillum rubrum* chromatophore is known, the structure of its active site and the electrostatic field it imparts are unknown. In this same vein, acid dissociation constants for aqueous pyrophosphate indicate that the $Mg{\cdot}P_2O_7^{2-}$ complex is the most abundant at the pH levels of biological systems. Nevertheless, it has recently been proposed that the true substrate is the neutral $Mg_2{\cdot}P_2O_7$ complex. This proposal is based on the high resolution (2.0 Å) X-ray determined structure of a soluble pyrophosphate from the budding yeast *Saccharomyces cerevisae*.[31] Furthermore, the corresponding mechanism of hydrolysis should involve two more metal ions and several water molecules in an arrangement quite different from bulk water. The theoretical approach can yield valuable information to discriminate the effects of coordination by two metal ions, or the effects of substituting the cation, as in the present study.

Structural changes were found to occur in the equilibrium geometries for the cation−pyrophosphate complexes and their isomerization products when calcium replaced magnesium. The structural differences can be generalized by noting that magnesium exhibited "tighter" coordination. That is, magnesium was found to consistently form shorter bonds with the coordinating oxygen ligands; this is consistent with the differences in the first hydration shells of calcium and magnesium.[24] The longer Ca•O bonds, which can be attributed to calcium's larger ionic radius, resulted in distortion of the PP_i chelator relative to its structure in the magnesium−pyrophosphates. However, the distortion affected by the calcium upon PP_i was moderated by hydrogen-bonding in the neutral and monoanionic complexes; this is evidenced by the greater divergence in $\rangle P{-}O{-}P\langle$ linkage angles between complexes of calcium and magnesium with fewer, or no, hydrogens avaliable to form weak bonds ($\Delta\angle P{-}O{-}P = 1^{\circ}_{\text{neutral}}, 5^{\circ}_{\text{mono}}, 17^{\circ}_{\text{di}}$). The stabilization of the pyrophosphate structure by hydrogen bonding is also apparent in the computed RMS differences of the PP_i portion of the complexes ($RMS_{\text{neutral}} = 0.12$ Å, $RMS_{\text{mono}} = 0.15$ Å, $RMS_{\text{di}} = 0.35$ Å).

Because one of our most important findings, the difference in free energies between the isomerizations of Mg−pyrophosphates and Ca−pyrophosphates is due to the differences in the correlation contributions; further calculations were performed to check the validity of the results. To confirm the convergence of the correlation energy, we performed calculations at the third (MP3//SCF) and fourth (MP4SDQ//SCF) orders of the perturbation theory for the SCF-optimized complexes with calcium. The amount of correlation captured at the MP3 and MP4SDQ levels resulted a small fraction of that captured at the MP2 level (see Supporting Information). To evaluate the cause of the differing extent of correlation energy captured at the MP2//SCF level, the geometries of the isomerization reactants and products of one of the dianionic conformers

$$[M{\cdot}P_2O_7]^{2-}_{\text{stag}} \rightarrow [PO_3{\cdot}M{\cdot}PO_4]^{2-}_{1\times 3}, \quad \text{where } (M = Mg, Ca)$$

were reoptimized at the MP2 level; these dianionic systems were examined because their electron-rich nature amplifies correlation energy effects. The amount of correlation energy captured at the MP2//MP2 level for the optimized geometries of the complexes with both calcium and magnesium are approximately the same (see Supporting Information). The computed (SCF+MP2)//MP2 isomerization energies for the dianionic complexes exhibit the same trend as those computed at the (SCF+MP2)//SCF level: The numerical value for the complex with magnesium does not change appreciably (2.8 kcal/mol *vs* the previous 3.9 kcal/mol) and for the complex with calcium it is still positive and large, though it becomes significantly smaller (30.4 kcal/mol *vs* the previous 74.1 kcal/mol). In terms of structure, the MP2-optimized geometries have the anticipated longer bond lengths than do the SCF-optimized geometries.

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What is significant is that the metal−oxygen bonds in the calcium complexes are elongated by ≈0.1 Å, while those in the magnesium complexes are elongated by less than half that amount (less than 0.05 Å). In other words, the SCF and MP2 equilibrium geometries for the magnesium complexes more closely coincide in structure than for the calcium complexes. The correlation energy for the isomerization products of the latter is smaller than for the other computed complexes.

From the amount of correlation energy captured, it is not immediately clear whether the MP2 method is equally applicable to both the magnesium− and the calcium−pyrophosphate system. However, the norm of the correction to the wave function for the calcium and magnesium complexes are very similar in value indicating that the second-order method should be reliable in comparing the two systems.

To further confirm that the MP2//SCF results are sufficiently accurate to enable conclusions to be drawn, various basis sets were used to perform MP2 single-point calculations for all calcium complexes. The contributions of correlation to their isomerization energies are consistently large and positive (see Supporting Information).

Thus we can have confidence on the most significant differences found between the studied calcium−pyrophosphates and their magnesium counterparts, their isomerization reaction enthalpies and energies, significantly higher for the former. Therefore the isomerizations of calcium−pyrophosphates are highly endothermic and nonspontaneous in the gas-phase, whereas the isomerizations of magnesium−pyrophosphates are more likely to occur.

As previously stated, the structural differences between the complexes of calcium and magnesium are dominated by the inherent coordination properties of their ions; these coordination properties are maintained in all complexes, independently of the pyrophosphate's charge. Therefore, it can be expected that they will prevail even within the active site of the enzyme. Even though the $Ca{\cdot}PP_i$ complexes may be structurally similar to the $Mg{\cdot}PP_i$ complexes, the differences in coordination seem to determine the differences in the energies of the isomerization reactions.

These results reveal that the proposed divalent metal cation assisted reaction mechanism for the gas-phase isomerization and subsequent hydrolysis of the pyrophosphate anions (*i.e.*, reactions 1−4) are consistent with observed pyrophosphatase selectivity.

Acknowledgment. This research was supported by the NSF, U.S.A.−CONACyT, Mexico, Cooperative Science Program, under Grant INT-931326, and by DGAPA-UNAM, Grant IN-112896. Part of the calculations were performed with the computers of DGSCA-UNAM.

Supporting Information Available: Tables with Hartree-Fock, MP2, MP3, and MP4SDQ energies and ΔE^{MP2} computed with various basis sets (12 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA972715G