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Where is Electronic Energy Stored in Adenosine Triphosphate?[†]

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The gas-phase electronic energy of the hydrolysis of methyl triphosphate, a model of adenosine 5′-triphosphate (ATP), is partitioned into local (atomic and group) contributions. A modified definition of Lipmann's "group transfer potential" is proposed on the basis of the partitioning of the total electronic energy into atomic contributions within the framework of the quantum theory of atoms in molecules (QTAIM). The group transfer potential is defined here as the sum of the atomic energies forming the group in ATP minus the sum of the energies of the same atoms in inorganic phosphate. It is found that the transfer potential of the terminal phosphate group in ATP is significantly reduced, from +241.7 to +73.1 kcal/mol, as a result of complexation with magnesium. This is accompanied by a concomitant change in the energy of reaction from −168.6 to −24.9 kcal/mol. Regions within ATP where the electronic energy changes the most upon hydrolysis are identified. The study is conducted at the DFT/B3LYP/6-31+G(d,p) level of theory.

Introduction

Phosphorylated nucleotides such as adenosine 5′-triphosphate (ATP) and guanosine 5′-triphosphate (GTP) have long been identified as "energy-rich" biological molecules.[1,2] These molecules are used by all living cells to store chemical energy in the so-called "high energy phosphate bonds" symbolized by P∼O.[1−4] The metabolic energy released from the oxidation of glucose (for example) is captured in the form of internal energy that is "distributed" and stored in several ATP molecules.[3] This energy, released during the hydrolysis of ATP, drives otherwise nonspontaneous biochemical reactions to completion, lending the designation of "energy currency of all living cells" to phosphorylated nucleotides.

When cellular energy reserves are low, the terminal P∼O bond in ATP is hydrolyzed to yield adenosine diphosphate (ADP) and inorganic phosphate (P_i):

$$\mathrm{ATP}^{4-}+\mathrm{H_2O}\rightarrow\mathrm{ADP}^{3-}+\mathrm{H_2PO_4}^{-} \qquad (1)$$

The net Gibbs energy change at standard conditions (ΔG^0) when reaction (1) takes place as written is −7 to −8 kcal/mol.[5]

The triphosphate tail of ATP exists predominantly in its fully deprotonated (anionic) form in aqueous medium at or near physiological pH (ca. 7.4), a form highly stabilized by solvation effects. Møller−Plesset second order perturbation calculations (MP2) used in conjunction with the polarized continuum solvation model (PCM) predict a solvation energy as high as −441.9 kcal/mol for the diphosphate anion ($HP_2O_7^{3-}$), an energy consistent in magnitude with semiempirical calculations and empirical estimates.[6] Moreover, discrete solvation calculations at the density functional theory (DFT/B3LYP) level on mononucleotides indicate a stabilization energy of −11.5 to −8.3 kcal/mol per water molecule in the first solvation shell.[7] Each mononucleotide is capable of accommodating up to four water molecules near the singly protonated phosphate group at 300 K, as revealed by mass spectrometry.[7] The inclusion of solvation effects is therefore necessary to reproduce experimental ΔG^{o} values.[5] However, this is *not* what this paper is about.

In the expression $\Delta G = \Delta H - T\Delta S = (\Delta E + \Delta E_{\mathrm{ZPE}} + \Delta E_{289\mathrm{K}}) - T\Delta S$, the term ΔE (the vibrationless 0 K electronic energy) is dominant in the case of reactions of pyrophosphates and their magnesium complexes.[8] It is this term, ΔE of the hydrolysis of ATP in the gas-phase (eq 1), which is brought to the fore in this work.

The present paper examines an atom-by-atom partitioning of the global ΔE of hydrolysis of ATP. In other words, this study aims at pinpointing the atoms or regions in the reacting system experiencing the largest changes in energy during the reaction.

The role of Mg^{2+} in changing the global and local electronic contributions to the energy of hydrolysis of ATP is examined in this paper since this metal cofactor is present in ATPase enzymes.[8−14] One of the goals of the paper is, thus, to address the question of how complexation with Mg^{2+} influences the contribution of each atom $\Delta E(\Omega)$ to the energy of hydrolysis (in the gas phase). This work will be extended in the future by including solvent effects and thermodynamic corrections, an extension necessary before comparisons with experimental ΔG^{o} can be made.

Atomic Contributions to the Energy of Reaction

We have recently studied atomic contributions to the bond dissociation energies (BDE) of C−C and C=C bonds in hydrocarbons[15] as well as the P−O(H) bond in HPO_4^{2-}.[16] The contribution of an atom to the BDE is defined as the energy of an atom in the dissociation products minus the energy of the same atom in the intact molecule.[15,16] This definition is extended to an atomic contribution to the (electronic) energy of reaction as

$$\Delta E(\Omega) = E(\Omega)_{\mathrm{products}} - E(\Omega)_{\mathrm{reactants}} \qquad (2)$$

where $\Delta E(\Omega)$ is the change in the energy of a particular atom Ω in a molecule, while $E(\Omega)_{\mathrm{reactant}}$ and $E(\Omega)_{\mathrm{products}}$ are the

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energies of this atom in the reactants and the products, respectively. The energy of reaction is thus the sum of these atomic contributions

$$\Delta E_{\text{electronic(0K)}} = \sum_{\Omega} \Delta E(\Omega) \qquad (3)$$

where the subscript in $\Delta E_{\text{electronic (0 K)}}$ will be dropped henceforth to read ΔE for simplicity.

A key step in the above approach is the definition of the energy of an atom in a molecule, $E(\Omega)$, a quantity readily calculated within the framework of the quantum theory of atoms in molecules (QTAIM).[17−19] In order to make this paper self-contained we present a brief summary of some key concepts of this theory.

Brief Summary of Some Concepts of QTAIM

QTAIM takes the electron density $\rho(\mathbf{r})$ as a starting point and focuses on the properties of atoms in a molecule such as charge; dipole or higher multipole moments; volume; total, kinetic, and potential energy; polarizability and magnetizability tensor; etc.

Bonding is present when a line of locally maximum electron density, termed a "bond path", links the nuclei of two atoms in real 3D space. The collection of bond paths in a molecule defines its molecular graph. The nature and strength of chemical bonding is reflected in the properties of the electron density and energy densities (kinetic [$G(\mathbf{r})$], potential [$V(\mathbf{r})$], and total [$H(\mathbf{r}) = G(\mathbf{r}) + V(\mathbf{r})$]) evaluated at the bond critical point (BCP), the point of minimum electron density along the bond path.

The electron density near a nucleus is characterized by the topography of its associated gradient vector field $\nabla\rho(\mathbf{r})$. The gradient vector field lines terminating at a particular nucleus constitute an "atomic basin" which, along with its associated nucleus, is defined as an atom in a molecule.

An atomic electron population can be obtained by integrating the electron density over the atomic basin

$$N(\Omega) = \int_{\Omega} \rho(\mathbf{r})\, \mathrm{d}\mathbf{r} \qquad (4)$$

which can be used to obtain a corresponding atomic charge by subtracting $N(\Omega)$ from the nuclear charge Z_{Ω}

$$q(\Omega) = Z_{\Omega} - N(\Omega) \qquad (5)$$

An atomic basin is bounded by a surface that is never crossed by the gradient vectors of the electron density. The collection of such surfaces splits the molecule into nonoverlapping atoms and each is referred to as an "inter-atomic" or "zero-flux" surface, the latter designation indicating the local satisfaction of the following boundary condition:

$$\nabla\rho(\mathbf{r}) \cdot \mathbf{n}(\mathbf{r}) = 0 \quad \text{for all points } \mathbf{r} \text{ belonging to the surface } S(\Omega) \qquad (6)$$

where $\mathbf{r}$ is the position vector and $\mathbf{n}(\mathbf{r})$ the unit vector normal to the surface $S(\Omega)$ of an atom Ω in a molecule.

Bader has shown that atoms satisfying the zero-flux condition (eq 6) obey an atomic virial theorem[17] (analogous to the molecular virial theorem), i.e., $V(\Omega) = -2T(\Omega)$ where $V(\Omega)$ and $T(\Omega)$ are the potential and kinetic energies of an atom in a molecule, respectively. From this theorem and the definition of total energy [$E(\Omega) = T(\Omega) + V(\Omega)$], the energy of an atom in a molecule is the negative of its kinetic energy [$E(\Omega) = -T(\Omega)$]. This (nontrivial) result is of considerable importance since it yields the energy of an atom in a molecule (indirectly but readily) from the mere knowledge of the integral of the electronic kinetic energy over the atomic basin without having to calculate the electron−electron repulsive, nuclear−nuclear repulsive, or electron−nuclear attractive contributions, explicitly.

Since our target is to study the atomic contributions to the energy of reaction involving relatively large (realistic) models of ATP, density functional theory is the sole practical option. A conceptual problem immediately arises due to this computational limitation: The atomic virial theorem always applies in principle but is computationally only approximately satisfied if the atomic kinetic energies are calculated with the use of molecular orbitals obtained from a Hartree−Fock (HF) or post-HF calculation. In contrast, an atomic energy calculated using Kohn−Sham (KS) orbitals (at the DFT level)[20] do not satisfy the atomic virial theorem, not even in principle, since the kinetic energy (K.E.) calculated with these orbitals is the noninteracting K.E. and misses the correlation K.E. (for a complete discussion, see Appendix B of ref 16).

To test the performance of DFT, a detailed comparison between atomic contributions to the BDE in a simple phosphate has been carried out at the MP2 and DFT levels.[16] A satisfactorily agreement between MP2 and DFT/B3LYP results has been found and analyzed theoretically[16] providing a basis for the use of the latter (more economical) level in the present investigation.

Method

Calculations Details. The B3LYP[21−24] hybrid DFT functional is used in this work along with the 6-31+G(d,p) Pople basis set, since this level of theory has been shown to be adequate for this system.[8,10] A set of diffuse functions (indicated by the "+") is included in this basis set to all non-hydrogen atoms. Diffuse functions are particularly important to improve the description of the electron density in highly anionic systems.

Thus, KS "wavefunctions" were obtained at the B3LYP/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory using the Gaussian 03 package.[25] Electron densities were subsequently analyzed using AIMPAC/AIMALL97[26,27] and molecular graphs were obtain with AIM2000.[28]

The isodensity envelope chosen for the atomic volume calculations is $\rho = 0.001$ au because it corresponds to the experimental van der Waals volumes in the gas phase, and usually encloses more than 99% of the electron population of the molecule.[17] This is the same isodensity envelope up to which the atomic integrations are carried out.

The Model. ATP consists of a triphosphate tail responsible for the energy storage, and a nucleoside (ribose sugar and an attached adenine base) believed to play a role in enzyme recognition but not in energy storage.[29] Thus, ATP is modeled by a triphosphate tail capped with a terminal methyl group in place the nucleoside to reduce the computational burden. A methyl group has been chosen to cap the phosphate tail instead of a hydrogen atom to eliminate the possibility of forming hydrogen bonds between a terminal hydrogen and the phosphate tail, hydrogen bonding that may favor coiled conformations. For the sake of simplicity, methyl triphosphate and methyl diphosphate will be referred to as ATP and ADP, respectively.

The Reaction. Experiments using ^{31}P NMR suggest that the hydrolysis of ATP involves an in-line nucleophilic attack of a water molecule on the terminal phosphate with inversion of configuration of Pγ.[30] Hydrolysis, according to this mechanism (the one considered in this paper), results in the formation of two bonds (O−Pγ and Oγ1−H′) and the breaking or two other (O−H′ and Pγ−O3; Figure 1). More elaborate mechanisms such as a "multicenter proton relay mechanism" involving more than

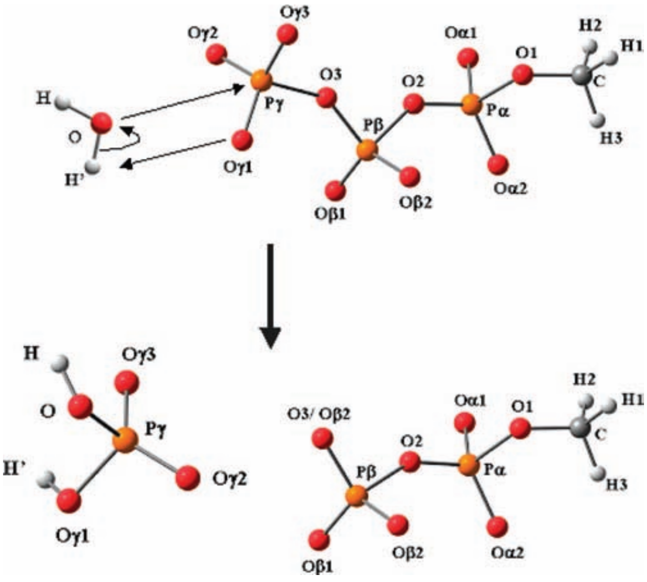

Figure 1. Numbering scheme on a ball and stick representation of the optimized geometries of the reactants and products of the model hydrolysis of ATP in the absence of Mg^{2+}.

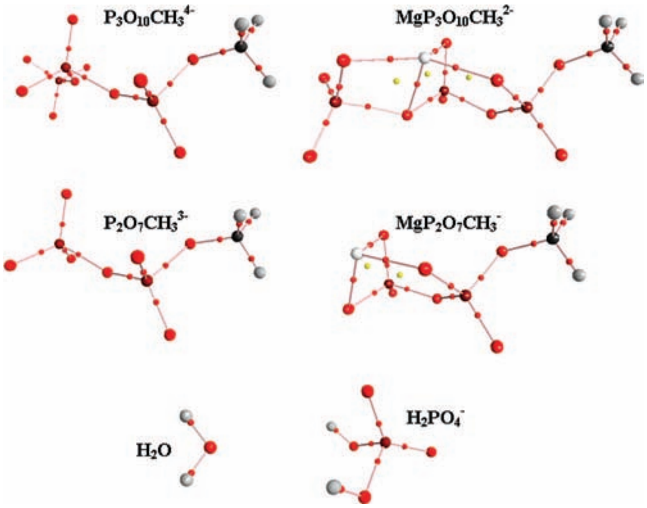

Figure 2. Molecular graphs of the species considered in this study: $P_3O_{10}CH_3^{4-}$, $MgP_3O_{10}CH_3^{2-}$, $P_2O_7CH_3^{3-}$, $MgP_2O_7CH_3^{-}$ (analogs of ATP, ATP complexed with Mg^{2+}, ADP and ADP complexed with Mg^{2+}, respectively), and $H_2PO_4^{-}$ is the inorganic phosphate. The positions of the bond critical points are indicated with the small red dots on the bond paths and those of the ring critical points by the (unconnected) yellow dots within each ring. The positions of the atomic nuclei are indicated by the spheres (P = dark red, O = red, C = black, H = gray, and Mg = white).

one water molecule have also been proposed more recently but are not considered here.[31−33]

Results and Discussion

Bond Properties and Molecular Graphs. The molecular graphs displayed in Figure 2 show that the metal is tetracoordinated in $MgATP^{2-}$ (to Oα2, Oβ1, Oγ2, and O3) but only tricoordinated in $MgADP^{-}$ (to Oα2, Oβ1, and O3/Oβ2). Table 1 lists the values of some bond properties evaluated at the BCP for the bonding interactions involving the metal. As can be seen from the table, the electron density at the BCP involving the metal in the $MgADP^{-}$ complex is very similar in all three bonds ranging from 0.052 to 0.059 au. This is consistent with a bonding of approximately equal strength (as also reflected in the bond lengths that range from 1.893 to 1.961 Å). In contrast, one of the four bonds to the metal in $MgATP^{2-}$, Mg−O3, is significantly longer and weaker than the others [bond length (BL) = 2.133 Å, ρ_{BCP} = 0.034 au], while the Mg−Oγ2 is considerably shorter (BL = 1.905 Å) and almost twice as strong (ρ_{BCP} = 0.057 au). The seven bonds listed in Table 1 exhibit the hallmarks of ionic bonding[34] since they are characterized by the following properties evaluated at the BCP: (1) Relatively small magnitudes of ρ_{BCP} and its laplacian $\nabla^2\rho_{BCP}$, potential energy density (V_{BCP}), and total energy density at the BCP (H_{BCP}); (2) $\nabla^2\rho_{BCP} > 0$ and $H_{BCP} > 0$. The ionic nature of the seven Mg−O bonding interactions is reflected in the substantial atomic charges carried by every atom involved in these seven interactions (discussed below in the section about the charge distribution).

Table 2 lists selected bond properties for the bonds along the O−P−O phosphate backbone. The entries of this table show that the metal induces a marked alternation in the bond lengths along the oxygen−phosphate backbone: The terminal O3−Pγ is (considerably) elongated while the consecutive O3−Pβ bond is shortened, etc. The observed alternation has already been noted by others [see for example refs 11 and 31]. The metal induces a 0.135 Å elongation of the O3−Pγ (from 1.826 to 1.961 Å) associated with a 0.020 au reduction in the electron density at the BCP (from 0.105 au to 0.085 au). Thus, as remarked by other authors,[31,35] the complexation with the metal is accompanied by a weakening of the terminal P−O bond, the bond which breaks in the hydrolysis reaction.

Energies of the Hydrolysis of ATP in the Absence and Presence of Mg^{2+}. All energies reported in this paper are electronic energies for motionless molecules in the gas phase at 0 K. The calculated energies of hydrolysis in absence and presence of the metal are, respectively

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{-} \qquad \Delta E = -168.6 \text{ kcal/mol} \quad (7)$$

$$MgATP^{2-} + H_2O \rightarrow MgADP^{-} + P_i^{-} \qquad \Delta E = -24.9 \text{ kcal/mol} \quad (8)$$

These results are similar to reaction energies for systems lacking one phosphate group (i.e., ADP instead of ATP, and AMP instead of ADP) calculated at the same level of theory [B3LYP/6-31+G(d,p)][10]

$$ADP^{3-} + H_2O \rightarrow AMP^{2-} + P_i^{-} \qquad \Delta E = -136.6 \text{ kcal/mol} \quad (9)$$

$$MgADP^{-} + H_2O \rightarrow \text{Mg-AMP} + P_i^{-} \qquad \Delta E = -15.6 \text{ kcal/mol} \quad (10)$$

The lower exothermicities of reactions (9) and (10) can be attributed to the lower electrostatic repulsion in the reactants since the shorter phosphate tail carries less charge in each case when compared to reactions (7) and (8), respectively.

In reactions (8) and (10), the metal significantly reduces the energy of hydrolysis in comparison with the respective metal-free reactions (7 and 9). This reduction reflects the preferential binding of Mg^{2+} to the reactant with respect to the products by 168.6 − 24.9 = 143.7 kcal/mol (in reaction 8) and by 121.0 kcal/mol (in reaction 10). One can, thus, interpret the difference of 143.7 kcal/mol in the values of ΔE of reactions (7) and (8) as the difference in the binding of the metal to the reactant (ATP^{4-}) and to the product (ADP^{3-}), i.e.,

TABLE 1: Bond Properties Evaluated at the Bond Critical Point in Analogues of $MgATP^{2-}$ and $MgADP^{-}$

bond	BL (Å)	ρ_{BCP} (au)	$\nabla^2\rho_{BCP}$ (au)	G_{BCP} (au)	V_{BCP} (au)	H_{BCP} (au)
ATP						
Mg−Oα2	1.943	0.051	0.407	0.087	−0.072	0.015
Mg−Oβ1	2.014	0.045	0.325	0.071	−0.061	0.010
Mg−Oγ2	1.905	0.057	0.476	0.103	−0.087	0.016
Mg−O3	2.133	0.034	0.220	0.049	−0.043	0.006
ADP						
Mg−Oα2	1.893	0.059	0.494	0.107	−0.090	0.017
Mg−O3/β2	1.958	0.053	0.405	0.089	−0.077	0.012
Mg−Oβ1	1.961	0.052	0.401	0.088	−0.076	0.012

TABLE 2: Bond Lengths, Bond Lengths Alternation, and Electron Densities at the Bond Critical Points in Analogues of ATP^{4-} and $MgATP^{2-}$ along the P−O−P Chain

bond	BL (Å)	Mg^{2+}-induced change in BL (Å)	BL alternation (Å)	ρ_{BCP} (au)	Mg^{2+}-induced change in ρ_{BCP} (au)
ATP^{4-}					
C−O1	1.390			0.281	
O1−Pα	1.748			0.132	
Pα−O2	1.581		−0.167	0.179	
O2−Pβ	1.812		0.231	0.109	
Pβ−O3	1.594		−0.218	0.175	
O3−Pγ	1.826		0.232	0.105	
$MgATP^{2-}$					
C−O1	1.416	0.026		0.257	−0.024
O1−Pα	1.664	−0.084		0.157	0.025
Pα−O2	1.615	0.034	−0.049	0.170	−0.009
O2−Pβ	1.720	−0.092	0.105	0.134	0.025
Pβ−O3	1.619	0.025	−0.101	0.171	−0.004
O3−Pγ	1.961	0.135	0.342	0.085	−0.020

$$ATP^{4-} + Mg^{2+} \rightarrow MgATP^{2-} \qquad \Delta E = -922.6 \text{ kcal/mol} \quad (11)$$

$$ADP^{3-} + Mg^{2+} \rightarrow MgADP^{-} \qquad \Delta E = -778.9 \text{ kcal/mol} \quad (12)$$

The stronger binding of Mg^{2+} to ATP may be attributed to its larger negative electrostatic charge than ADP.

Atomic Properties and their Changes upon Hydrolysis. Table 3 lists several atomic properties $P(\Omega)$ and their changes $\Delta P(\Omega)$ due to hydrolysis in the absence and presence of Mg^{2+} defined (similarly to eq 2) as

$$\Delta P(\Omega) = P(\Omega)_{\text{products}} - P(\Omega)_{\text{reactants}} \quad (13)$$

The listed properties include atomic electron populations $N(\Omega)$, atomic charges $q(\Omega)$, atomic energies $E(\Omega)$, and atomic volumes vol(Ω).

The table is organized in the following manner: The rows are labeled by atoms, group sums, or molecular sums. For example, data entered in the sixth row, labeled Σ_{CH_3O}, represent the sum of the properties of the atoms in the terminal methoxy group. The table is organized in four data blocks corresponding to the reactants and products of reactions (7) and (8), respectively.

Charge Distribution in the Absence and Presence Mg^{2+}. As illustrated in Figure 3 (and as can be seen from Table 3), the net charge of ATP, $q(\text{ATP}) = -4$ au, is approximately uniformly distributed among the three phosphate and the terminal methoxy groups. Each group bears approximately a unit of negative charge with a magnitude ranging from ca. 1.2 to 0.8 au with the most negative being the γ-phosphate group. Unlike the typical general biochemistry textbook depiction, the terminal γ-phosphate group does not bear a net charge of −2 au, but rather a charge slightly more negative than an atomic unit (∼ −1.2 au). The small deviation of the total charge of ATP from −4 (by 0.002 au) and other similar deviations from molecular values are due to small cumulative numerical integration errors.

The lower segment of Figure 3 and the data in Table 3 clearly show that Mg^{2+} complexed to ATP remains, to a good

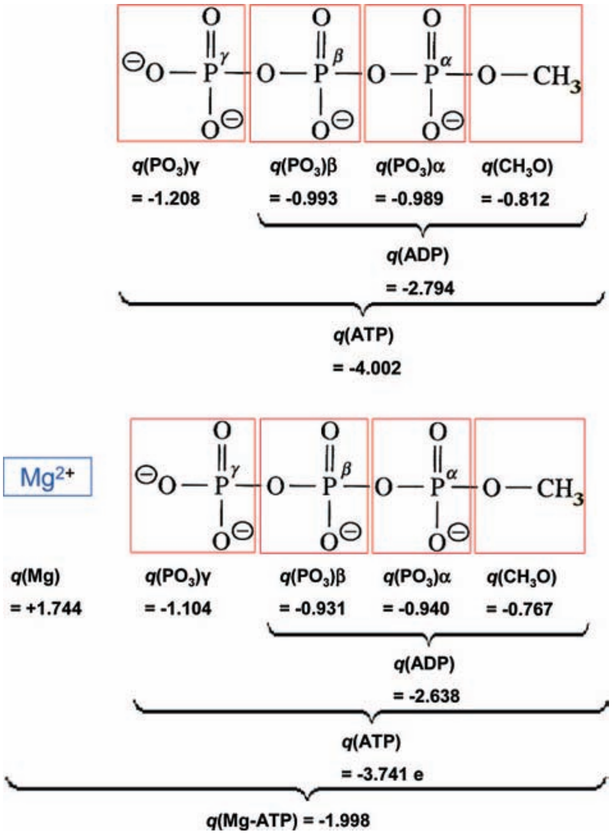

Figure 3. Charge distribution in ATP and in the absence (a) and presence (b) of the Mg^{2+}.

TABLE 3: Atomic Properties in Reactants $P(\Omega)$ and Their Changes $\Delta P(\Omega)$ after Hydrolysis in the Absence and Presence of Mg^{2+}

atom (Ω)	$N(\Omega)$ (au)	$\Delta N(\Omega)$ (au)	$q(\Omega)$ (au)	$E(\Omega)$ (au)	$\Delta E(\Omega)$ (kcal/mol)	$\Delta E(\Omega)_{Mg^{2+}}$[a] (kcal/mol)	vol(Ω) (au)	Δvol(Ω) (au)
				Without Mg^{2+}				
				$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^-$, $\Delta E = -168.6$ kcal/mol				
				Reactants				
ATP^{4-}								
C	5.365		0.635	−37.5080			58.1	
H1	1.106		−0.106	−0.6587			59.1	
H3	1.033		−0.033	−0.6329			52.6	
H2	1.072		−0.072	−0.6436			57.7	
O1	9.236		−1.236	−75.6449			111.4	
Σ_{CH_3O}	**17.812**		**−0.812**	**−115.0880**			**338.8**	
Pα	11.406		3.594	−340.1338			29.4	
Oα1	9.563		−1.563	−75.6120			166.9	
Oα2	9.552		−1.552	−75.6260			164.1	
O2	9.468		−1.468	−75.7376			114.7	
$\Sigma_{\alpha PO_3}$	**39.989**		**−0.989**	**−567.1094**			**475.2**	
Pβ	11.434		3.566	−340.1162			31.5	
Oβ1	9.547		−1.547	−75.6294			166.6	
Oβ2	9.546		−1.546	−75.6303			166.8	
O3	9.467		−1.467	−75.7022			117.4	
$\Sigma_{\beta PO_3}$	**39.993**		**−0.993**	**−567.0781**			**482.2**	
Pγ	11.465		3.536	−340.1499			32.1	
Oγ1	9.579		−1.579	−75.5793			174.1	
Oγ2	9.582		−1.582	−75.5708			173.6	
Oγ3	9.583		−1.583	−75.5707			173.6	
$\Sigma_{\gamma PO_3}$	**40.208**		**−1.208**	**−566.8707**			**553.4**	
$\Sigma_{molecule}$	**138.002**		**−4.002**	**−1816.14622**			**1849.6**	
H_2O								
O	9.165		−1.165	−75.7076			152.7	
H	0.417		0.583	−0.3632			22.2	
H′	0.417		0.583	−0.3632			22.3	
$\Sigma_{molecule}$	**10.000**		**0.000**	**−76.4341**			**197.2**	
				Products				
ADP^{3-}								
C	5.370	0.005	0.630	−37.5151	−4.5		58.2	0.1
H1	1.107	0.002	−0.107	−0.6595	−0.5		59.1	0.0
H2	1.065	0.032	−0.065	−0.6421	−5.8		56.7	4.1
H3	1.033	−0.039	−0.033	−0.6329	6.7		52.6	−5.1
O1	9.238	0.002	−1.238	−75.6412	2.3		112.4	1.1
Σ_{CH_3O}	**17.812**	**0.001**	**−0.812**	**−115.0909**	**−1.8**		**339.1**	**0.3**
Pα	11.408	0.002	3.592	−340.1677	−21.3		29.4	−0.0
Oα1	9.567	0.004	−1.567	−75.6132	−0.7		167.5	0.6
Oα2	9.554	0.002	−1.554	−75.6281	−1.3		164.2	0.1
O2	9.470	0.002	−1.470	−75.7271	6.6		118.7	4.0
$\Sigma_{\alpha PO_3}$	**39.998**	**0.009**	**−0.998**	**−567.1360**	**−16.7**		**479.8**	**4.6**
Pβ	11.472	0.038	3.528	−340.2037	−54.9		32.5	1.0
Oβ1	9.573	0.026	−1.573	−75.5915	23.8		171.7	5.2
Oβ2	9.569	0.024	−1.569	−75.5957	21.7		171.9	5.1
O3/Oβ3	9.576	0.108	−1.576	−75.5886	71.3		171.6	54.2
$\Sigma_{\beta PO_3}$	**40.189**	**0.196**	**−1.189**	**−566.9795**	**61.9**		**547.7**	**65.5**
$\Sigma_{molecule}$	**98.000**	**0.205**	**−3.000**	**−1249.2064**	**43.4**		**1366.6**	**70.4**
P_i^-								
Pγ	11.402	−0.063	3.598	−340.2452	−59.8		29.9	−2.2
Oγ1	9.343	−0.236	−1.343	−75.6589	−49.9		134.5	−39.5
Oγ2	9.518	−0.063	−1.518	−75.6827	−70.2		158.4	−15.1
Oγ3	9.549	−0.034	−1.549	−75.6691	−61.7		160.1	−13.5
$\Sigma_{\gamma PO_3}$	**39.812**	**−0.396**	**−0.812**	**−567.2559**	**−241.7**		**483.0**	**−70.4**
O	9.343	0.178	−1.343	−75.6589	30.6		134.4	−18.3
H	0.422	0.005	0.578	−0.3641	−0.5		22.4	0.1
H′	0.422	0.005	0.578	−0.3641	−0.5		22.4	0.1
Σ_{H_2O}[b]	**10.187**	**0.187**	**−0.187**	**−76.3870**	**29.5**		**179.2**	**−18.0**
$\Sigma_{molecule}$	**49.999**	**−0.209**	**−0.999**	**−643.6429**	**−212.2**		**662.2**	**−88.4**

TABLE 3: Continued

atom (Ω)	*N*(Ω) (au)	Δ*N*(Ω) (au)	*q*(Ω) (au)	*E*(Ω) (au)	Δ*E*(Ω) (kcal/mol)	$\Delta E(\Omega)_{Mg^{2+}}$[a] (kcal/mol)	vol(Ω) (au)	Δvol(Ω) (au)
With Mg^{2+}								
$MgATP^{2-} + H_2O \rightarrow MgADP^- + P_i^-$, $\Delta E = -24.9$ kcal/mol								
Reactants								
$MgATP^{2-}$								
C	5.428		0.572	−37.5527		−3.8	59.9	
H1	1.036		−0.036	−0.6417		4.1	52.0	
H2	1.003		−0.003	−0.6273		9.4	49.6	
H3	1.021		−0.021	−0.6336		−6.9	51.6	
O1	9.280		−1.280	−75.7056		−7.8	107.6	
Σ_{CH_3O}	**17.767**		**−0.767**	**−115.1609**		**−5.0**	**320.7**	
Pα	11.356		3.644	−340.0663		23.4	27.0	
Oα1	9.514		−1.514	−75.6705		−4.9	155.3	
Oα2	9.580		−1.580	−75.6986		13.9	141.7	
O2	9.490		−1.490	−75.8077		0.9	110.1	
$\Sigma_{\alpha PO_3}$	**39.940**		**−0.940**	**−567.2430**		**33.3**	**434.1**	
Pβ	11.399		3.601	−340.0982		34.3	29.2	
Oβ1	9.568		−1.568	−75.7159		6.4	141.5	
Oβ2	9.494		−1.494	−75.6687		−22.4	156.5	
O3	9.471		−1.471	−75.7022		−49.0	109.5	
$\Sigma_{\beta PO_3}$	**39.931**		**−0.931**	**−567.1849**		**−30.7**	**436.7**	
Pγ	11.514		3.486	−340.1970		29.6	33.6	
Oγ1	9.505		−1.505	−75.6454		41.4	159.0	
Oγ2	9.580		−1.580	−75.6543		52.4	149.2	
Oγ3	9.504		−1.504	−75.6428		45.2	158.3	
$\Sigma_{\gamma PO_3}$	**40.104**		**−1.104**	**−567.1394**		**168.6**	**500.1**	
Mg	**10.257**		**1.744**	**−200.1162**		**−22.1**	**42.0**	
$\Sigma_{molecule}$	**147.998**		**−1.998**	**−2016.8444**		**144.3**[c]	**1733.6**	
H_2O								
O	9.165		−1.165	−75.7076			152.7	
H	0.417		0.583	−0.3632			22.2	
H	0.417		0.583	−0.3632			22.3	
$\Sigma_{molecule}$	**10.000**		**0.000**	**−76.4341**			**197.2**	
Products								
$MgADP^-$								
C	5.447	0.019	0.554	−37.5659	−8.3		60.4	0.6
H1	1.017	−0.018	−0.017	−0.6360	3.6		50.7	−1.3
H2	0.984	−0.019	0.017	−0.6215	3.6		47.6	−1.9
H3	1.019	−0.002	−0.019	−0.6340	−0.2		51.6	−0.0
O1	9.287	0.008	−1.287	−75.7144	−5.5		107.4	−0.3
Σ_{CH_3O}	**17.753**	**−0.013**	**−0.753**	**−115.1717**	**−6.8**		**317.7**	**−3.0**
Pα	11.362	0.007	3.638	−340.0629	2.1		27.8	0.7
Oα1	9.500	−0.014	−1.500	−75.6795	−5.6		153.3	−2.0
Oα2	9.579	−0.001	−1.579	−75.6784	12.6		144.0	2.3
O2	9.487	−0.003	−1.487	−75.7958	7.5		110.2	0.1
$\Sigma_{\alpha PO_3}$	**39.929**	**−0.011**	**−0.929**	**−567.2165**	**16.6**		**435.2**	**1.1**
Pβ	11.424	0.025	3.576	−340.1310	−20.6		30.0	0.8
Oβ1	9.566	−0.002	−1.566	−75.6678	30.2		147.2	5.7
Oβ2	9.566	0.072	−1.566	−75.6697	−0.7		147.2	−9.3
O3/Oβ3	9.487	0.016	−1.487	−75.6666	22.3		156.2	46.6
$\Sigma_{\beta PO_3}$	**40.043**	**0.112**	**−1.043**	**−567.1351**	**31.2**		**480.5**	**43.8**
Mg	**10.276**	**0.019**	**1.724**	**−200.1513**	**−22.1**		**50.2**	**8.2**
$\Sigma_{molecule}$	**108.001**	**0.107**	**−1.001**	**−1449.6747**	**19.0**		**1283.6**	**33.8**
P_i^-								
Pγ	11.402	−0.112	3.598	−340.2452	−30.2		29.9	−3.7
Oγ1	9.343	−0.162	−1.343	−75.6589	−8.5		134.5	−24.5
Oγ2	9.518	−0.062	−1.518	−75.6827	−17.8		158.4	9.2
Oγ3	9.549	0.044	−1.549	−75.6691	−16.5		160.1	1.8
$\Sigma_{\gamma PO_3}$	**39.812**	**−0.291**	**−0.812**	**−567.2559**	**−73.1**		**483.0**	**−17.1**
O	9.343	0.178	−1.343	−75.6589	30.6		134.4	−18.3
H	0.422	0.005	0.578	−0.3641	−0.5		22.4	0.1
H′	0.422	0.005	0.578	−0.3641	−0.5		22.4	0.1
Σ_{H_2O}[b]	**10.187**	**0.187**	**−0.187**	**−76.3870**	**29.5**		**179.2**	**−18.0**
$\Sigma_{molecule}$	**49.999**	**−0.104**	**−0.999**	**−643.6429**	**−43.6**		**662.2**	**−35.2**

[a] Calculated according to eq 17. [b] References to H_2O in the products do not imply an isolated water molecule in this case, but rather the sum of the atomic properties of the three atoms that, in the product HPO_4^- molecule, came from water (O, H, and H′). [c] The small discrepancy with the value $\Delta E = 143.7$ kcal/mol (eq 16) is due to cumulative numerical integration errors.

approximation, a doubly charged cation, gaining no more than 0.256 au of electronic charge (since its positive charge is reduced from +2.000 to +1.744 au). In the Mg-ATP complex, the three phosphate and the capping methoxy groups all retain a negative charge that is close to unity, most of the charge gained by the metal being at the expense of the electron population of the terminal phosphate, the phosphate group that is transferred in the hydrolysis reaction (the charge of the γ-phosphate group drops from -1.208 au in free ATP^{4-} to -1.104 au in $MgATP^{2-}$).

In contrast with the metal, the oxygen atoms O3/Oβ3, Oγ1, and O experience a significant change in population and charge upon hydrolysis. Oγ1 looses 0.236 au to be gained primarily by the oxygen of water in the Mg^{2+}-free case, a value that drops to 0.162 au in the case of the Mg^{2+} complex.

Atomic Volumes in the Absence and Presence of Mg^{2+}. The change in the integrated atomic volumes due to hydrolysis is significant only for atoms experiencing the breaking or making of chemical bonds (Table 3). The O3/Oβ3 exhibits a significant increase in its volume [Δvol(O3/Oβ3) = 54.2 au in the metal-free case and 46.6 au in the presence of Mg^{2+}] since it loses a nearest neighbor (the γ-PO_3) and its electronic cloud is now bounded by infinity instead of the Pγ|O3/Oβ3 interatomic surface (the vertical bar indicates the zero-flux surface satisfying eq 6).

In the products of the metal-free reaction, Oγ2 shrinks by 15.1 au (~10% of its original volume) as it looses 0.063 au of electronic population. In the meantime, Oγ1 shrinks by 39.5 au due to the formation of the new Oγ1—H′ bond as it captures one of the protons of water. Oγ3 also shrinks as it looses 0.034 of its electron population upon hydrolysis. The entire γPO_3 group looses a population of 0.396 au accompanied with a decrease in the group volume by 70.4 au. Because of the transfer of electron population to the metal in the intact $MgATP^{2-}$ complex, the decrease in population and volume of the terminal phosphate group upon hydrolysis [$\Delta N(\gamma\text{-PO3}) = -0.291$ au and $\Delta\text{vol}(\gamma\text{-PO3}) = -17.1$ au] is less dramatic in the case of the complex.

In both the metal-free case as well as the metal complex, the two hydrogen atoms of water do not experience a significant change in electron population, volumes, or energies.

Group Properties and Group Transfer Potentials. The total energy of the three phosphate groups in ATP exhibits a trend similar to that in their total electron populations and volumes (Table 3). Electron populations of these groups have the trend $N(\alpha\text{-PO}_3) = 39.989 < N(\beta\text{-PO}_3) = 39.993 < N(\gamma\text{-PO}_3) = 40.208$, the corresponding volumes are 457.2, 482.2, and 553.4 au, and the respective group energies are -567.1094, -567.0781, and -566.8707 au (the terminal γ-phosphate being the least stable in ATP). Thus, the corresponding relative energies of the PO_3 groups (taking α-PO_3 as the reference) are 0.0, 19.7, and 149.8 kcal/mol for the α-, β-, and γ-groups, respectively.

In the case of the magnesium complex of ATP, the trends in group volumes and energies parallel those without magnesium but with a considerable reduction in the relative energy of the γ-PO_3. If we take the most stable phosphate group in $MgATP^{2-}$ as a reference (the α-PO3), the relative energies of the phosphate groups along the chain are 0.0, 36.5, and 65.0 kcal/mol for the α-, β-, and γ-groups, respectively. Thus, the metal has a general dampening effect and evens-out the variations in group energies along the phosphate tail of ATP and ADP.

Lipmann introduced the concept of "group potential" [1] which measures the degree of "activation of a group in a certain binding, comparing it to what might be called the 'ground state' or the 'free compound'".[36] QTAIM allows the determination of the energy of an atom or a group within a molecule unambiguously. We can take advantage of this fact to modify Lipmann's definition by calling the group potential as the energy of the group (the sum of the energies of the atoms constituting the group) in the molecule of interest minus the energy of that group in a reference compound (e.g., the product of hydrolysis). With this modified definition, the group potential (GP) of the γ-phosphate group in ATP is given by

$$\begin{aligned}\mathrm{GP}(\gamma\text{-PO}_3)_{\mathrm{ATP}^{4-}} &= \sum_{\gamma\text{-PO}_3} E(\Omega)_{\mathrm{ATP}^{4-}} - \sum_{\gamma\text{-PO}_3} E(\Omega)_{\mathrm{H_2PO_3}^-}\\ &= -566.8707 + 567.2559\ \text{(au)}\\ &= +241.7\ \text{(kcal/mol)}\end{aligned} \tag{14}$$

The group potential of the terminal γ-phosphate in the Mg-ATP complex is

$$\begin{aligned}\mathrm{GP}(\gamma\text{-PO}_3)_{\mathrm{MgATP}^{2-}} &= \sum_{\gamma\text{-PO}_3} E(\Omega)_{\mathrm{MgATP}^{2-}} - \sum_{\gamma\text{-PO}_3} E(\Omega)_{\mathrm{H_2PO_3}^-}\\ &= -567.1394 + 567.2559\ \text{(au)}\\ &= +73.7\ \text{(kcal/mol)}\end{aligned} \tag{15}$$

much lower in magnitude (by $241.7 - 73.1 = 168.6$ kcal/mol) than in the magnesium-free case indicating that this group has much less transfer tendency from $MgATP^{2-}$ than from metal-free ATP. The difference in the γ-phosphate group transfer potential between the free and metal-bound ATP is the most significant contribution to the energetic difference of 143.7 kcal/mol between the ΔEs of reactions (7) and (8).

Atomic Contributions to the Energy of Hydrolysis in the Absence and Presence of Mg^{2+}.

$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^-$. In the magnesium-free case, only 10 atoms contribute significantly (in excess of 10 kcal/mol in magnitude) to the total energy of reaction as can be seen in Table 3 and Figure 4. Six atoms, Pα, Pβ, Pγ, Oγ1, Oγ2 and Oγ3, favor ADP and P_i, whereas four atoms, Oβ1, Oβ2, O3/Oβ3, and O, favor ATP and H_2O. The sum of the contributions of these 10 atoms is -170.5 kcal/mol, ~2 kcal/mol less than the energy of reaction (-168.6 kcal/mol).

The γ-phosphate group has a dominant contribution favoring the products. This contribution is equivalent to the negative of γ-phosphate group transfer potential defined in eq 14, $\Delta E(\gamma\text{-PO}_3) = -241.7$ kcal/mol, with each atom in the group contributing between ca. -50 and -70 kcal/mol. Interestingly, the two groups flanking the γ-phosphate in the reaction, that is, the β-phosphate on one side and the incoming water molecule on the other, disfavor the reaction by 61.9 and 29.5 kcal/mol respectively. In the case of the incoming water molecule, the bulk of this effect is localized on the oxygen atom, the two hydrogen atoms being indifferent to the reaction within 0.5 kcal/mol. In the case of the β-phosphate group, the +61.9 kcal/mol is the result of the net balance of a contribution of -54.9 kcal/mol from the Pγ (favoring the products) countered and overwhelmed by a total contribution from the three oxygen atoms in this group $\Delta E(\text{O}\beta1,\text{O}\beta2,\text{O3/O}\beta3) = +116.8$ kcal/mol (favoring the reactants). It is interesting that group contributions to the energy of hydrolysis exhibits an alternation in sign, positive for H_2O and β-phosphate, and negative for γ- and α-phosphates.

$MgATP^{2-} + H_2O \rightarrow MgADT^- + P_i^-$. The bar graphs of Figure 4, (a) and (b), are plotted to the same scale. Mg^{2+} remarkably dampens all atomic contributions to the energy of reaction, $\Delta E(\Omega)$, as well as their sum, the energy of reaction ΔE.

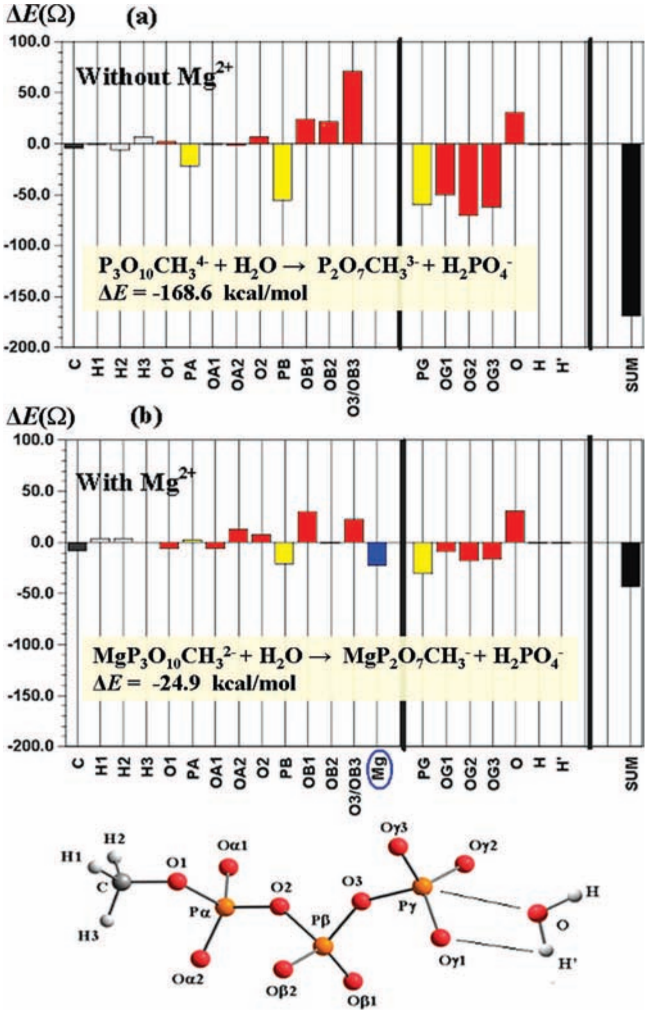

Figure 4. Atomic contributions to the energy of hydrolysis of ATP in the absence (a) and presence (b) of Mg^{2+}, along with the numbering scheme. The heavy vertical lines partition each bar graph into three regions: The one on the left corresponds to ADP, the middle to P_i, and the last with one black bar is the sum of all contributions. A negative $\Delta E(\Omega)$ indicates that the atom is more stable in the products of hydrolysis while a positive value indicates the reverse. (Changes in energies are in kcal/mol.)

Applying Hess' law to eq 12 and the reverse of eq 11 gives

$$\mathrm{ADP}^{3-} + \mathrm{MgATP}^{2-} \rightarrow \mathrm{ATP}^{4-} + \mathrm{MgADP}^{-} \qquad \Delta E = +143.7\ \mathrm{kcal/mol} \quad (16)$$

Equation 16 underscores the preferential binding of Mg^{2+} to ATP with respect to its binding to ADP as noted previously. The atomic contributions to the total binding energy of Mg^{2+} to ATP and ADP can be compared using reaction (16). To achieve this comparison, the change in the atomic contribution to the ΔE of hydrolysis due to complexation with Mg^{2+} is defined as

$$\Delta E(\Omega)_{\mathrm{Mg}^{2+}} = E(\Omega)_{\mathrm{MgADP}^-} + E(\Omega)_{\mathrm{ATP}^{4-}} - E(\Omega)_{\mathrm{MgATP}^{2-}} - E(\Omega)_{\mathrm{ADP}^{3-}} \quad (17)$$

noting that not all atoms are necessarily present in every one of the above four species.

Figure 5 displays the relative affinities of Mg^{2+} ($\Delta E(\Omega)_{\mathrm{Mg}^{2+}}$ in Table 3) to the various atoms in ATP. The metal itself has a negative $\Delta E(\Omega)$ and thus favors ADP, but once complexed with ATP, it significantly changes the contribution of the γ-phosphate group, $\Delta E(\gamma\text{-PO}_3)_{\mathrm{Mg}^{2+}} = +168.6$ kcal/mol in favor of ATP. The second major contributors favoring ATP are the two other phosphorus atoms (Pα and Pβ). In contrast, the presence of the metal significantly affects the contributions of seven atoms in favor of the hydrolysis product ADP, the most affected atoms being O3 and Oβ2 with a net Mg^{2+}-induced change in their combined contribution of -71.4 kcal/mol. The net balance of $\Delta E(\gamma\text{-PO}_3) + \Delta E(\mathrm{O3, O}\beta 2)$ represents ca. 68% of the total ΔE of reaction.

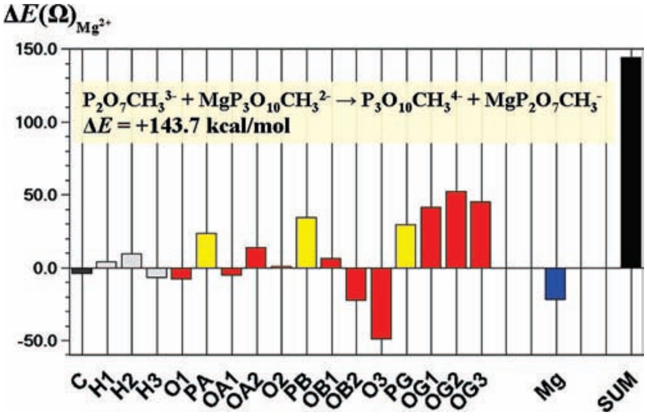

Figure 5. Partitioning of the energy of the displacement of Mg^{2+} from ATP to ADP. Negative $\Delta E(\Omega)$ values indicate a higher affinity of Mg^{2+} to ADP and positive values indicate a higher affinity to ATP. The right-most column is the sum of all contributions (+143.7 kcal/mol). (Changes in energies are in kcal/mol.)

Conclusions

The atomic partitioning of several molecular properties and their changes brought about by the hydrolysis of ATP have been investigated in the absence and presence of Mg^{2+}. The main conclusions of this paper can be summarized as follows:

- The metal shares four bond paths with ATP and only three with ADP.
- Consistent with the findings of other investigators (ref 31 for example), the Mg^{2+} is found to have a considerable effect on the bond properties of the triphosphate tail of ATP and ADP. The metal (1) induces a large alternation in the length and strength (ρ_{BCP}) of the P−O bonds in the backbone and (2) significantly weakens the terminal P−O bond (Pγ-O3 in ATP and Pβ-O2 in ADP), the bond that breaks to release the terminal phosphate in the hydrolysis reaction.
- The negative charge on each phosphate group in both complexed and free ADP and ATP is close to unity, in contrast to the typical biochemistry textbook depiction of formal charges in these molecules whereby a charge of −2 au is assigned to the terminal phosphate. (See Figure 3).
- Complexation with the metal does not dramatically alter the net atomic and group charges in ATP or ADP since Mg^{2+} itself remains essentially a doubly charged cation with a net charge of ca. +1.7e in both complexes.
- The atoms that exhibit the largest changes in electron population (or charges) and volume are found to be those directly involved in the bond making and breaking, namely, the oxygen atoms O3/Oβ3, Oγ1, and O and the γ-phosphate group of ATP.
- The energy of reaction changes from −168.6 to −24.9 kcal/mol upon complexation with magnesium (gas phase calculations at the DFT/B3LYP-6-31+G(d,p) level without ZPE and thermodynamic corrections).

- Mg^{2+} significantly dampens the individual contributions of all atoms and groups to the energy of reaction, particularly the atoms in the terminal (γ) phosphate group, the group released as inorganic phosphate (P_i) in the products of hydrolysis.
- In absence of the metal, the terminal phosphate group (γ-PO_3) is the dominant contributor to the energy of reaction and is, therefore, the region in the ATP molecule where the dominant fraction of the electronic energy released in hydrolysis resides. A similar conclusion is applicable in the metal-complex case but to a much reduced extent. These observations are quantified by a proposed modified definition of Lipmann's group transfer potential, found to be +241.7 and +73.1 kcal/mol, respectively, indicating that the terminal phosphate is a significantly better leaving group in the metal-free case. (The modified Lipmann's group transfer potential is defined here are the difference between the sum of atomic energies of a group in the compound of interest and the sum of the energies of the same atoms in a reference compound).
- The magnesium cation itself is more stable in its complex with ADP (rather than in its complex in ATP), but through the changes it induces in the energies of the rest of the atoms in the system, the binding of the metal to ATP ($\Delta E_{\text{Binding}} = -923$ kcal/mol) is significantly stronger than its binding to ADP ($\Delta E_{\text{Binding}} = -779$ kcal/mol).
- This study will be extended in the future to include solvation effects, ZPE, and thermochemical corrections, effects and corrections indispensible for comparison with experimentally determined Gibbs energy of reactions.

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