Ion-Radical Mechanism of Enzymatic ATP Synthesis: DFT Calculations and Experimental Control

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Received: October 19, 2009; Revised Manuscript Received: November 23, 2009

A new, ion-radical mechanism of enzymatic ATP synthesis was recently discovered by using magnesium isotopes. It functions at a high concentration of $MgCl_2$ and includes electron transfer from the $Mg(H_2O)_m^{2+}(ADP^{3-})$ complex ($m = 0-4$) to the $Mg(H_2O)_n^{2+}$ complex as a primary reaction of ATP synthesis in catalytic sites of ATP synthase and kinases. Here, the structures and electron transfer reaction energies of magnesium complexes related to ATP synthesis are calculated in terms of DFT. ADP is modeled by pyrophosphate anions, protonated ($HP_2O_7H^{2-}$, $HP_2O_7CH_3^{2-}$) and deprotonated ($HP_2O_7^{3-}$, $CH_3P_2O_7^{3-}$). The reaction generates an ion-radical pair, composed of $Mg(H_2O)_n^{+}$ ion and pyrophosphate anion-radical coordinated to Mg^{2+} ion. The addition of the latter to the substrate P═O bond results in ATP formation. Populations of the singlet and triplet states and singlet−triplet spin conversion in the pair are controlled by hyperfine coupling of unpaired electrons with magnetic ^{25}Mg and ^{31}P nuclei and by Zeeman interaction. Due to these two interactions, the yield of ATP is a function of nuclear magnetic moment and magnetic field; both of these effects were experimentally detected. Electron transfer reaction does not depend on m but strongly depends on n. It is exoergic and energy allowed at $0 \le n \ll \infty$ for the deprotonated pyrophosphate anions and at $0 \le n < 4$ for the protonated ones; for other values of n, the reaction is energy deficient and forbidden. The boundary between exoergic and endoergic regimes corresponds to the trigger magnitude n^* ($n^* = 4$ for protonated anions and $6 < n^* \ll \infty$ for deprotonated ones). These results explain why ATP synthesis occurs only in special devices, molecular enzymatic machines, but not in water ($n = \infty$). Biomedical consequences of the ion-radical enzymatic ATP synthesis are also discussed.

ATP synthesizing enzymes are known to be perfectly arranged molecular devices generating an energy carrier, a P−O chemical bond in ATP. Great progress in understanding the structure, molecular dynamics, and mechanical functioning of these enzymes has now been attained.[1–5] The generally accepted and quite evident mechanism of ATP synthesis implies a nucleophilic, catalyzed by Mg^{2+} ions, addition of inorganic phosphate (in ATP synthase) or phosphate group of phosphorylating substrates (in kinases) to ADP. The main function of Mg^{2+} ion was traditionally thought to coordinate phosphate reagents, to keep them along the reaction pathway and, perhaps, to slightly modify their chemical reactivity by complexation, accompanied by redistribution of charges in a complex. The Mg^{2+} ion was always considered as an assistant and never assumed that it participates in the reaction as a reagent.

Another universal property of the phosphorylating molecular machines is squeezing water molecules out of the catalytic site when protein domains of enzyme approach each other to unite substrate and ADP and stimulate ATP synthesis.[6–8] Particularly, this process was discussed in detail for the ATP synthase,[8] a rotary machine, which can both synthesize and hydrolyze ATP depending on the clockwise or anticlockwise rotation of the shaft. However, it is an open question, whether a release of water is accompanied by destruction of the Mg^{2+} ion hydrate shell and whether this process results in the change of the ion reactivity.

At last, the rate of enzymatic ATP synthesis was shown to strongly depend on the magnesium isotopes.[9–17] The activity of ATP synthase and phosphocreatine, pyruvate, and phosphoglycerate kinases, in which Mg^{2+} ion has a magnetic isotopic nucleus ^{25}Mg, was found to be 2−3 times higher than that of enzymes, in which Mg^{2+} ion has spinless, nonmagnetic isotopic nuclei ^{24}Mg or ^{26}Mg. There is no difference in the ATP yield for enzymes with ^{24}Mg and ^{26}Mg; it gives evidence that in this reaction the magnetic isotope effect operates rather than the classical, mass-dependent one.

The isotope effect was shown to be a function of the concentration of Mg^{2+} ions.[12,16] At low concentration, there is no isotope effect; i.e., the classical, generally accepted nucleophilic mechanism of the ATP synthesis dominates. If the concentration of Mg^{2+} ions exceeds the intracellular one by 50−100 times, a huge isotope effect appears, which evidences that the new, spin-dependent ion-radical mechanism of ATP synthesis is switched on, providing an additional and considerable *enzymatic* source of ATP. This conclusion is also strongly supported by the magnetic field dependence of the ATP synthesis by creatine kinase.[15]

Now we are faced with the problem to formulate such a chemical mechanism of ATP synthesis and to choose such reactions in a catalytic site which would demonstrate all of these

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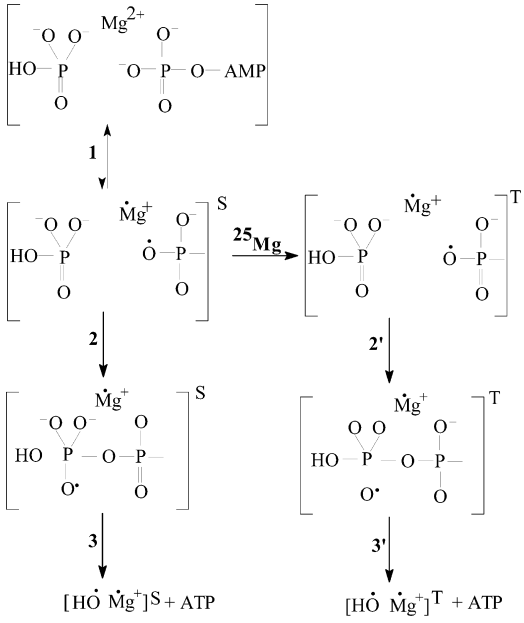
10.1021/jp909992z
Published on Web 01/22/2010

SCHEME 1: Reaction Scheme of the Nuclear Spin Selective Phosphorylation by ATP Synthase



fundamental properties of the phosphorylating molecular machines. This mechanism should include radical or ion-radical steps with participation of Mg^{2+} ion as a reagent, whose chemical reactivity is supposed to be dependent on the number of water molecules in its hydrate shell. The reaction mechanism in a catalytic site is not accessible to direct experimental testing; this is a reason that the focus of our work is a theoretical inspection of the reaction mechanism. The purpose of the paper is to present a theoretical analysis of the redox reactions of magnesium complexes, to calculate their structures and energies, to discriminate energy allowed and energy forbidden reactions, and ultimately formulate the ion-radical mechanism of ATP synthesis.

Computational Technique

Full optimization of the geometries and energy characteristics was performed with the Gaussian 03 program package[18,19] using the density functional theory (DFT) with the B3LYP three-parameter exchange-correlation functional and conventional 6-31G* split-valence basis sets.[19]

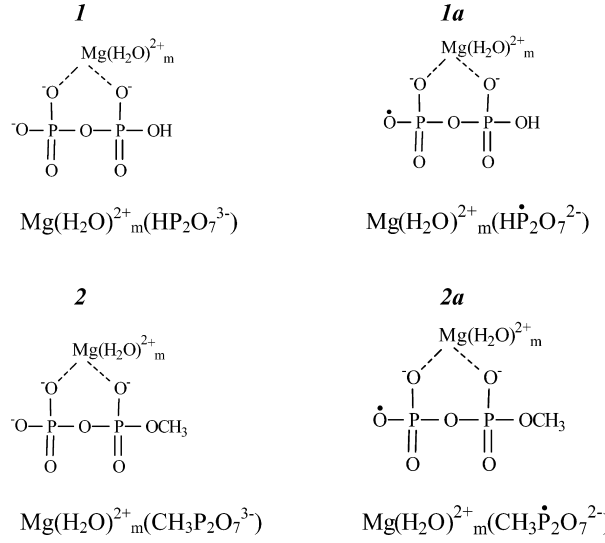
General Scheme of the Nuclear Spin Dependent Phosphorylation

As was mentioned in the introduction, a classical paradigm of the ATP synthesis is based on the nucleophilic addition of the phosphate group to ADP. Magnesium nuclear spin dependence of the ATP synthesis is a new, paradigm-shifting phenomenon. It means that besides the generally accepted nucleophilic mechanism there is another mechanism, which should be in conformity to the three postulates which follow from the experiment:

(i) Phosphorylation is an electron transfer reaction which generates an ion-radical pair comprised of Mg^+ ion and phosphate radical-anion of ADP as the partners.

(ii) Because of spin conservation, the chemical reactivity of triplet and singlet spin states of the ion-radical pair is different and results in a difference in the yield of ATP along the singlet and triplet channels.

CHART 1



(iii) The relative contribution of these spin channels into the ATP yield is controlled by electron−nuclear (hyperfine) magnetic coupling of unpaired electrons with magnetic nucleus ^{25}Mg in the Mg^+ ion and with ^{31}P in phosphate radical; it induces singlet−triplet spin conversion and results in the nuclear spin selectivity of the phosphorylation.

Such a mechanism was formulated earlier in a very general form;[16] for the particular case of ATP synthase, it is shown in Scheme 1, where AMP is an adenosine monophosphate residue of ADP.

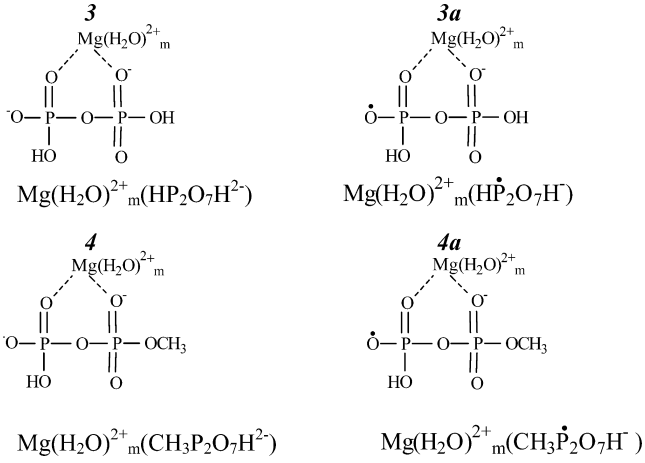
As a first step, the reaction scheme implies a transfer of electron from the terminal phosphate group of ADP to Mg^{2+} ion, generating a primary ion-radical pair, composed of monovalent radical cation Mg^+ and the oxyradical of ADP (reaction 1), in a singlet spin state due to the total spin conservation in this process. The next step is the phosphorylation itself which occurs as an attack of the P═O chemical bond of inorganic phosphate by ADP oxyradical (reaction 2). Generated in this addition reaction is another oxyradical that decomposes via β-scission of the P−O chemical bond (reaction 3) and generates ATP and the final ion-radical pair ($\overset{\bullet}{HO}\ \overset{\bullet}{M}g^+$), which regenerates Mg^{2+} in the reaction

$$(\overset{\bullet}{HO}\ \overset{\bullet}{M}g^+) \xrightarrow{H^+} H_2O + Mg^{2+} \qquad (I)$$

The rate of phosphorylation along a singlet channel (reactions 1−3 in Scheme 1) is suppressed by spin allowed back electron transfer in the primary ion-radical pair, which regenerates starting reagents and decreases ATP yield. However, in the presence of $^{25}Mg^{2+}$ ion, hyperfine coupling of an unpaired electron with the ^{25}Mg nucleus in Mg^+ stimulates singlet−triplet conversion of the primary ion-radical pair and transforms it into the triplet pair, in which back electron transfer is spin forbidden. This new, triplet channel of phosphorylation provides an additional yield of ATP, which increases by 2−3 times the total production of ATP. The final ion-radical pair in the triplet channel undergoes fast triplet−singlet conversion due to electron spin relaxation in OH radical (relaxation time is about 10^{-11} s) and again regenerates Mg^{2+} ion in the reaction I.

This reaction scheme explains magnesium isotope and magnetic field effects in ATP synthesis. Moreover, from the experimentally measured yield of ATP as a function of $^{25}Mg^{2+}$

CHART 2



content and the kinetic scheme, equivalent to the reaction in Scheme 1, the rate constants in the catalytic site were estimated:[20] 1.2×10^8 s^{-1} for reaction 1 in Scheme 1, 1.2×10^9 s^{-1} for the back electron transfer in the primary ion-radical pair, and 6×10^8 s^{-1} for the rate of the singlet−triplet spin conversion in the site with $^{25}Mg^{2+}$ ion. However, the most intriguing problem is that ATP synthesis as a redox reaction shown in Scheme 1 occurs only in enzymes and does not take place in water solutions. In this paper, we will focus on the key reaction 1 in Scheme 1. Our task is to answer the question, why this reaction proceeds only in enzymes and what conditions are required for this reaction to be exoergic and energy allowed.

Magnesium Complexes of ADP and Their Reactions

In the catalytic site, ADP is presented as magnesium complex $Mg^{2+}(ADP^{3-})$; for this reason, we have the calculated structure and energy of complexes **1** and **2** (Chart 1) in which the $Mg^{2+}(ADP^{3-})$ complex is modeled by hydrated pyrophosphate complexes $Mg(H_2O)_m^{2+}(HP_2O_7^{3-})$ and $Mg(H_2O)_m^{2+}(CH_3P_2O_7^{3-})$ with a hydrogen atom and methyl group, respectively, instead of adenosine residue.

Magnesium ion is supposed to be added to oxygen atoms of pyrophosphate anion, as shown in Chart 1, and donates two coordination bonds. The other coordination bonds may be used for addition of m water molecules, m being in the range from 0 to 4. The value $m = 4$ corresponds to the fully completed six-coordinated shell of Mg^{2+} ion.

Besides complexes **1** and **2**, we have also calculated the structure and energy of the ion-radicals **1a** and **2a** (Chart 1) generated from **1** and **2** by electron detachment.

Note that, in complexes **1** and **2**, **1a** and **2a** pyrophosphate anions are in deprotonated forms. However, in cells and mitochondria at the physiological pH, ADP is presented in both forms—deprotonated and partly protonated—almost equally, so that we have also calculated the structure and energy of partly protonated complexes **3** and **4** (Chart 2), as well as their ion-radicals **3a** and **4a** generated from **3** and **4** by electron detachment.

Figure 1 shows some typical structures for selected values of m. The withdrawal of an electron from complexes **1**−**4** results in redistribution of the electron density and small changes of the interatomic distances in complexes **1a**−**4a** with respect to those in **1**−**4**. In structures **1**−**4** and **1a**−**4a**, the formal charge of the Mg^{2+} ion is shown (Charts 1 and 2); the true magnitude of positive charge on the magnesium atom in **1**−**4** is in the range 0.66−0.77 and increases by 25−30% in structures **1a**−**4a** due to redistribution of electron density induced by electron detachment.

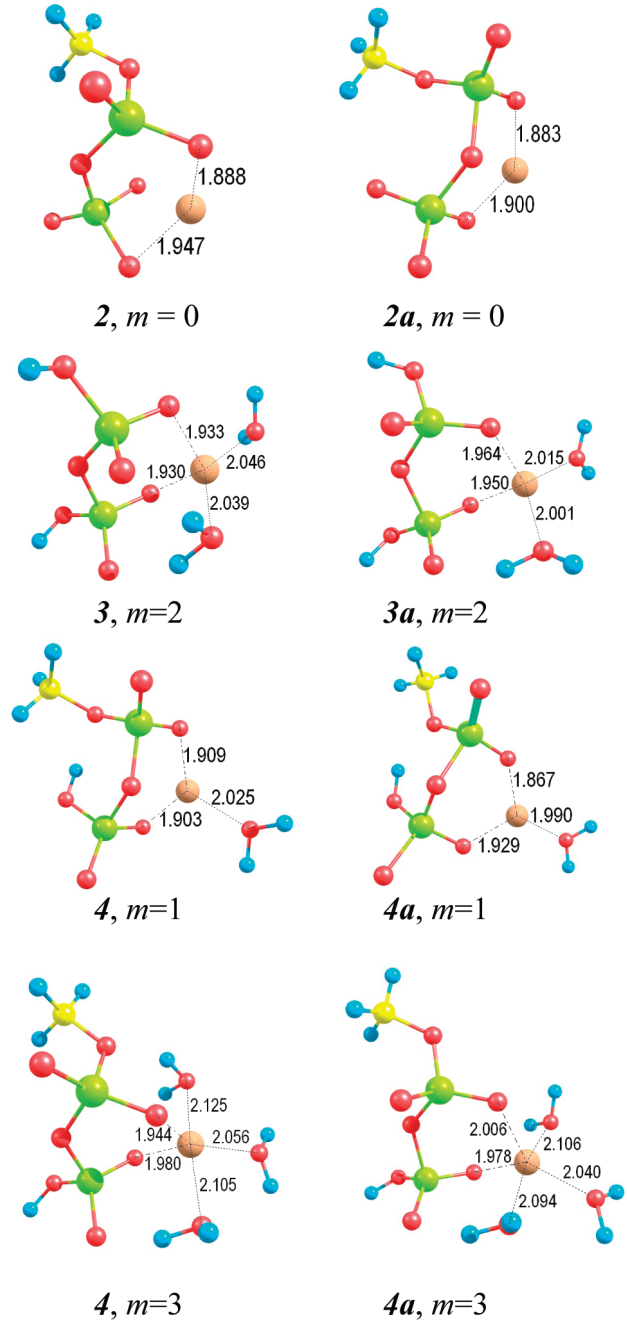


Figure 1. Representative structures of selected magnesium complexes. Distances are in Å; magnesium atoms are gold, phosphorus is green, oxygen is red, carbon is yellow, and hydrogen is blue.

At last, we have calculated the structure and energy of the hydrated complexes $Mg(H_2O)_n^{2+}$ and $Mg(H_2O)_n^{+}$ as a function of n, the number of water molecules in the coordination sphere of magnesium ion.

Now we may calculate the energies of the electron transfer from hydrated pyrophosphate complexes **1**−**4** shown in Charts 1 and 2 to the $Mg(H_2O)_n^{2+}$ complexes as a function of n and m:

$$Mg(H_2O)_n^{2+} + \mathbf{1} \rightarrow Mg(H_2O)_n^{+} + \mathbf{1a} \quad (1)$$

$$Mg(H_2O)_n^{2+} + \mathbf{2} \rightarrow Mg(H_2O)_n^{+} + \mathbf{2a} \quad (2)$$

$$Mg(H_2O)_n^{2+} + \mathbf{3} \rightarrow Mg(H_2O)_n^{+} + \mathbf{3a} \quad (3)$$

$$Mg(H_2O)_n^{2+} + \mathbf{4} \rightarrow Mg(H_2O)_n^{+} + \mathbf{4a} \qquad (4)$$

These four reactions model a key redox reaction, denoted in Scheme 1 as reaction 1, and generate pyrophosphate ion-radicals coordinated to the Mg^{2+} ions.

Energy of Electron Transfer Reactions

A total energy for the reactions 1−4 is determined as a difference between the summary energy of reactants and products; the reaction is exoergic if the difference is positive, i.e., the energy of reagents exceeds that of products. On the contrary, the reaction is endoergic and energy forbidden if the difference is negative. Such an approach has an important advantage in that it cancels any errors and compensates possible inaccuracy in calculations of individual reagent and product complexes.

The energies of reactions 1−4 for $n = 0-6$ and $m = 0-4$ were calculated by methods of quantum chemistry as mentioned in the Computational Technique section. To calculate energies of these reactions in water, i.e., under the conditions of the total hydration of magnesium ion (we will assume for this case that $n = \infty$), the following approach has been used. The total energy of every reaction 1−4 is an additive sum of energies for the two processes: detachment of an electron from complexes **1**−**4** with formation of **1a**−**4a** and attachment of an electron to $Mg(H_2O)_n^{2+}$; the latter refers to the reaction $Mg(H_2O)_n^{2+} + e \rightarrow Mg(H_2O)_n^{+} + \Delta E(n)$ as an electron affinity of hydrated magnesium complexes.

The calculated values of $\Delta E(n)$ for $n = 0-6$ are given in Table 1. At $n = 0$, the calculated value $\Delta E = 15.41$ eV quite satisfactorily agrees with the experimental magnitude 15.14 eV.[21]

For the reaction

$$Mg(H_2O)_\infty^{2+} + e \rightarrow Mg(H_2O)_\infty^{+} + \Delta E(\infty)$$

in water the energy $\Delta E(\infty)$ was derived from the thermodynamics of the reaction. The energy diagram for the hydrated magnesium ions Mg^{2+} and Mg^{+} taken from ref 22 is shown in Figure 2. It results in $\Delta E(\infty) = 0.32$ eV, and this value was used in calculations of energies for the reactions 1−4 in water.

The total energies of the reactions are presented in Figure 3 as functions of m and n; they result in the following conclusions.

(1) The energies do not depend on whether the adenosine residue is replaced by a hydrogen atom (in complexes **1** and **3**) or by a methyl group (in complexes **2** and **4**). It means that these energies may be certainly attributed to the reactions of the $Mg^{2+}(ADP^{3-})$ in native enzymes.

(2) The energies only slightly depend on m ($m = 0-4$), the number of water molecules in the first coordination sphere of the ions $Mg(H_2O)_m^{2+}$(pyrophosphate) and $Mg(H_2O)_m^{+}$-(pyrophosphate); therefore, they are almost independent of the hydration of the $Mg^{2+}(ADP^{3-})$ complex in the catalytic site.

(3) The energies of all reactions are strongly dependent on n, the number of water molecules in the hydrate shell of the $Mg(H_2O)_n^{2+}$ and $Mg(H_2O)_n^{+}$ (see Figure 3).

(4) No one reaction occurs in water, when the hydrate shell of the $Mg(H_2O)_n^{2+}$ is fully completed ($n = \infty$). This result is in perfect agreement with the fact that the ATP synthesis from ADP and substrate does not take place in water.

(5) In order for ATP synthesis to proceed in the catalytic site, it is necessary to partly remove water from the site and hydrate shell of the $Mg(H_2O)_n^{2+}$. Only at this condition, the key reaction, electron transfer from $Mg^{2+}(ADP^{3-})$ to $Mg(H_2O)_n^{2+}$, reaction 1 in Scheme 1, becomes energy allowed.

(6) The reaction of complexes **1** and **2** with $Mg(H_2O)_n^{2+}$ is exoergic at $n = 0-6$ and even at $n > 6$ (Figure 3). Switching over the reaction from the exoergic regime to the endoergic one takes place at n^* which lies somewhere between $n = 6$ and $n = \infty$. This magnitude $6 < n^* < \infty$ functions as a trigger, it determines

TABLE 1: Electron Affinity ΔE (in eV) of the $Mg(H_2O)_n^{2+}$ Complexes as a Function of n

n	0	1	2	3	4	5	6	∞
ΔE	15.4	13.2	11.1	9.5	7.8	7.3	6.5	0.32

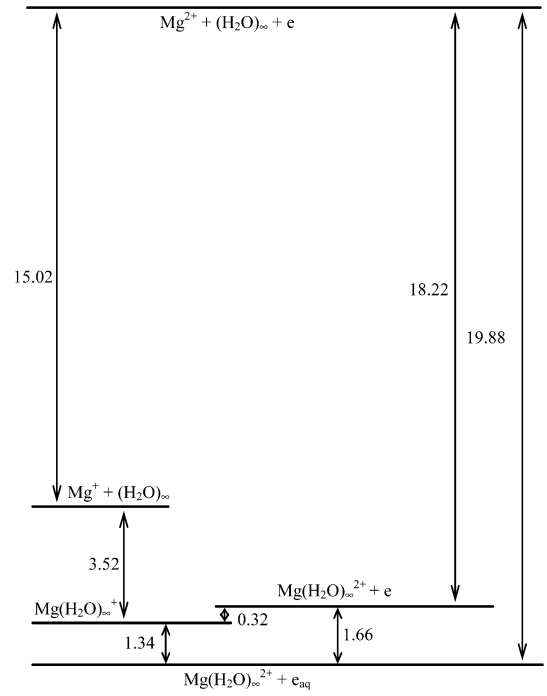


Figure 2. Energy diagram for the hydrated ions Mg^{2+} and Mg^{+}. Energies are given in eV, zero level refers to Mg^{2+} in water, and e_{aq} and e denote hydrated and dry electron, respectively.[22]

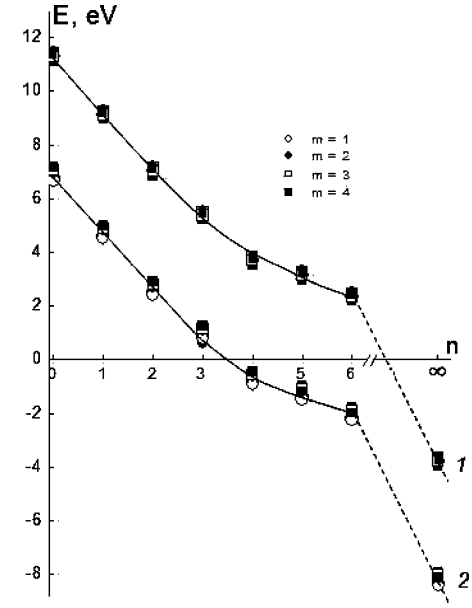


Figure 3. Energies of reactions 1 (curve 1) and 2 (curve 2) as a function of the number of water molecules n in hydrated complex $Mg(H_2O)_n^{2+}$.

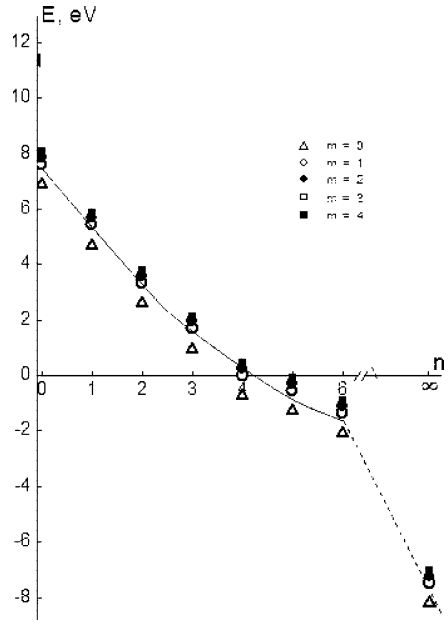


Figure 4. Energies of reactions 5 and 6 as a function of the number of water molecules n in hydrated complex $Mg(H_2O)_n^{2+}$.

the boundary between energy allowed and energy forbidden regimes, and the boundary is overcome as the compression of the catalytic site squeezes water molecules out of the site.

(7) For the reactions of complexes **3** and **4**, which include protonated pyrophosphate anions $HP_2O_7H^{2-}$ and $CH_3P_2O_7H^{2-}$, with the $Mg(H_2O)_n^{2+}$ the trigger value of n^* is estimated quantitatively, $n^* = 4$ (Figure 3); at $n > 4$, the reactions are endoergic, and at $n < 4$, they are allowed by energy.

Reactions of the Substrate Magnesium Complexes

Three types of magnesium complexes may be presented and react in the enzymatic site: hydrated $Mg(H_2O)_n^{2+}$, $Mg^{2+}(ADP^{3-})$, and Mg^{2+}(substrate) complexes; in the latter case, a substrate may be inorganic phosphate (as in ATP synthase) or phosphates of creatine, glycerate, and pyruvate (in phosphorylating kinases).

Besides reactions 1−4, which model interaction of hydrated complexes $Mg(H_2O)_n^{2+}$ with the $Mg^{2+}(ADP^{3-})$ complexes and result in ATP synthesis, we have also calculated energies of reactions modeling interaction between $Mg(H_2O)_n^{2+}$ and Mg^{2+}(substrate) complexes. It is not known exactly whether substrate is presented in the enzymatic site as a free molecule or as a magnesium complex; however, the latter possibility is very probable, taking into account a high strength of magnesium ion bonding with phosphate anions. For this reason we have calculated energies of the electron transfer reactions 5 and 6:

$$Mg(H_2O)_n^{2+} + Mg(H_2O)_m^{2+}(HPO_4^{2-}) \rightarrow Mg(H_2O)_n^{+} + Mg(H_2O)_m^{2+}(\dot{H}PO_4^{-}) \quad (5)$$

$$Mg(H_2O)_n^{2+} + Mg(H_2O)_m^{2+}(CH_3PO_4^{2-}) \rightarrow Mg(H_2O)_n^{+} + Mg(H_2O)_m^{2+}(CH_3\dot{P}O_4^{-}) \quad (6)$$

The former refers to the ATP synthase, and the latter models ATP synthesis by pyruvate kinase.

The energies of both reactions 5 and 6 are almost identical and at all values of m and n are much more endoergic (by ∼4 eV, see curve 1 in Figure 3) than the similar reactions of $Mg^{2+}(ADP^{3-})$. If the reactions with the substrates even happen, they do not result in ATP synthesis and would only decrease the efficiency of the phosphorylating enzyme. In particular, for ATP synthase with $^{24,26}Mg^{2+}$ in the catalytic site, the efficiency is about 10%, but it is twice higher for enzyme with $^{25}Mg^{2+}$ ion in the site, as derived from the kinetic analysis of the magnesium isotope effect in ATP synthesis by ATP synthase.[20]

These results convincingly demonstrate that the reactions of hydrated complexes $Mg(H_2O)_n^{2+}$ with Mg^{2+}(substrate) complexes may be neglected and the dominating reaction is an electron transfer from the $Mg^{2+}(ADP^{3-})$ to $Mg(H_2O)_n^{2+}$ complex as a starting reaction of the ion-radical ATP synthesis.

Conclusion

Theoretical monitoring of reactions between magnesium complexes reveals such reactions which perfectly model those of $Mg^{2+}(ADP^{3-})$ complexes in the catalytic site of phosphorylating enzymes and which unambiguously satisfy the full body of enzyme properties summarized in the introduction.

First, they are reactions of electron transfer from the partly hydrated Mg^{2+}(pyrophosphate) complex to the $Mg(H_2O)_n^{2+}$ complex, so that in modified Scheme 1 the key reaction 1 should be replaced by reaction

$$Mg(H_2O)_n^{2+} + Mg(H_2O)_m^{2+}(ADP^{3-}) \rightarrow Mg(H_2O)_n^{+} + Mg(H_2O)_m^{2+}(ADP^{2-})$$

It generates an ion-radical pair in which subsequent reactions, similar to reactions 2 and 3 in Scheme 1, accomplish ATP synthesis.

Now one can formulate a general idea about how magnesium ion functions in enzymatic ATP synthesis. When Mg^{2+} ions are presented in low concentration, they couple with ADP and stimulate nucleophilic addition of the $Mg^{2+}(ADP^{3-})$ complex to substrate accompanied by ATP synthesis. The invasion of the second Mg^{2+} ion in the catalytic site results in complexation of substrate; the latter decreases (more or less) its chemical reactivity and suppresses the nucleophilic channel of the ATP synthesis (it was convincingly demonstrated for pyruvate kinase[16]). However, the presence of the Mg^{2+} in excess switches on another, very effective ion-radical reaction of ATP synthesis which may be controlled by magnesium isotope substitution and magnetic fields, both permanent and oscillating.

Shortly, both mechanisms, nucleophilic and ion-radical, coexist and function independently; the former dominates at low concentration of the Mg^{2+} ions, the latter prevails at high content of magnesium ions.

Second, an ion-radical pair as a spin selective nanoreactor results in appearance of the two reaction spin channels, singlet and triplet; their relative contribution in ATP synthesis is controlled by hyperfine coupling of unpaired electrons with magnetic nuclei ^{25}Mg and ^{31}P and results in magnetic ^{25}Mg isotope and magnetic field effects.

Third, one can conclude that the compression of reagents in the catalytic site and squeezing water molecules out of the site is accompanied by partial dehydration of the $Mg(H_2O)_n^{2+}$ ion. The reaction of electron transfer from the Mg^{2+}(pyrophosphate) complex to the fully hydrated Mg^{2+} ion does not occur in water, it is energy forbidden by 4 and 8 eV for the deprotonated and protonated pyrophosphate complexes, respectively (Figure 3).

However, a removal of water from the coordination sphere of the $Mg(H_2O)_n^{2+}$ ion activates the ion, so that at some threshold value n^* the electron transfer reaction becomes exoergic and energy allowed. For the reaction of deprotonated pyrophosphate complex, n^* ranges in limits $6 < n^* \ll \infty$; for the reaction of protonated complex $n^* = 4$ (Figure 3).

The water molecule with number n^* in the complex $Mg(H_2O)_n^{2+}$ functions as a trigger; it switches over the reaction between endoergic and exoergic regimes. At $n > n^*$, electron transfer is endoergic, and at $n < n^*$, it is energy allowed and rather exoergic (see Figure 3). Note that the energy of electron transfer is independent of the hydration of the $Mg(H_2O)_m^{2+}$(pyrophosphate) complex; it is almost identical for $m = 0-4$ (Figure 3).

As the primary reaction, electron transfer generates an ion-radical pair composed of paramagnetic complexes $Mg(H_2O)_n^{+}$ and $Mg(H_2O)_m^{2+}(HOPO_2OPO_3^{2-})$. Magnetic parameters ($g$-factors, ^{25}Mg and ^{31}P hyperfine coupling constants) of the $Mg(H_2O)_m^{2+}(HOPO_2OPO_3^{2-})$ complex and $(HOPO_2OPO_3^{2-})$ radical were shown[23] to be identical; it unambiguously demonstrates that, namely, the pyrophosphate ligand (and, therefore, the ADP ligand in the catalytic site of the enzyme) in the $Mg(H_2O)_m^{2+}(HOPO_2OPO_3^{3-})$ complex donates an electron to the $Mg(H_2O)_n^{2+}$ ion. It means that the electron transfer reaction occurs as a detachment of an electron from the lone pair of the negatively charged terminal oxygen atom of the pyrophosphate ligand in the $Mg(H_2O)_m^{2+}(HOPO_2OPO_3^{3-})$ to the $Mg(H_2O)_n^{2+}$. The remaining unpaired electron is almost completely localized on the oxygen atom; the next step of the ATP synthesis is an addition of the complexed pyrophosphate radical to the double P═O bond of the substrate phosphate group which is further accompanied by ATP synthesis, as shown in Scheme 1.

The intracellular concentration of Mg^{2+} ions is in the limits 0.21−0.24 mM, so that the dominating source of ATP in the living organisms is the nucleophilic reaction. The ion-radical mechanism functions under conditions when the Mg^{2+} concentration is in a 50−100-fold excess over the intracellular one. We consider this conclusion with great satisfaction because the discovery of nuclear spin dependence of phosphorylation did not dismiss a generally accepted, classical nucleophilic mechanism of intracellular ATP synthesis.

It is worthy, however, to note that the ion-radical mechanism of enzymatic ATP synthesis may also function in the living organisms due to statistical fluctuations in distribution of magnesium ions in cells and mitochondria. When the two Mg^{2+} ions are presented in the catalytic site, they both are complexed with phosphate groups of ADP and substrate. However, the casual presence of the third, free (uncomplexed with phosphates) Mg^{2+} ion switches on the ion-radical mechanism of the ATP synthesis. Therefore, the contribution of the ion-radical mechanism of ATP synthesis cannot be completely excluded even in the living organisms.

The great advantage of the ion-radical mechanism is that it can be switched on artificially by injection of $MgCl_2$ (or, even better, of $^{25}MgCl_2$) in excess to stimulate ATP synthesis and prevent much pathology related to the deficiency of ATP, such as hypoxia, heart diseases, and other cardio toxic effects.

For these purposes, a specific nanocontainer, a magnesium ion carrier, was designed for targeted delivery of $^{25}Mg^{2+}$ in nanoamounts to the heart muscle. Being membranotropic cationites, these "smart" nanocontainers release $^{25}Mg^{2+}$ ions only in response to the metabolic, induced by hypoxia, acidic shift in cells but take them back after the normal cell functioning recovers. The energy stimulating and survival effects of $^{25}MgCl_2$ delivered by nanocontainers were demonstrated for many living organisms.[24–26] The new mechanism discovers a breakthrough in design of the new remedies for treatment of hypoxia and heart diseases.

Acknowledgment. This work was financially supported by Russian Ministry of Science and Education (grant NS-1468.2008.3) and by Russian Fund for Basic Researches (grants 08-03-00141 and 08-03-12003). The European INTAS-06/09 Magnetic Field Biological Effects Research Grant is also acknowledged.

Supporting Information Available: Cartesian coordinates for the optimized structures; complete ref 18. This material is available free of charge via the Internet at http://pubs.acs.org.

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JP909992Z