Full Articles

New versatile mechanism of enzymatic synthesis of adenosine triphosphate: theoretical foundation and experimental control

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The energies of electron transfer between pyrophosphate metal complexes and hydrated metal complexes, which is the key reaction that models the radical ion mechanism of adenosine triphosphate (ATP) synthesis, were calculated. A threshold dependence of the energy on the number of water molecules n in the ion hydration sphere was established: for $n < 4$ the reaction is exothermic, while for $n \geq 4$ it is endothermic. The switching of the energy regime accounts for the fact that ATP is synthesized only in molecular machines, enzymes, rather than in homogeneous aqueous solutions. It was predicted theoretically that the radical ion ATP synthesis may be catalyzed by not only magnesium ions as in living cells but also by calcium, zinc, barium, cadmium, and tin ions. This prediction was experimentally confirmed by the discovery of the magnetic isotope effect in the calcium- and zinc-catalyzed ATP synthesis by creatine kinase. The efficiency of ATP synthesis in the presence of ions with magnetic nuclei ^{43}Ca and ^{67}Zn is almost twice as high as that in the presence of the same ions with nonmagnetic nuclei ^{40}Ca and ^{64}Zn.

Keywords: hydrated metal complexes, pyrophosphate complexes, density functional theory, isotopes, isotope effects.

In living organisms, adenosine triphosphate (ATP) as the main molecular energy carrier is synthesized by enzymes according to the mechanism of nucleophilic addition of the phosphate group to adenosine diphosphate (ADP). The reaction is catalyzed by Mg^{2+} ions. This mechanism is generally accepted and versatile. Recently, it was

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shown with the use of magnesium isotopes[1—4] that at high ion concentrations, the nucleophilic mechanism is inhibited and a new, radical ion mechanism of ATP synthesis is switched on. This can be reliably detected based on both the magnetic isotope effect (replacement of the nonmagnetic isotopes ^{24}Mg and ^{26}Mg by the magnetic isotope ^{25}Mg induces an almost twofold increase in the ATP yield)[5] and the magnetic-field dependence of the ATP synthesis.[6] The contribution of this mechanism in living cells is insignificant due to the low concentration of Mg^{2+} ions in the cells (0.2—0.3 mmol L^{-1}); however, it can be used for medico-biological purposes when high concentration of Mg^{2+} ions is created in living cells and the radical ion mechanism of ATP synthesis is artificially switched on.[7—9] The key reaction of the radical ion mechanism is transfer of an electron from $Mg(ADP)^{2-}$ and $Mg(ADP)^{3-}$ complexes to the hydrated ion $Mg(H_2O)_n{}^{2+}$ in the enzyme catalytic site to give a radical ion pair serving as a spin-selective nanoreactor responsible for the magnetic effects in the ATP synthesis. The reactions in this pair are accompanied by ATP synthesis.[2,10]

The purpose of this work is to provide theoretical analysis of the energy behavior of the key electron transfer reaction, to establish the energy boundaries for functioning of the radical ion mechanism, and to find out whether this mechanism applies only to the magnesium ions or it is versatile and can be stimulated by other metal ions.

Calculation Procedure

The calculations with full geometry optimization for all structures were carried out by the density functional theory with the B3LYP three-parameter exchange correlation functional in the 6-31G* (for Mg, Ca, Zn) and LANL2DZ (for Ba, Cd, Sn) basis sets[11] using the Gaussian-03 program package.[12] The energies were determined as the differences ΔE between the total energies of the reactants and products. At $\Delta E > 0$, the reaction is exothermic, while at $\Delta E < 0$, the reaction is endothermic. An advantage of these calculations is compensation of all systematic errors and inaccuracies in the determination of the energies of individual reactants and products.

Results and Discussion

Hydrated complexes. The structures and energies of the complexes $M(H_2O)_n{}^{2+}$ and $M(H_2O)_n{}^{+}$ were calculated as functions of the number of water molecules n in the coordination sphere of the M ion. Singly charged hydrated ions are formed upon the addition of an electron to doubly charged ions. The addition energy calculated as the energy difference between the doubly and singly charged ions is the electron affinity energy E_a of the complexes $M(H_2O)_n{}^{2+}$. These energies are shown in Fig. 1 for M = Mg, Ca, Zn, Ba, Cd, and Sn.

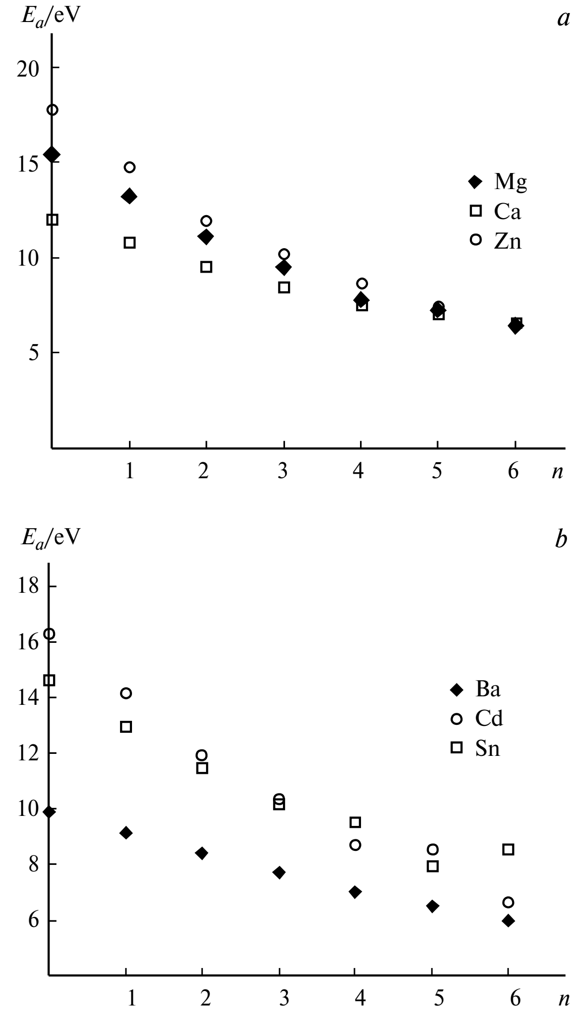
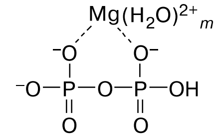


Fig. 1. Electron affinity energies E_a of the hydrated complexes of Mg, Ca, Zn ions (*a*) and Ba, Cd, Sn (*b*) ions plotted *vs. n*.

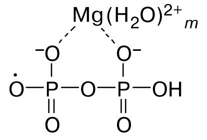
For all $M(H_2O)_n{}^{2+}$ ions, the electron affinity is positive and appreciably depends on n (especially at small n; for larger n, this dependence is less pronounced).

Pyrophosphate complexes. In the catalytic sites of native enzymes, ADP is present as the magnesium complex $Mg^{2+}(ADP)^{3-}$; therefore, we calculated the structures and energies of the complexes $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OH)^{3-}$ (**1a**) and $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OMe)^{3-}$ (**2a**). These species model the $Mg^{2+}(ADP)^{3-}$ complex by hydrated pyrophosphate complexes in which the hydrogen atom and the methyl group simulate the adenosine group of the ADP molecule. It is assumed that the Mg^{2+} ion adds to the pyrophosphate anion using two coordination bonds. Other coordination bonds are spent for attaching m water molecules ($0 \le m \le 4$). The value $m = 4$ corresponds to the fully occupied coordination sphere of the Mg^{2+} ion.

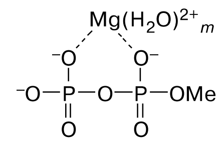
We also calculated the structure and the energy for the complexes $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OH)^{2-}$ (**1b**) and $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OMe)^{2-}$ (**2b**), which are formed



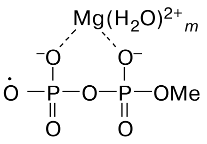
1a



1b



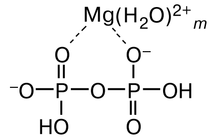
2a



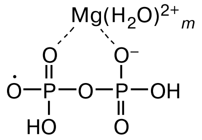
2b

from $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OH)^{3-}$ (**1a**) and $Mg(H_2O)^{2+}{}_m(OPO_2OPO_2OMe)^{3-}$ (**2a**) upon removal of an electron. The energy difference between the complexes **1a** and **1b** (**2a** and **2b**) is the ionization potential E_d of complex **1** (**2**).

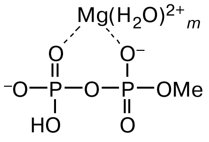
In complexes **1a** and **2a**, the pyrophosphate anion is triply charged. Since the pK values of the pyrophosphate anions are close to 7, the ADP molecules can exist in the cells and mitochondria at physiological pH ≈ 7 as both triply charged and doubly charged (with one proton attached) species in commensurable amounts. Therefore, we calculated the structures and the energies of the doubly charged complexes $Mg(H_2O)^{2+}{}_m(HOPO_2OPO_2OH)^{2-}$ (**3a**) and $Mg(H_2O)^{2+}{}_m(HOPO_2OPO_2OMe)^{2-}$ (**4a**), and also the complexes $Mg(H_2O)^{2+}{}_m(HOPO_2OPO_2OH)^{-}$ (**3b**) and $Mg(H_2O)^{2+}{}_m(HOPO_2OPO_2OMe)^{-}$ (**4b**), which are formed from **3a** and **4a** upon removal of an electron. The corresponding energy differences provide the ionization potentials E_d of complexes **3a** and **4a**.



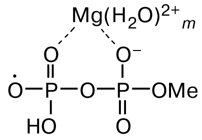
3a



3b



4a



4b

Structures of the types **3a**,**b** and **4a**,**b** were also calculated for pyrophosphate complexes of calcium, zinc, barium, cadmium, and tin ions; the ionization potentials of all complexes are summarized in Table 1.

Table 1. Ionization potentials (E_d) of pyrophosphate complexes

m	E_d/eV					
	Mg	Ca	Zn	Ba	Cd	Sn
$M(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{3-} \longrightarrow M(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{2-}$						
0	—	—	—	—	—	—
1	—	—	—	—	—	—
2	−4.11	—	—	—	—	—
3	−4.02	—	—	—	—	—
4	−3.99	—	—	—	—	—
$M(H_2O)_m{}^{2+}(OPO_2OPO_2OMe)^{3-} \longrightarrow M(H_2O)_m{}^{2+}(OPO_2OPO_2OMe)^{2-}$						
0	—	−3.9	−4.58	−3.57	−4.90	−5.62
1	−4.29	−4.0	−4.37	−3.56	−4.64	−5.60
2	−3.95	−3.7	−4.50	−3.90	−4.84	−4.71
3	−4.12	−3.7	—	−3.95	−4.55	−4.72
4	−4.07	−3.7	−4.40	−3.93	−4.41	−4.24
$M(H_2O)_m{}^{2+}(HOPO_2OPO_2OH)^{2-} \longrightarrow M(H_2O)_m{}^{2+}(HOPO_2OPO_2OH)^{-}$						
0	—	—	—	—	—	—
1	−8.62	—	—	—	—	—
2	−8.55	—	—	—	—	—
3	−8.52	—	—	—	—	—
4	−8.22	—	—	—	—	—
$M(H_2O)_m{}^{2+}(HOPO_2OPO_2OMe)^{2-} \longrightarrow M(H_2O)_m{}^{2+}(HOPO_2OPO_2OMe)^{-}$						
0	—	—	−9.64	−8.30	−9.14	−9.77
1	−8.72	−8.0	−8.84	−8.26	−8.78	−9.76
2	−8.43	−8.2	−8.45	−8.31	−8.79	−8.62
3	−8.35	−8.1	−8.42	−8.00	−8.75	—
4	−8.25	−8.0	−8.52	—	−8.54	Unstable

Electron transfer reactions. With known electron affinity E_a of the hydrated complexes $M(H_2O)_n{}^{2+}$ and the ionization potentials of the pyrophosphate complexes E_d, it is possible to calculate the total energies $\Delta E = (E_a + E_d)$ for electron transfer from the pyrophosphate complex to the hydrated complex:

$$M(H_2O)_n{}^{2+} + M(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{3-} \longrightarrow M(H_2O)_n{}^{+} + M(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{2-} \quad (1)$$

$$M(H_2O)_n{}^{2+} + M(H_2O)_m{}^{2+}(HOPO_2OPO_2OH)^{2-} \longrightarrow M(H_2O)_n{}^{+} + M(H_2O)_m{}^{2+}(HOPO_2OPO_2OH)^{-} \quad (2)$$

$$M(H_2O)_n{}^{2+} + M(H_2O)_m{}^{2+}(OPO_2OPO_2OMe)^{3-} \longrightarrow M(H_2O)_n{}^{+} + M(H_2O)_m{}^{2+}(OPO_2OPO_2OMe)^{2-} \quad (3)$$

$$M(H_2O)_n{}^{2+} + M(H_2O)_m{}^{2+}(HOPO_2OPO_2OMe)^{2-} \longrightarrow M(H_2O)_n{}^{+} + M(H_2O)_m{}^{2+}(HOPO_2OPO_2OMe)^{-} \quad (4)$$

The dependences of these energies on the numbers of molecules *m* and *n* in the coordination sphere of the ion are shown in Fig. 2 (for M = Mg, Ca, Zn) and Fig. 3 (for M = Ba, Cd, Sn). (Note that ΔE is not the activation energy, see Calculation Procedure). Analysis of the results reveals the following general trends:

1. Reactions (1) and (3) refer to the triply charged anions and reactions (2) and (4) refer to the doubly charged (with one proton attached) anions. Their energies are substantially different: for the former, they are positive at any *n* and *m*, while for the latter, they are 4—5 eV lower. This result reliably indicates that the major contribution to the energy behavior of the electron transfer is made by the Coulomb electrostatic interaction.

2. The energies ΔE depend considerably on the *n* value. The energies of reactions (1) and (3) remain positive at any *n*, while the energies of reactions (2) and (4) change the sign: at low *n* values, $\Delta E > 0$, while at high *n* values, $\Delta E < 0$. The inversion of the ΔE sign and switching of the reaction regime occurs at $n^* = 3$ or $n^* = 4$; these are the threshold values determining the boundary between two electron transfer regimes, namely, the exothermic (energetically allowed) and endothermic (energetically forbidden) regimes.

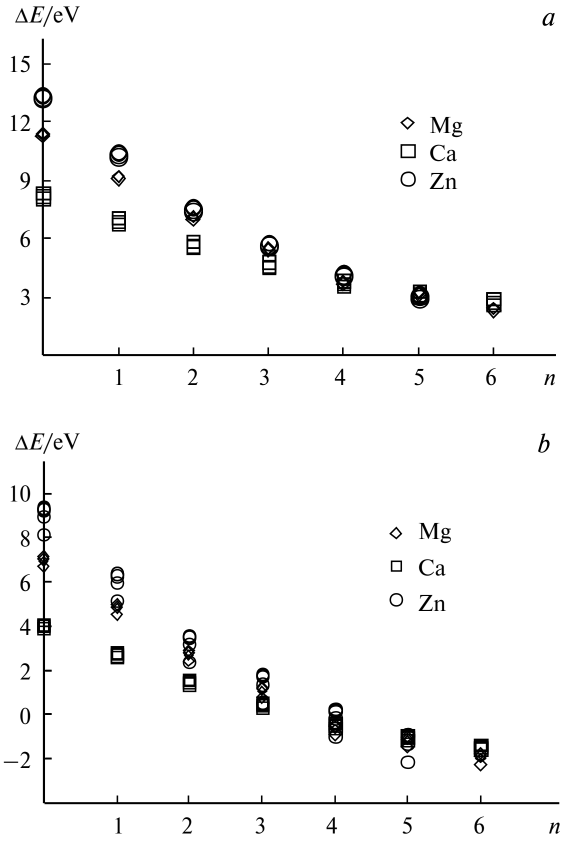


Fig. 2. Energies ΔE of reactions (3) (*a*) and (4) (*b*) plotted *vs.* *n* for Mg, Ca, Zn ions.

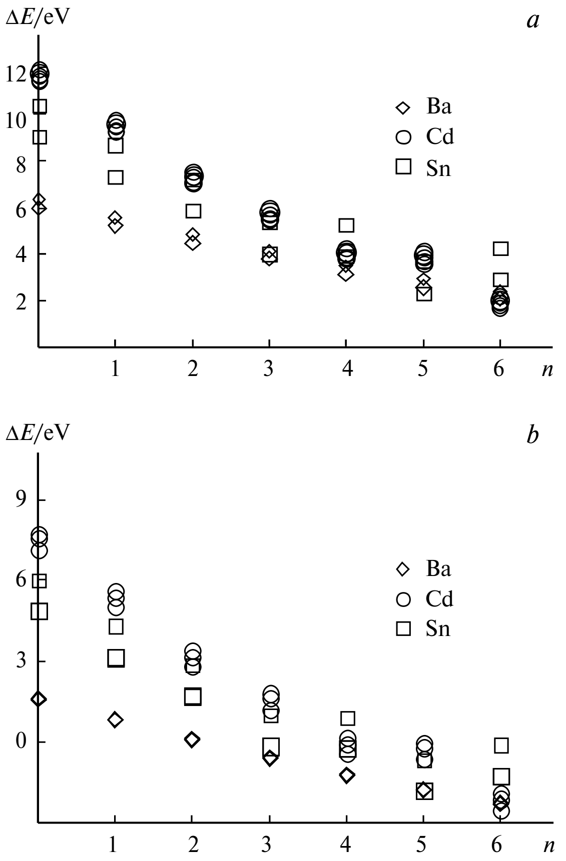


Fig. 3. Energies ΔE of reactions (3) (*a*) and (4) (*b*) plotted *vs.* *n* for Ba, Cd, Sn ions. Different dots at every *n* value correspond to different *m* values.

3. No calculated values of the energies of the hydrated complexes $M(H_2O)_{\text{Њ}\infty}{}^{2+}$ and $M(H_2O)_\infty{}^+$ in water ($n = \infty$) are available; however, the electron affinity of the $M(H_2O)_\infty{}^{2+}$ ions can be determined using thermodynamic data known currently only for magnesium ions.[13] It follows from these data that the electron affinity E_a of the $Mg(H_2O)_\infty{}^{2+}$ ion in water is 0.32 eV. Then the energies of reactions (1) and (2), *i.e.*, the values $\Delta E = (E_a + E_d)$, are −3.8 and −8.2 eV, respectively. This implies that the reactions of both triply and doubly charged magnesium pyrophosphate complexes in water are highly endothermic. Reactions (3) and (4) are also endothermic: $\Delta E = -3.9$ and −8.0 eV, respectively.

4. The dependence of the electron transfer reaction regime on the *n* value plays a fundamental role, as it accounts why these reactions become energy-consuming in aqueous solutions and occur only in enzymes, *i.e.*, molecular machines of ATP syntheses.[14] A remarkable feature of these machines is that their functioning is accompanied by compression of the catalytic site and displacement of water from it. The removal of water results in partial destruction of the hydration shell of the $M(H_2O)_n{}^{2+}$ ions. When the critical condition $n < n^*$ is fulfilled, the ion is

activated and the electron transfer becomes energetically allowed.

5. The energies ΔE of the electron transfer reactions do not depend on whether adenosine is replaced by hydrogen (in reactions (1) and (2)) or methyl group (in reactions (3) and (4)); this means that these energies can also be attributed with certaintly to reactions of the $M^{2+}(ADP)^{3-}$ and $M^{2+}(ADP)^{2-}$ complexes in native catalytic sites of the enzymes where M = Mg.

6. The energies ΔE almost do not depend on m (m = 0—4); this means that the electron transferred from the pyrophosphate to the hydrated complexes is removed from the pyrophosphate ligand, more precisely, from the lone pair of its terminal oxygen atom rather than from the magnesium ion. The reliability of this conclusion is confirmed by comparison of the magnetic parameters (g-factors and HFC constants for ^{31}P and ^{25}Mg nuclei) of the paramagnetic complex $Mg(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{2-}$ and the ligand, the $(OPO_2OPO_2OH)^{2-}$ radical. The magnetic parameters and the spin density distribution in both species are identical,[15] *i.e.*, the unpaired electron in the complex $Mg(H_2O)_m{}^{2+}(OPO_2OPO_2OH)^{2-}$ is actually localized on the pyrophosphate ligand.

7. The energies ΔE depend quantitatively on the metal ion; however, their functional dependences on n are identical for both triply and doubly charged ions. First, both of them decrease monotonically with an increase in n. Second, the threshold (trigger) values of n^* for the doubly charged ions of all metals are equal being in the range n^* = 3—4 (see Figs 2 and 3).

Experimental control of the radical ion mechanism

Calculations of the energies of electron transfer, the key reaction of the radical ion synthesis, predict that the radical ion ATP synthesis is a versatile phenomenon. The ions of all six metals that are analyzed in this work (M = Mg, Ca, Zn, Ba, Cd, Sn) can catalyze the radical ion enzymatic reaction. Moreover, it is easy to predict whether or not other ions would possess the same ability; it is sufficient to know only their electron affinities, which are most often well-known and tabulated (see, for example, Ref. 16).

The theoretically predicted radical ion mechanism of the ATP synthesis was proved experimentally for Mg^{2+} ions based on both the isotope and magnetic-field effects.[5,6] Moreover, in full agreement with the theoretical prediction, this mechanism is confirmed by the ATP synthesis catalyzed by Ca^{2+} and Zn^{2+} ions. The dependence of the ATP synthesis by creatine kinase on the magnetic moments of Ca and Zn nuclei (Figs 4 and 5) is a reliable proof for the radical ion mechanism (note that neither Ca nor Zn produce ATP in native cells). It can be predicted with certainty that the Ba^{2+}, Cd^{2+}, and Sn^{2+} ions would also catalyze the ATP synthesis and demonstrate the magnetic isotope effect similarly to the Ca^{2+} and Zn^{2+} ions. Catalysis of ATP synthesis by these ions in living organisms is hardly physiologically significant; however, it can be used for medical purposes, as is the case for magnesium.[7—9]

Thus, the energies of the reactions of electron transfer from pyrophosphate metal complexes to hydrated complexes were calculated; these energies were shown to correspond to the energies of the radical ion reactions of ATP

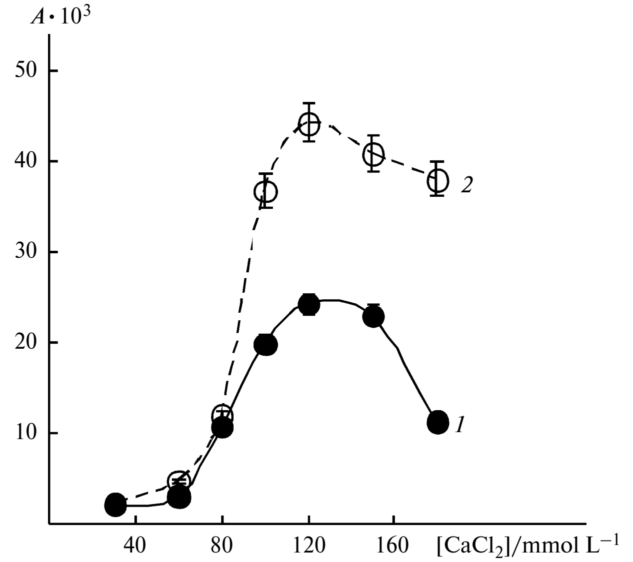


Fig. 4. Yield of ATP synthesized by creatine kinase plotted *vs.* concentration of $CaCl_2$ containing nonmagnetic ^{40}Ca isotope (*1*) and magnetic ^{43}Ca isotope (*2*). The yield of ATP was measured based on radioactivity A of ^{32}P nuclei that were delivered to ATP from the radioactive creatine phosphate substrate.

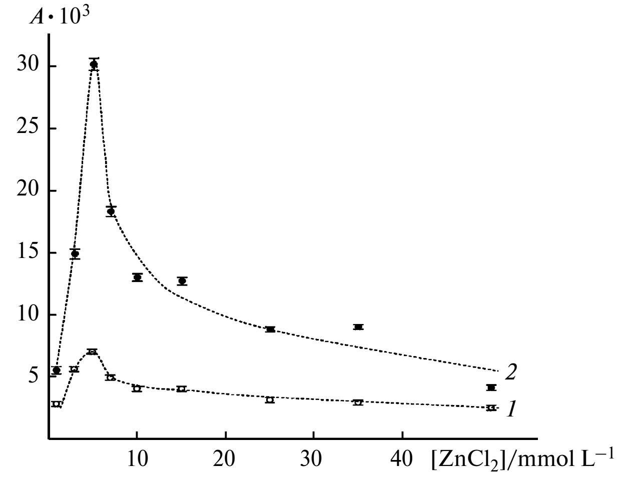


Fig. 5. Yield of ATP synthesized by creatine kinase plotted *vs.* concentration of $ZnCl_2$ containing nonmagnetic ^{64}Zn isotope (*1*) and magnetic ^{67}Zn isotope (*2*). The yield of ATP was measured based on radioactivity A of ^{32}P nuclei that were delivered to ATP from the radioactive creatine phosphate substrate.

synthesis in the enzyme catalytic sites. A threshold dependence of the energy on the number of water molecules n of the ion hydration shell was established: when $n < 4$, the reaction is exothermic, when $n \geq 4$, it is endothermic. The switching of the energy regime accounts for the fact that the synthesis of adenosine triphosphate occurs only in enzymes rather than in homogeneous aqueous solutions. It was theoretically predicted that the radical ion ATP synthesis can be catalyzed by not only magnesium ions, as in living cells, but also by calcium, zinc, barium, cadmium, and tin ions. This prediction was supported by the discovery of the magnetic isotope effect in the ATP synthesis catalyzed by calcium and zinc ions: ions containing magnetic nuclei performed the synthesis twice faster than the same ions with nonmagnetic nuclei.

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