ISSN 0006-3509, Biophysics, 2020, Vol. 65, No. 3, pp. 416–425. © Pleiades Publishing, Inc., 2020.
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MOLECULAR BIOPHYSICS

Magnetic-Isotope Effects of Magnesium and Zinc in Enzymatic ATP Hydrolysis Driven by Molecular Motors

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Received November 30, 2019; revised March 4, 2020; accepted March 6, 2020

Abstract—The effects of different magnesium and zinc isotopes on the enzyme activity of myosin subfragment-1 have been explored. The rate of the enzymatic ATP hydrolysis in reaction media enriched with the magnetic isotope, ^{25}Mg, is twice as high as it is in reaction media enriched with the nonmagnetic isotopes, ^{24}Mg or ^{26}Mg. A similar effect of nuclear spin catalysis has been detected in the experiments with zinc isotopes as cofactors of the enzyme. The rate of the enzymatic ATP hydrolysis with magnetic ^{67}Zn increases by 40–50% compared to that with nonmagnetic ^{64}Zn or ^{68}Zn. The magnetic-isotope effects have been observed at the physiological concentration of magnesium and zinc chlorides (5 mM). The catalytic effect of the magnetic magnesium isotope ^{25}Mg has been revealed in the experiments with Mg-dependent ATPase of myometrial plasma membranes. The magnetic-isotope effects indicate that there is a spin-selective rate-limiting step in the chemo-mechanical process driven by the “molecular motor” due to the energy of ATP hydrolysis and that nuclear spin catalysis causes acceleration of this stage. Some possible mechanisms of the nuclear spin catalysis are discussed.

Keywords: nuclear spin catalysis, myosin, ATPase activity, biomolecular motors, bioreliability, magnetic-isotope effect, magnesium, zinc

DOI: 10.1134/S0006350920030094

INTRODUCTION

Cells and tissues contain atoms of chemical elements, many of which have two types of stable isotopes, magnetic and nonmagnetic. As an example, magnesium has three stable isotopes, ^{24}Mg, ^{25}Mg, and ^{26}Mg with relative contents of 78.7, 10.1, and 11.2%, respectively. The ^{25}Mg isotope is magnetic because its atomic core has a nuclear spin ($I = 5/2$), which creates a magnetic field. The ^{24}Mg and ^{26}Mg isotopes are nonmagnetic because their nuclei do not have a nuclear spin ($I = 0$) and, accordingly, do not create a magnetic field. Another common element in nature, zinc, has five stable isotopes, i.e., ^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, and ^{70}Zn with relative contents of 48.6, 27.9, 4.1, 18.8, and 0.6%, respectively. Among these, ^{67}Zn is the magnetic isotope ($I = 5/2$); the other four isotopes are nonmagnetic ($I = 0$). Magnetic isotopes are also known to create internal magnetic fields, which can exceed the Earth’s magnetic field (≈0.05 mT) by 10 to 100 times at a distance approximately equal to the chemical bond length [1].

Recently, magnetic isotope effects (MIE) were detected in experiments with living cells enriched with the magnesium magnetic isotope. As an example, the activity of the superoxide dismutase antioxidant enzyme in *E. coli* cells that were grown on a medium enriched with ^{25}Mg was lower by 40% than in cells grown on a medium enriched with the nonmagnetic isotope of magnesium [2]. *S. cerevisiae* yeast cells, enriched with the magnetic magnesium isotope, recover after irradiation with a short-wave ultraviolet light or ionizing radiation twice as fast as cells, enriched with the nonmagnetic magnesium isotope [3]. In the works of our group, the magnesium MIE was first detected in the reaction of ATP hydrolysis catalyzed by myosin, which is one of the most important bioenergetic molecular motors [4, 5].

The goal of this work was to study the ATP-hydrolase activity of biomolecular motors in the presence of various isotopes of magnesium and zinc as the cofactors of the enzyme. In experiments with myosin we revealed significant effects of the acceleration of enzymatic hydrolysis in the presence of magnetic magnesium and zinc isotopes in comparison with those in the presence of the nonmagnetic isotopes of the same

Abbreviations: MIE, magnetic-isotope effect.

Table 1. The isotope composition of magnesium in reaction media for measuring ATP hydrolysis

Isotope	Isotopic enrichment, %			
	$^{24}MgCl_2$	$^{25}MgCl_2$	$^{26}MgCl_2$	$MgCl_2$
^{24}Mg	99.7 ± 0.1	0.17 ± 0.02	0.16 ± 0.03	79.2 ± 0.4
^{25}Mg	1.3 ± 0.1	98.2 ± 0.2	0.46 ± 0.1	10.1 ± 0.1
^{26}Mg	1.1 ± 0.1	0.22 ± 0.1	98.7 ± 0.3	10.7 ± 0.2

The solutions contained 20 mM Tris-HCl (pH 7.2), 0.01 mM $CaCl_2$, 100 mM KCl, 3 mM ATP, and 5 mM magnesium chloride of the following isotope composition: $MgCl_2$, $^{24}MgCl_2$, $^{25}MgCl_2$, and $^{26}MgCl_2$. Average values ± mean square deviation ($m \pm SD$) are presented; the number of independent measurements in each solution was $n = 5$.

elements (nuclear spin catalysis). A small catalytic effect of the magnetic isotope of magnesium was also detected in experiments with Mg-ATPase isolated from the myometrial plasma membranes.

MATERIALS AND METHODS

We used ^{24}MgO, ^{25}MgO, and ^{26}MgO magnesium oxides with isotope enrichment of 99.9, 98.8, and 97.7 atomic percentage and zinc oxides ^{64}ZnO, ^{67}ZnO, and ^{68}ZnO, with isotope enrichment of 99.3, 94.2, and 98.9 atomic percentage, respectively (Electrochimpribor, Russia). These oxides were used for the preparation of the solutions of the chlorides $^{24}MgCl_2$, $^{25}MgCl_2$, $^{26}MgCl_2$, $^{64}ZnCl_2$, $^{67}ZnCl_2$, and $^{68}ZnCl_2$ under the action of concentrated HCl of analytical grade according to the standard method [6]. Magnesium and zinc chlorides of natural isotope composition ($MgCl_2$ and $ZnCl_2$) and other reagents used in this work were from Sigma-Aldrich (United States), Perkin Elmer (United States), and Merck (Germany).

The concentrations of magnesium and zinc in the initial solutions were measured by inductively coupled plasma atomic emission spectroscopy; all solutions were leveled by the concentration of magnesium and zinc ions, respectively. The isotope compositions of the magnesium and zinc solutions and their elemental composition were evaluated by inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectroscopy at the Analytical Center of The Institute of Microelectronics Technology Problems and High Purity Materials of the Russian Academy of Sciences (Chernogolovka, Moscow oblast). The contents of the impurity elements Li, B, Na, Mg, Al, P, S, K, Ca, V, Mn, Fe, Cu, Zn, Sr, and Ba were evaluated by inductively coupled plasma atomic emission spectroscopy using an iCAP-6500 Duo spectrometer (Thermo Scientific, United States). The contents of Li, B, Be, Al, P, Sc, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, and U were determined by inductively coupled plasma mass spectrometry on an X-Series II spectrometer (Thermo Scientific, United States) according to the methods described earlier [7]. The evaluation of Li, B, Al, P, V, Mn, Cu, Zn, Sr, and Ba by two independent methods allowed us to monitor the accuracy of the analysis in each sample [7].

Biochemical experiments were performed in the Department of Muscle Biochemistry of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. Myosin subfragment-1 was prepared from smooth muscle (myometrium) according to the standard method [8, 9]. The myosin subfragment-1 is considered as a sufficient functional unit of myosin because it retains all its native properties, i.e., catalytic ATP-hydrolase activity and the ability to interact with actin [8–10]. Mg^{2+}-dependent plasma membrane ATPase (basal Ca^{2+}-independent Mg^{2+}-ATPase) was prepared from smooth muscle according to the method described in [11, 12]. The ATP-hydrolase activity of enzymes was measured at 37°C according to the amount of inorganic phosphate (Pi) released during the ATP hydrolysis [13]. This method (the Fiske–Subbarow method) is based on the reaction of inorganic phosphate with ammonium molybdate in the presence of ascorbic acid as a reducing agent, which results in the formation of a colored complex in an amount proportional to the amount of Pi formed during ATP hydrolysis [13]. Experimental data were analyzed using the standard methods of variance analysis (ANOVA) and the MS Office and Statistica 4 programs.

RESULTS

We analyzed the isotope composition of the reaction media used for the ATP hydrolysis. The results of the experiments with various magnesium and zinc isotopes (Table 1 and Table 2, respectively) indicate a high degree of isotope enrichment of the reaction media with magnesium (^{24}Mg, ^{25}Mg, or ^{26}Mg) and zinc (^{64}Zn, ^{67}Zn, or ^{68}Zn) isotopes.

The ATP-hydrolase activity of myosin subfragment-1 in the media with various magnesium isotopes was measured in a standard reaction medium that contained 5 mM (the physiological concentration)

Table 2. The isotope composition of zinc in reaction media for measuring ATP hydrolysis

Isotope	Isotopic enrichment, %		
	$^{64}ZnCl_2$	$^{67}ZnCl_2$	$^{68}ZnCl_2$
^{64}Zn	99.2 ± 0.1	0.74 ± 0.02	0.48 ± 0.02
^{66}Zn	0.54 ± 0.04	1.6 ± 0.02	0.42 ± 0.02
^{67}Zn	0.063 ± 0.009	93.9 ± 0.1	0.27 ± 0.01
^{68}Zn	0.20 ± 0.01	3.7 ± 0.03	98.8 ± 0.1
^{70}Zn	0.006 ± 0.001	0.045 ± 0.003	0.027 ± 0.004

The solutions contained 20 mM Tris-HCl (pH 7.2), 0.01 mM $CaCl_2$, 100 mM KCl, 3 mM ATP, and 5 mM zinc chloride of the following isotope composition: $^{64}ZnCl_2$, $^{67}ZnCl_2$, and $^{68}ZnCl_2$. Average values ± mean square deviation ($m \pm SD$) are presented; the number of independent measurements in each solution was $n = 5$.

magnesium chloride, i.e., $^{24}MgCl_2$, $^{25}MgCl_2$, $^{26}MgCl_2$, or $MgCl_2$ (the natural isotope composition) (Fig. 1a). Three independent series of experiments were performed with different myosin preparations isolated at different times from three different animals. Three to eight repetitions were performed with each isotope of magnesium and magnesium of a natural isotope composition. In all the experimental series, the same magnetic-isotope effect was observed, i.e., the activity of the enzyme in the presence of the magnetic isotope (^{25}Mg) was higher by factors of 2–2.5 than that in the presence of the nonmagnetic isotope (^{24}Mg or ^{26}Mg). However, there were no significant differences in the activity of the enzyme in experiments with nonmagnetic isotopes of magnesium. It is important to note that MIE is not observed in nonenzymatic (spontaneous) ATP hydrolysis. The rate of the ATP hydrolysis did not differ in the reaction media of the identical composition that contained all components except the enzyme with the use of $^{24}MgCl_2$, $^{25}MgCl_2$, $^{26}MgCl_2$, or $MgCl_2$ (Fig. 1b). The magnetic-isotope effect was observed only in the enzymatic hydrolysis of ATP.

The next figure shows the ATP-hydrolase activity of the same enzyme in reaction media that contained $^{25}MgCl_2$ and $^{24}MgCl_2$ in different ratios, i.e., the concentrations of the magnetic (^{25}Mg) and nonmagnetic (^{24}Mg) isotopes in the solution varied from 0 to 5 mM and from 5 to 0 mM, respectively. An almost linear increase occurred in the rate of ATP hydrolysis with an increase in the proportion of the magnetic isotope content in the reaction solution (Fig. 2).

We also studied the effects of various zinc isotopes on the ATP-hydrolase activity of myosin subfragment-1. The standard reaction medium contained zinc chlo-

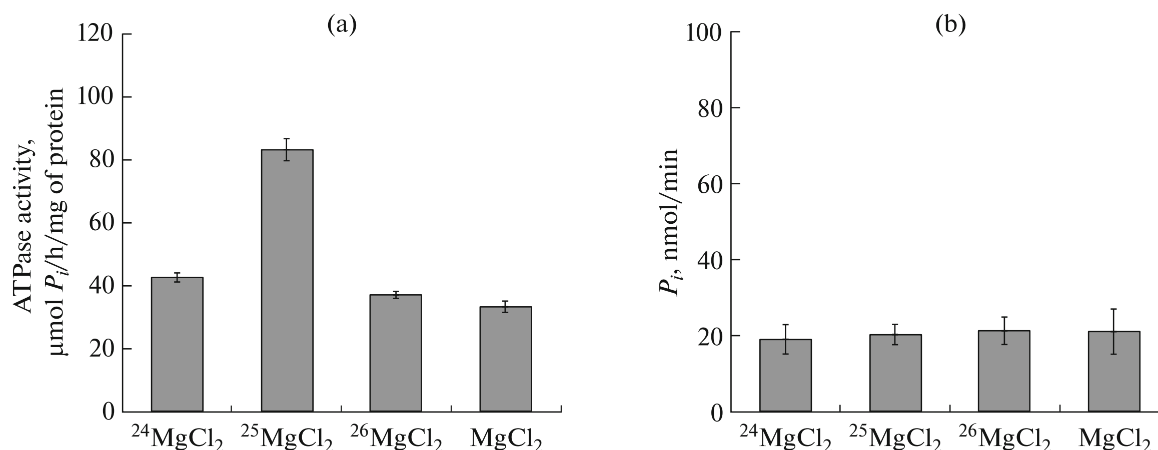

Fig. 1. (a) The ATPase activity (m ± SD) of myosin subfragment-1 in reaction media with different magnesium isotopes. The solutions contained 20 mM Tris-HCl (pH 7.2), 0.01 mM $CaCl_2$, 100 mM KCl, 3 mM ATP, myosin subfragment-1 (20 μg of protein/mL) and 5 mM of magnesium chloride of one of the following isotope composition: $MgCl_2$ (natural isotope composition), $^{24}MgCl_2$, $^{25}MgCl_2$ or $^{26}MgCl_2$. The differences between the average values in experiments with the magnetic isotope ^{25}Mg and nonmagnetic magnesium isotopes are statistically significant ($P < 0.01$). (b) Nonenzymatic hydrolysis of ATP in aqueous solutions in the presence of $^{24}MgCl_2$, $^{25}MgCl_2$, $^{26}MgCl_2$, and $MgCl_2$. According to [4].

Table 3. The results of the analysis of the elemental composition of reaction media with magnesium chloride of various isotope compositions

Element	Determination limit (DL), mg/L	Elemental composition mg/L			
		natural Mg	^{24}Mg	^{25}Mg	^{26}Mg
Li	0.00008	0.00027	0.00015	0.00017	0.00010
Be	0.00008	<DL	<DL	<DL	<DL
B	0.03	<DL	<DL	<DL	<DL
Na	0.3	158	149	135	137
Mg	0.1	154	159	142	139
Al	0.02	0.045	0.030	0.049	0.065
Si	0.3	0.17	0.17	0.15	0.13
P	0.5	224	229	227	230
S	2	<DL	<DL	<DL	<DL
K	0.3	6360	6247	6280	6313
Ca	0.7	2.0	2.0	2.4	2.1
Sc	0.001	<DL	<DL	<DL	<DL
Ti	0.02	<DL	<DL	<DL	<DL
V	0.002	<DL	<DL	<DL	<DL
Cr	0.02	<DL	<DL	<DL	<DL
Mn	0.003	0.029	0.031	0.037	0.080
Fe	0.1	<DL	<DL	<DL	<DL
Co	0.003	<DL	<DL	<DL	<DL
Ni	0.007	<DL	<DL	<DL	<DL
Cu	0.003	0.0056	0.0039	0.0067	0.011
Zn	0.009	0.065	0.050	0.070	0.063
Ga	0.001	<DL	<DL	<DL	<DL
Ge	0.002	<DL	<DL	<DL	<DL
As	0.002	<DL	<DL	<DL	<DL
Se	0.009	<DL	<DL	<DL	<DL
Br	0.3	2.2	2.4	2.6	2.6
Rb	0.0003	0.0860	0.0936	0.0950	0.0969
Sr	0.002	<DL	0.0029	0.0046	<DL
Y	0.0001	<DL	<DL	<DL	<DL
Zr	0.0001	0.0031	0.0013	0.0022	0.0028
Nb	0.0004	<DL	<DL	<DL	<DL
Mo	0.0003	0.0079	0.0093	0.0136	0.0168
Ru	0.0002	<DL	<DL	<DL	<DL
Rh	0.0002	<DL	<DL	<DL	<DL
Pd	0.0004	<DL	<DL	<DL	<DL
Ag	0.0002	0.00078	0.00068	0.00075	0.00109
Cd	0.0003	0.0037	0.0040	0.0043	0.0040
In	0.0001	<DL	<DL	<DL	<DL
Sn	0.0002	0.00081	0.00027	0.00063	0.00100
Sb	0.0002	<DL	<DL	<DL	<DL
Te	0.0002	<DL	<DL	<DL	<DL
Cs	0.00004	<ПО	0.00006	0.00008	0.0001
Ba	0.001	0.0040	0.0045	0.0045	0.0043

Table 3. (Contd.)

Element	Determination limit (DL), mg/L	Elemental composition mg/L			
		natural Mg	^{24}Mg	^{25}Mg	^{26}Mg
La	0.0002	<DL	<DL	<DL	<DL
Ce	0.0008	<DL	<DL	<DL	<DL
Pr	0.00002	<DL	<DL	<DL	<DL
Nd	0.0003	<DL	<DL	<DL	<DL
Sm	0.00002	<DL	<DL	<DL	<DL
Eu	0.000007	<DL	<DL	<DL	<DL
Gd	0.00001	<DL	<DL	<DL	<DL
Tb	0.00001	<DL	<DL	<DL	<DL
Dy	0.00001	<DL	<DL	<DL	<DL
Ho	0.00001	<DL	<DL	<DL	<DL
Er	0.00001	<DL	<DL	<DL	<DL
Tm	0.00001	<DL	<DL	<DL	<DL
Yb	0.00001	<DL	<DL	<DL	<DL
Lu	0.00001	<DL	<DL	<DL	<DL
Hf	0.0001	0.00120	0.00078	0.00062	0.00062
Ta	0.0002	<DL	<DL	<DL	<DL
W	0.0003	<DL	<DL	<DL	<DL
Re	0.00001	<DL	<DL	<DL	<DL
Os	0.00002	<DL	<DL	<DL	<DL
Ir	0.00001	<ПО	<ПО	<ПО	<ПО
Pt	0.00003	<DL	<DL	<DL	0.00067
Au	0.0001	<DL	<DL	<DL	<DL
Hg	0.0003	<DL	<DL	<DL	<DL
Tl	0.00001	0.0088	0.0091	0.0091	0.0094
Pb	0.0008	0.038	0.037	0.039	0.039
Bi	0.00003	0.00006	<DL	<DL	0.00004
Th	0.00002	0.00060	0.00036	0.00027	0.00025
U	0.00002	<DL	<DL	<DL	<DL

ride instead of magnesium chloride, i.e., 5 mM $^{67}ZnCl_2$ (the magnetic isotope of zinc) or 5 mM $^{64}ZnCl_2$ or $^{68}ZnCl_2$ (nonmagnetic isotopes of zinc). Two independent series of experiments were performed with the enzyme isolated from two animals; at least three repeats with each isotope of zinc and each enzymatic preparation were carried out. The results are shown in Fig. 3. The Zn^{2+} ion as the myosin cofactor is known to be less effective than Mg^{2+} [14, 15]. The activity of the enzyme in the presence of nonmagnetic zinc ions was lower than in the presence of magnesium ions (Fig. 3). In the experiments with various nonmagnetic zinc isotopes, we observed no differences in the rate of the ATP hydrolysis. However, the rate of the ATP hydrolysis in the presence of the magnetic zinc isotope (^{67}Zn), was higher by 50–70% compared to that in the presence of nonmagnetic zinc isotopes. Thus, the effect of the acceleration of the enzymatic ATP hydrolysis in the presence of the zinc isotopes was analogous to the MIE previously detected in the experiments with the magnesium isotopes.

In addition to the experiments with myosin, we studied the ATP hydrolysis by Mg^{2+}-dependent ATP-hydrolase of myometrial plasma membranes. This enzyme, the so-called basal Mg^{2+}-ATPase or Ca^{2+}-independent Mg^{2+}-ATPase, plays an important role in the regulation of both the concentration of protons outside and inside the cells and the concentration of Ca^{2+} ions in the cytoplasm of smooth muscle cells [11, 12]. Three independent series of experiments were carried out with preparations of this enzyme isolated from three animals. Typical results are shown in Fig. 4. The rate of the enzymatic ATP hydrolysis in the pres-

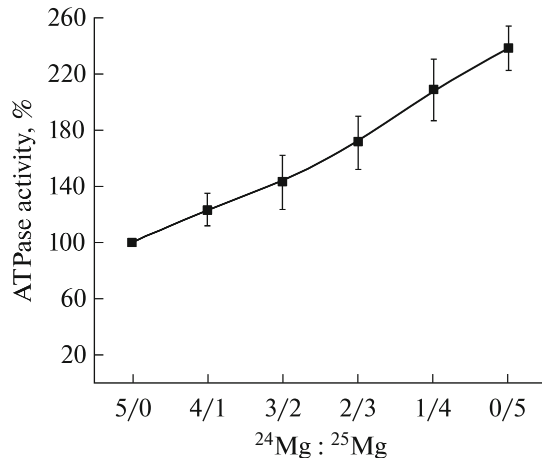

Fig. 2. The ATPase activity of myosin subfragment-1 as a function of the fraction of the ^{25}Mg magnetic magnesium isotope in the reaction mixture. Data ($m \pm SD$) are presented as a percentage of the activity of 5 mM $^{24}MgCl_2$ accepted as 100%.

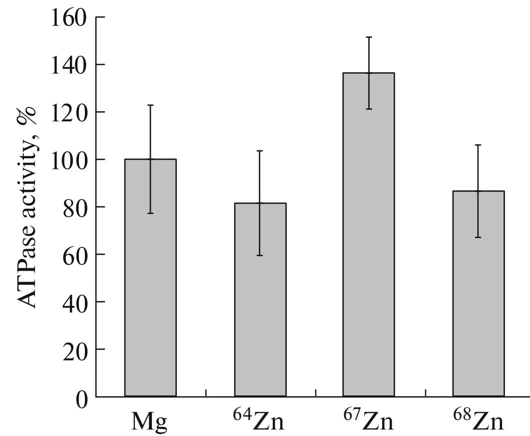

Fig. 3. The ATPase activity of the myosin subfragment-1 in reaction media containing various zinc isotopes. The solutions contained 20 mM Tris-HCl (pH 7.2), 0.01 mM $CaCl_2$, 100 mM KCl, 3 mM ATP, myosin subfragment-1 (20 μg protein/mL) and 5 mM zinc chloride ($^{64}ZnCl_2$, $^{67}ZnCl_2$ or $^{68}ZnCl_2$). The data ($m \pm SD$, $n = 3$) are presented as the percentage to enzymatic activity in the presence of 5 mM $MgCl_2$ of natural isotope composition, accepted as 100%. The differences between the average values in the experiments with the magnetic isotope, ^{67}Zn, and the nonmagnetic zinc isotopes are statistically significant ($P < 0.05$).

ence of the magnetic isotope ^{25}Mg was higher by 15–20% (on average) than in the presence of nonmagnetic isotopes. Thus, the catalytic effect of the magnetic magnesium isotope was also revealed in these experiments.

It could be assumed that the detected magnetic-isotope effects are caused by different contents of impurities of any foreign elements in the samples of magnesium or zinc isotopes. However, this is not confirmed by the data of elemental analysis of media that contained various isotopes of magnesium (Table 3) and zinc (Table 4). According to the data of atomic emission spectrometry and mass spectrometry, the elemental composition of the reaction media was the same, and the content of impurity elements was no more than a few micromoles per liter, regardless of the type of magnesium or zinc isotopes. As an example, the content of iron (Fe) in the solution with the magnetic magnesium isotope (^{25}Mg) is almost four times higher than in the solution with the nonmagnetic isotope (^{24}Mg) but two and a half times less than in the solution with another nonmagnetic isotope (^{26}Mg). Meanwhile, the activity of the enzyme in the presence of the nonmagnetic isotopes ^{24}Mg or ^{26}Mg was almost the same but two times lower, respectively, than that in the presence of the magnetic isotope of magnesium. Similarly, the possibility of the influence of other impurity elements is excluded. It should be taken into account that not only the initial oxides of magnesium and zinc but other reagents necessary for the experiments also contain impurities that are introduced into the reaction media in the quantities that significantly exceed the amounts of the same impurities introduced from the magnesium or zinc stock solutions.

DISCUSSION

Magnetic-isotope effects were detected in our experiments. It has been shown that the enzymatic

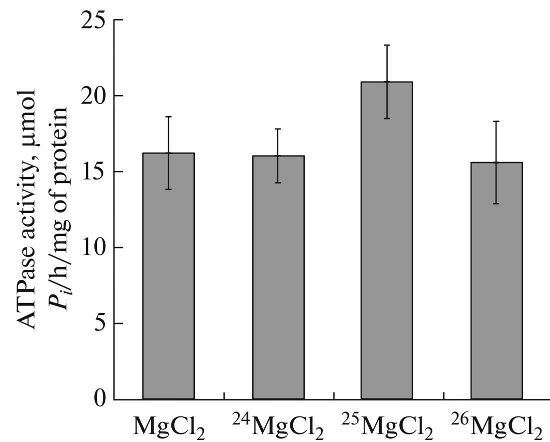

Fig. 4. The ATPase activity of Mg^{2+}-dependent ATP-hydrolase from the myometrial plasma membranes (μmol Pi/h/mg of protein) in reaction media containing various magnesium isotopes. The solutions contained 50 mM Tris-HCl (pH 7.4), 25 mM NaCl, 125 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM EGTA, 20 μg protein/mL, 3 mM ATΦ, and 5 mM magnesium chloride, correspondingly, natural isotope composition, $^{24}MgCl_2$, $^{25}MgCl_2$ or $^{26}MgCl_2$. The differences between the average values in experiments with the magnetic isotope and the nonmagnetic isotopes of magnesium (m ± SD, $n = 4$) are statistically significant ($P = 0.05$).

Table 4. The results of analysis of the elemental composition of reaction media with zinc chloride of various isotope compositions

Element	Determination limit (DL), mg/L	Elemental composition mg/L		
		^{64}Zn	^{67}Zn	^{68}Zn
Li	0.001	<DL	<DL	<DL
Be	0.0002	<DL	<DL	<DL
B	0.02	0.52	0.70	0.60
Na	0.3	0.57	1.0	0.77
Mg	0.05	<DL	0.069	<DL
Al	0.06	0.22	0.27	0.23
Si	0.6	<DL	<DL	<DL
P	0.6	<DL	<DL	<DL
S	2	<DL	<DL	<DL
K	0.1	<DL	<DL	<DL
Ca	0.3	<DL	<DL	<DL
Sc	0.008	<DL	<DL	<DL
Ti	0.01	<DL	<DL	<DL
V	0.003	<DL	<DL	<DL
Cr	0.01	<DL	<DL	<DL
Mn	0.003	<DL	<DL	<DL
Fe	0.09	<DL	<DL	<DL
Co	0.003	<DL	<DL	<DL
Ni	0.02	0.14	<DL	<DL
Cu	0.02	0.047	0.098	0.099
Ga	0.0004	<DL	<DL	<DL
As	0.06	<DL	<DL	<DL
Se	0.009	<DL	<DL	<DL
Rb	0.003	<DL	<DL	<DL
Sr	0.001	<DL	<DL	<DL
Y	0.001	<DL	<DL	<DL
Zr	0.002	<DL	<DL	<DL
Nb	0.001	<DL	<DL	<DL
Mo	0.000	<DL	<DL	<DL
Rh	0.001	<DL	<DL	<DL
Pd	0.001	<DL	<DL	<DL
Ag	0.002	<DL	<DL	<DL
Cd	0.001	<DL	<DL	<DL
Sn	0.004	<DL	<DL	<DL
Sb	0.001	<DL	<DL	<DL
Te	0.001	<DL	<DL	<DL
Cs	0.0002	<DL	<DL	<DL
Ba	0.001	0.0051	0.0025	0.010
La	0.0003	<DL	<DL	<DL
Ce	0.0003	<DL	<DL	<DL
Pr	0.0002	<DL	<DL	<DL
Nd	0.0007	<DL	<DL	<DL
Sm	0.0002	<DL	<DL	<DL

Table 4. (Contd.)

Element	Determination limit (DL), mg/L	Elemental composition mg/L		
		^{64}Zn	^{67}Zn	^{68}Zn
Eu	0.0001	<DL	<DL	<DL
Gd	0.0002	<DL	<DL	<DL
Tb	0.0001	<DL	<DL	<DL
Dy	0.0001	<DL	<DL	<DL
Ho	0.0001	<DL	<DL	<DL
Er	0.0001	<DL	<DL	<DL
Tm	0.0001	<DL	<DL	<DL
Yb	0.0001	<DL	<DL	<DL
Lu	0.0001	<DL	<DL	<DL
Hf	0.0001	<DL	<DL	<DL
Ta	0.0003	<DL	<DL	<DL
W	0.0003	<DL	<DL	<DL
Re	0.00003	<DL	<DL	<DL
Ir	0.0001	<DL	<DL	<DL
Pt	0.0006	<DL	<DL	<DL
Au	0.0002	<DL	<DL	<DL
Hg	0.0009	<DL	<DL	<DL
Tl	0.0001	<DL	<DL	<DL
Pb	0.002	0.0024	0.019	0.0082
Bi	0.0001	<DL	<DL	<DL
Th	0.0003	<DL	<DL	<DL
U	0.0001	<DL	<DL	<DL

hydrolysis of ATP, which is catalyzed by both myosins isolated from the myometrium and Mg-dependent ATPase of the plasma membranes of myocytes, is accelerated by the nuclear spin of the magnetic isotope, thus indicating nuclear spin catalysis. Previously, a similar catalytic effect (small but statistically significant) of the nuclear spin of the ^{25}Mg isotope was found in experiments concerning the effects of various magnesium isotopes on ATP hydrolysis catalyzed by H^+-ATPase (MF_0F_1 complex) isolated from yeast mitochondria and embedded in liposomes [16]. However, magnetic-isotope effects were not detected in experiments on the influence of various magnesium isotopes on ATP-dependent reactions catalyzed by creatine phosphate kinase and luciferase [17]. Nuclear spin catalysis appears to be observed only for enzymes that function as a molecular motor, i.e., that use the chemical energy of ATP to perform mechanical work. The ATP energy is used either completely, as in the case of myosin, or at least partially, as in the cases of mitochondrial H^+-ATPase and Mg-dependent ATPase of the myometrial plasma membrane.

Magnetic-isotope effects have long been known in chemical and molecular physics [19–21]. In chemistry, MIE is manifested via the fact that the rate and yield of the reaction with the involvement of free radicals and/or ion–radical pairs change significantly depending on whether the initial reagents contain the magnetic or nonmagnetic isotope of the same element. MIE is the direct consequence of the law of conservation of angular momentum, the electron angular momentum or electron spin in this case. The total electron spin (S) of the chemical reaction products must be equal to the total electron spin of the initial reagents. In physics, a similar spin ban occurs, for example, in singlet–triplet transitions in molecules and solids.

The magnetic-isotope effect indicates that there is a "bottleneck" in the studied process, i.e., a limiting stage, which is dependent on the electron spin state, and that the nuclear spin of the magnetic isotope accelerates this stage. In chemistry, the existence of a radical pair or ion–radical pair as an intermediate product ("bottleneck") of the reaction is usually assumed to explain MIE [20]. However, it is known that the reaction of the ATP hydrolysis with the formation of ADP and P_i follows an acid–base mechanism [22]. Accordingly, the ion–radical pair is unlikely to occur as an intermediate in this reaction. In fact, MIE

is not observed in nonenzymatic ATP hydrolysis. However, a different situation may occur in the case of ATP hydrolysis catalyzed by a molecular motor. It has been confirmed experimentally that ATP hydrolysis initiates electron-conformational interactions in the active center of the enzyme. Due to the energy released during ATP hydrolysis, conformational excitation, i.e., a deformation of the macromolecule, takes place [23, 24]. Moreover, during ATP hydrolysis catalyzed by myosin, the cycle of generating the mechanical stress of the macromolecule consists of several stages according to quantum-mechanical calculations [25]. At the first stage, the γ-phosphate of ATP is stabilized in the state of dissociated metaphosphate ($P_\gamma O_3^-$). In this case, the hydrolysis products, ADP and P_i, remain in the active center of the enzyme in close contact and are released only after myosin binds to actin filaments. This is consistent with the well-known fact of the reversibility of the ATP hydrolysis reaction with myosin. As long as the hydrolysis products remain bound to myosin in close contact, they can reform ATP [25]. It can be assumed that electron-conformational excitation of a macromolecule in the active center of the enzyme provides the transfer of the electron density on ADP or Mg^{2+} from, for example, the OH-group of the bound water molecule or from the NH_2 group of Glu-459 with the formation of the corresponding ion–radical pair. The first step is followed by the nucleophilic attack of the adenosine diphosphate oxyanion on the inorganic phosphate to form ATP. The stable spin state of the product, ATP–Mg (in experiments with zinc, ATP–Zn), must be a singlet (electron spin $S = 0$). Meanwhile, the nuclear spin of ^{25}Mg (^{67}Zn) can convert the myosin-bound ion–radical pair into a triplet state ($S = 1$) through the hyperfine interaction with the ion-radical pair's unpaired electron. The nuclear spin of the ^{25}Mg (^{67}Zn) isotope creates a spin ban, thus hindering the undesirable reverse reaction of the ATP synthesis and, consequently, contributing to the direct reaction of ATP hydrolysis. The hypothesis of the key role of a virtual ion–radical pair in the synthesis of ATP during oxidative phosphorylation was stated approximately 50 years ago [26].

An alternative explanation of nuclear spin catalysis in ATP hydrolysis catalyzed by molecular motors is possible [21]. It can be assumed that the energy released during the ATP hydrolysis (~0.54 eV) is not large enough for the electron-conformational transition of the macromolecule into the singlet excited state. This energy is sufficient for the transition to a lower triplet state ($S = 1$). However, this transition from the ground state ($S = 0$) is forbidden by the spin conservation law. The magnetic isotope (^{25}Mg or ^{67}Zn) changes the situation, i.e., the nuclear spin of the isotope eliminates the spin ban. Thus, the magnetic isotope provides the necessary spin conversion of the electron-conformational state of the macromolecule into the triplet state (coherent bosons), thus accelerating the chemomechanical cycle of the enzymatic reaction [21]. A similar mechanism was proposed in solid-state physics to explain the influence of magnetic fields on the mobility of dislocations [27].

Let us consider another possible explanation for the catalytic effect of the nuclear spin of ^{25}Mg/^{67}Zn isotopes. Hydrolysis of ATP catalyzed by a molecular motor is accompanied by a significant conformational rearrangement of the macromolecule. In this case, processes of dehydration and rehydration of the electrically charged amino-acid groups occur. Meanwhile, there are two isomers of water molecules, which differ in the mutual orientation of the hydrogen nuclear spins, i.e., *ortho*-H_2O and *para*-H_2O with parallel and antiparallel orientations of proton spins, respectively. According to quantum statistics, *ortho*-H_2O makes up 75% of the total volume at room temperature [28]. There is reason to believe that *ortho*-H_2O molecules have a predominant affinity for *L*-amino acids compared with the *para*-H_2O molecules [29]. In this case, the movement of the predominantly bound *ortho*-H_2O molecules is difficult during the conformational transformation of the macromolecule, and the spin-rotational interactions of protons are too weak to ensure the proper efficiency of *ortho–para* transitions. Again, the magnetic isotope ^{25}Mg (or ^{67}Zn) can significantly improve the situation, i.e., they can eliminate the problem of spin prohibition, thus ensuring the necessary conversion rate of the water isomers.

In molecular motors, which operate on the nonmagnetic isotope of magnesium, the function of spin catalysis can be performed by the nuclear spins of hydrogen (^{1}H, $I = 1/2$) and phosphorus (^{31}P, $I = 1/2$). The relatively high catalytic activity of the magnetic isotopes of magnesium and zinc is obviously caused by the fact that the nuclear spins of ^{25}Mg ($I = 5/2$) and ^{67}Zn ($I = 5/2$) are five times larger than the nuclear spins of ^{1}H and ^{31}P, thus creating the stronger local magnetic fields (the hyperfine interaction constant ≈21 mT) in the active center of the enzyme.

CONCLUSIONS

Experiments with the most important molecular motor of bioenergetics, myosin, demonstrated significant effects of acceleration of enzymatic ATP hydrolysis by the magnetic isotopes of magnesium and zinc. The effect of the nuclear spin catalysis was also revealed in experiments with Mg-dependent ATPase of the myometrial plasma membrane.

Detailed mechanisms of the nuclear spin catalysis with molecular motors are a task for further research.

This article is based on a report at the VI Congress of Biophysicists of Russia (Sochi, 2019) [30].

ACKNOWLEDGMENTS

The authors thank T.A. Veklich for the experiments with ATPase of the myometrial plasma membrane and S.A. Kosterin, academician of the National Academy of Sciences of Ukraine (head of the Department of Muscle Biochemistry of Palladin Institute of Biochemistry, NAS), and his colleges for their fruitful cooperation.

FUNDING

The work was supported by the Ministry of Science and Higher Education of the Russian Federation (theme AAAA-A19-119092390041-5).

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS

Experiments with animals were performed in full compliance with the European Convention for the protection of animals used for scientific experiments and other scientific purposes (Strasbourg, 18.III.1986).

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Translated by A.S. Levina