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Nuclear Spin Catalysis in Living Nature

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Abstract—Experiments with cells enriched in stable magnesium isotopes, magnetic ^{25}Mg or nonmagnetic ^{24}Mg and ^{26}Mg, are carried out. It is revealed that adaptation of bacteria *E. coli* to the growth media enriched in magnetic ^{25}Mg proceeds faster as compared to the growth media enriched in nonmagnetic magnesium isotopes. In experiments with another commonly accepted cell model, *S. cerevisiae* yeast, it is revealed that the rate constant of postradiation recovery of the cells after UV irradiation is two times higher for cells enriched in ^{25}Mg than for cells enriched in the nonmagnetic isotope. In collaboration with Ukrainian colleagues from the Palladin Institute of Biochemistry, the effects of different isotopes of magnesium on ATPase activity of myosin isolated from myometrium are studied. It is found that the rate of the enzymatic hydrolysis of ATP for ^{25}Mg is 2.0–2.5 times higher as compared to nonmagnetic isotopes ^{24}Mg and ^{26}Mg. Some possible mechanisms of magnetic isotope effects (nuclear spin catalysis) in biological objects are discussed.

Keywords: biocatalysis, nuclear spin catalysis, stable isotopes, magnetic isotope effect, reliability

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INTRODUCTION

Biopolymer catalysts, like other cell structures, are built of atoms of chemical elements. Many of them have magnetic and nonmagnetic stable isotopes. Internal magnetic fields created by atomic nuclei of several magnetic isotopes (at a distance of the chemical bond length) are 10–100 times greater than Earth's magnetic field (~0.05 mT) [1]. The question arises whether the magnetic field of an atomic nucleus has any effect on efficiency and reliability of biocatalysis. Magnesium as one of the most abundant elements in nature is of particular interest, since its cation Mg^{2+} serves as a cofactor for the enzymes of hydrolysis and synthesis of ATP and other important enzymes. Magnesium has three stable isotopes, ^{24}Mg, ^{25}Mg, and ^{26}Mg, with natural abundance of 78.7, 10.13, and 11.17%. Only ^{25}Mg is a magnetic isotope (nuclear spin $I = 5/2$) and creates a magnetic field, whereas ^{24}Mg and ^{26}Mg are nonmagnetic isotopes (nuclear spin $I = 0$) [1]. We carried out experiments with cells enriched in different isotopes of magnesium. Together with Ukrainian biochemists, we studied the effect of different magnesium isotopes on the enzyme myosin. Magnetic isotope effects were detected; namely, the magnetic magnesium isotope ^{25}Mg performs cofactor functions much more efficiently than the nonmagnetic isotopes ^{24}Mg and ^{26}Mg.

MATERIALS AND METHODS

Magnesium oxides ^{24}MgO, ^{25}MgO, and ^{26}MgO with isotope enrichment of 99.8, 98.8, and 97.7 at %, respectively, were purchased from Elektrokhimpribor (Rosatom, Sverdlovsk oblast).

Experiments with *Escherichia coli* cells were performed at the Institute of Problems of Chemical Physics and, thereafter, reproduced in the Department of Microbiology of Orenburg State University (head of the department, Prof. D.G. Deryabin) [2, 3]. In order to perform the isotopic enrichment of bacteria *E. coli*, we prepared three alternative liquid nutrient M9 media that, in addition to glucose (from Ekokhim, Russia) and other standard components (Sigma-Aldrich), contained magnesium isotopes as $^{24}MgSO_4$, $^{25}MgSO_4$ or $^{26}MgSO_4$ at a concentration of 2.2 mM/L. All other experimental conditions (elemental composition of the growth medium, pH, etc.) were identical. Equal aliquots of the cell suspension were incubated for 24 h in the M9 medium containing no magnesium and then were placed in the same medium but supplemented with ^{24}Mg, ^{25}Mg, or ^{26}Mg as the corresponding sulfate, and then were cultured under continuous aeration with shaking at 37°C. The experiments with the three isotopes were carried out simultaneously, three cell culture samples with each isotope; i.e., nine samples were studied under the same conditions in parallel experiments. The experiment was repeated twice with different inoculants. The kinetics of cell culture growth was monitored by measuring the optical density at 600 nm (OD_{600}). The viability of cells was evaluated according to their ability to form colonies on the standard solid nutrient medium (agar) using Petri dishes [2, 3].

Activity of the enzyme superoxide dismutase (SOD) in the cells was measured early in the stationary growth phase polarographically using standard methods.

Experiments with isotopically enriched *Saccharomyces cerevisiae* yeast cells were performed at the Petersburg Nuclear Physics Institute, National Research Center Kurchatov Institute, in the Department of Molecular and Radiation Biology (head of the department, Prof. V.G. Korolev) in conjunction with Ukrainian colleagues from the Institute of Cell Biology and Genetic Engineering of National Academy of Sciences of Ukraine, Kyiv [4, 5]. Several standard liquid nutrient M3 media containing various isotopes of magnesium in the form of magnesium sulfate were prepared at a concentration of 3.7 mmol/L. After three days of cultivation under conditions of continuous aeration at 30°C, the cells were washed from the nutrient medium, resuspended in a sterile phosphate buffer (pH 7.0) ("starvation medium"), and irradiated in this medium with short-wave UV light (λ = 240–260 nm, J = 100–300 J/m^2). To study the kinetics of postradiation recovery, the irradiated cells were incubated at 30°C in the same "starvation medium" for some time. Thereafter, the aliquots of the cells were seeded on agar in Petri dishes to estimate the ability of the cells to form colonies [4, 5].

The effects of magnesium isotopes on myosin subfragment-1 isolated from smooth muscle (myometrium) of pigs were studied at the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (Kyiv, Ukraine; head of the section on biochemistry of muscles, Academician S.A. Kosterin). The enzyme activity was measured in standard reaction solutions containing chlorides of different magnesium isotopes ($^{24}MgCl_2$, $^{25}MgCl_2$, or $^{26}MgCl_2$) at a concentration of 5 mM [6].

The elemental composition of the samples was analyzed by atomic emission spectrometry and high-resolution mass spectrometry at the Institute of Microelectronics Technology and High Purity Materials of the Russian Academy of Sciences (head of the analytical center, V.K. Karandashev).

RESULTS AND DISCUSSION

Growth kinetic curves of *E. coli* bacterial culture have a standard form. The period of relatively slow adaptation to the new liquid growth medium (lag phase) is followed by the fast exponential growth phase (log phase) and then the stationary phase, during which the growth of cells stops because of depletion of the substrate, primarily, that of glucose (Fig. 1). Results of quantitative analysis of the kinetic data are listed in Table 1.

It follows from the data that cells transplanted into a new liquid nutrient medium adapt significantly faster to the medium if it contains the magnetic magnesium isotope ^{25}Mg, as compared to parallel experiments with nonmagnetic isotopes ^{24}Mg or ^{26}Mg. No significant differences were found for the nonmagnetic isotopes ^{24}Mg and ^{26}Mg. During the exponential phase, the rate constants of cell culture growth for all three magnesium isotopes were approximately equal.

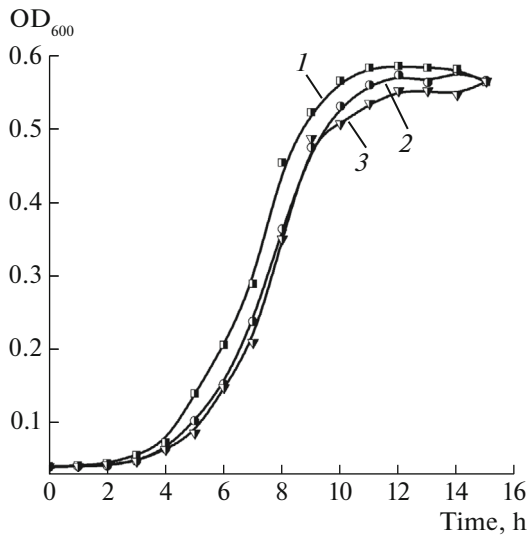

Fig. 1. Kinetic curves of growth of *E. coli* cells in liquid media enriched in (*1*) magnetic isotope $^{25}MgSO_4$ or nonmagnetic isotopes (*2*) $^{24}MgSO_4$ or (*3*) $^{26}MgSO_4$.

The striking effect of the magnetic isotope was found in the measurement of activity of the antioxidant enzyme superoxide dismutase (SOD) in cells that reached the stationary growth phase. SOD activity in the cells grown on the medium enriched in ^{25}Mg was

Table 1. Quantitative parameters of growth of *E. coli* cells depending on the type of magnesium isotope: T is the duration of the adaptation period (lag phase); k is the growth rate constant in the log phase. Here we present our results ($m \pm$ SD) of two parallel independent experiments with three samples simultaneously tested for each isotope in the same experiment, ^{24}Mg, ^{25}Mg, or ^{26}Mg (N = 3)

Magnesium isotope	T, h	k, h^{-1}
Experiment I		
$^{24}MgSO_4$	3.0 ± 0.2	1.63 ± 0.02
$^{25}MgSO_4$	2.3 ± 0.3*	1.68 ± 0.05**
$^{26}MgSO_4$	3.4 ± 0.2	1.55 ± 0.06
Experiment II		
$^{24}MgSO_4$	12.1 ± 0.9	2.0 ± 0.3
$^{25}MgSO_4$	9.8 ± 0.5*	2.3 ± 0.1**
$^{26}MgSO_4$	12.7 ± 0.8	2.1 ± 0.2

* The differences between average values for magnetic isotope ^{25}Mg and nonmagnetic isotopes ^{24}Mg and ^{26}Mg are statistically significant at $p \le 0.02$; ** the differences between average values for magnetic isotope ^{25}Mg and nonmagnetic isotopes ^{24}Mg and ^{26}Mg are statistically insignificant at $p \le 0.5$.

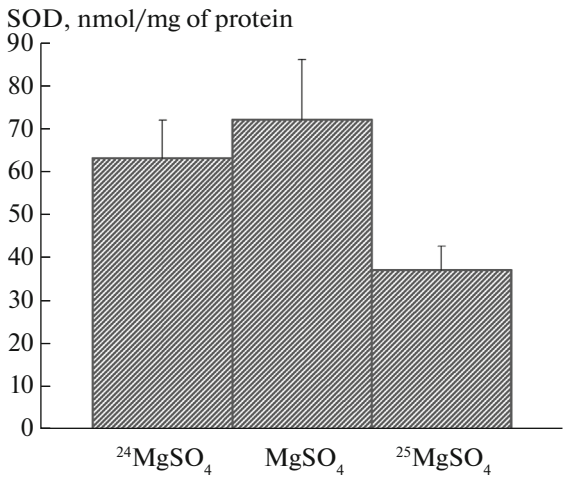

Fig. 2. SOD activity in *E. coli* cells grown in media with $^{24}MgSO_4$, $MgSO_4$ (natural abundance of isotopes), or $^{25}MgSO_4$. The differences between the average values for the ^{25}Mg magnetic isotope and the ^{24}Mg nonmagnetic isotope are statistically significant at $p \le 0.01$.

40% lower than in the cells grown on the medium enriched in the nonmagnetic isotope ^{24}Mg (Fig. 2).

It is known that there are two types of superoxide dismutase (Mn-SOD and Fe-SOD) in aerobically growing cells and that the total activity level of the enzyme is determined by the level of its substrate (level of superoxide radicals—oxygen anion radicals $O_2^{\bullet-}$) [7]. The cells enriched in the magnetic magnesium isotope as compared with the cells enriched in the nonmagnetic isotope are faster in not only achieving the stationary phase but also aging in this phase because of depletion of food substrates. With aging, cell metabolism slows down, resulting in a decrease in the level of generation of superoxide radicals as by-products of oxidative metabolism [8].

The magnetic isotope effect of magnesium was also observed in our experiments with another conventional cell model, *S. cerevisiae* yeast [4, 5]. We studied the effect of different magnesium isotopes on the kinetics of postradiation recovery of yeast. The survival rate of the cells transplanted into the nutrient medium (agar) immediately after irradiation did not exceed a few percent. After irradiation at this dose, the majority of cells have no time to repair damaged genetic structures before mitosis and cell division produces nonviable daughter cells. Incubation in the "starvation medium," where the cells do not divide, provides additional time for repair processes, providing an increased survival rate.

It was found that cells enriched in the isotope ^{25}Mg recovered more efficiently than cells enriched in the isotope ^{24}Mg. For cells enriched in ^{25}Mg, the recovery rate constant was found to be $0.058 \pm 0.004\ h^{-1}$, whereas it was almost two times lower ($0.032 \pm 0.003\ h^{-1}$) for cells enriched in ^{24}Mg, the difference between the average values being statistically significant at $p = 0.02$ [4, 5].

According to the data from atomic emission spectrometry and mass spectrometry, the elemental composition of the growth media was the same and the content of impurity elements was not more than a few micromoles per 1 L (regardless of the type of magnesium isotope). One would assume that the differences are caused by different content of impurities of any foreign elements falling into the growth medium with magnesium isotopes. However, data of the analysis of starting magnesium oxide formulations shown in Table 2 exclude this assumption.

One would also assume that the effect resulted from the high content of calcium impurities in ^{25}MgO (1.6 times more than in ^{24}MgO) rather than the nuclear spin of the isotope ^{25}Mg. However, the calcium content in ^{25}MgO is four times smaller than that in the other nonmagnetic oxide isotope, ^{26}MgO. The effect cannot be attributed to the different content of iron impurities. The iron content in ^{25}MgO is almost 4 times larger than that in ^{24}MgO, but is 2.5 times less than that in ^{26}MgO. Meanwhile, the effect of nonmagnetic ^{24}Mg is not statistically different from the effect of the other nonmagnetic isotope, ^{26}Mg. The influence of other trace elements, the content of which is different in ^{24}MgO and ^{26}MgO, is also excluded.

Nickel and selenium deserve separate consideration, because in oxide of the magnetic magnesium isotope the content of impurities of these elements is higher as compared with oxides of the nonmagnetic magnesium isotopes. The final concentrations of these elements added to 1 L of the medium containing ^{24}MgO, ^{25}MgO, and ^{26}MgO were $<0.15 \times 10^{-8}$, 0.30×10^{-8}, and 0.15×10^{-8} M for nickel and $<1.1 \times 10^{-8}$, 2.2×10^{-8}, and 1.1×10^{-8} M for selenium. Nickel ions are necessary for bacteria consuming hydrogen as the energy source [9]. However, *E. coli* bacteria are not among this group of bacteria. Under anaerobic growth conditions of *E. coli* cell culture, selenium presumably enhances the resistance of formiate against dehydrogenation [10]. Obviously, in our case, when the cells were grown in aerobic conditions, this factor is irrelevant.

Furthermore, it should be appreciated that not only magnesium oxides but other reagents required for experiments contain impurities which are introduced into the growth medium and other experimental media in amounts significantly greater than the amount of the same impurities introduced with much less magnesium additives. For example, the iron content in glucose produced by Ekokhim (Russia, GOST (State Standard) 6038-79) is about 0.002 wt %; in Na_2HPO_4 (Sigma-Aldrich, product # 71640, CAS # 7558-79-4), it is about 0.0005 wt %; in NaCl (Sigma-Aldrich, product # 71376, CAS # 764714-5) and NH_4Cl (Sigma-Aldrich, product # 31107, CAS

Table 2. Content of impurity elements (wt %) in magnesium oxides

Element	^{24}MgO	^{25}MgO	^{26}MgO	Element	^{24}MgO	^{25}MgO	^{26}MgO
Li	<0.0001	<0.0001	<0.0001	In	<0.0001	<0.0001	<0.0001
Be	<0.0005	<0.0005	<0.0005	Sn	<0.0001	<0.0001	<0.0001
B	<0.001	0.008	0.0026	Sb	<0.0001	<0.0001	<0.0001
Na	0.002	<0.002	0.004	Te	<0.0002	<0.0002	<0.0002
Al	0.0011	0.0008	0.031	I	<0.0005	<0.0005	<0.0005
Si	<0.005	<0.005	<0.005	Cs	<0.0001	<0.0001	<0.0001
P	<0.005	<0.005	<0.005	Ba	<0.0001	0.0003	0.0002
K	<0.005	<0.005	0.017	La	<0.0001	<0.0001	0.0003
Ca	<0.005	0.008	0.34	Ce	<0.0001	<0.0001	<0.0001
Sc	<0.0002	<0.0002	<0.0002	Pr	<0.0001	<0.0001	<0.0001
Ti	<0.0004	<0.0004	0.0015	Nd	<0.0001	<0.0001	<0.0001
V	<0.0001	<0.0001	<0.0001	Sm	<0.0001	<0.0001	<0.0001
Cr	<0.0005	<0.001	0.0030	Eu	<0.0001	<0.0001	0.0002
Mn	0.0032	0.0020	0.059	Gd	<0.0001	<0.0001	<0.0001
Fe	<0.005	0.019	0.048	Tb	<0.0001	<0.0001	<0.0001
Co	<0.0001	<0.0001	0.0011	Dy	<0.0001	<0.0001	<0.0001
Ni	<0.0001	<0.0002	<0.0001	Ho	<0.0001	<0.0001	<0.0001
Cu	0.0029	0.0021	0.0004	Er	<0.0001	<0.0001	<0.0001
Zn	0.0006	0.0005	0.0009	Tm	<0.0001	<0.0001	<0.0001
Ga	<0.0001	<0.0001	<0.0001	Yb	<0.0001	<0.0001	<0.0001
Ge	<0.0001	<0.0001	<0.0001	Lu	<0.0001	<0.0001	0.0003
As	<0.0001	<0.0001	<0.0001	Hf	<0.0001	<0.0001	<0.0001
Se	<0.001	<0.002	<0.001	Ta	<0.0001	<0.0001	<0.0001
Br	<0.005	<0.005	<0.005	W	<0.0001	<0.0001	<0.0001
Rb	<0.0001	<0.0001	<0.0001	Re	<0.0001	<0.0001	<0.0001
Sr	<0.0001	<0.0001	0.0002	Os	<0.0001	<0.0001	<0.0001
Y	<0.0001	<0.0001	<0.0001	Ir	<0.0001	<0.0001	<0.0001
Zr	<0.0001	<0.0001	<0.0001	Pt	<0.0001	0.0031	0.0002
Nb	<0.0001	<0.0001	<0.0001	Au	<0.0001	<0.0001	<0.0001
Mo	<0.0001	<0.0001	<0.0001	Hg	<0.0001	<0.0001	<0.0001
Ru	<0.0001	<0.0001	<0.0001	Tl	<0.0001	<0.0001	<0.0001
Rh	<0.0001	<0.0001	<0.0001	Pb	<0.0001	<0.0001	0.0015
Pd	<0.0001	<0.0001	<0.0001	Bi	<0.0001	<0.0001	<0.0001
Ag	<0.0001	<0.0001	<0.0001	Th	<0.0001	<0.0001	<0.0001
Cd	<0.0001	<0.0001	<0.0001	U	<0.0001	<0.0001	<0.0001

12125-02-9), it is about 0.0001 wt %. The amount of iron introduced with these salts and glucose in 1 L of the experimental medium is ~20×10^{-5} g, while iron introduced with ^{24}Mg, ^{25}Mg, and ^{26}Mg salts does not exceed 0.4×10^{-5}, 1.7×10^{-5}, and 4.3×10^{-5}, respectively. The same reagents according to the certificates contain heavy metals lead and nickel (~0.0005 wt % of each of them). With these reagents, ~70×10^{-8} mol/L of lead and 110×10^{-8} mol/L of nickel are added to the experimental solution. Meanwhile, addition of the most "contaminated" oxides ^{26}MgO and ^{25}MgO to the same solution results in only 0.65×10^{-8} mol/L of lead and 0.30×10^{-8} mol/L of nickel. Thus, the amount of impurities coming from the main components is significantly greater than the amount of the same impurities coming into the medium with the magnesium isotopes. It is also important that the impurities of the main components are added to each of the experimental media in the same amounts irrespective of the magnesium isotope contained in the medium.

Adenosine triphospahe (ATP) is the main source of energy in the cells; therefore, it can be assumed that in living cells the magnetic isotope effect (MIE) resulted from higher efficiency of "fuel and power" nanoreactors in cells enriched in the magnesium magnetic iso-

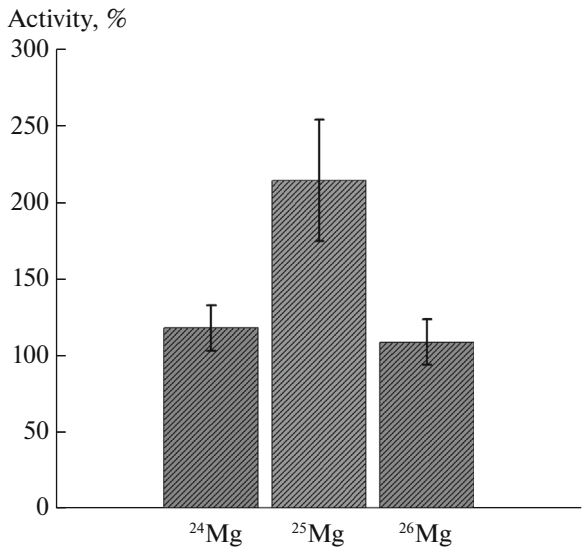

Fig. 3. ATPase activity of myosin subfragment 1 isolated from smooth muscle (myometrium) in reaction solutions containing different magnesium isotopes $^{24}MgCl_2$, $^{25}MgCl_2$, or $^{26}MgCl_2$ at a concentration of 5 mM. The activity value is given as a percentage of the enzyme activity in a solution containing 5 mM $MgCl_2$ (natural abundance of isotopes) [6].

tope. One of the most studied "molecular motors" is the muscle protein myosin. The enzyme provides the hydrolysis of the terminal phosphate bond in the ATP molecule: ATP + $H_2O \rightarrow$ ADP + P_i. The energy released therewith (under physiological conditions, ~0.54 eV) is used to carry out the work—muscle contraction. The ATPase activity of myosin obligatorily depends on the presence of the Mg^{2+} ions required for ATP binding in the active site of the enzyme and playing a significant role in catalysis of ATP hydrolysis [7].

Figure 3 shows the results of our experiments on the effect of different magnesium isotopes on ATPase activity of myosin subfragment 1. Myosin subfragment 1 is considered a sufficiently functional unit of myosin, since it retains all its native properties, namely, the ATPase activity and the ability to interact with actin. Several series of independent experiments with the enzyme isolated from smooth muscles of pigs at different times were carried out. Despite the variability in average values of the ATPase activity from one experimental series to the other, the same effect was observed in all experimental series. Namely, the enzyme activity in the presence of the magnetic isotope ^{25}Mg was 2.0–2.5 times higher than the activity of the same enzyme in the presence of the nonmagnetic isotopes ^{24}Mg or ^{26}Mg or in the presence of a natural mixture of magnesium isotopes. At the same time, no significant differences in activity were found in the case of nonmagnetic isotopes ^{24}Mg and ^{26}Mg. The effect is observed at physiological concentrations of magnesium chloride (5 mM). It is important to note that, in spontaneous ATP hydrolysis, i.e., in the absence of the enzyme, the magnetic isotope effect is not observed [6].

In chemistry, the magnetic isotope effect (MIE) is known for many elements having magnetic and nonmagnetic isotopes, including carbon, oxygen, silicon, sulfur, germanium, tin, mercury, and uranium [11–13]. MIE manifests itself experimentally by the fact that the reaction rate and the yield of products of the reaction involving free radicals and/or radical ion pairs substantially vary depending on whether the reactants contain magnetic or nonmagnetic isotope of the same element. This effect is a direct consequence of the law of conservation of angular momentum, a fundamental law of nature, which is as strict as the law of conservation of energy. Here, we are talking about the law of conservation of angular momentum of the electron (the electron spin): the total electron spin (S) of chemical reaction products must be equal to the total spin of the initial reagents. A similar spin ban occurs during singlet–triplet transitions in molecules, including macromolecules [11–13].

In order to eliminate the ban imposed by the law of conservation of spin, the spin state of the reagents must be changed. The spin conversion from the singlet state to the triplet state may be provided by the spin–lattice relaxation, for example, owing to spin–orbit interactions in the presence of many-electron paramagnetic ions (such as Fe^{3+}/Fe^{2+}). However, the spin–orbit coupling is weak in organic radicals, and the only way to ensure the spin conversion in the absence of paramagnetic ions is the application of magnetic field (external field or atomic nuclear spin field). MIE is a kinetic phenomenon, which clearly indicates that in the studied process there is a spin-selective bottle-neck and the process rate is limited by the spin evolution of the system [12, 13].

The Mg^{2+} ion is an obligatory cofactor of ATP enzymatic hydrolysis provided by myosin. However, in the active center of any ATPase, including myosin, complex [ATP^{4-} Mg^{2+}] hydrolyzes instead of ATP to form [ADP^{3-} Mg^{2+}] and inorganic phosphate (P_i, $H_2P_\gamma O_4^-$) [7]. The observed magnetic isotope effect indicates that, in the kinetics of ATP enzymatic hydrolysis catalyzed by myosin, there is a limiting step that is accelerated by the nuclear spin of the isotope ^{25}Mg. In chemical physics of free radical reactions, the existence of a radical pair or a radical ion pair as an intermediate (bottle-neck) is assumed to explain MIE. It is known, however, that the ATP hydrolysis reaction to form ADP and P_i proceeds according to the acid-base mechanism and the appearance of any radical ion pair as an intermediate in this reaction is unlikely. Indeed, MIE is not observed in experiments on nonenzymatic hydrolysis of complexes ATP–Mg^{2+} [6].

A different situation arises in the case of ATP hydrolysis catalyzed by myosin. It has long been known (proved experimentally) that ATP hydrolysis initiates electron-conformational interactions in the active site of the enzyme, resulting in a change in the

conformation of the enzyme macromolecule. Owing to the energy released by ATP hydrolysis (~0.54 eV), conformational excitation occurs; basically, the strain of the macromolecule is observed [14]. According to quantum-mechanical calculations of molecular dynamics, a mechanical stress generation cycle during the catalytic hydrolysis of ATP by myosin consists of several stages [15]. In the first stage, ATP γ-phosphate is stabilized in a state of dissociated metaphosphate ($P_\gamma O_3^-$). The hydrolysis products (ADP and P_i) remain in the active site of the enzyme in close contact and are released only after myosin binds to actin filaments. This agrees with the well-known fact of reversibility of the ATP hydrolysis reaction by myosin. The ATP hydrolysis reaction is reversible as long as the protein remains in the postrecovery-prepower stroke conformation [15]. As long as the hydrolysis products (ADP and P_i) remain bounded by myosin and are in close contact, they can form ATP again. We can assume that, in the conditions of electronic conformational excitation of the myosin macromolecule in the active site of the enzyme, there is the transfer of electron density from the OH^- group of bound water molecules or the NH_2 group of Glu459 to ADP^{3-} or Mg^{2+} to form the corresponding radical ion pair. This is followed by the nucleophilic attack of inorganic phosphate oxyanion to form ATP. The stable spin state of the product (ATP–Mg) should be singlet, i.e., the electron spin $S = 0$. Meanwhile, this pair bounded by myosin is converted into the triplet state ($S = 1$) owing to the hyperfine interaction of the ^{25}Mg nuclear spin with an unpaired electron of the radical ion pair. Creating this spin ban, the ^{25}Mg nuclear spin isotope prevents the undesired reverse reaction of ATP synthesis, thereby promoting the direct ATP hydrolysis reaction. The hypothesis of the crucial role of the virtual radical ion pair in ATP synthesis in oxidative phosphorylation was expressed about 50 years ago [16]. Quantum chemical calculations [13] indicate the possibility of realization of this mechanism at the appropriate time of the spin–spin and spin–lattice relaxation.

Alternatively, the catalytic effect of the ^{25}Mg nuclear spin can be explained as follows. The energy released during ATP hydrolysis (~0.54 eV) is not large enough for the electronic conformational excitation of the myosin macromolecule to the singlet state. This energy is sufficient to produce a low-lying triplet state, but the transition from the ground state ($S = 0$) to the triplet state ($S = 1$) is forbidden by the law of conservation of spin. The magnetic isotope ^{25}Mg changes the situation. The ^{25}Mg nuclear spin eliminates the problem of spin ban, providing the required spin conversion to the triplet state [17]. A similar mechanism has been suggested to explain the effects of the magnetic field on mobility of dislocations in solids [18].

Consider another possible explanation for the observed catalytic effect of the nuclear spin of ^{25}Mg. During the conformational transition, the position of many atomic groups of the macromolecule are changed. At the same time, there occur processes of dehydration and rehydration of electrically charged groups. It is known that there are two isomers of water molecules differing in mutual orientation of hydrogen nuclear spins, namely, *ortho*-H_2O with parallel orientation of proton spins and *para*-H_2O with antiparallel orientation of proton spins. According to quantum statistics, *ortho*-H_2O is 75% of the total volume at room temperature [19]. There is a reason to believe that *ortho*-H_2O molecules have a preferential affinity to L-amino acids as compared to *para*-H_2O molecules [20]. If this is true, the movement of mainly bound *ortho*-H_2O molecules with conformational transitions of the macromolecule is difficult. Spin-rotational interactions of protons are too weak to ensure adequate effectiveness of *ortho/para*-transitions. Magnetic ^{25}Mg is able to improve significantly the situation by eliminating the problem of the spin ban, providing the required conversion rate of water isomers.

In biochemistry, MIE was first found by A.L. Buchachenko and his coworkers. In experiments with mitochondria isolated from mouse hearts, they found that the oxidative phosphorylation with the ^{25}Mg isotope is 2–3 times more efficient than that with ^{24}Mg and ^{26}Mg [21]. Since the nonmagnetic magnesium isotopes ^{24}Mg (78.7%) and ^{26}Mg (11.17%) are the most abundant in nature, the authors of [21] had to remove natural magnesium from the mitochondria by the EGTA chelator and then add magnesium chloride of the desired isotope in order to detect MIE. By this treatment, it is impossible to avoid undesirable structural and functional changes in mitochondria [22]. Similar effects were found in the study of enzymes creatine phosphokinase and phosphoglycerate kinase: the ATP yield with magnetic ^{25}Mg was two times higher than that in similar reactions with nonmagnetic ^{24}Mg and ^{26}Mg (see the reviews [12, 13]). However, in similar experiments with creatine phosphokinase, British scientists failed to detect MIE [23]. Russian authors [13] noticed the high content of iron impurity in oxide ^{25}MgO (2% of the weight of magnesium used by the British group). Isotopic products purchased from Rosatom and used in Russian studies have a significantly higher degree of purification (in particular, they contain 200–1000 times less iron impurities). Meanwhile, at high concentration, the paramagnetic iron ions, if they do not "kill" the radical ion mechanism of ATP synthesis, eliminate the nuclear spin selectivity of the process, because in this case the singlet–triplet conversion is provided by strong spin–orbit interaction rather than the Zeeman hyperfine coupling of the electron spin with the nuclear spin. Consequently, it becomes impossible to observe the effects of magnetic isotopes or external magnetic fields [13].

There are the data on the magnetic isotope effects of magnesium and zinc in the experiments with isolated DNA polymerase. During the experiments with

Mg- and Zn-dependent isolated beta-polymerases, it was found that their enzymatic activity is inhibited by magnetic magnesium and zinc isotopes [24]. It is known that, in synthesis of oligonucleotide chains, the DNA polymerase uses ATP energy released in the couple reaction of trinucleotide hydrolysis catalyzed by the same enzyme [7]. Taking into account the magnetic isotope effect of acceleration of ATP hydrolysis by myosin [6] discovered recently, we can assume that the decelerating effect on DNA synthesis is caused by the kinetic imbalance resulting from the effect of nuclear spin of the magnetic isotope on kinetics of the ATP enzymatic hydrolysis.

In the "molecular motors" operating on the nonmagnetic magnesium isotopes, the spin catalysis function can be carried out by the nuclear spins of phosphorus ^{31}P and protons ^{1}H. The comparatively high catalytic activity of ^{25}Mg is probably due to the fact that the spin of the nucleus of the ^{25}Mg isotope is 5 times higher than that of the nuclei of hydrogen or phosphorus and due to localization features of the Mg^{2+} ion in the active site of the enzyme, resulting in a relatively large local magnetic field (hyperfine interaction constant ~21 mT) created by the magnesium nuclear spin. The ^{25}Mg isotope proved to be a successful "lamp" shedding light on details of the chemical mechanism of enzymatic catalysis.

Thus, it has been demonstrated experimentally that magnetic isotope effects occur not only in chemistry but also in biology. A detailed study of the physicochemical mechanisms of MIE in biocatalysis in addition to determination of biological mechanisms to enhance nuclear spin catalysis in living cells is the subject of further research.

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