Stable Magnetic Isotopes and Reliability of Biomolecular Nanoreactors

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ABSTRACT

The main line of creating reliable devices from unreliable components is prophylaxis of failures. In biomolecular nanoreactors, the prophylactic effects can be provided by stable magnetic isotopes. For example, among three stable magnesium isotopes, ^{24}Mg, ^{25}Mg and ^{26}Mg, only ^{25}Mg has the nuclear spin and nuclear magnetic moment. We have revealed the isotope differences in quantitative parameters of growth of the bacteria *E. coli* on the media contained different isotopes of magnesium. The cells which were previously enriched with ^{25}Mg demonstrate the essentially higher viability (determined by counting colony forming units) by comparison with the cells which were previously enriched with ^{24}Mg or ^{26}Mg. Besides, ^{25}Mg prevents the free-radical failures in the biomolecular nanoreactors. On the same nuclear-spin background, stable magnetic isotopes of other elements also hold considerable promise for magnetic-field control over efficiency and reliability of biomolecular nanoreactors.

Keywords: nanomedicine, nanoengineering, reliability, magnetic isotopes, magnetic fields

1. INTRODUCTION

Nanotechnology – a catchall term for engineering materials sized between 1 and 100 billionths of a meter – is widely seen as having enormous scientific and commercial potential. The idea that engineering can be extended towards the molecular scale, to electronic devices of high efficiency based on single molecules and so on, has become currency over the past decade. This trend of nanoscaling brings engineering down to the dimensions of molecular structures. At the same time, it brings technical devices down to intrinsic instability of functional parameters due to thermal, mechanic and other fluctuations. That raises the question as to whether operation of the nanoscaling devices can be reliable. One can lower temperature up to helium values at which thermal fluctuations are less. The alternative way is to follow the general theory of reliability [1]. The systems reliability may be essentially magnified by the inserting of redundant amount of the functional components which fulfill one and the same function. Another line of creating the highly reliable systems from unreliable components is preventive maintenance or prophylaxis of failures [1–3]. Keeping in mind the possible free-radical damages, researchers who fabricate new composite materials embedded with organic dye molecules and paramagnetic metal atoms should provide the composites with appropriate antioxidants as well. Along this nanoengineering line the general principles of spin-chemistry, amongst them – control of chemical reactivity by selective isotope modification, hold considerable promise for basic research as well as for creating reliable optoelectronic and nanophotonic devices.

2. MAGNETIC SOTOPES IN MOLECULAR NANOREACTORS

Apart from energy control (the law of conservation of energy), a chemical reaction as electron-nuclear rearrangement of reactants into products is controlled by angular momentum, spin, of reactants. The total spin of products must be identical to that of reactants. This law of spin conservation immediately follows from quantum mechanics, from the fundamental and universal Pauli principle: no two electrons may occupy the same quantum state simultaneously (see [4, 5] and references therein). For example, a pair of free radicals, each with electron spin $S = 1/2$, can form a chemical bond ($S = 0$) if the spin state of the pair is singlet, i.e. spins of the two electrons are subtracted to give the net $S = 0$ (spin multiplicity is one). If the spin state of the radical pair is triplet, i.e. spins of two electrons are added up to give the net $S = 1$ (spin multiplicity is three), then the radicals cannot react immediately. It means that only one-quarter of encounters, when the radical pair is in the singlet state, gives the recombination product while three-quarters of the initial radical pairs are inhibited from the reaction. To lift the ban on the reactions forced by the law of spin conservation, spins of the reactants must be changed.

Magnetic fields are the only means that are able to change the spin states. Accordingly, the probability (P) of chemical reaction is a function of parameters of these interactions [4, 5]:

$$P = f (H; \omega; H_1; J; a; I; m_I; \mu_n) \qquad (1),$$

where H is applied constant magnetic field (Zeeman interaction that produces magnetic field effect); ω and H_1 are frequency and amplitude of microwave magnetic

fields); J is exchange energy (the exchange interaction). The equation also contains the parameters of hyperfine coupling a, nuclear spin I, nuclear spin projection m_I, and nuclear magnetic moment μ_n, i.e. the parameters of interactions of electron spins with magnetic nuclei (Fermi coupling).

Correspondingly, acceleration of the free-radical reactions can be achieved through changes in the total electron spin of reactants by interaction with magnetic fields of magnetic nuclei. This is known as magnetic isotope effect, a new trend in chemical physics within recent years [5, 6].

The magnetic isotope effect (MIE) is a purely kinetic phenomenon and manifests itself as the dependence of the reaction rate on the nuclear spins of the reactants. Namely, the reaction will show different reaction rates and different yields of products according to whether the reagents contain magnetic or nonmagnetic isotopes. In chemistry, MIE has been discovered for a number of magnetic isotopes, among them ^{1}H and ^{2}D, ^{13}C, ^{17}O, ^{29}Si, ^{33}S, ^{73}Ge, ^{117,119}Sn, ^{199,201}Hg, and ^{235}U [5]. MIE selects isotopes by spin and magnetic moment while classical isotope mass effect (CIE) selects isotopic nuclei in accordance with their masses. For example, it has been recently found that the photochemical reduction of Hg-compounds by natural sunlight in aquatic ecosystems leads to both mass-dependent fractionation of Hg isotopes, due to the CIE, and mass-independent fractionation of the odd-mass Hg isotopes, due to the MIE [7].

3. MAGNETIC ISOTOPES IN BIOMOLECULAR NANOREACTORS

The enhancement of the reaction rate by nuclear spins of the reactants can be denoted as the 'nuclear spin catalysis' [8]. Such the phenomenon, magnetic isotope effect of ^{25}Mg, has been recently discovered for biomolecular nanoreactors of the living cell [9–11]. It was revealed that bacteria *Escherichia coli*, the commonly accepted cell model, can perceive the difference between magnetic and nonmagnetic nuclei of magnesium. Among three stable magnesium isotopes, ^{24}Mg, ^{25}Mg and ^{26}Mg with natural abundance 78.99, 10.00 and 11.01 %, only ^{25}Mg has the nuclear spin ($I = 5/2$) and, therefore, the nuclear magnetic moment. Two other isotopes are spinless ($I = 0$) and, hence, have no magnetic moment. We revealed the isotope differences in quantitative parameters of growth of *E. coli* on the media contained different isotopes of magnesium. The length of adaptation (lag-phase) of bacteria to the liquid media supplied with magnetic isotope, ^{25}Mg, was found to be essentially shorter than the adaptation to the media supplied with nonmagnetic isotopes, ^{24}Mg or ^{26}Mg. Furthermore, on the solid nutrient media the cells, which were previously grown on the liquid medium supplied with ^{25}Mg, demonstrate essentially higher viability (determined by counting colony forming

units) by comparison with the cells which were previously grown on the media supplied with ^{24}Mg or ^{26}Mg [9–11].

It is generally known that energetic demands of every operation in living systems are met by molecules of adenosine triphosphate (ATP). Most of ATP is produced by specific biomolecular nanoreactors, the enzymes organized in respiratory electron transport nanoreactors (ETR) [12]. Normal function of the ETR enzymes, be it mitochondrial nanoreactors in eukaryotic cells of animals or similar nanoreactors of bacteria cells, is in the transport of electrons, one by one, from the electron donor molecules to the end enzyme, cytochrome oxidase, from which the electrons are transferred to molecules of oxygen with two-electron reduction of oxygen into water. Free energy released during the electron transport is used for synthesis of ATP from adenosine 5'-diphosphate (ADP) and inorganic phosphate (P_i), the so-called "oxidative phosphorylation [12]. It is also well known that the ATP synthesis depends on the ion of Mg^{2+} as obligatory cofactor. In experiments with isolated mitochondria, it has been discovered that the rate of synthesis of ATP with the magnetic ^{25}Mg was essentially higher than that with the nonmagnetic ^{24}Mg or ^{26}Mg, while there was no difference between the effects of ^{24}Mg^{2+} and ^{26}Mg^{2+} on the ATP synthesis [6]. Inasmuch as ATP is the main source of energy for the biomolecular nanoreactors of bacteria too, the similar MIE obviously takes place in the adaptation processes of the living cells [11].

Factual evidence of MIE, on its own, indicates that there is a spin-selective "bottle-neck" of the process under investigation. To explain the MIE of magnesium-25 in the ATP synthesis, a reversible electron transfer in the catalytic center of the ATP synthase from the terminal phosphate anion of ADP to Mg^{2+} was suggested [5, 6]. Due to the hyperfine coupling of unpaired electron with magnetic nucleus, the nuclear spin of ^{25}Mg induces the transition of the ion-radical pair, ADP-Mg^+, from the initial singlet state ($S = 0$) into the triplet state ($S = 1$), the lifespan of which is longer thereby providing more time and higher yield for the reaction of the ATP synthesis.

4. NUCLEAR SPIN AS THE FACTOR OF RELIABILITY IN BIOMOLECULAR NANOREACTORS

The biopolymer nanoreactors of ETR have very ancient evolutionary origin and, hence, seem to be ones of the most reliable biomolecular machines. But yet their reliability characteristics are not perfect because these molecular machines experience conformational fluctuations. In consequence, normal elementary acts of the electron transport alternate with random malfunctions when an electron, rather than waits for transport to the next enzyme of the ETR, goes directly to an adjacent oxygen molecule resulting in production of the free radical (superoxide, $O_2^{\bullet-}$). Meanwhile, chemical products of $O_2^{\bullet-}$, the so-called reactive oxygen species, are toxic and initiate free-radical

damages in the biopolymer nanoreactors. Therefore, the malfunctions in ETR followed by production of $O_2^{\bullet-}$ are considered as the failures of vital importance for living cells [1–3].

Since ^{25}Mg is more effective cofactor of oxidative phosphorylation, it transpires that the ATP synthase is essentially faster acting enzyme with the magnetic nucleus by comparison with the nonmagnetic ones. The retardation of electron transport in the sites of the electron-transport nanoreactors which are coupled with phosphorylation of ADP increases probability of malfunctions resulting in the electron leakage onto oxygen with formation of reactive $O_2^{\bullet-}$. Hence, the yield of $O_2^{\bullet-}$ as the faulty by-products of electron transport is bound to be much lower with ^{25}Mg-ADP by comparison with ^{24}Mg-ADP or ^{26}Mg-ADP. Indeed, we have revealed that the cells of *E. coli* grown on ^{25}Mg exhibit less activity of superoxide dismutase (SOD), by about 40 per cent, when compared to the cells grown on nonmagnetic ^{24}Mg [9–10]. Meanwhile, SOD is the special antioxidant enzyme eliminating the $O_2^{\bullet-}$ radicals and the level of the SOD activity is normally adjusted to the intracellular level of $O_2^{\bullet-}$ [12]. Hence, the lower level of SOD activity testifies the lower production of $O_2^{\bullet-}$ in the case when the cells are supplied with the magnetic isotope.

Thus, with magnetic isotope ^{25}Mg, the biopolymer nanoreactors of oxidative phosphorylation operate not only more effective but more reliable too, in comparison with their operation on non-magnetic isotopes ^{24}Mg and ^{26}Mg. Down-grading production of $O_2^{\bullet-}$ and its reactive chemical products, the stable magnetic isotope of magnesium actually produces the preventive antioxidant effect. The antioxidant effect of the nuclear spin, that ^{25}Mg favors the less production of reactive oxygen species, should obviously increase longevity of the electron-transport nanoreactors. Therefore, it can reveal itself in nature as kinetic nuclear-spin selection of the favorable isotope, namely, kinetic isotope enrichment with magnetic ^{25}Mg in the processes of recycling and regeneration of the electron-transport nanoreactors. For example, one can expect for enrichment with the favorable magnetic ^{25}Mg in recycling and regeneration of mitochondria with aging.

Contrary, in the case of photosynthetic nanoreactors, one can predict undesirable pro-oxidant effect of the magnetic ^{25}Mg. Indeed, it is known that the function of the vast majority of chlorophyll molecules (Chl) as the derivatives of the magnesium-protoporphyrin complexes is to absorb light energy and transfer it to the specific energy sinks, the so-called reaction centers of the photosynthetic nanoreactors [12]. While performing this energy-transfer function, the light-exited Chl molecules are in the singlet state ($^1Chl^*$, electron spin $S = 0$). However, there is probability of the radiationless relaxation into the triplet state (^3Chl, $S = 1$) followed by formation of singlet oxygen, $^1O_2$, the molecules of which are substantially more reactive by comparison with the usual triplet O_2 molecules and, thereafter, produce oxidative damages [12]. As nuclear spin of ^{25}Mg can catalyze the conversion of $^1Chl^*$ into the

triplet ^3Chl, one can expect for the higher yield of $^1O_2$ and, thereafter, more photodynamic damages with chlorophyll molecules containing ^{25}Mg instead of the spinless ^{24}Mg or ^{26}Mg. Correspondingly, it is beyond reason to hope for selection of the magnetic isotope, ^{25}Mg, in the case of algae or green plants. In addition, the functional disadvantage of ^{25}Mg can be followed by increased synthesis of carotenoids and other natural antioxidants. Indeed, measurements of magnesium isotopic composition of the chlorophylls extracted from cyanobacteria and similar analysis of the chlorophyll forms in the leaves of English Ivy (*Hedera helix L.*) have revealed the isotope distribution following usual classical mass-isotope effect with no evidence for the depletion of ^{25}Mg [13].

Apart from magnesium, there are many other elements which have both kinds of stable isotopes, nonmagnetic and magnetic ones, including carbon (^{12}C with $I = 0$ vs. ^{13}C with $I = 1/2$), oxygen (^{16}O, ^{18}O with $I = 0$ vs. ^{17}O with $I = 5/2$), silicon ($^{28, 30}Si$ with $I = 0$ vs. ^{29}Si with $I = 1/2$), calcium ($^{40, 42, 44, 48}Ca$ with $I = 0$ vs. ^{43}Ca with $I = 7/2$), iron ($^{54, 56, 58}Fe$ with vs. ^{57}Fe with $I = 1/2$), zinc ($^{64, 66, 68}Zn$ with $I = 0$ vs. ^{67}Zn with $I = 5/2$), etc. [14]. For example, it was found that deuterated polyunsaturated fatty acids protect yeast cells against the toxic effects of lipid autoxidation products [15]. In passing, the nuclear spin of ^{17}O should lift the spin ban over the reaction of the mitochondrial ubisemiquinone radicals with oxygen, thereby catalyzing formation of $O_2^{\bullet-}$. As a result, one can expect for more reactive oxygen radicals and more free-radical damages due to ^{17}O in comparison with nonmagnetic ^{16}O and ^{18}O. The pro-oxidant action of ^{17}O can reveal itself as selective enrichment of free-radical peroxidation products with this unfavorable magnetic isotope as compared with ordinary metabolites.

5. CONCLUSIONS AND OUTLOOK

The trend of nanoscaling brings functional elements of molecular dimensions down to intrinsic instability due to thermal, mechanic and other fluctuations. The most prospect line of creating the highly reliable systems from unreliable components is preventive maintenance or prophylaxis of failures. Keeping in mind the possible free-radical damages, researchers who fabricate new composite materials embedded with organic dye molecules and paramagnetic metal atoms should provide the composites with appropriate antioxidants as well. Along this nanoengineering line the general principles of spin-chemistry, amongst them − control of chemical reactivity by the selective modification with stable magnetic isotopes, hold considerable promise. The magnetic isotope effect, as such, always and unambiguously indicates that the reaction under study is spin-selective process with participation of paramagnetic intermediates, such as a free radical pair, ion-radical pair or triplet state that undergo the spin conversion. The preventive antioxidant effect of ^{25}Mg opens the ways toward novel medicine based on this magnetic isotope including anti-aging drugs, and radio-protectors. Up to date,

there have been no efforts to detect magnetic isotope effects for other elements, except magnesium, in biopolymer nanoreactors. Meanwhile, inasmuch as the electron spin moments can be changed by external magnetic fields, it makes possible to exert control over nanoengineering devices with the help of the relevant magnetic and electromagnetic fields. Thus, based on the same nuclear spin-catalysis background, stable magnetic isotopes hold considerable promise for control over efficiency and reliability of molecular and biomolecular devices in optical communications, quantum information processing, computational schemes and the like.

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REFERENCES

[1] D.M. Grodzinsky, V.P. Vojtenko, Y.A. Kutl-akhmedov, V.K. Koltover, "Reliability and Aging of Biological Systems", Kiev, Naukova Dumka, 1987.

[2] V.K. Koltover, J. Theor. Biol. 184, 157, 1997.

[3] V.K. Koltover, Control Sciences. 4, 40, 2004.

[4] B. Brocklenhurst, Chem. Soc. Rev. 31, 301, 2002.

[5] A.L. Buchachenko, "Magnetic Isotope Effect in Chemistry and Biochemistry", New York, Nova Science Publishing, 2009.

[6] A.L. Buchachenko, D.A. Kouznetsov, N.N. Breslavskaya and M.A. Orlova, J. Phys. Chem. B. 112, 2548, 2008.

[7] B.A. Bergquist and J.D. Blum, Science. 318, 417, 2007.

[8] V.K. Koltover, Free. Radical Biol. Med. 45, S61, 2008.

[9] T.N. Bogatyrenko, E.A. Kudryashova, U.G. Shevchenko, L.V. Tumanova and V.K. Koltover, "Nutrition, Oxygen Biology and Medicine", Paris, 57, 2009.

[10] T.N. Bogatyrenko, E.A. Kudryashova, L.V. Tumanova and V.K. Koltover, "Low and Superlow Fields and Radiations in Biology and Medicine", Saint-Petersburg, 92, 2009.

[11] V.K. Koltover, U.G. Shevchenko, L.V. Avdeeva, E.A. Royba, V.L. Berdinsky, and E.A. Kudryashova, Doklady Biochemistry and Biophysics. 2011 (in press).

[12] D.L. Nelson and M.M. Cox, "Lehninger Principles of Biochemistry", New York, Freeman, 2008.

[13] J.R. Black, Q.Yin, J.R. Rustad and W.H. Casey, J. Am. Chem. Soc. 129, 8690, 2007.

[14] D.M. Grant and R.K. Harris, eds., "Encyclopedia of Nuclear Magnetic Resonance", Chichester, Wiley, 1996.

[15] S. Hill, K. Hirano, V.V. Shmanai, B.N. Marbois, D. Vidovic, A.V. Bekish, B. Kay, V. Tse, J. Fine, C.F. Clarke, M.S. Shchepinov, Free. Radical Biol. Med. 50, 130, 2011.