Annalen der Chemischen Forschung
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Original Research Article

Thermodynamics and quantum mechanics of an ATP enzymatic production-Is it crucial for enzymatic phosphate transfer? : Analytical review and simulation Model

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Abstract

Nucleotide phosphorylation paths are the key metabolic routes requiring to overcome a tough energy barrier which exceeds by 3-4 times of accumulated or released energy as itself (~10 kcal/mol). This energy is supposed to be taken from the mechanical compression of the enzyme catalytic site and used to form P–O chemical bond by direct nucleophilic addition of phosphate to either nucleoside or nucleotide residue. In the present review, both fundamental background and a presumable applied perspectives of the phenomena are described.

Keywords: Enzymatic phosphorylation, metal dependent catalysis, ion-radical reactions, nucleotide synthesis, singlet-triplet nuclear spin conversion.

Cite this article as
Maria A. Dryagina, Dmitry A. Kuznetsov, Maxim E. Grigoriyev, Anatoly L. Buchachenko. Thermodynamics and quantum mechanics of an ATP enzymatic production-Is it crucial for enzymatic phosphate transfer? : Analytical review and simulation Model. Annalen der Chemschen Forshung. 2014; 2(2): 41-53

Introduction

Three fundamental properties of atomic nuclei – mass, spin (and related magnetic moment) and volume – are the source of isotope effects. The mostly deserved and popular, with almost hundred-year history, is the *mass-dependent* isotope effect. The first *mass-independent* isotope effect which chemically discriminates isotopes by their nuclear spins and nuclear magnetic moments rather than by their masses was detected in 1976 [1]. Photo-chemical decomposition of dibenzyl ketone at 20□C in benzene was shown to result in its ^{13}C isotope enrichment which strongly exceeds that expected from *mass-dependent* isotope effect. Even more convincing was magnetic field dependence of the effect which was a direct evidence of its magnetic nature. Turro was the first who used micelles as the micro-reactors for the photolysis reactions; he has shown that the reactions in micelles result in enormously large isotope separation [2]. The effect was named as magnetic isotope effect (MIE) because it is controlled by magnetic interaction, i.e. electron-nuclear hyperfine coupling in the paramagnetic species, the reaction intermediates [3-7].

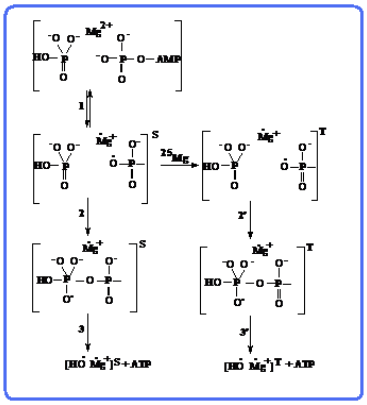
MIE certifies *nuclear spin selectivity* of chemical reactions, i.e. the dependence of the reaction rates on the nuclear spin and nuclear magnetic moment of the reactants [8-10]. It follows from the universal physical property of chemical reactions to conserve angular momentum (spin) of electrons and nuclei. MIE as a highlight in spin chemistry is based on the fundamental and universal principle: all chemical reactions are spin selective, they are allowed only for those spin states of reactants whose total spin is identical to that of products [10,11]. It is unique, it introduces in chemistry magnetic interactions. Contributing almost nothing in chemical energy, being negligibly small and traditionally ignorable, magnetic interactions are the *only ones* which are able to change electron spin of reactants and switch over the reaction between spin-allowed and spin-forbidden channels controlling chemical reactivity. The effect is now detected for oxygen, silicon, sulfur, germanium, tin, mercury, magnesium, calcium, zinc, and uranium [11,12].The most important consequence of the nuclear spin selectivity is the fractionation of magnetic and nonmagnetic isotopes which is known to be much more efficient than that of light and heavy isotopes induced by mass-dependent isotope effect [12].

Recently, a new function of magnetic isotopes was revealed in biochemical reactions: the activity of magnesium-dependent ATP-producing enzymes, in which Mg^{2+} ion has a magnetic isotopic nucleus ^{25}Mg, was found to be 2-3 times higher than that of enzymes in which Mg^{2+} ion has nonmagnetic, spinless isotopic nuclei ^{24}Mg or ^{26}Mg. There was no difference in the ATP yield for enzymes with ^{24}Mg and ^{26}Mg, i.e. in these reactions MIE functions [13-15]. On the basis of this discovery a new, ion-radical mechanism of the ATP synthesis, coexisting with traditional

nucleophilic mechanism, was suggested [16]. It was definitely proved by existence of magnetic isotope effect on the ATP synthesis catalyzed also by calcium and zinc ions: the yield of ATP increases by 2-3 times when catalytic site carries magnetic nuclei ^{43}Ca and ^{67}Zn in $^{43}Ca^{2+}$ and $^{67}Zn^{2+}$ ions instead of nonmagnetic, spinless nuclei ^{40}Ca and ^{64}Zn [17,18]. Another confirmation of the ion-radical mechanism follows from the magnetic field dependence of the ATP synthesis [19]. This effect was shown to function in isolated mitochondria and in living organisms [20,21]. Such *in vivo* effect is used to stimulate ATP synthesis in heart muscle in order to prevent hypoxia and other pathologies related to the deficiency of ATP (for details see review [22] and references therein). So far as the ions in the catalytic sites of enzymes are not depleted but regenerated and conserved this phenomenon, i.e. acceleration of the ATP synthesis by the ions with magnetic nuclei, is in fact nuclear spin catalysis which is similar to the electron spin catalysis [23,24].

Thermodynamis and quantum mechanics of an ATP enzymatic production

In terms of the only so far accepted paradigm, enzymatic ATP synthesis is a nucleophilic reaction. It proceeds as an attack of the attaching phosphate residue towards a terminal P atom in ADP to form terminal P□O bond accumulating in ATP the energy ~10kcal/mol. The reaction is known to be catalyzed by Mg^{2+} ion. The latter is considered to coordinate reactants in the catalytic site keeping them on the reaction trajectory to facilitate nucleophilic attack and probably slightly modify their reactivity via partial redistribution of charges in the reactants. According this paradigm, the nucleophilic attack occurs as a pressing of one closed electronic shell into another one; this event needs to overcome a huge energy barrier caused by the repulsive potential of the exchange forces between molecules under reaction. The exchange potential is known to sharply (exponentially) increase on the short distances needed for the reaction to occur; any long-distance Coulomb attracting potential even if it exists is not capable to compensate the short-distance repulsive potential.



Scheme-1: Ion-radical mechanism of enzymatic ATP synthesis

This undisputable physical argument has been proved by numerous calculations of the phosphorylation energy barriers on the basis of quite reliable methods of quantum chemistry including combined quantum chemistry/molecular dynamics techniques. The nucleophilic transfer of phosphoryl group requires 42-46 kcal/mole [25]; nucleophilic attack of hydroxyl to hydrolyze ATP needs to overcome an energy barrier 39 kcal/mole [26]. It follows that the nucleophilic mechanism is strongly energy deficient: both accumulation and release of relatively small portion of energy (~10 kcal/mole) take high energy cost which is by 3-4 times higher than the accumulated or released energy itself.

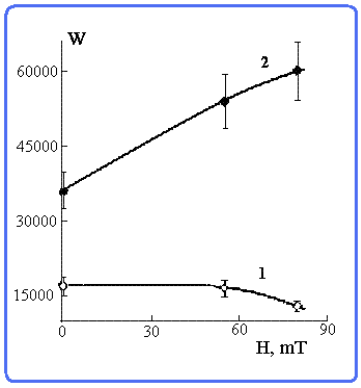


Figure-1: The rate w of the ATP synthesis by creatine kinase with $^{24}Mg^{2+}$ (1) and $^{25}Mg^{2+}$ (2) ions as a function of magnetic field. Note the two-fold difference in the rate of ATP synthesis in the Earth magnetic field.

A source of energy needed to cover the energy deficit for energy-consuming ATP synthesis is a compression of the catalytic site which is accompanied by conversion of the protein mechanical energy into the chemical energy of P□O bond in ATP. Energy pumping of the catalytic site is maintained by trans-membrane electric potential (like in the case of ATP-synthase) or by thermal fluctuations in kinases; the latter were called by Oster as the Darwin's Brownian motors [27].

A low cost energy path in the ATP synthesis

As mentioned above the ATP synthesis depends on which magnesium isotope presented in catalytic site. The rate of ATP synthesis by enzymes in which Mg^{2+} ions possess magnetic nuclei ^{25}Mg was found to be 2-3-fold higher than the rates exhibited under the same conditions by the same enzymes but possessing non-magnetic, spinless nuclei ^{24}Mg or ^{26}Mg.[13-15]A discovery of such unusual mass-independent, nuclear-magnetic isotope effect reliably demonstrates that the ATP synthesis is a radical (or ion-radical) process in which paramagnetic intermediates – ion-radicals and ion-radical pairs – participate [16]. This new universal mechanism is shown to operate in various enzymes both in oxidative and substrate phosphorylation. Scheme-1 illustrates it by example of the ATP synthase functioning.

As a first step, Scheme 1 implies electron transfer from the terminal phosphate group of ADP^{3-} to Mg^{2+} ion (AMP stands for adenosine monophosphate residue); it generates primary ion-radical pair, composed of the radical-cation Mg^{+} and oxy-radical ADP^{2-} (reaction 1). Due to the total spin conservation

primary ion-radical pair, as any other thermally generated radical pair, is in a singlet spin state. The next step is the phosphorylation itself in which the ADP^{2-} oxy-radical attacks the P=O chemical bond of inorganic phosphate (reaction **2**). Generated in this reaction oxy-radical decomposes via □-scission of the P–O chemical bond (reaction **3**, well known reaction in chemistry of the oxy-radicals) generating ATP and the final ion-radical pair (HO Mg^{+}). The latter regenerates Mg^{2+} in the reaction of back electron transfer.

The rate of phosphorylation and the ATP yield along the singlet channel (reactions 1-3 in Scheme 1) are suppressed by spin allowed reverse electron transfer in the primary ion-radical pair which regenerates starting reactants. However, in the presence of $^{25}Mg^{2+}$ ions hyperfine coupling of the unpaired electron with magnetic nucleus ^{25}Mg in the radical-cation $^{25}Mg^{+}$ stimulates singlet-triplet spin conversion of the primary ion-radical pair and transforms it into the triplet pair, in which back electron transfer is spin forbidden. This new triplet channel of phosphorylation (reactions **2'** and **3'**) provides an additional yield of ATP which increases the total production of ATP by 2-3 times. The final ion-radical pair in the triplet channel undergoes fast triplet-singlet conversion due to electron spin relaxation (in OH the spin relaxation time is about 10^{-11} s) and again regenerates Mg^{2+} ion in the reaction (I). Thus, the essence of the magnetic isotope effect in the ATP synthesis is that the magnetic interaction of the unpaired electron in $^{25}Mg^{+}$ radical with magnetic nucleus induces singlet-triplet spin conversion and switches on a new, additional reaction channel of the ATP synthesis. It certifies nuclear spin control of chemical reactivity.

In terms of nucleophilic paradigm ion-radical mechanism seems to be unexpected and unbelievable, however, it is definitely proved by existence of magnetic isotope effect on the ATP synthesis catalyzed also by calcium and zinc ions: the yield of ATP increases by 2-3 times when catalytic site carries magnetic nuclei ^{43}Ca and ^{67}Zn in $^{43}Ca^{2+}$ and $^{67}Zn^{2+}$ ions instead of nonmagnetic, spinless nuclei ^{40}Ca and ^{64}Zn.[17,18]Another confirmation of the ion-radical mechanism follows from the magnetic field dependence of the ATP synthesis (Figure-1)[19].

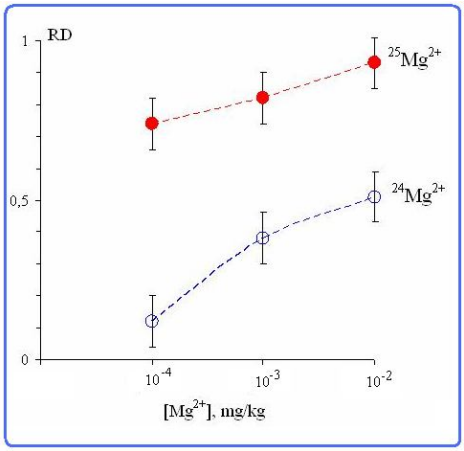


Figure-2: Recovery degree (RD) of ATP production in rats as a function of amount of the magnesium ions delivered into the heart muscle.

RD stands for the extent of restoration of a hypoxia-suppressed myocardium tissue ATP content, i.e. zero RD means a total ATP depletion, while RD=1.0 shows a

complete restoration of normal pre-hypoxia myocardium ATP level.
This effect is an unambiguous argument in favor of the ion-radical, spin selective mechanism of enzymatic phosphorylation; it may be observed exclusively in the ion-radical mechanism, it is excluded in the nucleophilic reaction (physical reasoning of the effect is explained in quantum theory is presented in [18, 28,29].An additional convincing argument in favor of the ion-radical mechanism is observation of the in vivo MIE on the ATP synthesis in living organisms (rats, rabbits, goats, Figure-2). It shows that the yield of ATP in the heart muscle is by 3-6-fold higher when $^{25}Mg^{2+}$ ions are delivered in the heart muscle instead of $^{24}Mg^{2+}$ ions [21,30].

ATP synthesis: A Molecular machine in action

Scheme 1 is nothing but an idea to illustrate of how ion-radical mechanism looks like and why the MIE appears. But for at least two reasons it is hardly possible to apply this Scheme to ATP synthesis anyway. First, ADP as an electron donor, in the catalytic site is coupled with hydrated Mg^{2+} ion (it is not shown in Scheme 1). Second, magnesium ion accepting electron from ADP^{3-} is solvated by surrounding amino acid residues and, predominantly, by water molecules (they are also not shown in Scheme 1).Ion-radical mechanism presented by Scheme 1 seems to be unbelievable, because electron transfer (reaction 1) does not occur in water where metal ions are highly hydrated; the first coordination sphere of an ion takes six water molecules. The remarkable property of enzymes is that in the reactive state, when the enzyme domains are drawn together to unite substrate and ADP, they squeeze water molecules out of the catalytic site [31] and partly dehydrate Mg(H2O)n2+ ion. The removal of the water molecules increases both positive charge q(Mg) on the magnesium and its electron affinity, i.e. the energy of electron attachment Ea (calculated at the B3LYP/6-31G* level of DFT theory [32,33], Table-1).Ionization potential of ADP^{3-} was calculated to be 4.1 eV as the energy E_d of electron detachment from hydrated magnesium pyrophosphate complex modeling $[ADP^{3-}]\cdot Mg(H_2O)_m$; it was shown to be independent on m [16]. Taking Ea from Table-1 one can calculate a total energy of electron transfer E as $E_a–E_d$ which is in fact the energy of reaction

$$\mathbf{Mg(H_2O)_n^{2+}+[ADP^{3-}]\cdot Mg(H_2O)_m \longrightarrow Mg(H_2O)_n^{+}+ [ADP^{2-}]\cdot Mg(H_2O)_m \quad (II)}$$

It is shown in Figure-3 as a function of n, the number of water molecules in coordination sphere of magnesium ion. Figure-3 exhibits remarkable property. In water, i.e. at the conditions of the total hydration of magnesium ion (we will assume for this case n = □) electron transfer is endothermic, so that the reaction (II), i.e. the reaction **1** in Scheme 1, is energetically forbidden and ATP synthesis does not occur. When the value of n decreases and the ion becomes partly dehydrated, electron affinity of $Mg(H_2O)_n^{2+}$ ion increases. At n = 12 the energy E becomes positive, the reaction becomes exothermic and ATP synthesis is allowed. These arguments provide answers to the questions: what really the molecular

machines are needed for and why do they (and only they) have an exclusive possibility to produce ATP?

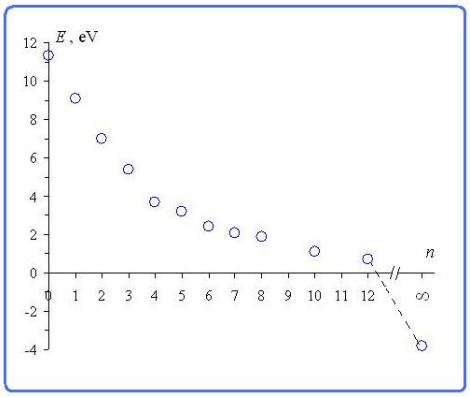


Figure-3: Energy of the electron transfer as a function of *n*, the number of water molecules in the $Mg(H_2O)_n^{2+}$ ion.

According to this mechanism, the compression energy of enzyme macromolecule is spending on the removal of water out of the ion hydrate shell which activates this ion as an electron acceptor. There is no need to destroy the first, tightly bound, coordination shell with n = 6. In order to make allowed electron transfer as well as the ATP synthesis, it is merely enough to remove weakly bound water out of the external hydrate shell with n = 12. In this process, a total energy deficit does not exceed the level of 3–5 kcal/mol, i.e. it takes about 2-3 times less energy than the accumulated amount of energy itself.

Thus, physical activation of magnesium ion achieved by its partial dehydration and following electron transfer is a low energy consuming process. The reaction (II) is a key reaction where molecular dynamics and chemistry works together, a point where mechanics of the ATP-synthesizing molecular machines cross chemistry. Dependence of the ATP synthesis on the nuclear magnetic moment of magnesium and magnetic field is a specific signature of the ion-radical mechanism. It is reliable test to distinguish two major routs of the ATP synthesis, nucleophilic and ion-radical.

Table-1: The charges *q*(Mg) and energies of electron detachment Ea for $Mg(H_2O)_n^{2+}$ ions

n	*q*(Mg)	E_a, eV
0	2.00	15.4
1	1.73	13.2
2	1.51	11.4
3	1.31	9.5
4	1.17	7.8
5	1.09	7.3
6	1.01	6.5
7	0.99	6.2
8	0.95	6.0
10	0.88	5.2
12	0.84	4.8
∞		– 0.3 [a]

[a] Taken from the reference [16]

Nucleophilic reaction Vs Ion-radical path:Parallel or alternative ?

Ion-radical mechanism of enzymatic ATP synthesis has three crucial advantages. First, in contrast to uncontrollable nucleophilic mechanism, it can be controlled by magnesium ion concentration, by

isotope substitution and external magnetic field. Second, being perfectly controllable, ion-radical channel can be artificially switched on by delivering of $MgCl_2$ (or, even better, of $^{25}MgCl_2$) into a hypoxia-suffering heart muscle tissue to stimulate ATP synthesis in vivo and prevent or correct numerous metabolic heart muscle disorders of many sorts [21,30] related to the deficiency of ATP. For these purposes a special magnesium ion carrier based on some new adduct of porphyrin with fullerene-C_{60} (PMC) has been designed [34].

The ability to stimulate ATP synthesis in the heart muscle of living organisms was demonstrated by in vivo experiments. Injection of doxorubicin was to promote a significant damage to the local myocardial ATP synthesis making it suppressed by about 70%. Then the PCM loaded with $^{24}MgCl_2$ or $^{25}MgCl_2$ was injected and after that a recovery of the ATP production up to initial, pre-doxorubicin, level was observed. The extent of recovery as a function of magnesium concentration is shown in Figure-2. Evidently, there is a large isotope effect: [^{25}Mg]PMC stimulates ATP synthesis by 3-6 times more efficiently than [^{24}Mg]PMC. This is a first observation of the isotope effect manifesting itself in living organism and, hence, showing the potential of this specific effect to be applied as an efficient remedy for heart diseases treatment. Recently [^{25}Mg]PMC was shown to be very effective in preventing different kinds of toxicity, cardiac failures and diabetic neuropathy [22, 35,36].

The third advantage of the ion-radical mechanism is its low energy cost. Indeed, this mechanism does not require overcoming high energy nucleophilic barrier and strong compression. Even low level of compression is sufficient to remove a weakly bound water molecules out of the magnesium ion hydrate shell and, hence, to induce electron transfer as a starting, key reaction of the ATP synthesis. A low energy cost is the reason of high efficiency of ATP synthesis along the ion-radical path. The increased efficiency induced by $^{25}Mg^{2+}$ ions means increasing of the ATP production per each revolution of the γ-shaft in the F1 fragment of the ATP synthase. It makes pharmacological potential of $^{25}Mg^{2+}$ ions even more attractive because high level of ATP production is reached without an additional increase of the hazardous superoxide radical yield.

A discovery of the ion-radical mechanism does not exclude a nucleophilic path in enzymatic ATP synthesis. However, it creates a new enigma: why these two routes, energy cheap and energy expensive, highly efficient and low efficient, coexist? No doubt, the source of energy needed to overcome high energy barrier in a nucleophilic attack is a compression of the catalytic site. In order to attach the phosphate group to ADP this compression must be quite significant. But on the way of a hard compression of reactants the ion-radical mechanism lies since it is supposed to be switched on even at rather weak compression. In other words, ion-radical mechanism should always precede nucleophilic one. We need to answer question, under what conditions one or another mechanism dominates. The

answer is expected to come from the inspection of the ATP synthesis as a function of the magnesium ion concentration.

There are two states of Mg^{2+} ions inside the catalytic site: in one of them the ion is tightly bound with ADP pyrophosphate residue, in another one metal ion is weakly bound being coordinated by amino acid residues and water molecules. Namely this ion is supposed to be responsible for the ion-radical mechanism since it accepts electron donated by ADP^{3-}.

It is clear that the Mg^{2+} ions are to get tightly bound to the ADP pyrophosphate residues at low magnesium concentrations. In this case, the weakly bound hydrated ions are absent. Under these conditions, a nucleophilic mechanism operates while the ion-radical one is switched off. At the high magnesium concentrations, on the contrary, an excess of catalytic ions switches ion-radical mechanism on.

The boundary between these two mechanisms is the point where free, not bound with phosphate, carboxyl or other chelating groups, metal ions appear in catalytic site. Since the catalytic sites are different for different ATP synthesizing enzymes the point where free metal ions appear are also different. It gives an answer, why ion-radical mechanism is switched on at different concentrations of metal ions in different enzymes.

This prediction is in a perfect agreement with experimental observations. It has been shown that at the low magnesium concentrations, isotope effect is either small (creatine kinase) or is absent at all (pyruvate kinase, glycerophosphate kinase) [15]. This leads to the conclusion that at low ion concentrations the nucleophilic mechanism dominates. On the opposite, the high magnesium concentrations exceeding the normal physiological values stimulate ion-radical mechanism. This property is also exhibited in experiments with Ca^{2+} and Zn^{2+} ions [17,18].

The intracellular concentration of metal ions is rather low [31], so that the dominating source of ATP in living organisms is thought to be a nucleophilic reaction. Ion-radical mechanism functions under conditions when the concentration of metal ions is rather high. However, due to local concentrations induced by fluctuations or by inhomogeneous distribution of metal ions in cells and mitochondria the contribution of the ion-radical mechanism of ATP synthesis in living organisms may appear to be not negligible. The relative contributions of nucleophilic and ion-radical mechanisms may be regulated by concentration of magnesium; this conclusion is of paramount importance in understanding medicinal effects of treatment by magnesium-containing drugs, food, drinks, etc. Moreover, both [^{24}Mg]PMC and [^{25}Mg]PMC are already used to treat diabetic retina pathology and stimulate growth of microorganisms for the biotechnological purposes.

The great advantage of the ion-radical mechanism is that it might be used for medicinal purposes to prevent and/or compensate some cell/tissue ATP losses caused be variable pathogenic factors and, therefore, to either treat or prevent a

number of essential cardiology-related syndromes.

Accumulation and release of energy in the ATP synthesis and hydrolysis, according to the generally accepted nucleophilic mechanism are energy expensive processes. They require overcoming large energy barrier which exceeds by 3-4 times accumulated or released energy as itself (~10 kcal/mole). This energy is supposed to be taken from the mechanical compression of the catalytic site and used to form P–O chemical bond by direct nucleophilic addition of phosphate to ADP. Recently discovered new, low energy ion-radical mechanism of the ATP synthesis is proved by observation of magnetic isotope and magnetic field effects on the ATP synthesis. In terms of this mechanism compression energy is used to partly dehydrate magnesium ion; energy cost of this process is 3-5 kcal/mole, i.e. by 2-3 times less than accumulated or released energy. Dehydration of the ion is shown to increase its electron affinity and stimulates electron transfer from ADP^{3-} to Mg^{2+} ion. This reaction is a starting point of the ion-radical mechanism, the point where molecular mechanics of enzymatic machines cross enzymatic chemistry. In contrast to uncontrollable nucleophilic mechanism, ion-radical mechanism can be switched on artificially by delivering magnesium ions directly into the heart muscle to stimulate ATP synthesis *in vivo* and prevent cardiac failures, toxicity effects and other medical disorders related to the deficiency of ATP.

Conclusions and perspectives

Ion-radical mechanism of the nucleotides and, hence, RNA and DNA synthesis is a, most likely, the preface for a novel biochemical paradigm. Staying certainly out-of-mainstream, this paradigm may look “heretic” to a contemoprary academic community. Nonetheless, this no doubt deserves a clear understanding of a real significance of such a quantum mechanical approach to a wide range of aspects dealing with what the metal engaging enzymatic catalysis is all about. Noteworthy, McArthur was certainly right remarking on the occasion of the longevity gene false discovery that “*... the more surprising the result seems to be, the less likely it is to be true*”. At a first glance, the ion-radical paradigm deserves to be treated the same way. So the mentioned above suspicious attitude looks natural.However, it should be taken into account that this paradigm has been firmly supported by magnetic isotope effects shown in experiments with magnetic nuclei of a number of metals - mercury, magnesium, zinc and calcium. This paradigm is also unambiguously proven by a magnetic field effect on the ATP synthesis. Besides, this paradigm is no doubt manifests itself in isolated mitochondria and in the whole organisms as well. Moreover, a reliable and directly observed data reveals both conditions and extents of the ion-radical mechanism expression including a possibility of its turning off using the ions of iron and isotopes. Last but not least, the mammalian tissues and organs with a most efficient functioning of the ion-radical mechanism were also

found. It is quite clear how this mechanism could be used to stimulate the ATP synthesis and then to exclude the ATP deficiency in cardiac diseases as well as how to apply the magnetic-isotope ions as medicinal agents for myocardial hypoxia and heart failure therapy, malignant cell destruction, apoptosis and cell proliferation control. This is true indeed as long as the magnetic metal ions are to be targeted towards the certain enzyme possessing cell compartments; the task of such a targeted delivery was shown of being solved using some non-toxic nanocationites like the water soluble porphyrine-based carriers (PMC16 and its derivatives). Dependence of the ATP synthesis on the nuclear magnetic moment and magnetic field is a specific signature of the ion-radical mechanism. It is reliable test to distinguish two major routs of the ATP synthesis, nucleophilic and ion-radical. The discovery of magnetic isotope/magnetic field effects simultaneously discloses a new, ion-radical mechanism and proves classical, nucleophilic mechanism.

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