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Zinc-Related Magnetic Isotope Effect in the Enzymatic ATP Synthesis: A Medicinal Potential of the Nuclear Spin Selectivity Phenomena

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Abstract: The rate of enzymatic ATP synthesis is shown to depend on the zinc isotopes. The ATP producing activities of creatine kinase and pyruvate kinase in which Zn^{2+} ions have magnetic nuclei ^{67}Zn are found to be 2-6 times higher than that of enzymes in which Zn^{2+} ions have nonmagnetic nuclei ^{64}Zn. The isolated rat heart muscle mitochondria exhibit a similar effect. An expression of this magnetic isotope effect in enzymatic ATP synthesis processed in the presence of Zn^{2+} ions is in a favor to the ion-radical mechanism of ATP production occurred within a high zinc concentration range. Both fundamental aspect and a possible pharmacological significance of the phenomenon described are under discussion.

Key words: Hyper activation of the ATP synthesis, magnetic isotope effects, mitochondria function control, phenomenon, Zn^{+2} ion, nuclei

INTRODUCTION

The rate of enzymatic ATP synthesis, a Mg involving process was recently shown to strongly depend on the magnesium isotopes. Activity of ATP synthase and ATP producing kinases in which Mg^{2+} ion has magnetic nucleus ^{25}Mg was found to be 2-3 times higher than that of enzymes in which Mg^{2+} ion has nonmagnetic nuclei ^{24}Mg or ^{26}Mg (Buchachenko *et al*., 2005a-c; Buchachenko, 2009).

The effect was shown to be a function of the concentration of Mg^{2+} ions at low concentration there is no isotope effect, i.e., classical generally accepted nucleophylic mechanism of the ATP synthesis dominates. If concentration of Mg^{2+} ions exceeds intracellular one by a few tens a huge isotope effect appears which gives evidence that the new, spin-dependent ion-radical mechanism of ATP synthesis is switched on (Buchachenko *et al*., 2008). Providing additional and considerable enzymatic source of ATP.

Similar effect was also observed for the calcium ions: the activity of creatine kinase with catalytic sites, loaded with $^{43}Ca^{2+}$ ions having magnetic nuclei ^{43}Ca was found to be 2.0±0.3 times higher than that of enzyme in which Ca^{2+} ions have even, nonmagnetic nuclei ^{40}Ca, ^{42}Ca or ^{44}Ca. (Kuznetsov *et al*., 2010).

Since ATP syntheses, catalyzed by magnesium and calcium are very similar in concentration dependences and isotope effects one can suppose that ion-radical mechanism is a universal phenomenon and may be detected for other metals as catalysts (Zn for instance).

MATERIALS AND METHODS

To verify this idea which would be useful to stimulate ATP production and prevent biomedical pathologies related to deficiency of ATP in the living organisms, we prepared two series of samples of Creatine Kinase (CK), Pyruvate Kinase (PK) and mitochondria. In one series the enzymes were loaded with Zn^{2+} ions of natural isotope composition in the other one the enzymes were loaded with Zn^{2+} ions strongly (by 78.4%) enriched with magnetic isotope ^{67}Zn (nuclear spin 5/2, magnetic moment+0.8 mB). Then both series were tested for their enzymatic activities in the identical conditions.

CK, E.C.2.7.3.2 was isolated from the *Vipera xanthia* lyophilized venom and purified according to (Kuznetsov *et al*., 2004). Rabbit reticulocyte PK, E.C.2.6.9.17 was purchased in the ammonium sulfate precipitated form from Worthington, Inc., Durham. The substrates, [^{32}P] phosphocreatine, 26.6-29.2 Ci $mmoL^{-1}$ and [^{32}P] phosphoenolpyruvate, 33.6-37.4 Ci $mmoL^{-1}$ were

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manufactured by the Amersham Radiochemical Centre, UK. The enzyme activity A was conventionally evaluated as the amounts of radioactive ^{32}P decays per minute found in the HPLC-separated nascent [^{32}P] ATP pool produced by 1.0 mg of pure enzyme during the 40 min incubation time in the Mg^{2+}/Ca^{2+} free samples. This time was shown to be enough for the ATP yield to reach a limiting value. For controls, the metal-free samples incubation at +37°C as well as ice-cold incubation tests were carried out (Buchachenko *et al*., 2005a; Kuznetsov *et al*., 1986) the yield of ATP in these experiments was shown to be small in comparison with that in the presence of Zn^{2+} ions.

The rat heart muscle mitochondria were isolated according to Randall with their following 60 min long incubation as described by Buchachenko *et al*. (2005b) to conduct then the oxygen consumption measurements (Rezayat *et al*., 2009) and the total ATP yield estimations (Buchachenko *et al*., 2005c). The optimum-balanced mitochondria incubation mixtures (Buchachenko *et al*., 2005a, b; Rezayat *et al*., 2009) were employed as they are as well as in their metal-lacking and Zn-supplemented modified forms, same like in experiments with pure enzyme specified above. For protein and DNA quantitative estimations, conventional colorimetric procedures were applied (Bradford, 1976).

Two samples of $ZnCl_2$ were prepared using a routine acidic treatment of the two sorts of zinc oxides: ZnO with natural isotope composition (^{64}Zn, 48.6%; ^{66}Zn, 27.9%; ^{67}Zn, 4.1%; ^{68}Zn, 18.8%; ^{70}Zn, 0.6%) and ^{67}ZnO enriched with magnetic nuclei (78.4% of ^{67}Zn versus 4.1% of that in natural ZnO), respectively. Enzymatic activity of the free metal CK, PK and mitochondria (the contents of Ca^{2+} and Mg^{2+} in the latter were negligibly small, 30-35 and 16-18 μg mg^{-1} of DNA, respectively) was measured as a function of the $ZnCl_2$ concentration.

RESULTS AND DISCUSSION

The yield of ATP produced by CK as a function of $ZnCl_2$ concentration is shown in Fig. 1. It exhibits the following remarkable features:

- Zn^{2+} ions actually catalyze ATP synthesis with the efficiency comparable with that of Mg^{2+} ions
- ATP yield increases as concentration of $ZnCl_2$ increases then reaches maximum and gradually decreases
- There exists enormously large isotope effect in the ATP synthesis by CK differing in isotope composition
- At high concentration of $ZnCl_2$ both ATP yield and isotope effect are almost completely suppressed

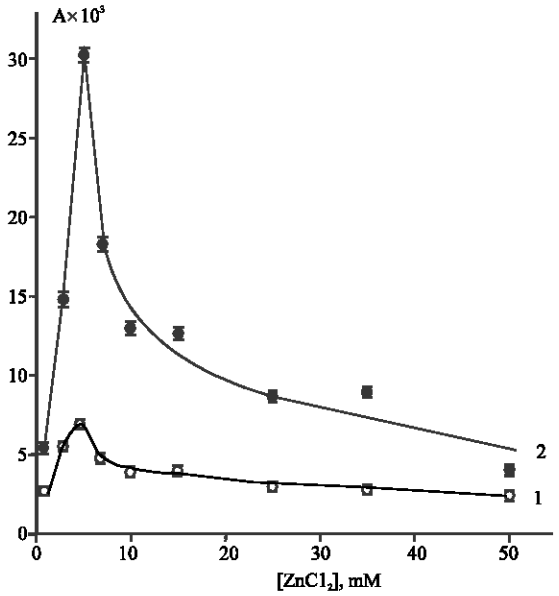

Fig. 1: The yield of ATP produced by CK as a function of $^{64}ZnCl_2$ (1) and $^{67}ZnCl_2$ (2) concentration

Both samples of CK contain zinc isotopes in different shares. In the samples with natural $ZnCl_2$ the share of magnetic nuclei ^{67}Zn is 4.1%; the total share of nonmagnetic isotopes is 95.9% (further we will conventionally denote the set of nonmagnetic isotopes as ^{64}Zn). In the samples loaded with enriched $^{67}ZnCl_2$ the shares of ^{67}Zn and ^{64}Zn are 78.4 and 21.6%, respectively. Now the activity A of the both samples of CK may be presented as a sum of additive contributions coming from catalytic sites carrying ^{67}Zn and ^{64}Zn:

$$A_1 = 0.041A\ (^{67}Zn)+0.959A\ (^{64}Zn) \quad (1)$$

$$A_2 = 0.784A\ (^{67}Zn)+0.216A\ (^{64}Zn) \quad (2)$$

Here A (^{67}Zn) and A (^{64}Zn) characterize the true values of enzymatic activity of catalytic sites with $^{67}Zn^{2+}$ and $^{64}Zn^{2+}$ ions, respectively. Substituting into the (Eq. 1, 2) $A_1 = 7000$ (Fig. 1, curve 1) and $A_2 = 30200$ (Fig. 1, curve 2) for the $ZnCl_2$ concentration 5 mM, it is easy to derive A (^{67}Zn) 37000 and A (^{64}Zn) = 5753. Their ratio A (^{67}Zn)/A (^{64}Zn) = 6.4±0.3 is the magnitude of isotope effect in enzymatic ATP synthesis by CK. It demonstrates that the CK catalytic sites with $^{67}Zn^{2+}$ ions produce ATP by 6 times more efficiently than those with $^{64}Zn^{2+}$ ions.

In order to compare both nuclear spin dependences of the ATP synthesis directed by CK from *Vipera xanthia* venom and that of the mitochondrial CK promoted one, some additional experiments were carried out using the

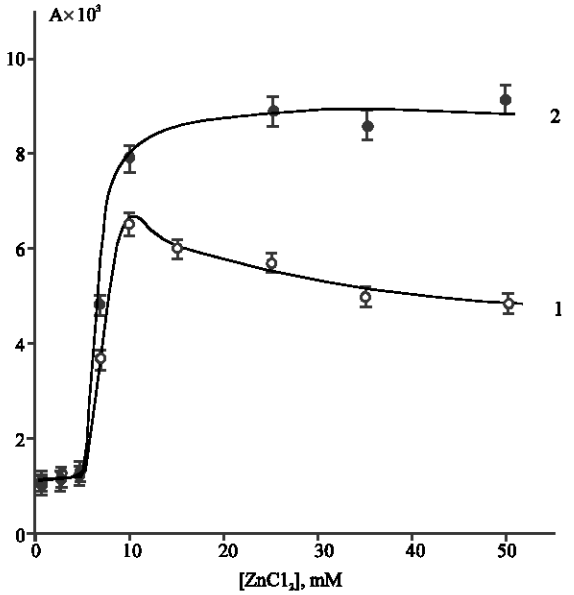

Fig. 2: The yield of ATP produced by PK as a function of $^{64}ZnCl_2$ (1) and $^{67}ZnCl_2$ (2) concentration

above specified identical standard conditions. As a result, no marked difference found whatsoever. Both CK species tested promotes the very same isotope-related specific activity response whatever metal concentration range studied (0.5-50.0 mM). There is no magnetic isotope effect revealed in the isolated mitochondria oxygen consumption at all it means that isotope effect arises in substrate ATP synthesis itself and has no relation to the NAD-engaging oxidative mitochondrial processes.

ATP synthesis by PK exhibits generally similar but not identical behavior (Fig. 2). The differences are quite evident (Fig. 1 and 2) they assumed to refer to those in molecular mechanics of these two enzymes, CK and PK. At the concentration of $ZnCl_2$ 5 mM there is no isotope effect for PK (unlike of CK for which it is 6.4±0.3) however at 50 mM of $ZnCl_2$ it reaches 2.2±0.3 (calculated according to Eq. 1 and 2).

Observation of magnetic isotope effect in ATP synthesis catalyzed by Zn^{2+} ions evidently demonstrates that the ion-radical mechanism of enzymatic ATP synthesis is a universal phenomenon. It includes electron transfer from Zn $(ADP)^{3-}$ complex to the hydrated Zn $(H_2O)^{2+}_n$ complex as a primary reaction of ATP synthesis. The reaction generates ion-radical pair composed of Zn $(H_2O)^{+}_n$ ion and ADP anion-radical coordinated to Zn^{2+} ion. The addition of the anion-radical to the substrate P = O bond results in ATP formation. Populations of the singlet and triplet states and singlet-triplet spin conversion in the pair are controlled by hyperfine coupling of unpaired electrons with magnetic ^{67}Zn and ^{31}P nuclei. Due to this interaction the yield of ATP is a function of nuclear magnetic moment as discussed in detail for magnesium induced ATP synthesis (Buchachenko *et al*., 2010). For the same reason ATP yield depends on the magnetic field (Buchachenko and Kuznetsov, 2008).

Two results need to be commented. First, the three enzymes, CK from the *Vipera xanthia* venom, mitochondrial CK and PK exhibit specific isotope effects. Both CK demonstrate ion-radical mechanism of ATP synthesis in the same range of $ZnCl_2$ concentration, however, isotope effects for these two enzymes are slightly different. These differences may be attributed to the differences in molecular structure and dynamics of protein domains in catalytic sites.

Second, large isotope effects are not unexpected and suspicious because they are induced by spin dynamics rather than chemical reactions themselves. Their magnitudes are controlled by the rates of singlet-triplet spin conversion in the ion-radical pairs and depend on the electron-nuclear (hyperfine) coupling of unpaired electrons with magnetic nuclei. In principle, there is no limit on the magnitude of magnetic isotope effect, (Buchachenko, 2009) in contrast to classical, mass-dependent one which is known to be limited on the ratio of nuclear masses.

CONCLUSION

Apart from its obvious fundamental significance, the phenomenon described possesses some clear pharmacological potential. Thus, the low toxic cation exchanging nanoparticles formed on a basis of the fullerene-C60 porphyrinic adducts were found to be the reliable carriers for magnetic bivalent metal isotopes suitable for a targeted delivery of the latter's ions *in vivo* followed then by a marked local hyper-activation of the pre-suppressed ATP synthesis (Rezayat *et al*., 2009; Amirshahi *et al*., 2008a).

This alone made a remarkable contribution to either prevention or treatment of several hypoxia related syndromes including the ones associated with some myocardial and lymphoid tissue energy metabolism disorders (Buchachenko, 2009; Kuznetsov *et al*., 2010; Amirshahi *et al*., 2008b). As per the phenomena described in a present study, they are no doubt worthy the further nanopharmacological testing in a way offered and pre-programmed recently (Buchachenko, 2009; Kuznetsov *et al*., 2010; Rezayat *et al*., 2009; Amirshahi *et al*., 2008a, b).

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