The tissue-specific “ferromagnetic attack” on hyperactivation of ATP synthesis by magnesium-25 in mitochondria

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Abstract. A 25Mg2+-operated hyper-activation of ATP synthesis has been investigated in mitochondria (Mt) isolated from iron-rich and iron-poor rat tissues: spleen, liver, skeletal muscle, myocardium, kidneys, brain. Both magnetic (25Mg) and non-magnetic (24Mg) magnesium isotopes were separately administered to estimate the degree of the ATP production related to the magnetic isotope effect (MIE) of 25Mg2+ as a function of the amount of Mt-endogenous iron ions. A strong but negative (r = -0.88) correlation between the 25Mg-MIE degree and the Mt[Fe2+] values was found. The physical and biophysical mechanisms behind these phenomena, as well as the possible impact of these data on further biochemical and pharmacological studies involving 25Mg-promoted nuclear spin selectivity in mitochondrial function, are under discussion.

Key words: magnesium-25, ferromagnetism, mitochondria, ATP

It is hardly possible to exaggerate the role played by magnesium in the control of enzymatic phosphorylation and, particularly, in kinases/ATP synthase-directed ATP production in Mt. However, there is a remarkable difference between the ATP synthesis rates induced by stable, paramagnetic (25Mg: nuclear spin +5/2, nuclear magnetic moment 0.85 Bohr magnetons, natural abundance 10%), and non-magnetic (24Mg, 26Mg: zero spin for both) magnesium isotopes. This magnetic isotope effect (MIE) remains enigmatic [1]. According to the most recent findings, this unique physical phenomenon involves an ion-radical, i.e. quantum mechanical not thermodynamic (nucleophilic) mechanism [2]. Although many of the details of this mechanism remain obscure, the biotechnological/pharmacological potential of 25Mg-related MIE looks quite promising [2-4].

Despite a nearly 10-year-long history of 25Mg-MIE studies in chemical enzymology and related areas [1, 2], no special attention has yet been paid to the possible in situ impact of endogenous, mitochondrial, free iron cations on the nuclear spin selectivity of 25Mg-promoted hyper-activation of ATP synthesis. Taking into account that such an impact might pharmacologically affect significant routes for magnesium involvement at the very heart of mitochondrial function, it makes sense to evaluate any possible correlation between
concentrations of free, endogenous, bivalent iron ions in Mt, and the rates of ATP synthesis reached in these organelles in vitro, under optimal conditions (20 mM MgCl₂), and in the case of separate use of either ²⁵MgCl₂ or ²⁴MgCl₂ species.

The specific aim of this work was to to employ a known methodological background [5] to provide:

– a better understanding of MIE biophysical validity
– and to predict the possible impact of this phenomenon on the pharmacodynamics of ²⁵Mg²⁺-releasing drugs [2-4, 6] once they are unequally (“asymmetrically”) distributed between iron-deficient and iron-overloaded body compartments.

Methods and materials

Animals

Healthy adult male Wistar Albino Glaxo (WAG/Sto2J and WAG/Qas3S strains) rats (180-220 g) were given a standard diet based on the ARAGVI 13 rat meal set purchased from Tsarskaya Okhota Ltd (Moscow, Russia). They were starved for 24 h prior to the experiment. To confirm reproducibility of the data, all measurements were replicated six times. Each Mt isolation was performed on organs/tissues of at least three animals. All ethical principles and veterinary requirements were fulfilled according to the NIH Office of Animal Care and Use Regulations and Standards.

Fine chemicals

ATP sodium salt and all buffer compounds were purchased from Serva (Heidelberg, GmbH, Germany), while Triton X100 and acetone were from Bio-Rad (Moscow, Russia). All reagents were of analytical grade.

Both magnesium isotopes tested (²⁵MgCl₂ and ²⁴MgCl₂), with an isotope enrichment degree of not less than 94%, were from GammaLab SA, (Alicante, Spain). To determine the ATP synthesis rate values, [³²P]orthophosphate with a specific activity of 6.70-8.40 Ci/mmol (Amersham Radiochemical Centre, Leeds, UK) was used as described in [6].

Isolation of mitochondria

All mitochondrial samples tested were conventionally isolated from the rat tissue homogenates listed below, and prepared using iron-free homogenization media, 1:4 v/w, 4°C, in a tightly-fitting, Teflon-glass RCJ200 homogenizer with a fixed speed for the Teflon pestle rotation (Biomedlab Ltd, Minsk, Republic of Byelorussia), adjusting the 7.8-8.4 pH range with either HEPES or Tris-based buffer systems containing 2.5 mM EDTA, 1.5 mM NaCl, 0.25 M sucrose, 0.1% glutathione, 0.001% soy bean trypsin inhibitor as specified in [7-12]; 800-15,000 × g centrifugation of homogenates was performed at +4°C (Spinco L5-85B Ultracentrifuge, rotor SW40, Beckman, Austria). These procedures were specifically adapted for each of the particular rat tissues studied: spleen [7], liver [8], skeletal muscles [9], heart muscle [10], kidneys [11], and brain [12].

Endogenous Mt iron measurements

To obtain the intra-Mt free iron pool samples, the pre-suspended (1:5 w/v, 4°C, 15 mM Tris-HCl (pH 7.85)/1.5 mM EDTA/10 mM KCl/0.25M sucrose) Mt pellets were first subjected to Triton X100 (adjusted to 2.0%, v/v), and then to a triple, ice-cold acetone treatment (10:1, v/v, overnight storage), followed by separation of the acetone-soluble fraction by centrifugation at 800 × g, 4°C, 15 min (rotor SV16E, Sorvall Q570 Lab High Speed Centrifuge (Sorvall Instruments, Inc., Ann Harbor, USA). The resulting supernatants consisting of the Mt-associated total low molecular mass compounds pool [5] were carefully collected and lyophilized, and then used to measure the Fe²⁺ content using energy-dispersive X-ray fluorescence spectrometry with a Fe-Zr performance element range employing the XEPOM-HE Analytical System according to the manufacturer’s recommendations (SPECTRO Analytical Instruments, GmbH, Kleve, Germany).

ATP synthesis rate measurements

The isolated Mt were first pre-incubated in an aerated suspension prepared by mixing the Mt pellets (1:10, w/v) in 215 mM mannitol/75 mM sucrose/1% BSA/20 mM MgCl₂ (either
Fe^{2+} suppress the $^{25}\text{Mg}$-operated ATP hyperproduction

$^{25}\text{MgCl}_2$ or $^{24}\text{MgCl}_2$ species, separately)/25 mM KH$_2$PO$_4$/20 mM HEPES, +37°C, for 15 min [13]. Once pre-incubation was completed, the ATP-triggering primer was added to the mixture, adjustment to a final concentration of 2.5 mM was performed simultaneously with the addition of succinate at 1.5 mM and $[^{32}\text{P}]$orthophosphate at 1.0 mM [5, 13]. The mixture was incubated for a further 60 min at the same temperature. Immediately after the incubation had finished, the Mt-containing mixtures were instantly subjected to the ice-cold Triton X100 treatment (2.0%, v/v)/10 mM Tris-HCl (pH 7.45) for two hours. The low molecular mass compound pool was extracted by a triple precipitation-rewashing with acetone (10 vol. per sample), using the same centrifugation procedure at 800 g, +4°C, for 20 min, in a Sorvall QS70 Lab High Speed Centrifuge, rotor SV16E. The resulting acetone-soluble material was concentrated in a rotor evaporator (1.5-2.5 mL, final volume) and then fractionated using the HPLC procedure (ODS-S5CN stationary phase, 10-60% linear pyridine elution gradient based on 10% water/methanol, Altex-1800 15 × 280 mm column, 22°C, 2,000 psi, 2.0 A$_{254}$ in a 50 µL sample for injection, Gilson W100 UV$_{254}$-detecting HPLC System (Gilson Ltd, Taijon, Republic of Korea). The ATP peaks were recorded for further $[^{32}\text{P}]$-measurements in dioxane-based Koch Light BetaSL20 scintillation liquid using the WALLAC JR880 Liquid Scintillation Counter (WALLAC, Finland) [5]. The values for the ATP production rate were expressed in $[^{32}\text{P}]$ATP cpm per mg Mt-protein [5]. For protein measurements, a routine Bradford colorimetric method was used [14]. For all control tests, the same incubation procedures were carried out at +4°C.

### Statistical analysis

Statistical analysis was performed with a two-tailed, unpaired Student’s t-test using Sigma BioStat A6 software (Sigma Co., Palo Alto, USA). The t-test was followed by a Bonferroni–Dunn test for multiple comparisons, allowing the determination of a rank correlation $r$ parameter for the $[\text{Fe}^{2+}] = f(\text{MIE})$ data row [13, 15]. All data are presented as the mean values ± the standard errors of the mean ($n$ is lower than or equal to 6). Only the data with a probability level $p$ smaller than 0.05 are listed in the table 1, as they are considered to be significantly different.

### Results

As shown in table 1, a highest level of tissue-specific, Mt-endogenous iron content (spleen) is associated with almost zero $^{25}\text{Mg}$-MIE, while the lowest (brain) “allows” as high as nearly 1.8-fold MIE difference in the $^{25}\text{Mg}/^{24}\text{Mg}$-promoted ATP synthesis rates. It should be noted that a rank comparative analysis of the latter as a function of Mt[$\text{Fe}^{2+}$] levels shows a strong negative ($r = -0.88$) correlation between the increase in values for tissue-specific Mt-iron content within the brain-spleen “line” and the decline of the extent of MIE observed.

### Discussion

The data obtained (table 1) are in a good accord with the DFT computational simulation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>[Fe$^{2+}$], pg/mg protein</th>
<th>ATP synthesis rate, $[^{32}\text{P}]$ATP cpm/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>62 ± 9</td>
<td>15,864 ± 176 $^{24}\text{Mg}^{2+}$ 28,816 ± 203 $^{25}\text{Mg}^{2+}$</td>
</tr>
<tr>
<td>Kidneys</td>
<td>97 ± 8</td>
<td>13,614 ± 188 $^{24}\text{Mg}^{2+}$ 24,870 ± 191 $^{25}\text{Mg}^{2+}$</td>
</tr>
<tr>
<td>Heart</td>
<td>384 ± 12</td>
<td>12,088 ± 173 $^{24}\text{Mg}^{2+}$ 21,561 ± 197 $^{25}\text{Mg}^{2+}$</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>575 ± 14</td>
<td>16,231 ± 201 $^{24}\text{Mg}^{2+}$ 19,617 ± 211 $^{25}\text{Mg}^{2+}$</td>
</tr>
<tr>
<td>Liver</td>
<td>3,096 ± 58</td>
<td>9,638 ± 167 $^{24}\text{Mg}^{2+}$ 9,439 ± 184 $^{25}\text{Mg}^{2+}$</td>
</tr>
<tr>
<td>Spleen</td>
<td>8,640 ± 67</td>
<td>10,833 ± 188 $^{24}\text{Mg}^{2+}$ 11,060 ± 160 $^{25}\text{Mg}^{2+}$</td>
</tr>
</tbody>
</table>

Table 1. Mg$^{2+}$-dependent ATP synthesis in mitochondria isolated from rat tissues with different endogenous iron contents.
models indicating that slight “iron pollution” (80-100 ppb) does not demolish the ion-radical path in ATP synthesis catalyzed by purified, major nucleotidyl kinases (creatine kinase, phosphoenolpyruvate kinase, alpha-glycerophosphate kinase) [2, 16]. On the other hand, this “pollution” – or, “ferromagnetic attack” – is nonetheless capable of destroying the $^{25}\text{Mg}^{2+}$-induced nuclear spin selectivity in the given enzymatic reactions. This “destruction”, in turn, can delete MIE from the ATP-producing pathways. Making no impact on a “classical” nucleophilic channel of nucleotide phosphorylation in Mt [1, 2].

The fact that the $^{25}\text{Mg}$-MIE has no or little chance of being expressed in iron-rich compartments of mammalian organism (table 1) is worthy of consideration in further MIE and $^{25}\text{Mg}$-focused biomedical research. This should be taken into consideration in any biotechnological production schemes dealing with $^{25}\text{Mg}$-related, overstimulation of recombinant protein synthesis such as those reported recently [17]. The current trend in these and related studies involves variable efforts to take advantage of the low toxicity of $^{25}\text{Mg}^{2+}$-releasing nanocarrier porphyrin-fullerene nanoparticles as pharmacophores [2-6].

Returning to our results (table 1), we must recall and again emphasize that the biophysical manifestation of MIE in mitochondrial function can best be described by the fact that nucleotidyl kinases/ATP synthase-directed ATP synthesis – as well as the ATP production occurring in isolated Mt or even in a whole mammalian organism – depends on which type of magnesium isotope coordinates the enzyme’s catalytic site. The resulting rate of ATP synthesis promoted by phosphate-transferring magnesium-containing enzymes is found to be 2-3-fold higher in $^{25}\text{Mg}^{2+}$-operated catalysis as compared to the same enzyme loaded with non-magnetic magnesium nuclei, $^{24}\text{Mg}^{2+}$ and $^{26}\text{Mg}^{2+}$ [1, 2, 5, 6, 16]. It should be outlined that there was no difference in the ATP-producing activities of enzymes with non-magnetic $^{24}\text{Mg}^{2+}$ and $^{26}\text{Mg}^{2+}$ ions [1, 2, 16].

A discovery of such a huge, mass-independent, nuclear-magnetic isotope effect seems to be reliable evidence that ATP synthesis is indeed radical or ion-radical driven, and therefore a very fast process. Paramagnetic intermediates (ion-radicals and ion-radical pairs) participate in these reactions. A dependence of the ATP production rate on the nuclear spin of magnesium is itself a firm argument supporting the statement on the $^{25}\text{Mg}$-promoted conversion of the enzyme protein domain compression energy into the energy of the P-O chemical bond in the energy-rich triphosphate chain of ATP [2, 16].

The crucial, truly critical, role of iron contamination existing in the purified creatine kinases in vitro reaction media was found to be sufficient to prevent or turn off the $^{25}\text{Mg}$-MIE [18], which was then re-interpreted in terms of the developing nuclear spin selectivity paradigm [2]. Thus, even a slight excess of iron (Fe$^{2+}$ or Fe$^{3+}$) ions might not “kill” an ion-radical mode, but instead suppress the $^{25}\text{Mg}$-induced singlet-triplet conversion of the magnetically originated ion-radical pairs playing the role of intermediates in the accelerated ATP formation route; this spin conversion act, truly a milestone on a nuclear spin selectivity expressway, is known to be controlled predominantly by spin relaxation “blink” and not by Coulomb hyperfine coupling or by the Zeeman interaction [1, 2].

Conclusions

In order to explore the pharmacological or biotechnological potential of the $^{25}\text{Mg}$-related magnetic isotope effect, a specific correction for quantitative iron parameters in all in vitro and in vivo research is no doubt required. This kind of correction will be particularly required in pharmacokinetic models describing the distribution of $^{25}\text{Mg}^{2+}$-carrying/releasing pharmaceutical agents and biotechnological nanotools between compartments with different endogenous iron levels.

Disclosure


References


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Fe$^{2+}$ suppress the $^{25}$Mg-operated ATP hyperproduction through the isotope window. *Chem Rev* 2012; 112: 2042-58.


