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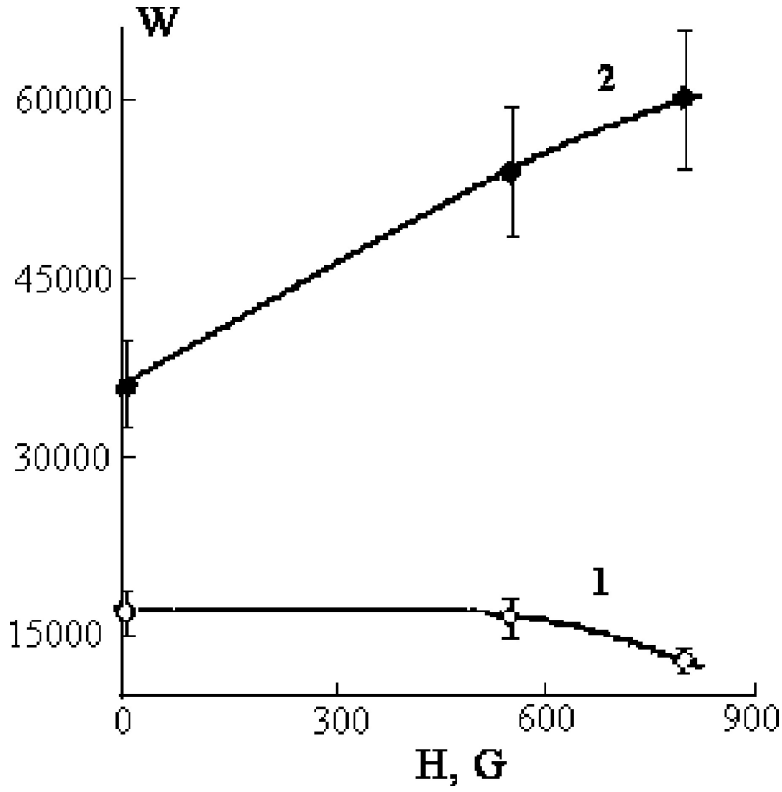
J. Am. Chem. Soc., **2008**, 130 (39), 12868-12869 • DOI: 10.1021/ja804819k • Publication Date (Web): 06 September 2008



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Published on Web 09/06/2008

Magnetic Field Affects Enzymatic ATP Synthesis

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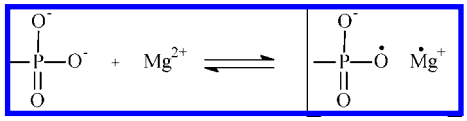
The results of numerous studies of the magnetic field effects on biological phenomena remain controversial and ambiguous. Their detailed analysis was presented by Grissom in 1995.[1] Since this time almost nothing has changed. An exception is the discovery of reliable magnetic field effects in the functioning of B_{12}-dependent enzymes [2−4] and enzymatic oxidation of organic substrates induced by horse radish peroxidase.[5−8] In all these cases enzyme biochemistry is controlled by reactions of transition metal ions (Co, Fe), as the key structural elements of the enzyme molecule, and accompanied by the generation of radical intermediates.

A recent discovery of the nuclear-magnetic isotope effect of magnesium in the ATP synthesis by phosphorylating enzymes (ATP synthase, creatine kinase, phosphoglycerate kinase, pyruvate kinase) undoubtedly proves that the ATP synthesis is an ion-radical reaction.[9−11] Its first step is an electron transfer from the ADP phosphate anion to Mg^{2+} ion generating an ion-radical pair composed of the ADP oxyradical and Mg^{+} radical-cation (of course,



both magnesium ions are supposed to be hydrated):

In this pair the two spin states, singlet and triplet, contribute differently to the ATP synthesis. In a singlet state, the reverse electron transfer regenerates initial reagents and suppresses the ATP production. In a triplet state, the reverse electron transfer is spin forbidden and, hence, the triplet phosphorylation channel is more efficient than the singlet one.[12]

Singlet−triplet spin conversion is induced by hyperfine coupling of unpaired electrons with magnetic nuclei of radical partners in the pair. Once a $^{25}Mg^{2+}$ ion with a magnetic nucleus ^{25}Mg is present in the catalytic site of the enzyme, one of the partners in the ion-radical pair is the radical-cation $^{25}Mg^{+}$. Magnetic coupling of the unpaired electron with the magnetic nucleus ^{25}Mg stimulates singlet−triplet spin conversion of the pair and switches on an additional activity, the triplet channel of ATP synthesis. As a result, the activity of enzymes with $^{25}Mg^{2+}$ ions is 2−3 times higher than that of enzymes carrying in catalytic sites $^{24,26}Mg^{2+}$ ions with spinless, nonmagnetic nuclei ^{24}Mg and ^{26}Mg (the activity of enzymes with $^{24}Mg^{2+}$ and $^{25}Mg^{2+}$ ions in catalytic sites was proved to be identical[10]). This unexpected effect was thought to be unbelievable, so many efforts were applied to prove it, to be convinced and to convince others that the magnesium isotope effect is a fact of irrefutable reliability.[12]

Both spin conversion of the ion-radical pair and the ratio of singlet/triplet channels, and, ultimately, the yield of ATP may be controlled not only by the internal magnetic field (hyperfine coupling) of ^{25}Mg nucleus but also by an external magnetic field. To verify this statement we have studied the yield of ATP synthesized by creatine kinase as a function of magnetic field.

Table 1. ATP Yield As a Function of Magnetic Field **H**

H, mT	*Y*(*Mg)	*Y*(^{25}Mg)
0	18700	34800
55	20100	52600
80	17500	58300

The crystallized electrophoretically homogeneous *V. xanthia* venom creatine kinase, 40 kDa active monomer, has been employed. Its catalytic activity was determined by HPLC as the amount of labeled [^{32}P] ATP formed in 1 min at 30 °C under optimal conditions of incubation with [^{32}P] phosphocreatine (160−180 mCi/mmol) in a medium containing Tris-HCl (pH 6.35), 20 mM $MgCl_2$, 12.5 μM ATP, 100 μg enzyme, 15 mM potassium phosphate, 160 μM [^{32}P] phosphocreatine, and 160 μM ADP at a total incubation duration of 30 min.[10,13]

In the incubation mixtures two isotopic forms of $MgCl_2$ were used: one has a natural isotope abundance (10% ^{25}Mg, 90% nonmagnetic isotopes $^{24,26}Mg$; we will label it as *Mg), another, labeled as ^{25}Mg, was composed of 96.7% ^{25}Mg and 3.3% of nonmagnetic isotopes ^{24}Mg and ^{26}Mg. Magnetic field was supplied by constant magnets. The yield *Y* of the ATP produced in 30 min incubation has been measured by [^{32}P] ATP radioactivity taken as a number of counts per minute (cpm) referred to 1 mg enzyme. The yield of ATP as a function of magnetic field is presented in Table 1.

In the table, *Y*(*Mg) stands for the yield of ATP produced by creatine kinase with a natural abundance of magnesium isotopes; *Y*(^{25}Mg) means the yield of ATP generated by creatine kinase enriched with magnetic ^{25}Mg isotope (96.7%).

Table 1 exhibits two important results. First, in zero magnetic field (to be exact, in the earth field) the ATP yield *Y*(^{25}Mg) is almost twice higher than *Y*(*Mg); this result was known from the earlier studies,[9−11] it is just reproduced here. Second, a magnetic field unambiguously affects ATP synthesis by both kinases: the ATP yield with *Mg kinase increases by 7−8% at 55 mT and then decreases at 80 mT, for kinase with ^{25}Mg it increases by 50% and 70% in the fields 55 and 80 mT, respectively.

Both *Y*(*Mg) and *Y*(^{25}Mg) are additive sums of contributions provided by both kinases:

$$Y(^{*}\mathrm{Mg}) = 0.1w(25) + 0.9w(24,26) \quad (1)$$

$$Y(^{25}\mathrm{Mg}) = 0.967w(25) + 0.033w(24,26) \quad (2)$$

where *w*(25) and *w*(24,26) are the rates of ATP synthesis by enzymes containing in catalytic sites magnetic and nonmagnetic magnesium isotopes, respectively. Combining eqs 1 and 2 with values *Y*(*Mg) and *Y*(^{25}Mg) from Table 1, one can derive the true values *w*(25) and *w*(24,26) characterizing activity of enzymes with magnetic and nonmagnetic magnesium nuclei in catalytic sites; they are shown in Figure 1 as a function of magnetic field.

The magnetic field dependence of the ATP synthesis is an unambiguous argument in favor of an ion-radical, spin selective

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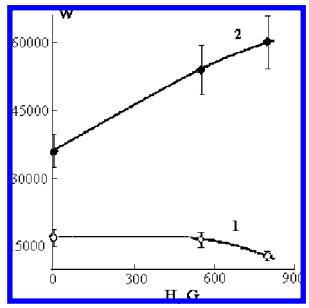

Figure 1. The rates of ATP synthesis $w(25)$ (black points) and $w(24,26)$ (open circles) by creatine kinases with $^{25}Mg^{2+}$ and $^{24,26}Mg^{2+}$ ions, respectively, in catalytic sites as a function of magnetic field.

mechanism of enzymatic phosphorylation. It is also in perfect agreement with a magnesium isotope effect in phosphorylation. Its physical meaning is rather simple and clear. In zero magnetic field electron and nuclear spins of the ion-radical pair are coupled by hyperfine interaction resulting in a total spin J_{max} as a sum of individual electron S and nuclear I spins of the partners:

$$J = \sum_{i=1,2} S_i + \sum_{j=1,2} I_j \qquad (3)$$

In the pairs with spinless magnesium isotopes $^{24,26}Mg$ only single magnetic nucleus, ^{31}P ($I = {}^1/_2$) in ADP oxyradical, is present, and $J_{max} = {}^3/_2$. In the pairs with ^{25}Mg ($I = {}^5/_2$) both spin carring nuclei, ^{31}P and ^{25}Mg, are present, and $J_{max} = 4$. These states are combinations of electronic/nuclear spin states involving the electron spin triplet exclusively and at the same time they are (degenerate) eigenstates of the total spin Hamiltonian. Thus they are conserved in time and not subject to triplet-singlet mixing. In a magnetic field their degeneracy is lifted and they are coupled to the singlet manifold. This opens new intersystem crossing channels of spin conversion (for details see ref 14). Altogether, the competition of Zeeman and hyperfine interactions in low magnetic fields results in an increase of the rate of singlet−triplet conversion and, consequently, in an increase of the ATP yield. This effect is much more pronounced for the pairs with ^{25}Mg nuclei than for those with nonmagnetic magnesium nuclei. This so-called low-field-effect reaches a maximum at $\mathbf{H} \approx a$, where the Zeeman interaction is comparable with the hyperfine coupling constant a. However, at $\mathbf{H} \gg a$, the Zeeman interaction dominates, and singlet−triplet spin conversion is suppressed by a magnetic field because the Zeeman splitting of T_+, T_0, and T_- spin substates cuts off the $S-T_+$ and $S-T_-$ channels from singlet−triplet conversion and results in a decrease of the ATP yield along the triplet channel.

For the pairs with a single magnetic nucleus ^{31}P (with $^{24.26}Mg^{2+}$ ions in catalytic sites) the opening of new spin channels is expected to occur at $a(^{31}P) \approx 3$ mT and further, at $\mathbf{H} \gg a(^{31}P)$, the yield of ATP should decrease. This effect is observed experimentally at fields of 55 and 80 mT (Figure 1). In the pairs with two magnetic nuclei, ^{31}P and ^{25}Mg, the total hyperfine coupling is by almost 1 order of magnitude larger, about 25 mT, because $a(^{25}Mg)$ in the $^{25}Mg^+$ ion is ∼21 mT.[15] The opening of spin conversion channels by the Zeeman interaction in this case is more effective (the number of channels is larger) and occurs at $\mathbf{H} < a(^{25}Mg)$. It results in the increase of the ATP yield observed experimentally. A suppression of singlet−triplet spin conversion and decreasing ATP yield is expected to occur in fields where $\mathbf{H} \gg a(^{25}Mg)$ which was not attained in our experiments (Figure 1). These qualitative considerations are in a perfect agreement with rigorous theoretical calculations.[16] Nevertheless, further detailed studies of the magnetic field effect in the ATP synthesis, both in low magnetic fields, at $\mathbf{H} \approx a(^{31}P)$, and in high fields, $\mathbf{H} \gg a(^{25}Mg)$, are required in order to determine the reaction rate constants in the catalytic site as it is pointed out in.[16] Note that the observation of a magnetic field effect on a vitally important biochemical process, such as ATP synthesis, represents a solid physical basis for the further development of magnetobiology.

Acknowledgment. This work was financially supported by Russian Ministry of Science and Education (Grant NS-1468.2008.3), by Russian Fund for Basic Researches (Grant 08.03.00141), and by INTAS (Project No 05-1000008-8070).

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JA804819K