



The effect of magnetic field on the activity of superoxide dismutase

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Abstract

The effects of magnetic field on superoxide dismutase activity were investigated. All living systems are affected by magnetic field and electromagnetic field in a way of their response systems. Since magnetic field has an impact on biochemical reactions that involve more than one unpaired electron, in our study SOD activity, one of the enzyme responsible for antioxidant system, was measured under magnetic fields using an apparatus explained at material methods. There has been a significant increase of SOD activity when passed 0, 1, 9 and 15 times at 2.9-4.6 mT magnetic field density for 0, 2.2, 19.8 and 33.0 seconds respectively.

Key words: Superoxide dismutase, magnetic field, enzyme activity

Manyetik alanın süperoksit dismutaz aktivitesi üzerine etkisi

Özet

Süperoksit dismutaz aktivitesi üzerine manyetik alan etkileri araştırılmıştır. Bütün yaşayan sistemler, yanıt sistemlerine bağlı olarak elektrik ve manyetik alan ve elektromanyetik alandan etkilenirler. Manyetik alan, ortaklamamış elektron içeren biyokimyasal reaksiyonlar üzerinde etkili olduğundan, çalışmamızda, antioksidan sistemlerinden biri olan, SOD aktivitesi manyetik alan altında ölçüldü. 2.9-4.6 mT da 0, 1, 9 ve 15 kez, sırasıyla 0, 2.2, 19.8 ve 33.0 sn lik zamanlarda geçirildiğinde SOD aktivitesinde önemli bir artış tespit edildi.

Anahtar sözcükler: Süperoksit dismutaz, manyetik alan, enzim aktivitesi

Introduction

Superoxide dismutase (SOD) is one of the most important antioxidant enzymes and present in all oxygen-metabolizing cells. Therefore, research on SOD activity will be important for the understanding of antioxidant mechanisms of life. Superoxide dismutases are divided in four classes considering their metal content. One of the classes is Cu²⁺-Zn²⁺ enzyme which is found in the cytosol of eukaryotes, in chloroplasts, and in the periplasm of some prokaryotes; the second class is Mn³⁺ protein which is found in bacteria, archaea, mitochondria and chloroplasts whereas Fe³⁺ enzyme is present in both

aerobic and anaerobic bacteria, archaea and plants (Parker and Blake, 1988; Beyer et al., 1989; Grace, 1990). The fourth type Ni-SOD has been discovered in several Streptomyces species (Cannio et al., 2000). In this study we used Cu-Zn superoxide dismutase that consists of two subunits of identical molecular weight joined by a disulfide bond with total molecular weight of 32,500 (Keele et al., 1971). In Cu-Zn SOD there are two Cu²⁺ and two Zn²⁺ atoms per molecule (Bannister et al., 1971). According to Forman and Fridovich (1973) Zn²⁺ has a structural, stabilizing role, while Cu²⁺ is directly involved in the catalytic activity. The mechanism of action of Cu-Zn SOD involves alternate reduction and reoxidation of the Cu²⁺ at the active site

during successive interactions with O₂.

Superoxide dismutase is an enzyme that catalyzes the conversion of superoxide free radicals to oxygen and hydrogen peroxide. In biological systems the sources of the electrons are generally enzymes and reducing substances. While reducing substances act as antioxidants by reducing more reactive species, electron-donors act as pro-oxidants by reducing less reactive species via reactions which are typically mediated by the cyclical reduction/oxidation of transition-metal ions. Superoxide dismutase and catalase catalyse the dismutation of superoxide and hydrogen peroxide, respectively. Peroxide and superoxide can also react in the presence of a metal ion to produce hydroxyl radical and molecular oxygen. Such agents as heavy metals or quinones produce free radicals and related activated electronic species in biological systems in antimicrobial defense, through the action of the mixed function monooxygenases, by various oxidative enzymes such as xanthine oxidase, and by auto oxidations mediated. By altering the concentration of O₂⁻, SOD helps prevent both direct toxicity from O₂⁻ and secondary toxicity from ·OH and H₂O₂. Cu-Zn SOD is inactivated by cyanide and H₂O₂ (Symonyan and Nalbandyan, 1972; Fielden et al., 1973). *In vivo* systems the adverse affect of H₂O₂ may be protected by catalase (Bray and Calabres, 1974) with which it is usually associated.

Free radicals are short-lived reactive chemical species having one or more electrons with unpaired spins and are generally highly reactive. Some radicals such as O₂ are stable and long-lived. Oxygen is a diradical, as it has two unpaired electrons. Its electrons rotate about an axis called as "spin". If outermost pair of electrons of oxygen molecule have parallel spins, they (↑↑) are in the "triplet" state and if oxygen molecules whose outermost pair of electrons have antiparallel spins, they (↑↓) are in the "singlet" state. The triplet state oxygen has two unpaired electrons with parallel spins, a characteristic that, according to rules of physical chemistry, does not allow them to react with most molecules. Thus, triplet oxygen is not very reactive. However, triplet oxygen can be activated by the addition of energy, and transformed into reactive oxygen species. If triplet oxygen absorbs sufficient energy to reverse the spin of one of its unpaired electrons, it forms the singlet state. Singlet oxygen, has a pair of electrons with opposite spins; though not a free radical it is highly reactive.



Singlet oxygen is produced either as a result of biological reactions or by photosensitization by the absorption of light energy. According to rules of physical chemistry, the "relaxation" (excess energy loss) of singlet oxygen back to the triplet state is "spin forbidden" and thus singlet oxygen has a long lifetime for an energetically excited molecule, and must transfer its excess energy to another molecule in order to relax to the triplet state. Reduction of molecular oxygen to superoxide, and of peroxide to hydroxyl radical is also "spin forbidden" and thus is slow unless catalyzed by a heavy ion. Alternative spin-permitted pathways for the reduction of O₂ include interaction of molecular oxygen with the excited triple state of another molecule to produce singlet oxygen or an excited state molecule and jumps to a higher energy orbital on the same atom.

An orbital electron may be viewed as a moving negative charge which generates a magnetic field as moving charges generate magnetic fields. Depending on the direction of its motion ("spin") about the orbital axis, this magnetic field will have its north magnetic pole oriented either "up" or "down" relative to its orbit. Stable compounds, especially those composed of low atomic weight atoms, have even numbers of electrons arranged two to each "orbital". The two electrons in each orbital are "paired". That is, their "spin"-derived magnetic fields are of opposite polarity, effectively cancelling them. A free radical with one or more of its electrons unpaired has either an odd number of orbital electrons, with one unpaired - a free radical *per se* - or of pairs of electrons of the same spin isolated singly in separate orbital. These uncanceled spins give radical species a net magnetic moment.

Living systems are affected by magnetic field (MF) and electromagnetic field (EMF), which are generated from both external (MF and EMF) and internal sources (natural metabolisms of organisms). Several studies concluded that magnetic and electromagnetic fields have different responses for biosystems such as neural and neuromuscular activity, tissue growth and repair, glandular secretion, and cell membrane function. Although electromagnetic field has an impact mostly on charged units and related metabolisms, magnetic field usually affects

biochemical reactions that involve more than one unpaired electron. Most enzymes do not involve radical intermediates and should be unaffected by a change in magnetic field. However, more than 50 enzymes are believed to generate free radical intermediates during catalysis and may be subject to the influence of external magnetic fields. The Grissom research group has shown that the activity of the B_{12} -dependent enzyme ethanolamine ammonia lyase changes with magnetic field of 100 mT, but not at weaker magnetic fields (Harkins and Grissom, 1994). Experiments are also being carried out with the heme enzymes, horseradish peroxidase and cytochrome P-450. The rate of horseradish peroxidase increases by 20% at fields as low as 1 mT (Taraban et al., 1997). Using electromagnetic field (50 Hz, 5.8 mT), Batcioglu et al. (2002) determined a significant decrease in catalase activity and an increase in superoxide dismutase activity. However, in our study we aimed to identify the activity of superoxide dismutase under magnetic field simulated by the apparatus explained at material methods.

The effect of an external magnetic field on enzymatic reaction rates can be determined in the same way classical enzyme kinetic parameters are determined. Enzymes with chromogenic substrates or products can be followed spectrophotometrically. The objective of this pre-study is to observe the influence of magnetic fields on SOD alone with radical intermediates in order to understand how magnetic field might influence biological processes through changes in radical pair recombination.

Materials and methods

Materials and devices

Bovine erythrocytes SOD, L-methionine, riboflavin, NBT were purchased from Sigma, Na_2CO_3 was purchased from Merck and EDTA was purchased from Amresco. Shimadzu 1601 spectrophotometer was used to measure absorbances. For application of magnetic field, a device prepared by JINR Laboratories and gifted to University of Istanbul was used (Atak et al., 2000).

Determination of SOD activity

SOD activity was determined by using a

spectrophotometric method adapted the procedure of Dhindsa et al. (1981). 1.0 mg of SOD (3000 unit) was dissolved in 10 ml of dH_2O to prepare stock solution. Magnetic field was applied as mentioned below on the enzyme dissolved in dH_2O . To compare the activities of SOD, one set of experiment employed at the same conditions but no magnetic field. The blank test was set up with all including but the enzyme. For the preparation of samples for the assay of SOD activity, 5 ml of enzyme sample was added into the solution including 0.1 ml of 1.5 M Na_2CO_3 , 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM NBT, 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM PBS (pH 7.5) and 1 ml of dH_2O . Just after, 0.1 ml of 60 mM riboflavin solution was added. The prepared enzyme samples were kept under the fluorescence light for 15 minutes and then the reactions were stopped taken the samples in a dark place. Their absorbances were measured at the wavelength of 560 nm against the blank. Enzyme samples were kept on ice till the treatment.

Magnetic field experiment

Magnetic field experiment was carried out by the procedure adapted from Atak et al. (2000). In our study, 10 magnets of $0.45 \times 0.065 \times 0.022$ m dimensions were used. The magnets were mounted onto the belt system that rotated with a rate of 1 m/second. The height of the magnets from the belt was 0.060 m. Samples of enzyme have been passed at 2.9-4.6 mT magnetic flux density 0, 1, 9 and 15 times at 0, 2.2, 19.8 and 33 seconds.

Statistics

Each set of experiment was repeated ten times. We applied Duncan's multiple range test method to compare experimental results of the groups exposed to magnetic field and control enzyme samples.

Results

SOD uses superoxide radicals and hydrogen to produce hydrogen peroxide and oxygen. None of the products or reactants is easily monitored by direct methods. Therefore the activity of SOD has been determined in the way of inhibition by the enzyme of an O_2^- dependent reaction. For the production of O_2^- , which is a substrate of SOD, the solution of riboflavin

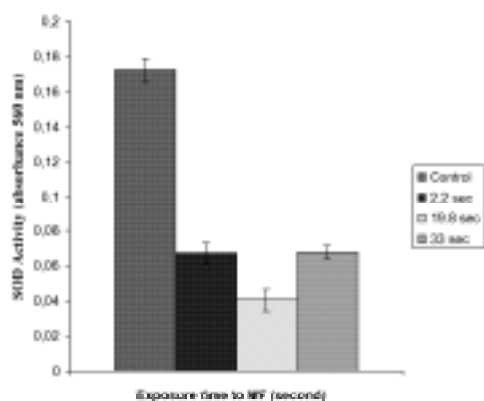


Figure 1. A comparison of the effect of magnetic field on SOD activity.

is utilized. O_2^- generated by the riboflavin reaction is spontaneously transformed to oxygen and hydrogen peroxide by superoxide dismutase. One of the colorimetric probes, NBT, was used to react with the excess of O_2^- that has not enough SOD activity to react with. Since the NBT method is based on the generation of water-insoluble blue formazan dye (λ_{max} : 560 nm) by a reaction with O_2^- , NBT was used to detect the occurrence of the superoxide anion radical to detect the activity of SOD (Winterbourn et al., 1975).

In this study, the absorbance values of magnetised and unmagnetised enzyme (control) samples were measured. Enzyme solutions were subjected to the magnetic field density for 2.2, 19.8 and 33 seconds respectively. The effects of magnetic field applied on SOD were shown on a graph plotted the absorbance values (Figure 1). Mean absorbance values of samples for control and magnetised enzyme solutions are also summarized.

Discussion

In our study, we aimed to understand that whether SOD has any influence due to magnetic field and if there is, what could be the magnitude of magnetic field that affect the activity of SOD. Some previous works were shown that electromagnetic field on living systems could have some effects on enzymes related to growth regulation, on calcium balance in the cell and on gene expression. Only biochemical reactions that involve more than one unpaired electron will be

affected by a magnetic field. SOD is one of the enzyme that is involved the reaction with superoxide radicals. The enzyme has Zn^{2+} and Cu^{2+} metal ions that may be subject to the influence of external magnetic fields due to electron distribution of mentioned metals. Indeed, the results of the effect of magnetic field intensity on the experimental saturation temperature for crystallization of Cu^{2+} and Zn^{2+} sulfates showed that the crystallization parameters were observed for diamagnetic Zn^{2+} sulfates but not for paramagnetic Cu^{2+} sulfates (Freitas et al., 1999).

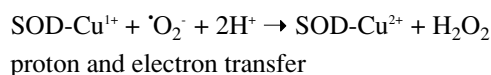
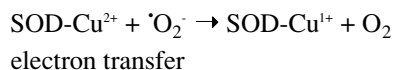
In our study, the absorbance measurements resulted in a differentiation between the control-enzyme samples that have no magnetic field on and the magnetized enzyme samples. At this point, it may be possible to consider two aspects for the effect of magnetic field on SOD. First, magnetic field on the enzyme may cause the unpaired electrons on metal ions to orient at the same direction with applied magnetic field and so gained more energy. Indeed this energy may be transferred onto the other molecules that cause more radicals that may affect to form more superoxide radicals via radicalic chain reactions. Then SOD could have more peroxide ions to react. Moreover, there are some results to explore the effect of magnetic field on water. Since water is diamagnetic, the effect of magnetic field can be seen at very high magnetic field (10 T, cf. Earth's magnetic field 30 μ T) (Ikezoe et al., 1998) Lower magnetic fields (0.2 T) have been shown, in simulations, to increase the number of monomer water molecules but, rather surprisingly, they increase the tetrahedrality at the same time (Zhou et al., 2000). Static magnetic effects have been shown to cause an increase in the ordered structure of water formed around hydrophobic molecules and colloids (Ozeki et al., 1991). The increase in refractive index with magnetic field has been attributed to increased hydrogen bond strength (Hosoda et al., 2004). This reinforces the view that it is the movement through a magnetic field, and it associated electromagnetic effect, that is important for disrupting the hydrogen bonding. The effect of the magnetic field on the hydrogen bonding has been further supported by the rise in the melting point of H_2O (5.6 mK at 6 T) and D_2O (21.8 mK at 6 T) indicating greater ordering (lower entropy) in the liquid water within a magnetic field (Inaba et al., 2004). Magnetic fields can also increase proton spin

relaxation, which may speed up some reactions dependent on proton transfer (Madsen, 2004) Unstructured water with fewer hydrogen bonds is a more reactive environment (Symons, 2001).

In summary, it is possible to conclude that the application of magnetic field resulted in an increase of the activity of SOD, when the enzyme was treated with substrate. The observations we had in this study showed that the response of SOD under different magnetic fields is noteworthy. The activities of enzymes changed related to magnetic fields that were exposed; the enzyme that had magnetic field for 19.8 seconds gave the lowest absorbance value that indicates the highest activity for enzyme. The enzymes that had magnetic fields at 2.2, 33.0 seconds showed decreased activities and the control enzyme had minimum activity. It is clear that there is no linear increase of activities depending on increase of magnetic fields. Indeed, in a study to display the effect of magnetic field on *Paulownia* seeds, seeds were passed 1, 3 and 9 times through a magnetic field of 4.7-5.7 mT and the number of seedlings was controlled on the 3rd, 5th, 10th and 12th days. The seedling numbers which were passed through 1 and 9 times were increased according to control. The best seedling value was obtained at 3 times passing (Atak et al., 2000). Although there might be different responses in living systems, SOD activity may follow similar pathway under magnetic fields for both *in vivo* and *in vitro* systems.

It seems that there is a discrepancy when we evaluate the data. If any increase on the magnetic field gives extra energy to activate the enzyme what could be the reason for the decrease of enzyme activity at highest magnetic field that we applied. The previous studies showed that SOD is inactivated by H₂O₂ (Symonyan and Nalbandyan, 1972; Fielden et al., 1973) if there is no catalase in medium to remove H₂O₂. Because SOD catalyses the formation of H₂O₂ and there is no catalase as there is in living systems, occurred H₂O₂ may accumulate by the time and causes the inhibition of enzyme, SOD. It is known that hydrogen peroxide slowly but irreversibly inactivates the enzyme superoxide dismutase (Bray and Calabres, 1974). Moreover, it is shown that at low concentrations of H₂O₂ cupric (Cu²⁺) changes to cuprous (Cu¹⁺) and cause reversible inhibition while at high concentrations or long exposures cause irreversible inactivation. When we consider the

catalytic mechanism of SOD, it may possible to conclude that accumulation of H₂O₂ might cause inactivation of SOD.



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