



RESEARCH ARTICLE

Effect of Different Wavelengths on Superoxide Dismutase

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Abstract

Introduction: Superoxide dismutase (SOD) is one of the most important antioxidant enzymes present in all oxygen-metabolizing cells. This enzyme eliminates toxins from our body, so it is vital to understand its action and activity under the influence of different wavelengths. The effects of different wavelengths of light in the visible range on SOD activity were investigated.

Methods: Enzyme samples were irradiated with five different wavelengths for chromotization. The absorbance values of the control and treated enzymes were subsequently measured.

Results: A wavelength of 644nm (red) showed the maximum increase in absorbance compared with all other color wavelengths used. Yellow showed least absorption.

Conclusion: Red color wavelength actually provides additional energy to the enzyme and hence the activation energy is lowered, compared with untreated enzyme.

1. Introduction

Superoxide dismutase (SOD) is one of the most important antioxidant enzymes present in all oxygen-metabolizing cells. A previous chromotherapy study [1] has shown encouraging results, therefore research into SOD activity was considered important for the understanding of antioxidant mechanisms. As this enzyme eliminates toxins from our body it would be valuable to understand its action and activity under the influence of different wavelengths. SOD is an enzyme that catalyzes the conversion of superoxide free radicals to oxygen and hydrogen peroxide. In biological systems, the sources of 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. SOD and catalase catalyze 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 [2]. Such agents like 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 mono oxygenases, by various oxidative enzymes such as xanthine oxidase, and by auto oxidations. By altering the concentration of oxygen, SOD helps prevent both direct toxicity from O₂ and secondary toxicity from OH and H₂. Cu-Zn SOD is inactivated by cyanide and H₂O₂ [3].

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Table Dominant wavelength of monochromatic light measured by Hitachi U-2000 UV-Vis double beam spectrophotometer, spectral bandwidth 0.1 nm, and ordinates selected 10

	Color	Dominant Wavelength (nm)	Hue	Purity (%)	Transmission (%)
1	Purple	464	Violet	36	32
2	Blue	483	Blue-green	52	52
3	Green	538	Greenish-yellow	15	37
4	Yellow	590	Reddish-yellow	40	82
5	Red	644	Red	41	51

In *in vivo* systems the adverse affect of H₂O₂ may be prevented by catalase, which is usually associated with SOD. In this study, we aimed to identify the activity of SOD under different wavelengths of light in the visible range.

2. Materials and Methods

SOD activity was determined by using the methodology described by Dhinda [4]. Bovine erythrocyte SOD was purchased from ICN, nitro blue tetrazolium (NBT) was purchased from Sigma and EDTA was purchased from Amresco. L-methionine and riboflavin was purchased from Fluka. Na₂CO₃ was purchased from Merck. A Hitachi spectrophotometer was used to measure absorbance. 1.0mg of SOD (3000 units) was dissolved in 10mL of dH₂O to prepare stock solution. To compare the activities of SOD, one sample was not exposed to any wavelength of light. For the assay of SOD activity, 10mL of enzyme sample was added into the solution containing 0.2 mL of 1.5 M Na₂CO₃, 0.4 mL of 200mM methionine, 0.2 mL of 2.25mM NBT, 0.2 mL of 3 mM EDTA, 3 mL of 100mM PBS (pH 7.5) and 2 mL of dH₂O. Immediately after the addition of enzyme, 0.2 mL of 60mM riboflavin solution was added. The methodology of Shamsudin was adopted for chromatization of enzyme samples [5]. Seven test tubes containing 2.5mL of solution were prepared for the experiment. Five were wrapped with multipurpose cellophane filter sheets of green, blue, purple, red and yellow (dominant wavelengths given in Table). One unwrapped and five wrapped samples were exposed, under the same conditions, to 12 watt full spectrum incandescent light bulb from a distance of 0.66m for 10 minutes. The absorbance was measured after exposing samples to the particular wavelengths of light at 560 nm.

3. Results

Application of colors (wavelengths in the visible region) resulted in increase of the activity of SOD

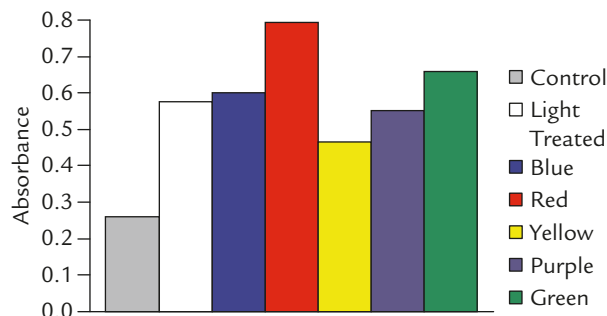


Figure Effect of irradiation in different color wavelengths on SOD.

when the enzyme was treated with substrate. The study shows that the response of SOD with different wavelengths is significant. In other words, we are dealing with photoactivation of the SOD enzyme. It is evident from the comparison (Figure) that different wavelengths in the visible region showed different results. Red showed the maximum increase in absorbance level compared with all other colors (wavelengths) used, while yellow showed least absorption. SOD responded to red color wavelength (644nm), implying that free radical elimination may become very easy and rapid with an application of red color in the body. The result also shows that activation energy of SOD lowers on exposing it to different wavelengths in the visible region.

4. Discussion

Living systems are affected by magnetic (MF) and electromagnetic fields (EMF), which are generated from both external and internal sources (natural metabolism of organisms). Several studies concluded that magnetic and electromagnetic fields have different responses in biosystems such as neural and neuromuscular activity, tissue growth and repair, glandular secretion and cell membrane function [2].

In our study, we aimed to understand whether superoxide has any reaction under different color

wavelengths and, if so, which wavelength affects the activity of this enzyme. Some previous studies showed that the electromagnetic field on living systems has some affect on enzymes related to growth regulation, calcium balance in the cell and gene expression [1]. SOD is one of the enzymes that are involved in the reaction with superoxide radicals. In our study, the absorbance measurements were different in untreated and chromotized samples.

Reduction of molecular oxygen to superoxide, and of peroxide to hydroxyl radicals is “spin forbidden” and thus is slow unless catalyzed by a heavy ion [6]. At this point, two aspects for the effect of color vibrations on SOD may need to be considered. First, color vibrations on the enzyme may cause the unpaired electrons on metal ions to orient at the same direction with the vibrations (wavelengths) and so gain additional energy. Indeed, this energy may be transferred onto the other molecules that cause more radicals that may form more superoxide radicals in radical chain reactions. The increase in vibrational energy has been attributed to increased hydrogen bond strength [7]. This reinforces the view that electromagnetic effect is important for disrupting hydrogen bonding. Electromagnetic fields can also increase proton spin relaxation, which may speed up some reactions dependent on proton transfer [8].

Conclusion

SOD responded to red color wavelength (644nm). This implies that free radical elimination may become very easy and rapid by using a red color wavelength on the human body.

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