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The effect of nano-TiO_2 photocatalysis on the antioxidant activities of Cu, Zn-SOD at physiological pH

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ABSTRACT

Security issues of nanoparticles on biological toxicity and potential environmental risk have attracted more and more attention with the rapid development and wide applications of nanotechnology. In this work, we explored the effect and probable mechanism of nano-TiO_2 on antioxidant activity of copper, zinc superoxide dismutase (Cu, Zn-SOD) under natural light and mixed light at physiological pH. Nano-TiO_2 was prepared by sol-hydrothermal method, and then characterized by X-ray Diffraction (XRD) and Transmission electron micrographs (TEM). The Cu, Zn-SOD was purified by sephadex G75 chromatography and qualitatively analyzed by sodium dodecyl sulfate polypropylene amide gel electrophoresis (SDS-PAGE). The effect and mechanism were elucidated base on Fourier Transform Infrared Spectrometer (FT-IR), Circular Dichroism (CD), zeta potential, and electron spin resonance (ESR) methods. Accompanying the results of FT-IR, CD and zeta potential, it could be concluded that nano-TiO_2 had no effect on the antioxidant activity of Cu, Zn-SOD by comparing the relative activity under natural light at physiological pH. But the relative activity of Cu, Zn-SOD significantly decreased along with the increase of nano-TiO_2 concentration under the mixed light. The results of ESR showed the cause of this phenomenon was the Cu(II) in the active site of Cu, Zn-SOD was reduced to Cu(I) by H_2O_2 and decreased the content of active Cu, Zn-SOD. The reduction can be inhibited by catalase. Excess $O_2^{\cdot -}$ produced by nano-TiO_2 photocatalysis under mixed light accumulated a mass of H_2O_2 through disproportionation reaction in this experimental condition. The results show that nano-TiO_2 cannot affect the antioxidant activity of Cu, Zn-SOD in daily life. The study on the effect of nano-TiO_2 on Cu, Zn-SOD will provide a valid theory support for biological safety and the toxicological effect mechanism of nanomaterials on enzyme.

1. Introduction

With the introduction of nanotechnology, metal oxide nanoparticles are widely and largely used [1]. In particular, titanium dioxide nanoparticles (nano-TiO_2) with high stability, anticorrosive and photocatalytic properties [2] have been tremendously used in daily living and consumer products, such as drug delivery systems, antibacterial materials, cosmetics, sunscreens, food additives, and so on [3–5]. It is unavoidable to lead to human exposure and contact with the TiO_2 material environment for the numerous applications of nano-TiO_2, so it is important to consider the potential hazards of nano-TiO_2. Some reports showed that TiO_2 nanoparticles could be accumulated in the bodies such as liver, spleen, kidney, brain, lung and reproductive organ [3–7], which might cause inflammation, asthma, Crohn's disease, and so forth [8–10] by the absorption and oxidative damage of reactive oxygen species (ROS, including $O_2^{\cdot -}$, $HO_2^{\cdot}$, $^{\cdot}OH$) generated by photocatalysis of nano-TiO_2. Some studies showed that the electrons (e^-) were excited into the conduction band of the nano-TiO_2, leaving a hole (h^+) in the valence band, and formed electron hole pair (e^-/h^+ pair) [11] under irradiation of UV light at wavelengths < 380 nm. When these e^-/h^+ pairs migrated to the surface/interface of the nano-TiO_2 particles and participated in the redox reactions with H_2O, OH^-, O_2, highly active reactive oxygen species (ROS) were produced. The overall reaction occurring can be written as

$$TiO_2 + hv \rightarrow TiO_2(e^- + h^+) \quad (1)$$

$$TiO_2(e^-) + O_2 \rightarrow TiO_2 + O_2^{\cdot -} \quad (2)$$

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (3)$$

$$TiO_2(e^-) + H_2O_2 \rightarrow TiO_2 + OH^- + {}^{\cdot}OH \quad (4)$$

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$$TiO_2(h^+) + H_2O \rightarrow TiO_2 + {}^{\cdot}OH + H^+ \quad (5)$$

$$TiO_2(h^+) + {}^{-}OH \rightarrow TiO_2 + {}^{\cdot}OH \quad (6)$$

The major product in the series redox on the surface/interface of nano-TiO_2 is superoxide anion ($O_2^{\cdot -}$), which is the precursor of the other reactive oxygen species (H_2O_2, $^{\cdot}OH$). These ROS have a toxic effect on lipids, carbohydrates, proteins and DNA [12,13].

Copper, zinc Superoxide dismutase (Cu, Zn-SOD, EC: 1.15.1.1), as a kind of metalloenzyme, widely existed in living organism. Cu, Zn-SOD is a highly conservative enzyme, comparing Cu, Zn-SOD from human erythrocyte with that from bovine erythrocyte, the full sequence identity is up to 80% [14]. Cu, Zn-SOD is a homodimer of molecular weight approximately 32 kDa. Each monomer consists of a copper atom and a zinc atom, and 151 amino acid residues, which folds into eight antiparallel β-strands. The special structure is a very important determinant in stability of the enzyme. Superoxide dismutase, glutathione peroxidase, glutathione s-transferase and catalase constitute the first line of defense against oxidative stress in living organism. Cu, Zn-SOD, a unique enzyme with scavenging superoxide anion ($O_2^{\cdot -}$) in the line [15], can catalyze the dismutation of $O_2^{\cdot -}$ into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) [16]. The accepted catalysis mechanism for Cu, Zn-SOD is mutual transformation between Cu (I) and Cu (II) by successive superoxide anion:

$$O_2^{\cdot -} + Cu^{2+} - Zn - SOD \rightarrow Cu^{+} - Zn - SOD + O_2 \quad (7)$$

$$O_2^{\cdot -} + Cu^{+} - Zn - SOD + 2H^+ \rightarrow Cu^{2+} - Zn - SOD + H_2O_2 \quad (8)$$

In the catalysis process, Cu (II) is reduced to Cu (I) by superoxide anion, the Cu (I) is re-oxidized to Cu (II) by the other superoxide anion, the products are molecular oxygen and hydrogen peroxide.

Abundant experiments in vivo about the effect of nano-TiO_2 on Cu, Zn-SOD found that nano-TiO_2 could induce oxidative stress in oral administration to mouse, and ROS level was up and Cu, Zn-SOD activities were down. In some papers, ROS generated by photocatalysis of nano-TiO_2 could reduce the activity of total SOD (including cytosolic and mitochondrial SOD) and decrease protein levels of Cu, Zn-SOD and Mn-SOD, oxidative stress of ROS can induce ectopically expression of Mn-SOD, lower the gene expression of Cu, Zn-SOD and Mn-SOD in some tissues [17–21]. The experiments in vitro indicated that the mechanism of the interaction between nano-TiO_2 and protein was adsorption by electrostatic attraction. In the research of interaction between nano-TiO_2 and lysozyme at pH 7.4, nano-TiO_2 could not affect the activity of free lysozyme, but could inactivate lysozyme by transforming the structure of lysozyme bridged with nano-TiO_2 from α-helix to β-sheet [22]. The main mechanism of adsorption of bovine plasma fibrinogen onto nano-TiO_2 was electrostatic interactions at pH 7.4. The α-helix content of fibrinogen dramatically decreased and the structural changes were time dependent and irreversible during the absorption and desorption by nano-TiO_2 [23]. The depression of activities of pepsin adhered to the nano-TiO_2 by spontaneous non-covalent reactions at pH 6.5 was attributed to their physical binding and unfolding of the secondary structure of pepsin [24]. In the studies of interaction between bovine serum albumin or human serum albumin and nano-TiO_2, the results indicated that the adsorption behavior related to the pH of solution and the specific surface area of TiO_2 [25–27]. The study of interaction between nano-anatase and SOD from rat erythrocytes at pH 7.8 indicated that nano-anatase directly bound to SOD by coordination of Ti—O (N) and Ti—S bond, and created a new metal ion-active site form in SOD, explaining the phenomenon that low concentration of nano-anatase could increase the SOD activity and high concentration of nano-anatase could inhibit the SOD activity [28]. Although some experiments had investigated that the nano-TiO_2 had potential toxic effects on protein and some tissue in vitro and in vivo, the biological effect and mechanism of nano-TiO_2 were very far away from completely being understood. Due to the function of scavenging oxygen free radical of antioxidant enzyme systems, it is still interesting to study the effect of nano-TiO_2 on the antioxidant activities of the enzymes at physiological pH (pH = 7.4) from the point of photocatalysis and adsorption. Thus, in this work, we chose the widely distributed antioxidant enzyme in the tissues, Cu, Zn-SOD [29], to study the effect on the antioxidant activity caused by nano-TiO_2 at physiological pH. All these contents would clarify that nano-TiO_2 had significant meaning to the security of the human body.

2. Materials and Methods

2.1. Materials

Superoxide dismutase (Cu, Zn-SOD) from bovine erythrocyte and catalase (CAT) from bovine liver were purchased from Beijing BioDee Biotechnology Co., Ltd. Sephadex G75 was obtained from Amersham Pharmacia Biotech AB Co., Ltd. Anhydrous ethanol, Tetrabutyl titanate and other chemicals were of analytical regent grade.

2.2. Preparation of Nano-TiO_2 Particles

The synthesis of nano-TiO_2 particles in the work employed a sol-hydrothermal method [30]. The mixed solution of 5 mL of tetrabutyl titanate and 5 mL of anhydrous ethanol, kept stirring until mix evenly, and then slowly dropped into the other mixed solution consisting of 20 mL of anhydrous ethanol, 5 mL of deionized water and 1 mL of 70% nitric acid, stirred uniformly at room temperature to carry out hydrolysis. After fully stirring for 1 h, a pale yellow transparent sol was obtained. The prepared sol was added into the stainless-steel reaction kettle and kept for 6 h at 160 °C, then cooled to room temperature. The precursor was dried at 60 °C for 24 h, and then the nano-TiO_2 particles were obtained by calcining of the sol-hydrothermal production for 2 h at 450 °C. The characteristic of the nano-TiO_2 particles was characterized by X-ray Diffraction (XRD, XRD-6000 from Shimadzu) and Transmission electron micrographs (TEM, JEM-200CX from JEOL).

2.3. Determination of Photocatalytic Activity of Nano-TiO_2

To investigate the photocatalytic performance of nano-TiO_2, the photocatalytic activity was carried out at room temperature in a quartz flask. 20 mg nano-TiO_2 was added into 20 mL of 10 mg/L methyl orange aqueous, and ultrasonic dispersed for 5 min, then kept for 30 min with magnetic stirring under the dark condition to ensure the adsorption-desorption equilibrium [31,32]. Balanced solution was exposed to mixed light irradiation (UV–visible light) from 500 W xenon lamp (CHFXQ, Beijing Changtuo Technology Co., Ltd). Time intervals were 15 min, analytical samples were taken from the suspension and TiO_2 was eliminated by centrifugal (micro-12, HANIL) at 10,000 rpm for 5 min. The concentration of methyl orange solution was analyzed by measuring its main absorption peak at 465 nm through UV–visible spectrometer (UV 1800, SHIMADZU).

2.4. Protein Purification

Cu, Zn-SOD was purified in this experiment by gel filtration on a Sephadex G75 [33] columns (2.6 cm × 60 cm), with the flow rate of 1.0 rpm, collected into a tube every 4 min, and samples were determined with the BCA method, respectively. The target protein was collected from test and lyophilized by Alpha1-2 (CHRiST, Germany). SDS polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify protein purity and the protein characteristics were characterized by UV–visible spectrometer (UV 1800, SHIMADZU).

2.5. Antioxidant Activity Assays of Cu, Zn-SOD

Antioxidant activity of Cu, Zn-SOD in this work was analyzed by using total SOD assay kit (Nanjing Jiancheng Biotech Inc., China) by