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Investigation of the photocatalytic effect of zinc oxide nanoparticles in the presence of nitrite

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HIGHLIGHTS

- Nitrite enhanced the photo-damage by ZnO nanoparticles to BSA and HaCaT cells.
- Protein nitration was induced by nitrite in photo-damaged BSA and HaCaT cells.
- The effects of photo-damage on BSA were affected by various factors.
- 50-nm ZnO induced more apoptosis than 90-nm ZnO in HaCaT cells.

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ABSTRACT

Zinc oxide nanoparticles are widely used in sunscreen products because of their chemical stability and capability of blocking harmful ultraviolet rays. However, zinc oxide nanoparticles can also generate reactive species under ultraviolet irradiation. Because nitrite can form reactive nitrogen species under oxidative stress and because it exists in perspiration and cosmetics, we studied the effects of nitrites on the photocatalytic damage of zinc oxide nanoparticles (50 nm and 90 nm) to bovine serum albumin and human keratinocyte cells under ultraviolet irradiation (365 nm and 254 nm). The results indicate that nitrite plays an enhancing role in photocatalytic damage by breaking amino acid residues and promoting protein oxidation and nitration. The concentrations of zinc oxide and nitrite, the irradiation light and duration, and the pH of the medium are important factors influencing this photocatalytic damage. Size effects of ZnO nanoparticles on bovine serum albumin and keratinocyte cells are different. It is speculated that the extent of photo-damage is partially dependent on the aggregation of zinc oxide. These findings may be valuable for understanding potential risks of applying zinc oxide nanoparticle-containing sunscreens to human skin under sunlight exposure.

1. Introduction

Zinc oxide nanoparticles have been widely recommended as a mineral-based ultraviolet (UV) filter for their excellent performance as a sunscreen [1]. Recent research has focused on the photocatalytic ability of semiconductor materials such as nano TiO_2 and ZnO nanoparticles [2,3]. It has been demonstrated that ZnO nanoparticles generate reactive species under ultraviolet irradiation [4], and the species can involve oxidation–reduction reactions [5,6]. Toxicology research has indicated that ZnO nanoparticles can induce oxidative damage in some model organisms, such as zebra fish [7], tetrahymena [8], *Escherichia coli* [9,10] and yeast [11].

Nitrite (NO_2^-) exists in various body fluids, including perspiration, saliva and blood. In general, the concentration of NO_2^- can reach up to 15 μM on the skin surface [12], and it is possible that the NO_2^- concentration becomes higher due to the evaporation of liquid. Furthermore, nitrite is commonly found in cosmetic products as a preservative. Previous reports showed that NO_2^- reduced the survival rate of skin fibroblast cells under the irradiation of ultraviolet-A rays [13], and the addition of NO_2^- enhanced DNA damage caused by ultraviolet light [14]. Several studies indicated that reactive nitrogen species (RNS) (e.g., nitric oxide and peroxynitrite) were generated in the photocatalytic reaction in the presence of NO_2^- [15–18]. These reactive species damaged proteins in the skin through protein oxidation and nitration and caused permanent injury [19,20].

The risk of applying ZnO nanoparticles to living organisms depends on the environment. It was found that ZnO nanoparticles resulted in the photo-degradation of organic contaminants in water, and its photocatalytic activity was affected by factors

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such as particle size and pH [21]. Considering the wide inclusion of ZnO nanoparticles in sunscreens, it is important to evaluate the photocatalytic damage of human skin under ultraviolet irradiation in the presence of ZnO nanoparticles and NO_2^-. In this study, in the presence of NO_2^-, bovine serum albumin (BSA) was used as a model protein to investigate the photocatalytic damage induced by 50-nm and 90-nm ZnO particles under different wavelengths of ultraviolet irradiation. Furthermore, we characterized the cytotoxicity and photo-damage induced by ZnO nanoparticles in human keratinocyte cells.

2. Materials and methods

2.1. Reagents

ZnO nanoparticles with particle sizes of 50 and 90 nm (ZnO-50 and ZnO-90) were bought from Aladdin Chemistry Co. Ltd. (Shanghai, China), and the average diameter and purity are provided in Table 1. BSA and a rabbit polyclonal antibody against dinitrophenol (DNP) were purchased from Sigma–Aldrich Company (St Louis, MO, USA). A mouse monoclonal antibody against nitrotyrosine (3-NT) was purchased from Upstate Corporation (Billerica, MA, USA). Peroxidase conjugated ImmunoPure goat anti-rabbit IgG (H + L) and Peroxidase conjugated ImmunoPure goat anti-mouse IgG (H + L) were bought from Biofly Technology Co. Ltd. (Wuhan, China), and Cy3-conjugated AffiniPure goat anti-mouse IgG (H + L) was from Jackson (West Grove, PA, USA). SuperSignal West Femto Maximum Sensitivity Substrate was purchased from the Pierce Biotechnology Company (Rockford, IL, USA). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA). Minimum essential medium (MEM) was obtained from Hyclone (San Jose, CA, USA), and fetal bovine serum (FBS) was purchased from Gibco BRL/Life Technologies (Grand Island, NY, USA). Other reagents and solvents were the highest purity commercially available.

2.2. Apparatus

A ZF-7 ultraviolet lamp (Yuhua, Shanghai, China) was used as the 365- and 254-nm UV source in this study. The UV–visible Spectrophotometer (Shimadzu, Kyoto, Japan) and the Spectrofluorophotometer (Shimadzu, Kyoto, Japan) were used to evaluate the photocatalytic damage of BSA. A Western blot system (Tanon, Shanghai, China) was applied to detect BSA oxidation and nitration. A fluorescence inverted microscope (Olympus, Tokyo, Japan) was used to observe cell morphology.

2.3. Experimental procedures

2.3.1. ZnO nanoparticle characterization

ZnO powders were detected by transmission electron microscopy (TEM) (Tecnai G2 20, FEI, Hillsboro, OR, USA) for morphological characterization. Each sample of ZnO nanoparticle solution was diluted to a concentration of 0.2 mg/ml and subjected to TEM analysis. The BET surface area was calculated using N_2 adsorption data by the standard calculation routines in its software, and N_2 adsorption (77.3 K) was measured using a Micromeritics ASAP 2020M surface area and porosity analyzer (Norcross, GA, USA). Before analysis, the samples were degassed at 110 °C for 8 h under vacuum (10^{-5} bar). The diameter of ZnO was confirmed by dynamic light scattering (DLS) (Nano-ZS 90, Malvern, UK), and the zeta potential of ZnO was evaluated by electrophoretic light scattering (ELS) (Nano-ZS 90, Malvern, UK).

2.3.2. Preparation of BSA samples

BSA, ZnO nanoparticles, and NO_2^- solutions were prepared with phosphate buffered saline (PBS). BSA solutions were added to ZnO nanoparticles and/or NO_2^- and then subjected to further analysis. Solutions containing ZnO nanoparticles were ultrasonicated before use to minimize aggregation. After the UV irradiation treatment, samples were immediately centrifuged to remove ZnO nanoparticles, and the supernatants were collected.

2.3.3. UV–vis and the fluorescence analysis

The UV–vis absorbance and fluorescence intensity of each sample were determined to evaluate the damage of BSA samples. For synchronous fluorescence analysis, the excitation wavelengths for tyrosine (Tyr) and tryptophan (Trp) residues were 270 nm and 250 nm, respectively. The difference values ($\Delta\lambda$) between excitation and emission wavelengths were stabilized at 15 nm for Tyr residues and 60 nm for Trp residues [22–24]. The slit width was 3.0 nm. The ratios (R_{SFQ}) of synchronous fluorescence quenching were calculated using the equation $R_{SFQ} = 1 - F/F_0$, where F_0 was the fluorescence intensity of BSA without ultraviolet irradiation, and F was the fluorescence intensity with ultraviolet irradiation for different periods of time.

2.3.4. Western blotting analysis

Western blotting was used for the semi-quantitative detection of the oxidation and nitration damage in BSA. For detection of protein oxidation, the samples were first derivatized with 2,4-dinitrophenylhydrazine in the presence of sodium dodecyl sulfate (SDS). After 1-h incubation at room temperature, the reaction was stopped with the addition of neutralization solution, and samples were mixed with loading buffer and subjected to 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) to detect protein tyrosine nitration. After electrophoresis, proteins in the gels were transferred to nitrocellulose membranes. The membranes were blocked in 5% skim milk/TBST (20 mM Tris–HCl, 150 mM NaCl and 0.1% Tween-20) overnight and incubated with either rabbit polyclonal anti-dinitrophenol or mouse monoclonal antibodies against 3-NT for 2 h. Afterwards, the membranes were incubated with horseradish peroxidase conjugated secondary antibody after being washed three times with TBST, and the proteins were visualized by the SuperSignal West Chemiluminescence Substrate System. The densitometric analysis of nitrated protein was performed with Quantity One software (Bio-Rad, Hercules, CA, USA).

2.3.5. Cell culture

Human keratinocyte cells (HaCaT) were purchased from the China Center for Type Culture Collection (CCTCC, Wuhan, China) and cultured in a humidified incubator at 37 °C in MEM containing 10% FBS. During ultraviolet irradiation, HaCaT cells were cultured in 24-well plates at a density of 1×10^5 cells per well, maintained in PBS and incubated with freshly prepared NO_2^- solution dissolved in PBS and the ZnO nanoparticles suspension for 1 h.

2.3.6. UV irradiation

UV treatment was carried out with a ZF-7 ultraviolet lamp, which contained two UV lamps with a continuous emission spectrum, one with a peak wavelength at 365 nm and another with a peak wavelength at 254 nm. The samples were placed 20 cm below the irradiation source with a UV irradiation intensity of 3.5 mW/cm^2 determined by a UV radiometer (Photoelectric Instrument Factory of Beijing Normal University, Beijing, China). The actual UV energy received by the BSA solution or HaCaT cells in plates was 12.6 J/cm^2 for 1 h of irradiation.