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Effects of metal oxide nanoparticles on the structure and activity of lysozyme

Yu-Hong Cheng[a,1], Chia-Min Lai[a,1], Kuen-Song Lin[b,**], Steven S.-S. Wang[a,*]

[a] Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan
[b] Department of Chemical Engineering and Materials Science/Environmental Technology Research Centre, Yuan Ze University, No.135, Yuandong Rd., Zhongli Dist., Taoyuan City 320, Taiwan

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ABSTRACT

We investigated the effects of nanoparticles (NPs) on the structure and activity of hen egg-white lysozyme (HEWL) using CeO_2 and ZnO NPs. Our results showed that CeO_2 NPs triggered the transition of lysozyme secondary structure from α-helix to β-sheet. CeO_2 NPs also induced the hydrophobic region of lysozyme to become exposed to the solvent. In contrast, the secondary structure content and hydrophobic region of lysozyme were only slightly changed in the case of ZnO NPs. In addition, the activity of the lysozyme was observed to decrease upon adsorption on CeO_2 NPs, whereas the effect of ZnO NPs on activity was negligible. The glutaraldehyde crosslinking results indicated that the percentage of the dimeric form of lysozyme was greatly enhanced by the addition of both NPs. Furthermore, the adsorption capacity, degree of favorability of adsorption, and surface heterogeneity for CeO_2 NPs were found to be greater than those on ZnO NPs. Given that CeO_2 NPs exhibit a higher surface area/mass than ZnO NPs, the surface concentration of lysozyme on CeO_2 NPs was lower than that on ZnO NPs. This result suggested that more direct interactions were involved between CeO_2 NPs and lysozyme, thereby leading to a more significant effect. Moreover, higher surface curvatures may also cause destruction of lysozyme's structure and thus affect its activity. In addition, taking into account the surface properties and protein properties, the Toth adsorption model along with the generated site energy distribution was further used to exaplain the difference between the results (e.g., structure, stability, and activity) of lysozyme adsorption on CeO_2 and ZnO NPs. The results reported here may aid in better understanding the beneficial or harmful impacts of nanoparticles on the biological systems.

1. Introduction

Currently, nanoparticles (NPs) are widely used in various fields, with the use of nanomaterial increasing exponentially today. The size-dependent physical properties and the optical and magnetic effects of NPs are mostly applied in the biomedical field in drug and gene delivery [1], fluorescent biological labels [2,3], probing of DNA structure [4], tissue engineering [5], imaging [6], and detection of protein [7]. The dimensional similarities with biomolecules have led to NPs being the focus of intensive research. However, NPs have become a threat to human health due to the fact that the smallest unit to form a living organism, a cell, is only 10 μm [8], which opens the potential for NPs crossing the various biological barriers within the body and threatening human health. In addition, people may be exposed to nanomaterial through ordinary biological processes such as inhalation, dermal uptake, ingestion, and injection [9]. Consequently, the characterization of NPs, as well as the examination of their effects on biomolecules such as proteins and DNA from a microscopic view, are crucial for understanding the NPs' potential risk to living systems.

Recent nanotoxicity studies have addressed the influence of nanomaterials in organs, cells, and biomolecules (e.g., protein, DNA, and lipids), specifically in respect to organs such as the lungs and skin, the mucosal membranes that are the portal-of-entry for toxicity, and the target organs for toxicity such as the endothelium, blood, spleen, liver, nervous system, heart, and kidneys [10]. At the cellular-level, a wide range of nanomaterial species have been linked to the creation of reactive oxygen species (ROS), which may affect the intracellular calcium concentrations, activate

* Corresponding author at: Department of Chemical Engineering, National Taiwan University, No.1, Sec.4, Roosevelt Rd, Taipei 10617, Taiwan.
** Corresponding author.
E-mail addresses: kslin@saturn.yzu.edu.tw (K.-S. Lin), sswang@ntu.edu.tw (S.S.-S. Wang).
[1] Both authors contributed equally to this work.

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transcription factors, and cause DNA damage, lipid peroxidation, and activation of signaling networks associated with loss of cell growth, fibrosis, and carcinogenesis [9,11,12]. Blood plasma typically contains several thousand different proteins with 12 order of magnitude differences in the concentration of these proteins [13], and the large surface area of NPs are capable of adsorbing these proteins; however, this greatly affects the bioactivity of biomolecules, such as enzymes, by inducing the conformational changes in proximity to solid surfaces. The unfolding kinetics of lysozyme, albumin, cytochrome c, and β-lactoglobulin were investigated. The proteins showed conformational changes in both the secondary and tertiary structures upon adsorption onto NPs [14–17], and amongst them, lysozyme was found to undergo a reduction in both α-helix content and enzymatic activity.

Lysozyme is one of the most studied enzymes due to its ease of purification and crystallization. The structure of lysozyme was established in 1965 when it was the second protein structure and the first enzyme structure to be deciphered by X-ray diffraction methods [18]. The catalytic domain and action mechanisms have also been investigated in detail. Thus, lysozyme has been widely used as a model protein to conduct amyloid research, crystallography, and nanomaterial research [19,20]. The widely used metal oxide NPs, e.g., TiO_2, ZnO, and CeO_2, have been produced in high tonnage. While CeO_2 and ZnO NPs have been extensively utilized in various industrial applications (e.g., used as a UV absorber, catalyst, and fuel additive), evidence suggests that these NPs might exhibit cytotoxic activities toward environmental organisms. Using lysozyme as a model biomolecule, we set out to understand how metal oxide NPs, CeO_2 and ZnO, affetced the structure and biological function of biomolecules and further examined the mechanism underlying the interaction(s) between NPs and biomolecules. We believe the outcome from this work may contribute to a better understanding of the potential beneficial or harmful impacts of nanoparticles on the biological systems.

2. Materials and methods

2.1. Materials

Hen egg-white lysozyme (HEWL) (EC 3.2.1.17) with a purity of ≥ 90% and glutaraldehyde solution of 25% were purchased from Merck (Darmstadt, Germany). Cerium dioxide (CeO_2) and zinc oxide (ZnO) nanoparticles were provided by Dr. Kuen-Song Lin (Yuan Ze University, Taiwan). Nile red, sodium phosphate dibasic, sodium dodecyl sulfate (SDS), N,N,N',N'-tetra-methyl-ethylenediamine, Tris, brilliant blue R, ammonium persulfate, bromophenol, 2-mercaptoethanol, glycine, and *Micrococcus lysodeikticus* ATCC were purchased from Sigma-Aldrich (St. Louis, MO, USA); sodium dihydrogen phosphate anhydrous was obtained from Nacalai Tesque, Inc. (Kyoto, Japan); acetic acid, sodium hydroxide, and hydrochloric acid were purchased from Tomita Pharmaceutical Co., Ltd., (Tokushima, Japan); acrylamide/bis-acrylamide 40% solution 37.5:1 and guanidine hydrochloride (GdnHCl) were purchased from Bio Basic Inc. (Taichung City, Taiwan); methanol was purchased from Mallinckrodt Baker (USA); 2-propanol was purchased from J.T. Baker (Center Valley, PA, USA); and Protein Marker (mid-range) was obtained from Genemark (Taiwan). All chemicals and reagents used in the experiments were of analytical grade and the water was de-ionized (D. I. water).

2.2. Synthesis of nanoparticles (NPs)

The CeO_2 NPs were synthesized through a hydrothermal process. Cerium ammonia nitrate was dissolved in water and ammonia hydroxide solution ($NH_4OH_{(aq)}$) was added to precipitate the cerium salt. A milky slurry was formed, dried, and was heated at 300 °C for 5–6 h. The resulting CeO_2 nanoparticles were decanted and washed several times with D.I. water. The ZnO NPs were prepared by using precipitation transformation method. $Zn(NO_3)_2$ and $(NH_3)_2CO_3$ solutions were well prepared of the same concentration before mixing. $Zn(NO_3)_{2(aq)}$ was added into $(NH_3)_2CO_{3(aq)}$ while being vigorously stirred and thereafter a $NaOH_{(aq)}$ also of the same concentration was added. The concentration ratio of $Zn(NO_3)_2$:$(NH_4)_2CO_3$ = 1:1.25 and the reaction was performed at 25 °C. The white ZnO slurry was washed with D.I. water, filtered, and redispersed in ethanol several times. The pure ZnO NPs were dried and formed after several washings at 320 °C for 3 h.

2.3. HEWL sample preparation with NPs

The stock solution of NPs at 0.1 g/L was prepared in 0.01 M phosphate buffer (pH 7.4). The solution was sonicated for 30 min and then stirred for 24 h before the following experiments were conducted. For the spectroscopic measurement, all the samples were prepared in different concentrations of NPs immediately after 10 min sonication, with or without HEWL protein, in 0.01 M phosphate buffer (pH 7.4), followed by incubation of samples for 120 min on the rotator with the speed of 10 rpm at 37 °C. To understand if the sonication and incubation steps affected the conformation of the HEWL alone, we performed far-UV CD measurements on the freshly prepared HEWL sample (the native HEWL) and the HEWL sample upon treatment of 10-min sonication followed by 120-min incubation. Our far-UV CD results showed that the steps/procedures of sonication (10 min) and incubation (120 min) did not cause any noticeable change in the secondary structure content of HEWL (see Fig. S1).

2.4. Measurement of zeta potential, FE-SEM, and HR-TEM

CeO_2 or ZnO NPs solution at 0.05 g/L was first prepared in a phosphate buffer (pH 7.4), followed by a titration from pH 10–2. The zeta potential was then measured with ZetaSizer Nano-ZS (Malvern Instruments, Malvern, UK). The morphology, microstructure, and particle size of the as-synthesized CeO_2 or ZnO NPs was determined by field emission-scanning electron microscopy (FE-SEM) (Hitachi, S-4700 Type II). High resolution-transmission electron microscopy (HR-TEM) analysis was conducted to investigate the crystallinity and particle size distribution of CeO_2 or ZnO NPs with a model Zeiss 10C using 300 kV accelerating voltage.

2.5. Circular dichroism absorption spectroscopy

The far-UV circular dichroism (CD) spectra of samples were measured using a JASCO J-815 CD Spectropolarimeter (Tokyo, Japan) with a 2 mm path length quartz cuvette. The far-UV region was scanned over the range of 190–260 nm at a scanning rate of 100 nm/min. Deconvolution of the far-UV CD spectra for estimation of secondary structure contents was conducted using the CDSSTR algorithm with reference database SP175 fitting.

2.6. Intrinsic fluorescence spectroscopy and fluorescence quenching

The fluorescence spectra were measured with a Cary Eclipse Fluorescence

Spectrometer (Varian Medical Systems Inc., Palo Alto, CA, USA). The excitation wavelength was at 280 nm, and the emission was at 300–400 nm with a 10 nm slit. The setting of PMT voltage was 530 V at a scanning rate of 600 nm/min. All samples were placed in the spectrometer for 2 min for thermostating and then measured. The