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INTERACTION OF CATALASE WITH DIFFERENT NANOCERAMICS

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Abstract

Catalase when in vivo is responsible for the breakdown of hydrogen peroxide to water and molecular oxygen. In industry, catalase is used for the removal of hydrogen peroxide after sterilization steps in dairy product processing. In this study, the interaction of catalase with different nanomaterials was monitored spectroscopic methods (absorbance, fluorescence and circular dichroism). Results showed different results among the nanomaterials with some showing significant reduction in absorbance and emission of the enzymes. Changes in the conformation of the enzyme was also observed upon mixing with the nanomaterials.

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Introduction

It is expected that nanotechnology will play an important role and have socioeconomic impact in nearly all industrial activity fields. There is a constant increase in the use of nanomaterials in the industrial sector. This is especially prominent in biomedicine with applications in diagnostics and therapeutics.[1,2] However, knowledge about the effects of nanoscale objects on biological systems and their potential toxicity is unknown and it has been found recently the interaction of nanoparticles with protein has emerged as a key parameter in nanomedicine and nanotoxicology.[3]

Nanomaterials or nanoparticles are materials with morphological features on the nanoscale, with special properties stemming from their nanoscale dimensions. Due to the unique properties of nanomaterials, they are used in almost every field of science. They have additionally found applications in improving products in medicine, electronics, energy, and the environment sector.

When nanoparticles interact with proteins, they can alter protein conformation and cause a chain of cascade effects leading to toxicity. Enzymes are proteins that catalyze biological reactions. Catalase which is regarded as one of the most common enzymes in plant and animal tissues is a tetrameric iron porphyrin protein and plays an important role in the antioxidant defense system. It protects cells from the toxic effects of hydrogen peroxide by converting it to oxygen and water without the formation of free radicals. The intake of exogenous environmental chemicals such as organic pollutants, heavy metals, and nano-materials, is likely to trigger the destruction of protein conformation and/or affect the catalytic activity of enzymes.[4,5] The conformational disorders and activity inhibition of catalase can cause enhanced generation of the free reactive oxygen species (ROS), which induces various deleterious effects such as cell dysfunction, death, malignant transformation and cancers.[6]

In this study, catalase was utilized as a model protein to investigate the effect of commercially available nanoceramics on proteins by using molecular spectroscopic techniques, including UV–visible absorption, fluorescence emission, and circular dichorism (CD) spectroscopy. This study provides a better understanding of the biosafety and biocompatibility issues of nanomaterials with important proteins.

Materials and Methods

The commercially available nanoceramics: aluminum nitride (AlN), aluminum oxide (AlO), silicon carbide (SiC), silicon nitride (SiN), silicon oxide (SiO), titanium carbide (TiC), titanium nitride (TiN), titanium oxide (TiO) and zinc oxide (ZnO) [from Sun Innovations, Inc.] were individually weighed (5.0 mg) and placed in Eppendorf tubes.

A stock solution of 1 uM catalase from bovine liver (Sigma Aldrich) in 0.010 M Tris buffer at pH 7.4 was prepared. Catalase solution (1.5 mL) was added to each nanomaterial. The resulting mixture was incubated for 10 minutes. Each mixture was centrifuged for 2 minutes at 6600 rpm using a Mini Centrifuge (Benchmark Scientific) which allowed the nanoceramic to settle in the Eppendorf tube.

The liquid component of each solution was collected and monitored for its absorbance, fluorescence and circular dichroism (CD) to determine the interaction of nanoceramic to catalase. The absorbance spectra from 200-500 nm were acquired using a Jasco V-570 spectrophotometer (Easton, MD) with 1.0 cm quartz cuvet. Emission spectra (290-500 nm) were obtained using a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ) with an excitation wavelength of 280 nm. The CD spectra (185-250 nm) were obtained using a Jasco 815 circular dichroism spectrometer. In all spectroscopic readings, blank solutions were prepared and subtracted with the sample spectra. These blanks are made up of just 0.010 M Tris buffer at pH 7.4 and the buffer added with nanoceramics.

Results

This study looked on the interaction of commercially available nanomaterials with catalase using different spectroscopic techniques. Figure 1 shows the UV-Vis spectra of catalase mixed with different nanoceramic and the control (catalase solution). Three peaks were observed for the control solution. These peaks can be found in the 230, 280 and 405 nm. There is a drastic change in the UV-Vis absorption spectra upon mixing the catalase with nanoceramics. The spectra of all solutions mixed with nanoceramics except for SiO show the disappearance of peak at the 405 nm region. There are reductions in the peaks at the 230 and 280 nm regions in all nanoparticles except for ZnO. Greatest reduction was

observed in SiC, AlN, AlO and TiO. For the ZnO, a broad peak is observed at the 250 nm region which extends to the other two peak regions (230 and 280 nm).

For the fluorescence spectra (Figure 2), a decrease in the emission intensity is observed in all solutions containing nanoceramics. The trend in the reduction of intensity is from 56.4% to 97.4%. The ranking in terms of increasing reduction in fluorescence intensity is SiO, TiC < SiN < TiN < TiO < SiC < AlN < ZnO < AlO. In addition, ZnO and AlO are observed to shift to 350 nm in terms of peak maximum.

Lastly, the circular dichroism (CD) spectra were obtained for all samples. The far-UV CD spectra of catalase incubated with nanoceramics are shown in Figure 3. Table 1 shows the changes in conformation in terms of the tertiary structure of catalase upon mixing with nanoceramics. Based on the figures and table, there is a distinct change in conformation of catalase upon the addition of nanoceraics. The changes in conformation can be divided in three groups. The first group has resulted in 100% alpha-helix conformation and this group includes AlO, SiC and TiN. The next group has around 70% alpha-helix conformation and 30% beta-turn. This includes AlN, SiO, TiC, ZnO. The last group which includes TiO and SiN has 83% alpha-helix.

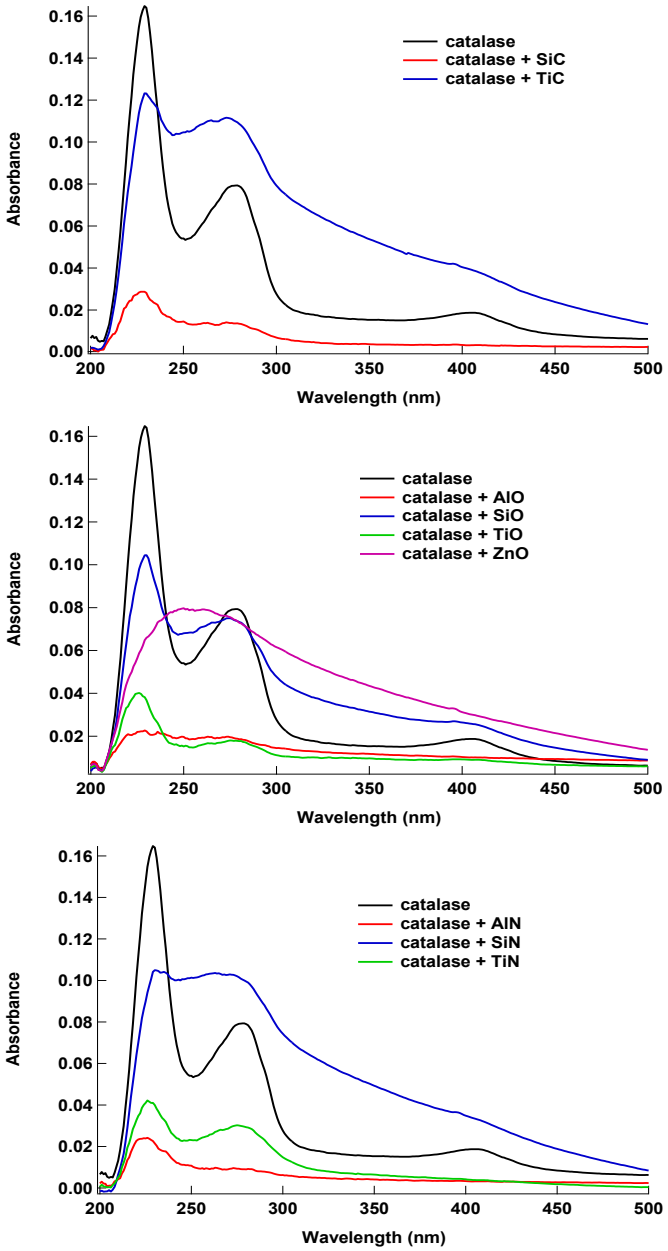

Figure 1. Absorbance spectra of catalase with and without the nanoceramics.

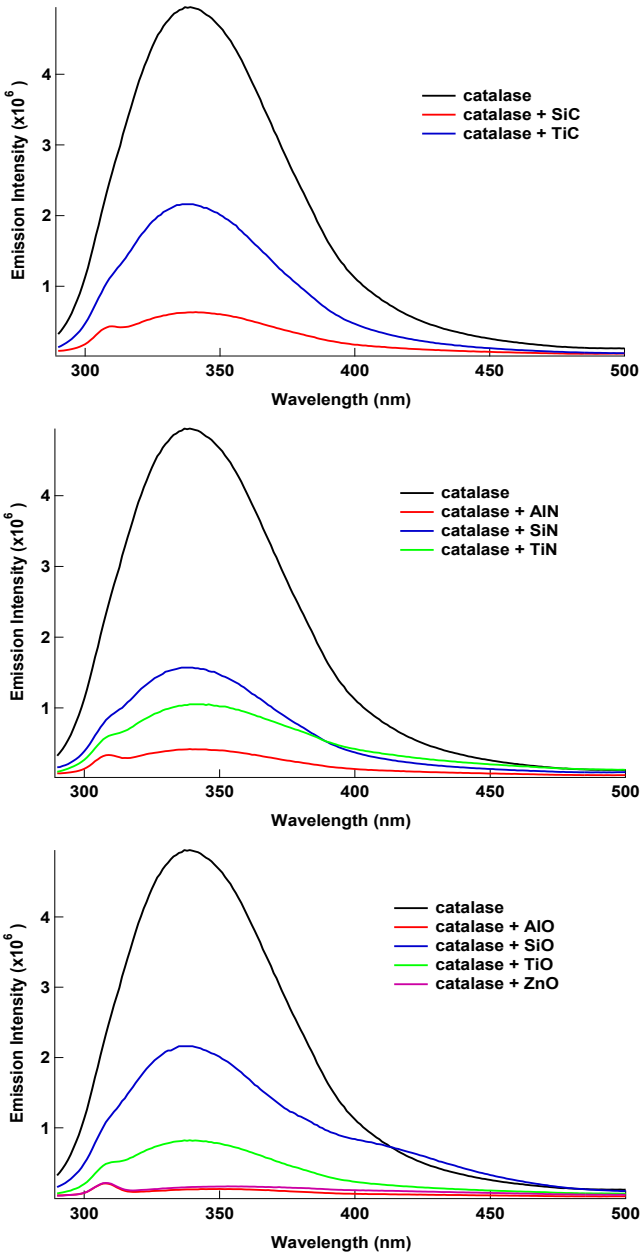

Figure 2. Fluorescence spectra of catalase with and without the nanoceramics.

Table 1. Conformation of catalase with and without nanoceramics.

	Helix	Beta	Turn	Random
Catalase	27.6	32.9	14.2	25.3
Catalase + AlN	69.2	0	30.8	0
Catalase + AlO	100	0	0	0
Catalase + SiC	100	0	0	0
Catalase + SiN	83.1	4	12.9	0
Catalase + SiO	69.6	0	30.4	0
Catalase + TiC	70.3	0	29.7	0
Catalase + TiN	100	0	0	0
Catalase + TiO	83.4	0	16.6	0
Catalase + ZnO	67.4	0	32.6	0

Discussion

The UV-Vis absorption spectroscopy technique can be used to explore the structural changes of a protein and to investigate protein-ligand complex formation. It is a useful conformational probe that is widely used in the determination of structural changes of proteins. The reduction of the absorbance or signal at different peaks means that the nanoceramics interact with the catalase since each peak corresponds to an absorption band. The interaction may affect the structure of catalase and its effect can be seen by looking at the changes in absorbance in each peak.

The peak at 405 nm is due to the Soret absorption band belonging to the π→π* transitions of hematoporphyrin in catalase.[7] The peak at 230 nm is due to the framework conformation of catalase[8] and any changes to it is related to the alterations in polypeptide backbone other than those associated with tyrosine and tryptophan residue.[9] The peak at 280 nm on the other is due to the aromatic amino acids (tryptophan, tyrosine and phenylalanine) of the enzyme.[10] The different trends observed on the absorbance of catalase upon addition of nanoceramics is consistent with what has been reported in literature. For instance, multiwalled carbon nanotubes (MWCNT) showed increase in absorbance in both the 280 nm and 405 nm region in one study[11] while another study using MWCNTs showed a decrease in absorbance in the 280 nm region.[12]

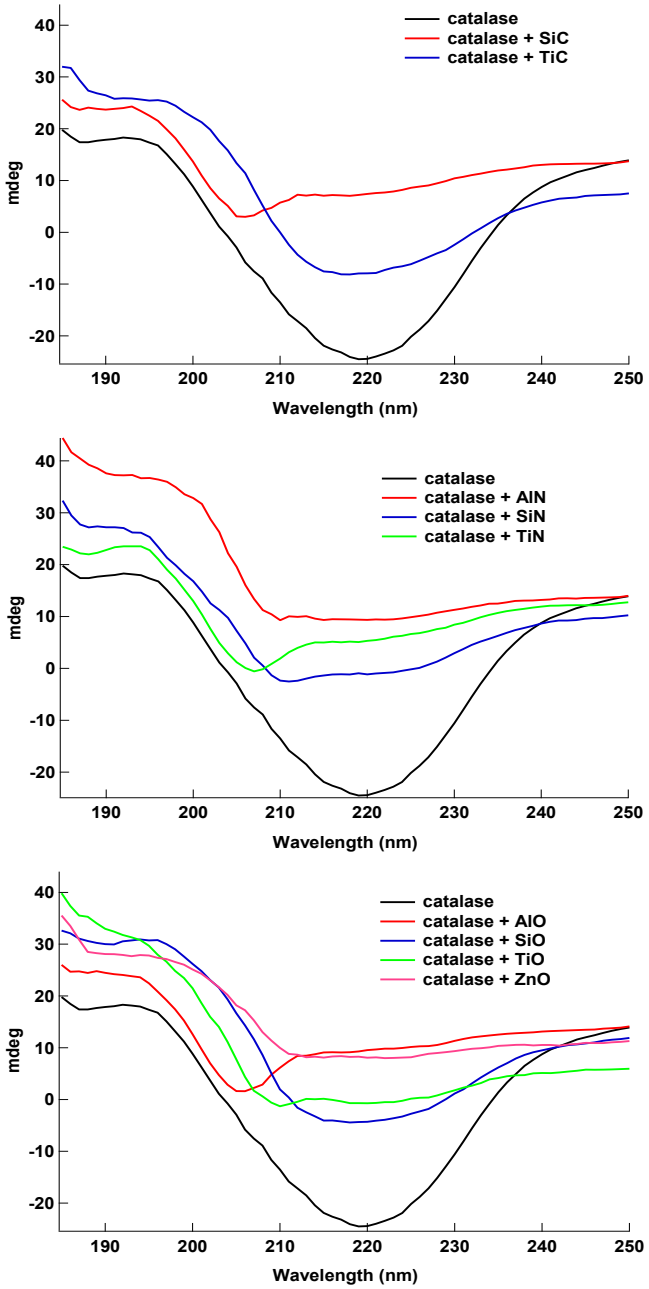

Figure 3. Circular dichrosim (CD) spectra of catalase with and without the nanoceramics.

The fluorescence intensity obtained by exciting the sample at 280 nm is due to the aromatic amino acids; particularly the intrinsic fluorescence emission of tryptophan and tyrosine present in catalase. The catalase from beef liver consists of 20 tyrosine and 5 tryptophan residues per subunit and each subunit contains one heme with Tyr 357 occupying the fifth coordination site.[13] The emission intensity and spectral characteristics of tyrosine and tryptophan residue mainly depends on the exposed environment.[14] The decrease in the intensity can only be due to the quenching of the intrinsic fluorescence of catalase upon mixing with nanoparticles. The red shift as exhibited by ZnO and AlO suggests that the aromatic amino acids residues in catalase were exposed to a less hydrophobic environment.[15] This is probably due to the restructuring of the surrounding water.[16]

CD spectroscopy is a common tool for the analysis of the interaction between protein and other molecules. The CD spectrum in the far UV region (180–250 nm) presents the information about the secondary structure of proteins.[17] The result means that nanoparticles affected the sceondary structure fractions. This suggests that the nanoparticles can affect the skeleton structure of catalse and prompt the protein unfolding, which can eventually lead to the alteration of the enzymatic activity. This is consistent with what have been observed in the interaction MWCNTs with catalase[11] where changes in the cd spectra could be due to induced secondary structural change and destruction of the hydrogen bond network.[18]

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