Chinese Chemical Letters Vol. 16, No. 8, pp 1105-1108, 2005
http://www.imm.ac.cn/journal/ccl.html

Sonocatalytic Damage of Bovine Serum Albumin (BSA) in the Presence of Nanometer Titanium Dioxide (TiO_2) Catalyst

Jun WANG*, Jing WU, Zhao Hong ZHANG, Xiang Dong ZHANG, Lei WANG,
Liang XU, Bao Dong GUO, Hong LI, Jian TONG

Department of Chemistry, Liaoning University, Shenyang 110036

Abstract: The sonocatalytic damage of bovine serum albumin (BSA) was studied in the presence of nanometer titanium dioxide (TiO_2) powders by low frequency (80 kHz) ultrasound. The destruction of secondary structure and change of α-helical structure of BSA were reflected by ultraviolet (UV) and circular dichroism (CD) spectroscopies.

Keywords: Bovine serum albumin (BSA), sonocatalytic damage, nanometer titanium dioxide (TiO_2).

In recent years, semiconductor titanium dioxide (TiO_2) as photocatalyst has been applied to kill or suppress tumor cells[1-5], because TiO_2 particles can react with water molecules adsorbed on the surface of tumor cells to produce hydroxyl (·OH) radicals with strong oxidation ability under ultraviolet (UV) light irradiation. Otherwise, TiO_2 particles can be easily engulfed by macrophage cells around blood vessel and fleetly eliminated from normal tissue. But the UV lights used as excitation resource do not possess strong penetration ability and high utilization efficiency. Recently, in order to explore a new method of treating tumour cells, we made use of ultrasound instead of UV lights and carried out a series of investigations on sonocatalytic reactions in the presence of nanometer TiO_2 powders owing to the good penetration ability and less energy consumption[6,7].

Proteins are the most abundant macromolecules in biological cell. The function of a protein molecule is decided by its space structure to a great extent, so the change of the specific space structure might cause the forfeiture of its function, even the death of a whole cell. In this work, the sonocatalytic damage of bovine serum albumin (BSA) as a model protein was investigated in the presence of nanometer TiO_2 powders by UV and CD spectroscopies. It was found that the α-helical secondary structure gradually disappeared and linear primary structure stretched with the time of ultrasonic irradiation.

The nanometer TiO_2 powders were purchased from Haerbin Chemistry Reagent Company (Haerbin, P. R. China). At first, the TiO_2 powders were treated at 200 °C. Their XRD and TEM were determined, respectively. The nanometer TiO_2 powder is

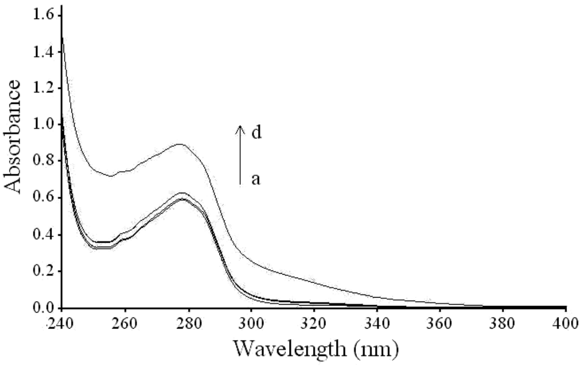
---

* E-mail: wangjun890@sina.com

predominantly anatase (90% anatase and 10% rutile) and its density and size are about 3.56 g/cm^{-3} and 20-30 nm, respectively. BSA was purchased from Sigma Company. All the other chemicals were in analytical grade. Doubly distilled water was used as a solvent.

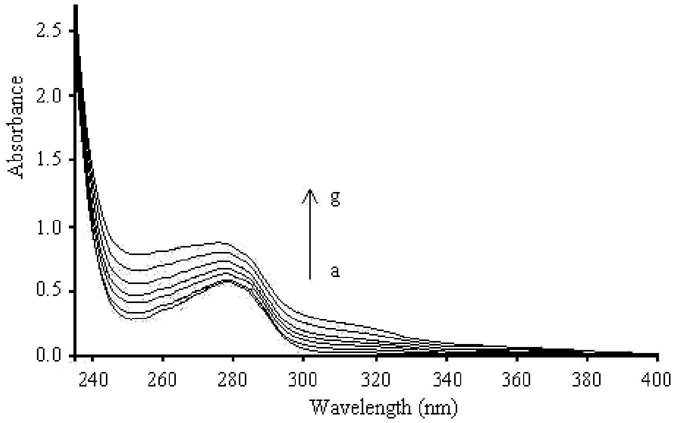
The prepared BSA solution (50 mL) and TiO_2 powders were placed into a glass reactor (about 50 cm^2 area bottom) that was put in the KQ-160TDB ultrasonics apparatus (Kunshan ultrasonics apparatus company). The ultrasonic frequency of 80 kHz, output power of 50 W and temperature of 37.0 °C were used throughout the experiment. The sonocatalytic damage of BSA was followed by intermittent sampling during the reaction process and each sample was immediately centrifuged and its UV spectra (LAMBDA-17 UV-Vis spectrophotometer) and CD spectra (Jasco J-810 spectropolarimeter) were determined using 1.0 cm and 1.0 mm path length quartz cuvettes, respectively. The effects of the amount of TiO_2 and initial pH value on the sonocatalytic damage of BSA were observed within 3.0 h by means of UV spectra.

Figure 1 UV spectra of BSA (1.5×10^{-2} mmol/L in phosphate buffer solutions (pH = 6.86)



a: original solution; b: only TiO_2 (1.5 g/L); c: only ultrasound; d: ultrasound+TiO_2 (1.5g/L)

Figure 2 UV spectra of BSA (1.5×10^{-2} mmol/L) during irradiation in the presence of TiO_2 (1.5 g/L)



a: 0.0 h; b: 1.0 h; c: 2.0 h; d: 3.0 h; e: 4.0 h; f: 5.0 h; g: 6.0 h.

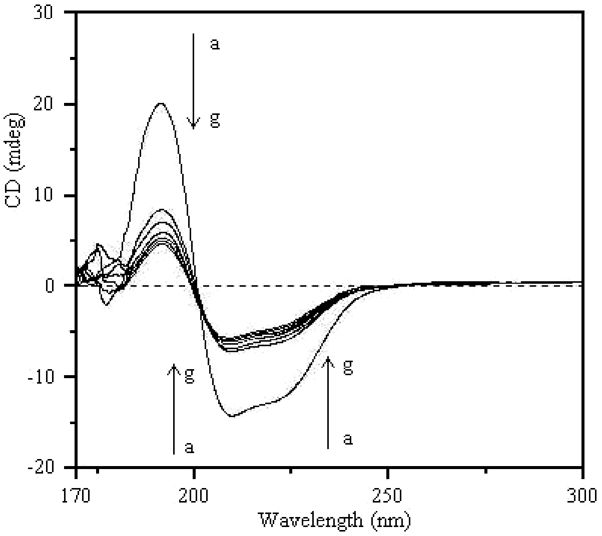
The associated action of ultrasound and TiO_2 powders on BSA was compared with that of TiO_2 and ultrasound only as shown in **Figure 1**, which showed that the hyperchromic effect under the confederate function of TiO_2 powders and ultrasonic irradiation are very obvious compared with only using TiO_2 or only using ultrasound, in the latters the hyperchromic effects could be hardly detected.

UV and CD spectra of the BSA solutions at 1.0 h intervals were recorded within 6.0 h to follow the process of the sonocatalytic damage of BSA.

As shown in **Figure 2**, the maximum absorption peak at approximately 278 nm rose gradually and slightly shifted to the blue area with the increase of irradiation time. As shown in **Figure 3**, the positive peak at 190 nm declined and the two negative peaks at 208 nm and 222 nm ascended along with irradiation time, but the shapes and positions of these peaks were not changed.

As well known, the UV absorption peak of BSA at 278 nm mainly attributes to the absorption of aromatic amino acid (for example: tyrosine, tryptophane, phenylalamine and so on) residues. Under ultrasonic irradiation the ·OH radicals produced by excitated TiO_2 powders and cavitation effect would attack the bisulfur bonds (-S-S-) maintained the secondary (mainly α-helical) structure of BSA. The bisulfur bonds between two cysteines are oxidized and cut off. In the result the peptide chain in α-helical structure of BSA extended and became linear primary structure. Going with the extension of peptide chain more and more aromatic amino acid residues expose from the interior of BSA, so the hyperchromic was more and more obvious in the UV spectra. At the same time, the stacking interaction between aromatic rings became weak after the bisulfur bonds broke in the result the energy of aromatic ring rose and the absorption peak shifted to blue area.

Figure 3 CD spectrum of BSA (1.0×10^{-3} mmol/L) under irradiation in the presence of TiO_2 (0.3 g/L)



a: 0.0 h; b: 1.0 h; c: 2.0 h; d: 3.0 h; e: 4.0 h; f: 5.0 h; g: 6.0 h

The changes of typical secondary α-helical structure of BSA also can be judged by the CD spectra. The decrease of positive peak at 190 nm and increase of two negative peaks at 208 and 222 nm indicated that the α-helix was reduced and the secondary structure was gradually destroyed along with irradiation time, which is consistent with the results determined by UV spectra.

The effects of the amount of TiO_2 powders and the initial pH value on the sonocatalytic damage of BSA have also been reviewed. The amount of TiO_2 powders exhibited saturation behaviour at the concentration of 1.5 g/L and the sonocatalytic damage of BSA in both acidic and basic solutions was more serious than in neutral condition.

Acknowledgment

We greatly acknowledge the National Natural Science Foundation of China for financial support.

References

1. H. Wang, W. K. Zhao, *et al., China J. Catal.*, **1995**, *20*, 373.
2. R. Cai, K. Hashimoto, *et al., Bull. Chem. Soc. Jpn.*, **1991**, *64*, 1268.
3. R. Cai, Y. Kubota, *et al., Cancer Res.*, **1992**, *52*, 2346.
4. R. Cai, K. Hashimoto, Y. Kubota, A. Fujishima, *Chem. Lett.*, **1992**, 427.
5. H. Sakai, R. Baba, *et al., Chem. Lett.*, **1995**, 185.
6. J. Wang, B. D. Guo, *et al., Ultrason. Sonochem.*, **2004,** *11*, 968.
7. J. Wang, B. D. Guo, *et al., J. Environ. Sci.*, **2004,** *16*, in press.

Received 23 September, 2004