Journal of Photochemistry and Photobiology B: Biology 128 (2013) 12–19

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Binding investigation on the interaction between Methylene Blue (MB)/TiO_2 nanocomposites and bovine serum albumin by resonance light-scattering (RLS) technique and fluorescence spectroscopy

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ARTICLE INFO

Article history:
Received 23 May 2013
Received in revised form 26 July 2013
Accepted 31 July 2013
Available online 8 August 2013

Keywords:
Resonance light scattering
Fluorescence
MB/TiO_2 nanocomposites
Bovine serum albumin
Interaction

ABSTRACT

The interaction between Methylene Blue (MB)/TiO_2 nanocomposites and bovine serum albumin (BSA) was investigated by resonance light scattering (RLS), fluorescence, three-dimension spectra and UV–vis absorbance spectroscopy. Several factors which may influence the RLS intensity were also investigated before characterizing MB/TiO_2–BSA complex. It was proved that the mechanism of MB/TiO_2 nanocomposites binding to BSA was mainly a result of the formation of MB/TiO_2–BSA complex. The binding constant of MB/TiO_2–BSA is 0.762×10^{-5} L mol^{-1} at 298 K. By calculating the binding constant at different temperature, the thermodynamic parameters ΔH, ΔG, and ΔS can be observed and deduced that the hydrophobic interactions played an important role to stabilize the complex. The distance r (3.73 nm) between donor (BSA) and acceptor (MB/TiO_2) was obtained according to fluorescence resonance energy transfer (FRET). The binding site for MB/TiO_2 on BSA was mainly located in sub-domain IIA. The UV–vis absorbance, circular dichroism and three dimension fluorescence have also been used to investigate the effect of MB/TiO_2 on the conformation of BSA.

1. Introduction

Proteins are the basis of life and their quantitative determination is extremely important in life sciences, clinical medicine, and biochemistry. Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, and are the major macromolecules contributing to the osmotic blood pressure [1]. In addition to blood plasma, serum albumins are also found in tissues and bodily secretions throughout the body; the extra vascular protein comprises 60% of the total albumin [2]. In this work, bovine serum albumin (BSA) is selected as our protein model because of its medically important, low cost, ready availability, unusual ligand-binding properties [3].

Proteins analysis continues to be an important and active area of investigation in the fields of biochemistry and clinical medicine. Tajmir-Riahi and coworkers [4–9] carried out systematically study of the mechanism of interaction between series of matter and BSA, these results of research have a good guide for other researchers. However, the most widely used methods for protein determination are Lowry [10], Bradford [11], Biuret [12], Bromocresol Green [13,14] and Bromophenol blue [15,16] assays. Most of these methods suffer from some disadvantages such as poor selectivity, low sensitivity, complicated procedure and instability of some reagents. Therefore, some other methods have been developed in recent years, such as the resonance light-scattering (RLS) technique which is simple, sensitive and selective for the determination of proteins.

In recent years, sensitive methods of determining proteins have been developed on the basis of enhanced RLS. The determination is mainly based on the fact that aggregation of a dye chromophore induced by a biological macromolecule can give rise to strong RLS. Most of these methods use protein–dye systems to determine the proteins [17–20]. Only a few cases have studied protein–dye–nanocomposites systems for protein determinations [21,22].

Methylene Blue (MB, formula: $C_{16}H_{18}N_3ClS$; CAS registry number: 61-73-4), which is also named as methylenum, caeruleum, tetramethylthionine chloride or swiss blue, is a cationic dye. Its redox formal potential, comprised between 0.08 and −0.25 V (versus SCE) in solution at pH 2–8, respectively, is close to those of several biomolecules [23]. MB has been widely used in electrochemical applications like catalyst and mediator in electrochemical biosen-

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http://dx.doi.org/10.1016/j.jphotobiol.2013.07.027

sors [23]. Moreover, TiO_2 has been sensitized with MB for photodegradation of various halocarbons using visible light [24]. Recently, a mixture of commercial TiO_2 powder and MB has been used to fabricate an intelligent ink sensible to oxygen [25,26]. More and more reports focus on nanocrystalline TiO_2 catalyst and are evaluated by measuring degradation rates of Methylene Blue (MB) under UV or visible light. The interaction of BSA and TiO_2 also aroused the interests of scientists. MB/TiO_2 nanocomposites were prepared by the LPD technique. It is not a simply mixture of two single materials, but a formation of composite, which can be considered as a new material. When the nanocomposite binds to BSA, the complex formation can be considered as a combined contribution from both MB and TiO_2. TiO_2 as a nanoparticle, is very sensitive to resonance light scattering, and in the MB/TiO_2 nanocomposite, TiO_2 can be considered as a carrier of MB, so MB/TiO_2 complex also has the high sensitivity of resonance light scattering. However, the conformational behavior of BSA changed by TiO_2 is still unclear thoroughly at present; especially the interaction of BSA and MB/TiO_2 is not reported now. It is of great importance in the application of these bioconjugated nanomaterials. Therefore, a general analytical tool for studying the interaction and binding of biomolecules with nanomaterials is crucial to address the challenges of nanobiotechnology.

Fluorescence spectroscopy is a powerful tool for detecting the reactivity of chemical and biological systems. The aim of our work was to determine the affinity of MB/TiO_2 to BSA and to investigate the thermodynamics of their interaction. The detailed investigation of MB/TiO_2–BSA association was carried out by using fluorescence spectroscopy, UV–vis absorbance spectroscopy. The effect of MB/TiO_2 on energy transfer and the environment adjacent to the tryptophan residue in BSA resonance raylei scattering spectroscopy, fluorescence resonance energy transfer (FRET) and synchronous fluorescence spectroscopy. The stoichiometry of MB/TiO_2 and BSA binding process has also been investigated.

2. Experimental

2.1. Apparatus

UV spectrum was recorded at room temperature on a UNiCO-4802 UV–vis double beam spectrophotometer (UNiCO Analytic Instrument Ltd., Shanghai, China) equipped with 1.0 cm quartz cells. The fluorescence spectra were recorded on LS55 Spectrofluorimeter (PerkinElmer, USA) equipped with 1.0 cm quartz cells and a thermostat bath. RLS spectra were obtained by synchronously scanning the excitation and emission monochromators ($\Delta\lambda = 0$) of the spectrofluorimeter through the wavelength range 300–450 nm on LS55 Spectrofluorimeter (PerkinElmer, USA). The RLS intensity was measured at 380 nm in a 1.0 cm quartz cell with a slit width of 5.0 nm for excitation and emission respectively. All pH measurements were made with a PB-10 acidity meter (Sartorius, Germany).

2.2. Reagents

BSA and MB, being electrophoresis grade reagents, were both purchased from Sigma (St. Louis, MO, USA). TiO_2 (Degussa P-25 Germany). TiO_2, a mixture of anatase and rutile (8:2), has a BET surface area of ca. 50 m^2/g and its primary particle size is ca. 27 nm for anatase and ca. 16 nm for rutile. The PBS buffer solution had a purity of no less than 99.5% and NaCl, KCl, Na_2HPO_4, KH_2PO_4, etc. were all of analytical purity (Shanghai Chemical Reagent Plant, China). The samples were dissolved in PBS buffer solution (pH 7.0 ± 0.1). According to the report by Gutiérrez-Taust [27] have described the preparation of MB/TiO_2 complex by the LPD technique to prepare the MB/TiO_2 nanocomposites suspension. All solutions were prepared with doubly distilled water. Sample masses were accurately weighted on a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

2.3. Procedures and methods

Fluorescence spectra at different temperatures (298 K, 302 K, 306 K and 310 K) were recorded on LS55 Spectrofluorimeter excitation wavelength was 280 nm. The widths of the excitation slit and the emission slit were set to 15 nm, 2.5 nm, respectively. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background. The RLS spectra were obtained by synchronously scanning the excitation and emission monochromators ($\Delta\lambda = 0$) of the spectrofluorimeter through the wavelength range 300–450 nm. The RLSintensity was measured by keeping the response width of the fluorimeter at 5 nm in a 1.0 cm quartz cell. RLS spectra were obtained by scanning simultaneously the excitation and emission monochromators ($\Delta\lambda = 0$) with the excitation and emission slits 5.0 nm. The wavelength for excitation and emission located at 380 nm. RLS intensity I for the reaction product and I_0 for the reagent blank were measured at the maximum scattered wavelength, $I = I – I_0$. All RLS measurements were made against blank solutions treated in the same way without proteins.

3. Results and discussion

3.1. TEM images of MB/TiO_2 nanocomposites

Fig. 1 shows the TEM images of TiO_2(A), MB/TiO_2 (B) nanocomposites and the X-ray diffraction cycles of MB/TiO_2 nanocomposites (C). The diameter of the P-25 TiO_2 nanoparticles is about 20 nm. But the TiO_2 nanoparticles diameter is about 100–200 nm (Fig. 1(A)). It shows that TiO_2 nanoparticles take place the evident phenomena of aggregation. The diameter of the MB/TiO_2 nanocomposites is about 20 nm (Fig. 1(B)) and put up the better dispersing and crystal character than single P-25 TiO_2 nanoparticles. From Fig. 1(C), we can see the perfect X-ray diffraction cycles of MB/TiO_2 nanocomposites.

3.2. The interaction between BSA and MB/TiO_2 nanocomposites

3.2.1. Characteristics of the RLS spectra

Resonance light scattering (RLS), a Rayleigh scattering phenomenon at a wavelength near or at the absorption bands of the scattering molecules, can provide a high signal level and good selectivity [28,29]. In order to understand the interaction between BSA and MB/TiO_2 nanocomposites, resonance light scattering spectroscopy was used in this work. The light scattering spectra for MB/TiO_2 and BSA–MB/TiO_2 at pH7.0 are shown in Fig. 2. In this experiment, the concentrations of BSA were stabilized at 1.0×10^{-7} - mol L^{-1}, and the concentration of MB/TiO_2 varied from 0 to 1.8×10^{-6} mol L^{-1} at increments of 0.2×10^{-6} mol L^{-1}. The effect of MB/TiO_2 on BSA RLS intensity is shown in Fig. 2. As can be seen from Fig. 2, addition of MB/TiO_2 with increasing concentrations caused a progressive and notable increasing of the RLS intensity, which indicates that an interaction between BSA and MB/TiO_2 occurred. As well known, protein folding is affected by many forces and associated with related interactions, such as hydrophobic interactions, electrostatic interactions (charge repulsion and ion pairing), hydrogen bonding interactions, intrinsic propensities, and Vander Waals forces, etc. Hydrophobic interactions are the repulsion between water and non-polar residues in proteins, lead-