Journal of Luminescence 130 (2010) 611–617

Contents lists available at ScienceDirect

Journal of Luminescence

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

Spectroscopic Studies on the Interaction of Acid Yellow With Bovine Serum Albumin

Xingren Pan, Rutao Liu*, Pengfei Qin, Li Wang, Xingchen Zhao

Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Shandong University, 27 Shanda South Road, Jinan 250100, P.R.China

ARTICLE INFO

Article history:
Received 19 April 2009
Received in revised form
29 October 2009
Accepted 5 November 2009
Available online 12 November 2009

Keywords:
Bovine serum albumin
Acid yellow
Fluorescence quenching
Toxicity

ABSTRACT

Azo dyes, which are common in the environment, can be toxic to various organisms. In order to determine the molecular mechanism of acid yellow 11(AY) toxicity, we studied the effect of AY exposure to the common protein bovine serum albumin (BSA) by several spectroscopic techniques including fluorescence spectroscopy, ultraviolet spectrophotometry (UV) and circular dichroism (CD). It could be concluded from the fluorescence spectra that the quenching effect of BSA by AY was mainly due to complex formation which was unrelated to the absorption of AY. The enthalpy change (ΔH) and entropy change (ΔS) were found to be -21.94 kJ/mol and 30.04 $Jmol^{-1}$ K^{-1}, respectively. The results confirm that electrostatic attraction was the predominant intermolecular force between BSA and AY. Furthermore, the binding distance (r) between AY and the inner tryptophan residue of BSA was determined to be 3.541 nm on the basis of Forster theory of non-radiative energy transfer. In addition, the conformational changes of BSA in the presence of AY were also analyzed by UV and CD. These results indicated that AY could interact with BSA by complex formation, which also affected the structure of BSA.

1. Introduction

Azo dyes are the largest group of synthetic colorants used in many fields, such as natural textile fibers, paper, drugs, food, leather, and cosmetic, etc[1]. They are separated into two types according to their solubility in water, liposoluble, and water-soluble[2,3]. Liposoluble dyes are transported directly into the liver and accumulate there, where they can do great harm if they are toxic or carcinogenic. The toxicity of the water-soluble dyes is mainly caused by the aromatic amines generated via their decomposition by azo reductase bacteria[4,5]. However, the toxicity produced by their direct interaction with biological macromolecules in vivo has not been explored previously. Consequently, a thorough study on the mechanisms of toxicity of the water-soluble dyes is needed. In this paper, we select the Acid yellow 11 (AY) dye having good water solubility as the investigated object. Its molecular structure is illustrated in supplemental Fig. 1.

Serum albumin, the most abundant protein constituent in blood plasma[6], plays a fundamental role in the disposition and transportation of various molecules and can react with many different ligands in vivo and in vitro [7]. As the structure of protein determines its important biological functions, the resultant structural alternations due to its interaction with ligands can influence the transport, metabolism and availability of serum albumin for other ligands[8–10]. Normally pollutants will interact with serum albumin after they enter the bloodstream. BSA was selected as the model protein because of its water-solubility, its stability, as well as its similarity to HSA (human serum albumin) for evaluating the AY toxicity to health.

In this paper, we designed a devise to verify the inner filter effect by experiment, which was generally proved by the theoretical calculation before[11–13]. The mechanism of interaction of AY to BSA has been investigated under simulated physiological conditions using fluorescence, ultraviolet absorption and circular dichroism (CD) spectroscopies. These methods combined further with other experiments like cell membrane transport of contaminants and toxicological testing [14,15]are useful for evaluating the interaction of AY with this protein.

2. Materials and methods

2.1. Materials

BSA (Beijing Chemical Reagent Corp., electrophoretic grade reagents) was made up as a stock solution of concentration 1.0×10^{-5} mol/L, then preserved at 0–4 °C and diluted as required. Acid Yellow 11 (Yanshou Knitting Co.) was prepared as a 1.0×10^{-4} mol/L stock solution. Britton-Robinson (BR) buffer (0.2 M), pH 7.4

* Corresponding author. Tel./fax: +86 531 88364868.
E-mail address: rutaoliu@sdu.edu.cn (R. Liu).

0022-2313/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jlumin.2009.11.004

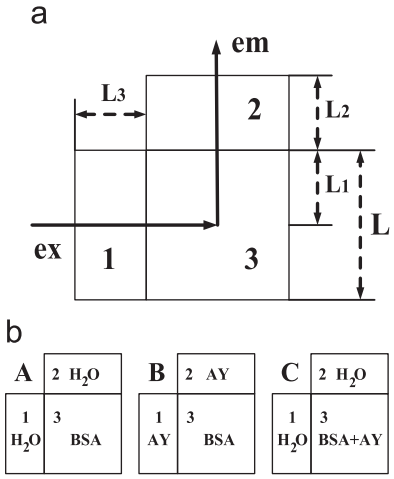

Fig. 1. Principles of optical path and the effects of AY absorption on the fluorescence spectra Conditions: (a) L=10 mm, L_1=L_2=L_3=$L/2$=5 mm; (b) c(BSA)=1×10^{-6} mol/L, c(AY)=4×10^{-6} mol/L.

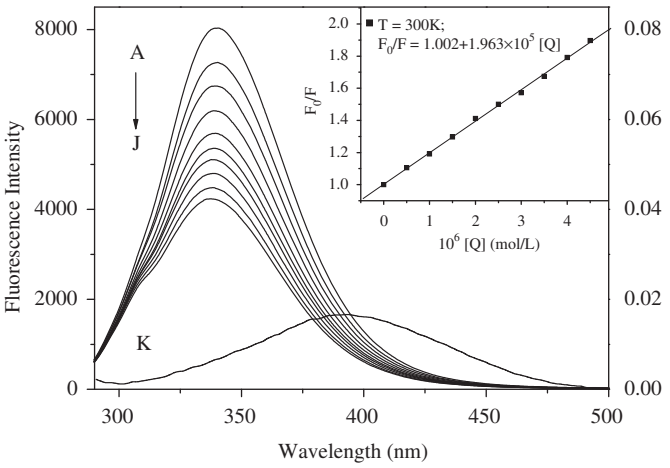

Fig. 2. Emission spectra of BSA in the presence of various concentrations of AY. Conditions: T=300 K, λ_{ex}=278 nm and B–R buffer: pH=7.4, c (BSA)=1×10^{-6} mol/L. C (AY) ($\times10^{-6}$ mol/L), A–J : 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5. Curve K shows the absorption spectrum of AY only, c (AY)=1×10^{-6} mol/L.

was used in making these solutions. All other chemicals were of analytical grade. Ultrapure water was used throughout the experiments.

2.2. Fluorescence measurements

All fluorescence spectra were recorded on an F-4600 fluorophotometer (Hitachi, Japan) equipped with a 10 mm quartz cell and a 150 W Xenon lamp. For the fluorescence measurements, 1 ml BR buffer at pH 7.4, different concentrations of AY, and 1 ml BSA solution were added in turn to a series of 10 ml colorimetric tubes and made up to the mark with water. After equilibration for 20 min, the fluorescence spectra were obtained.

The fluorescence emission spectra were measured at 290, 300, and 310 K, maintaining the temperature of the samples in a thermostatted water bath. The width of the excitation and emission slits were both set at 5.0 nm. The excitation wavelength was chosen at 278 nm, the emission wavelength was recorded from 290 to 500 nm. The synchronous fluorescence spectra were recorded at $\Delta\lambda$=15 and 60 nm. The wavelength range scanned from 250 to 310 nm.

In order to determine the inner filter effect of AY, a verification experiment was performed based on the principles shown in Fig. 1.

2.3. UV–vis absorption and circular dichroism spectroscopic measurements

The UV-vis absorption spectra of BSA in the presence and absence of AY were recorded at room temperature on a UV-vis-2450 spectrophotometer (Shimadzu, Japan) equipped with 10 mm quartz cells in the range from 200 to 310 nm.

Circular dichroism spectra were recorded from 200 to 250 nm on a J-810 Circular Dichroism Spectrometer (Jasco, Tokyo, Japan) using a quartz cell with path length of 10 mm. The scanning speed was set at 200 nm min^{-1}. Each spectrum was the average of two successive scans.

3. Results and discussion

3.1. The mechanism of quenching of BSA fluorescence by AY

Proteins are considered to have intrinsic fluorescence due to the presence of amino acids, mainly tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe)[16]. The fluorescence quenching spectra of BSA in the presence of different concentrations of AY at 300 K are illustrated in Fig. 2. It can be observed that the fluorescence intensity of BSA at a fixed concentration decreases regularly with the increased addition of AY, but there is no significant emission wavelength shift. These results suggest that AY interacts with BSA and quenches its intrinsic fluorescence.

Since the relative absorption band of AY lies in the vicinity of the fluorescence peak, a verification experiment (Fig. 1) was carried out to test if the absorption of AY had an impact on the fluorescence quenching of BSA. It is known that the fluorescence emitted in the cross section of the quartz cells is uniformly generated, but not at every point because of the different optical paths. According to the Lambert-Beer law, the average light path is half of the quartz cell's length. The fluorescence spectra of BSA and BSA with AY are shown in Fig. 3.

The fluorescence intensity of BSA alone, based on optical path A in Fig. 1, is 7464. The fluorescence intensity of BSA was slightly weaker than that in Fig. 2 due to the increase of optical path in the verification experimental setup. The total intensity of BSA and AY fluorescence is 7257, while a mixture of BSA and AY in the same fluorescence cell decreased to 3636. From the equation$(F_A-F_B)/(F_A-F_C)$, the contribution of the fluorescence quenching owing to the absorption of AY is 5.4%. So the absorption of AY has little effect on the fluorescence quenching of BSA and can be ignored. The reduction of the fluorescence intensity is mainly caused by the interaction between BSA and AY.

Fluorescence quenching, the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions, is usually classified into two mechanisms, static quenching and dynamic quenching according to their differing dependence on temperature and viscosity[17]. Dynamic quenching is attributed to the collisional encounters between the quencher and the fluorophore during the excited state. Static quenching is due to the formation of a nonluminescent ground-state complex between the fluorophore and quencher[18]. Both mechanisms can lead to a decrease of the fluorescence intensity. The temperature increase has different impacts on the two types of quenching constants. On the one hand, the higher the temperature, the larger the diffusion coefficients, therefore, higher temperature will lead to a larger dynamic quenching constant. On the other hand, the stability of the complex is decreased at higher temperatures; consequently, the values of the static quenching will be smaller[18–20]. To elucidate the