Chinese Chemical Letters Vol. 11, No. 3, pp. 251–254, 2000 

# The Photochemical Study of HSA and BSA with Resonance Light-Scattering and Fluorescence Spectra

Hong LIANG*, Xing Can SHEN, Fang LI, Zhi Liang JIANG

Institute of Bioinorganic Chemistry, Guangxi Normal University,Guilin 541004

**Abstract:** The resonance light-scattering (RLS) of human serum albumin (HSA) and bovine serum albumin (BSA) is reported for the first time, and applied to study photochemical reaction of HSA and BSA. The fact of photocrosslinking self-association effect in HSA and BSA solutions is identified by the enhancement of RLS. The fluorescence quenching at about 350 nm and 700 nm proves that tryptophan (Trp) residues are one of the photochemical activity sites in HSA and BSA molecules. The Rayleigh scattering (RS) spectra of HSA and BSA that were neglected in fluorescence spectra before are found at about 296 nm, 592 nm and 888 nm for the first time, and are of adventageous to studying the aggregation of HSA or BSA. The possible photochemical reaction mechanism is also proposed.

**Keywords**: Photochemical reaction, photocrosslinking self-association effect, resonance light-scattering, serum albumin.

In recent years, people have paid close attention to the physiological harms induced by ultraviolet (UV) irradiation. The serum albumin, which constitutes 60% of blood plasma, has very important physiological functions. Therefore, to study their photochemical reaction is of great significance. The metal ions, little molecules and medicines *etc* interacting with HSA or BSA have been reported [1,2,3,4], but it has not been reported about using RLS to study the photochemical reaction of HSA or BSA.. The RLS [5,6,7,8] is a new method put forward recently, and is of great advantageous to analysis of biochemical molecules.

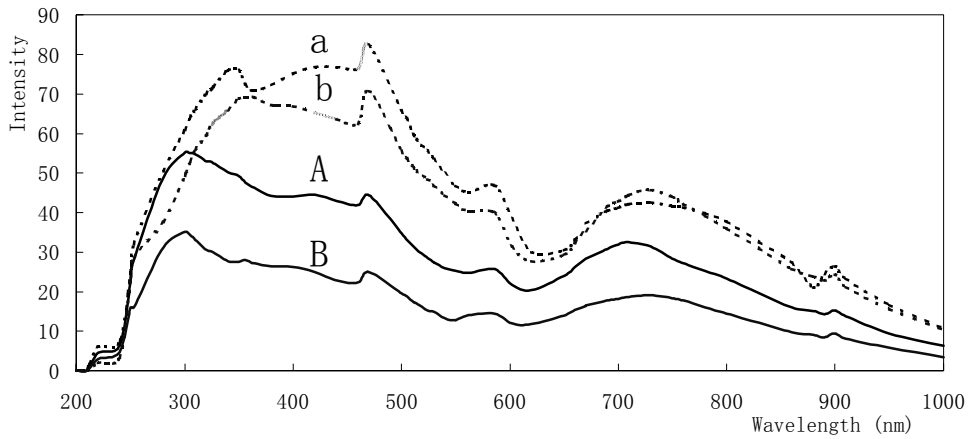
**Experimental:**

HSA, BSA and Trp are all electrophoresis grade reagents. All solutions containing 0.1 mol/ L NaCl and 0.1 mol/ L Tris-HCl (pH=7.43$\pm$0.02) were prepared with deionized water. The photochemical reactions were processed in photochemical reaction device [9] made by ourselves. The RLS data were obtained by scanning the excitation and emission monochromators ($\Delta \lambda$ =0.0 nm) with a shimadzu RF-540 spectrofluorophotometer (Kyoto Japan), within the wavelength region from 200 nm to 1000 nm. The excitation and emission slits were set to 10 nm.

As shown in **Figure 1**, there are five characteristic peaks in the RLS of HSA or BSA, two high intensity peaks at about 296 nm and 470 nm, a broad peak at about 690 nm, and two shoulder peaks at 590 nm and 900 nm respectively. The intensities of these

peaks are enhanced greatly after photochemical reaction (**Figure 1.** a and b). Meanwhile, the peaks at 300 nm shift to 350 nm. The blank tests of 0.1 mol/ L NaCl and 0.1 mol/ L Tris-HCl buffer solution and Trp solution show no evidence of enhancement under the same condition. According to the principle of RLS [5,6,7], the significant enhancement of RLS will occur if the biological macromolecules are aggregated. Therefore it can be concluded that there is photocrosslinking self-association effect in HSA and BSA dilute solutions. We also found that the enhancement of RLS is directly proportional to the concentrations of HSA or BSA solutions, the UV-irradiation time and intensity.

**Figure 1.** RLS a, b (---) are RLS of HSA, BSA with UV-irradiation respectively. A, B (—) are RLS of HSA, BSA respectively. The concentrations of HSA, BSA are both $1.0\times10^{-5}$mol/ L.

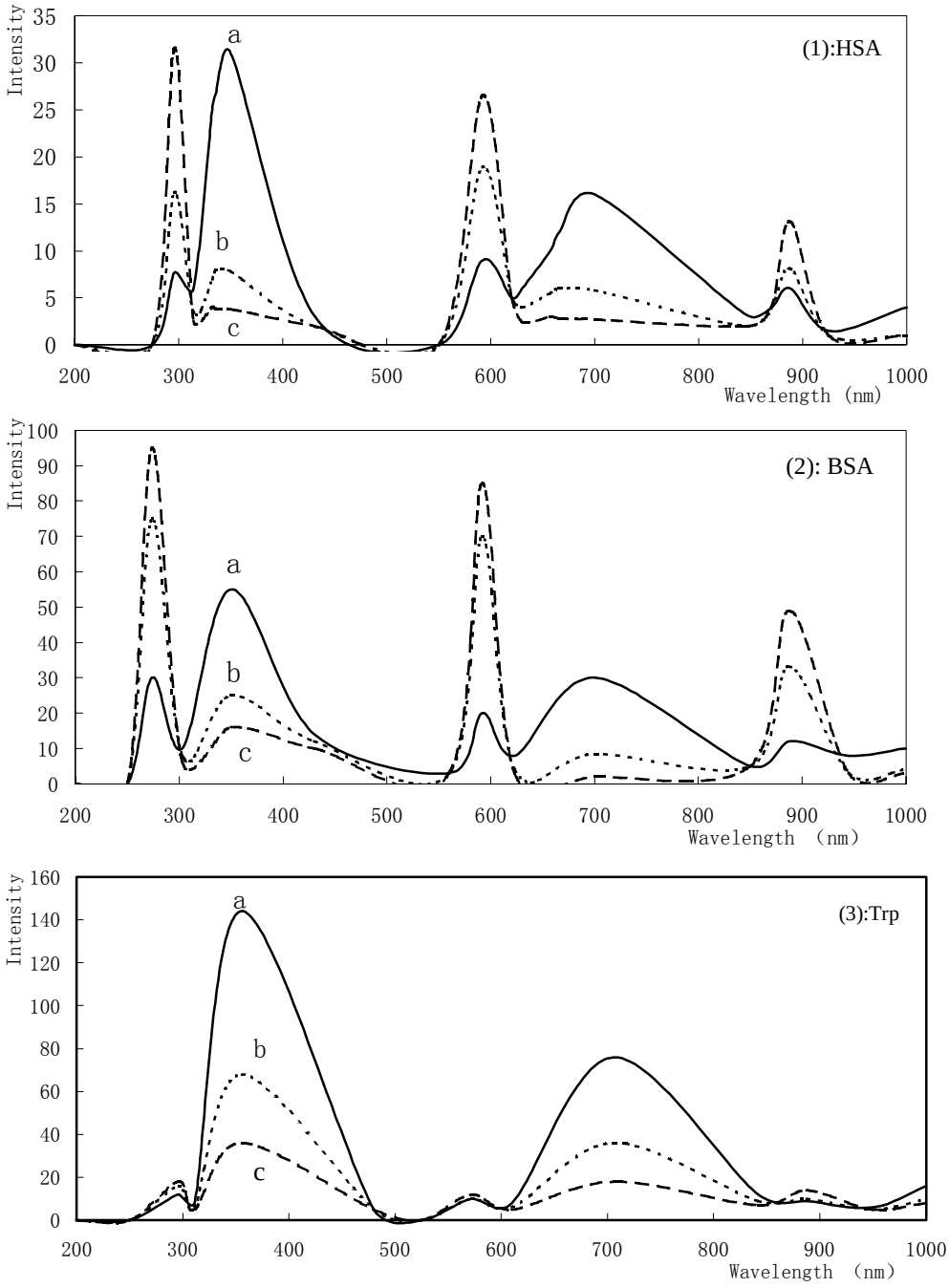



As the (214-Trp) residue in HSA and the (213-Trp, 134-Trp) residues in BSA, Trp-residues are the intrinsic fluorescence sites, and characterized significantly by $\lambda_{ex}/\lambda_{em}$ = 296/350 nm [2,3,10,11]. With $\lambda_{ex}$=296 nm, scanning the emission wavelength region from 200 nm to 1000 nm, we found that except the emission peaks at 350 nm, there are characteristic emission peaks at about the 296 nm, 592 nm, 700 nm and 888 nm (**Figure 2.** (a)). The intensities of fluorescence spectra of HSA, BSA, or Trp solutions are decreased at 350 nm and 700 nm after 5 min UV-irradiation (**Figure 2.** (b)), and become lower after 10 min UV-irradiation (**Figure 2.** (C)). This fact indicates that Trp-residues are one of the photochemical activity sites in HSA or BSA. The behaviors of the peaks at 700 nm are the same as those at 350 nm, whereas the intensities of former are lower than the latter. Considering 700 nm is double 350 nm, it is inferred that the peaks at 350 nm and 700 nm are the first and second fluorescence emission peaks, respectively[12].

The emission peaks of HSA and BSA at 296 nm, 592 nm and 888 nm are different from those at 350 nm and 700 nm. After UV-irradiation the peaks at 296 nm, 592 nm and 888 nm were not decreased in the intensity, but become even much higher. However, there is little change of Trp at 296 nm, 592 nm and 888 nm after UV-irradiation (**Figure 2.** (3)). This fact can be used to distinguish Trp form HSA or BSA. The behaviors of the peaks at about 296 nm, 592 nm and 888 nm are the same, and the relations of these peaks

are as follows: λ em = λ ex, λ em = 2 λ ex, and λ em = 3 λ ex. So these peaks are inferred to be the first, second, and third RS peaks respectively[12].

**Figure 2.** Fluorescence spectra a (—): HSA, BSA and Trp solutions without UV-irradiation; b (---): UV-irradiation for 5 min; c (— —): UV-irradiation for 10 min. The concentrations of HSA, BSA and Trp solutions are all $1.0\times10^{-5}$mol/ L



The mechanism of RS is similar to that of RLS [8], so it can be proved further that the photocrosslinking association effect exists in HSA or BSA molecules by the enhancement of RS.

We infer that the number of fluorescence emission peaks and RS peaks is more than that we have observed. The relations of these unobserved peaks are as following: $\lambda$ em = n $\lambda$ $em_0$ (350 nm), or $\lambda$ em = n $\lambda$ ex (296 nm), respectively, and the intensities of these peaks are reduced in order. But the peaks at about 296 nm for RS and 350 nm for fluorescence are the most sensitive. It makes possible to provide a sensitive way to distinguish HSA and BSA and to analyze the aggregate degree of them.

With Hitachi UV-3400 spectrophotometer, the absorption spectra of HSA, BSA and Trp show that the intensities of absorption enhance too, but the characteristic peaks at about 280 nm do not shift after UV-irradiation. The IR studies of solid photochemical reactions of Trp and BSA indicate that the structure of chemical bond almost has no change after photochemical reaction. From above experimental results, we can infer that Trp residues are probably one of the photochemical targets in HSA or BSA molecules, and the photochemical products of Trp are probably aqueous electrons ($e_{aq}$). The $e_{aq}$ can disconnect -S-S- to $-RS^-$, RS • , and induce -CONH to form Kety group $-C(OH)NH^-$. The $e_{aq}$ probably transfer to the amino residues such as His, Met, Phe *etc*, and other unsaturated residues[13]. These active groups all can be the sites of cross-linking and association with other molecules. But the photochemical reaction of HSA or BSA macromolecules with 584 or 581 amino acid residues must be very complex. The photochemical mechanisms and related physiological effect need further studies.

## Acknowledgment

This project is supported by the National Natural Science Foundation of China (NO. 29961001) and the Foundation for Talents Striding across the Century of Guangxi.

## Reference

1. H. Liang; X. J. Wang; B. G. Xing, *et al.*, *Chinese Sci. Bull.*, **1998**, *43*, 404.
2. H. Liang; B. G. Xing; Q. X. Wu; *et al.*, *Acta Chimica Sinica*, **1999**, *57*, 161.
3. B. Z. Zhang; W. Q. Wang; R. R. Yuang, *Acta Chimica Sinica*, **1994**, *52*, 1213.
4. C. Q. Ma; K. A. Li; F. L. Zhao; S. Y. Tong, *Acta Chimica Sinica*, **1999**, *57*, 389.
5. R. F. Pasternack; C. Bustamante, *et al.*, *J.Am.Chem.Soc.*, **1993**, *115*, 5393.
6. R. F. Pasternack; P. J. Colling, *Science*, **1995**, *269*, 935.
7. Y. F. Li; C. Z. Huang; X. L. Hu, *Chinese J. Anal. Chem.*, **1998**, *26*, 1508.
8. S. P. Liu; Q. Liu; F. L. Zhong; *et al.*, *Anal Chim Acta*, **1999**, *379*, 53.
9. Z. L. Jiang; X. H. Liu, *et al.*, *Anal. Chim. Actc*, **1997**, *354*, 359.
10. P. S Yang; P. Yang; *et al.*, *Prog. Biochem. Biophys.*, **1992**, *19*(2), 110.
11. G. B. Ma, *et al.*, *Chinese Biochem. J.*, **1990**, *17*(4), 290.
12. G. Z. Zheng, *et al.*,*Flourescence Analysis*, Beijing: Science Press, **1975**, P66.
13. S. J. Li, *et al.*, *The principle and application of macromolecules photochemistry*, Shanghai: Fudan University Press, **1993**, P426.

Received 28 June 1999