Polym. Bull. (2011) 67:1907–1915
DOI 10.1007/s00289-011-0577-x

ORIGINAL PAPER

Fluorescence study of interaction between an anionic conjugated polyelectrolyte and bovine serum albumin

Yani Wang · Jie Dong · Chuanzhen Liu · Biqing Bao · Lianhui Wang · Xiaowei Zhan · Huai Yang · Guojie Wang

Received: 16 March 2011 / Revised: 6 July 2011 / Accepted: 10 July 2011 / Published online: 19 July 2011
© Springer-Verlag 2011

Abstract The interaction between an anionic conjugated polyelectrolyte, poly[9,9-bis(3′-butyrate)fluoren-2,7-yl] sodium (BBS-PF), and bovine serum albumin was investigated by fluorescence spectroscopy. The emission of BBS-PF was effectively quenched by BSA with a quenching constant K_{SV} of 3.1×10^7 L/mol when BSA was at nanomolar concentrations, but the emission increased when the concentration of BSA was at micromolar level. The excitation band of BBS-PF blue-shifted when the emission was quenched where the negatively charged BSA induced the aggregation of BBS-PF, yet the excitation band of BBS-PF red-shifted when the emission increased where the BSA acted as a surfactant and formed hydrophobic interaction with BBS-PF. BBS-PF could also quench BSA through energy transfer by resonance with a quenching constant K_{SV} of 1.1×10^6 L/mol. The emission band changes of BSA reflected the conformation transitions of BSA from class II to class I and the binding of BBS-PF with BSA made the BSA more folded.

Keywords Fluorescence · Biosensor · Conjugated polyelectrolyte · Protein · Probe

---

Y. Wang · J. Dong · H. Yang · G. Wang (✉)
School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, China
e-mail: guojie.wang@mater.ustb.edu.cn

C. Liu
Beijing BOE Display Technology Co., Ltd, Beijing 100176, China

B. Bao · L. Wang
Laboratory of Advanced Materials, Fudan University, Shanghai 200433, China

X. Zhan
CAS Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

Introduction

Conjugated polyelectrolytes (CPEs), which are π-conjugated polymers with ionic side chains and can bind nucleic acid or protein surfaces through multivalent electrostatic interactions, have attracted much attention as optical probes in biomacromolecular detection [1–4]. Three types of signal transduction mechanisms of CPEs for biosensing have been reviewed as förster resonance energy transfer or electron transfer, analyte-induced aggregation of CPEs, and analyte-induced conformational changes of CPEs. Bovine serum albumin is a typical protein for research study because of its stability, water solubility, and versatile binding capacity. The interaction between the serum albumin and surfactants, photosensitizing agents and drugs has been studied extensively [5–9]. The binding of these small molecules to BSA is through ionic and hydrophobic interactions to the protein. The interaction of proteins such as BSA with polyelectrolytes has also attracted considerable attention in the polyelectrolyte-mediated protein adsorption [10–13]. However, few studies have been carried out by fluorescence on the interaction between the polyelectrolyte and the protein which are like-charged. Here we report the interaction between an anionic CPE and negatively charged bovine serum albumin and their fluorescence was explored.

Experimental section

Materials

All materials and reagents used in this work are commercial products of high grade employed as received without further purification unless otherwise stated. Bovine serum albumin and the sodium phosphate buffer (pH 7.5) were purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China).

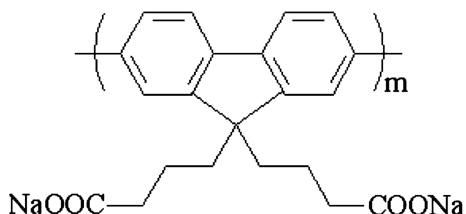
Characterization

UV–Vis absorption and fluorescence spectra were measured using a Shimadzu UV-3150 spectrophotometer and a Shimadzu RF-6301PC spectrophotometer, respectively. All optical measurements of BBS-PF (1.0×10^{-7} M) were performed in 10 mM sodium phosphate buffer at pH 7.5 at room temperature (around 25 °C). In this work, the sodium phosphate buffer (pH 7.5) was used as media to study the interaction of BBS-PF and BSA.

Results and discussion

The anionic CPE, poly[9,9-bis(3′-butyrate)fluoren-2,7-yl] sodium (BBS-PF), was synthesized by Suzuki coupling reaction, followed by hydrolysis [14]. Scheme 1 shows the chemical structure of BBS-PF, the number-average molecular weight

Scheme 1 Chemical structure of BBS-PF



(M_n) and distribution of which are 1.38×10^4 and 3.2, respectively, measured by gel permeation chromatography using polystyrene as a standard and tetrahydrofuran as the eluant. The absorption and emission spectra of BBS-PF in phosphate buffer (pH 7.5) are shown in Fig. 1. The absorption and emission bands are centred in the region of 375 and 415 nm, respectively. The BSA used here is a polyanion, since its isoelectric pH is 5.4 and the experiment is carried out in phosphate buffer at pH of 7.5. The absorption and emission bands of BSA are centred in the region of 278 and 344 nm, respectively, shown in Fig. 1, which are attributed to the absorption and emission of the tryptophan residues in BSA [15].

High-efficiency fluorescence quenching of a conjugated polyanion by cytochrome *c* has been reported [16], which is attributed to a combination of photoinduced electron transfer and the formation of bound complexes between the cationic and anionic polyelectrolytes. The anionic BSA can also quench the fluorescence of the anionic polyelectrolyte BBS-PF at low concentrations. The emission spectra of BBS-PF in the presence of BSA at different concentrations are shown in Fig. 2a and b. The emission intensity decreased when the concentration of BSA increased from 0 to 8.0×10^{-8} M, shown in Fig. 2a. Effective quenching is observed at low concentrations of 10^{-9} M and less effective quenching occurs at concentrations of 10^{-8} M, which can be seen from the Stern–Volmer plot for BBS-PF with BSA, shown in Fig. 2c. The quenching constant K_{SV} can be obtained by the Stern–Volmer equation [17],

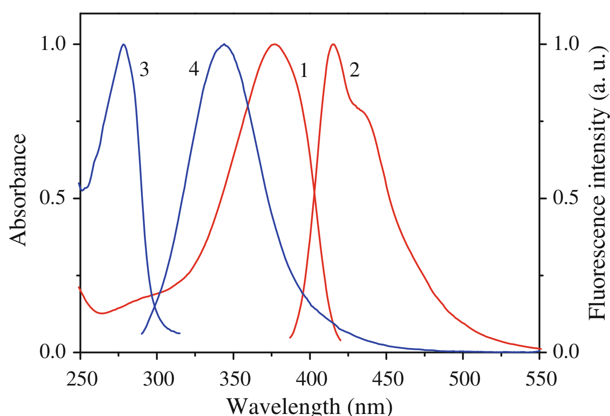


Fig. 1 UV–vis absorption and emission spectra of BBS-PF (*curve 1* and *2*) and BSA (*curve 3* and *4*) in phosphate buffer at pH 7.5, excitation at 375 nm for BBS-PF and excitation at 280 nm for BSA

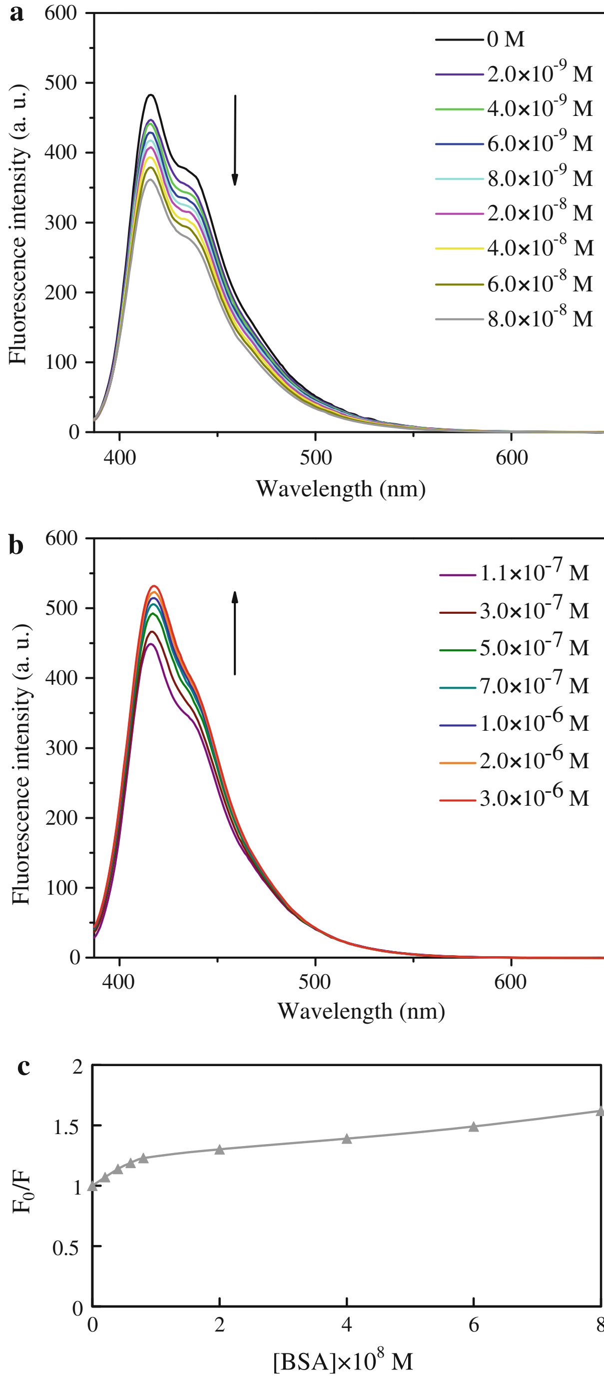


Fig. 2 **a**, **b** Emission spectra of BBS-PF ([BBS-PF] = 1.0×10^{-7} M) in the presence of BSA at different concentrations, excitation at 375 nm; **c** F_0/F plot for BBS-PF with BSA

$$F_0/F = 1 + K_{sv}[Q]$$

where F_0, F are the fluorescence intensity of the conjugated polymer before and after addition of a given molar concentration of quencher $[Q]$, respectively. The quenching constant K_{SV} for BSA is 3.1 × 10^7 L/mol at the low concentrations of 10^{-9} M, which is less than that of cytochrome *c* (3.2 × 10^8 L/mol) [16]. The less quenching might be due to its lack of electron transition from BBS-PF to BSA and lack of counter ions to form bound complexes. When the concentration of BSA increased from 8.0 × 10^{-8} to 3.0 × 10^{-6} M, the emission increased and exceeded its original intensity. The excitation spectra of BBS-PF reveal the different interaction between the CPE and BSA. At nanomolar concentrations of BSA, the excitation bands blue-shifted a little, however, the bands red-shifted when the

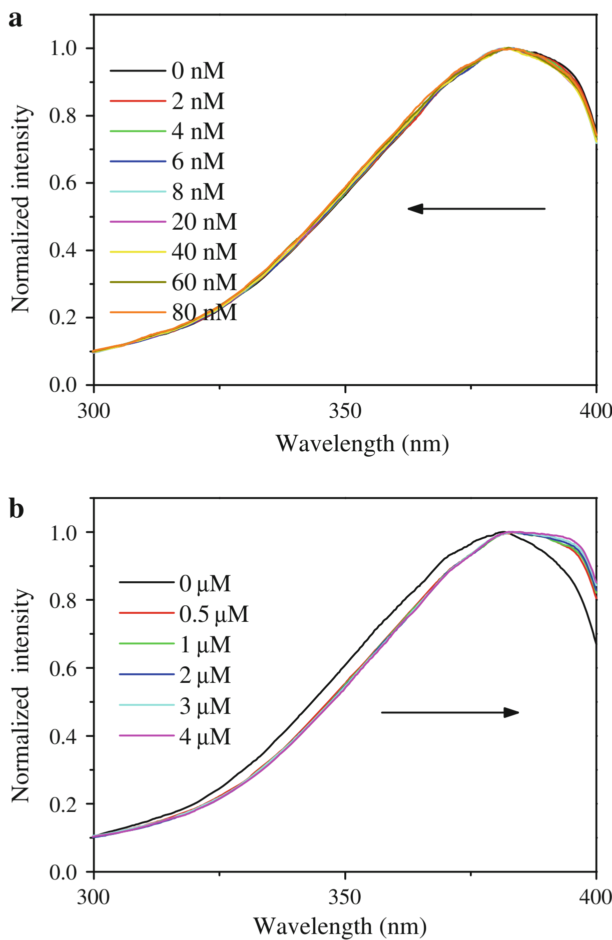


Fig. 3 **a** Excitation spectra of BBS-PF for 416 nm emission with different concentrations of 0, 2, 4, 6, 8, 20, 40, 60, and 80 nM of BSA; **b** excitation spectra of BBS-PF for 416 nm emission with different concentrations of 0, 0.5, 1, 2, 3, 4 μM of BSA

amount increased to micromolar concentrations, shown in Fig. 3a and b. The blue-shift indicates a π-stacking and interchain interaction of BBS-PF by the addition of proteins, as resembles the aggregation of MEH-PPV in solvent [18]. The

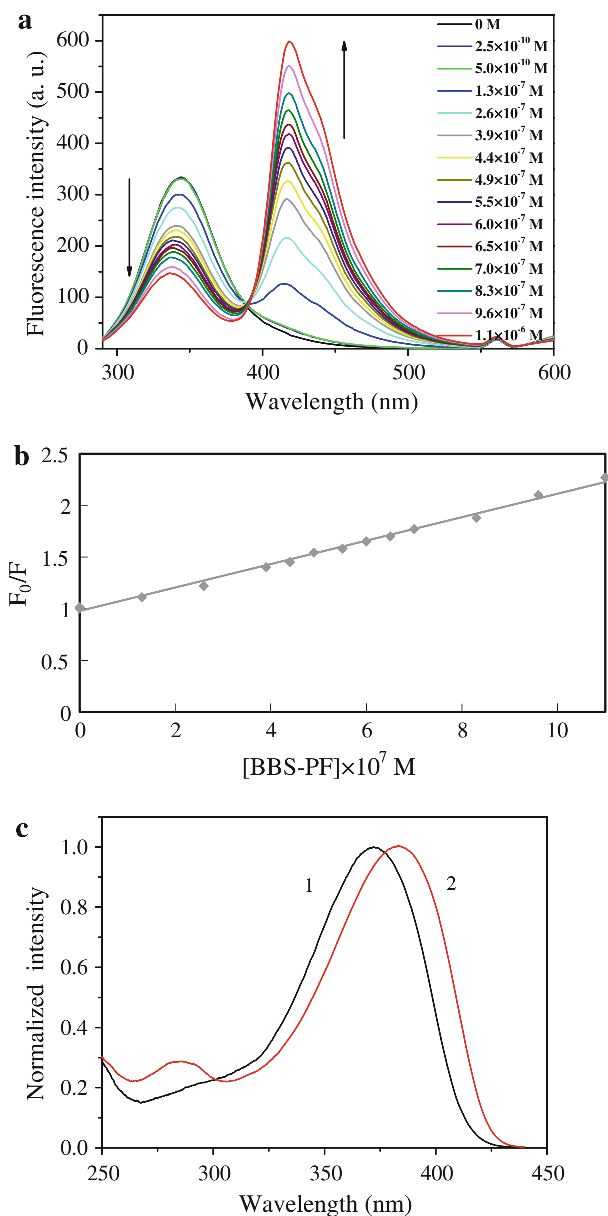


Fig. 4 **a** Emission spectra of BSA ([BSA] = 1.7 × 10^{-5} M) in the presence of BBS-PF at different concentrations, excitation at 280 nm; **b** F_0/F plot for BSA with BBS-PF; **c** excitation spectra of BBS-PF ([BBS-PF] = 1.0 × 10^{-7} M, curve 1) and the BBS-PF/BSA complex ([BBS-PF] = 1.0 × 10^{-7} M, [BSA] = 5.0 × 10^{-7} M, curve 2) for 450 nm emission

aggregation induced by the negatively charged BSA, results in the interchain interaction between the conjugated polymers, which in turn results in fluorescence quenching due to π-stacking of the backbones of the polymers [19]. At micromolar concentrations of BSA, the red-shift indicates that the aggregation of BBS-PF in the solvent becomes weak, resulted from the formed complex with the BSA surfactant, where the negative charge does not play a significant role [20]. The hydrophobic interactions of BSA with BBS-PF weaken the aggregation of BBS-PF in the system and the fluorescence increases.

The interaction between BBS-PF and BSA was further investigated by the fluorescence of BSA with different concentrations of BBS-PF. The emission intensity of BSA decreases with the increase of the concentration of BBS-PF, shown in Fig. 4a. The Stern–Volmer plot shows the quenching constant K_{SV} for BBS-PF is 1.1×10^6 L/mol, shown in Fig. 4b. Figure 4c shows the excitation spectra of the polymer BBS-PF and the BBS-PF/BSA complex for BBS-PF emission at 450 nm. For the excitation spectrum of the complex, a new band at 280 which is associated to the protein tryptophan appears, as can confirm the FRET from BSA to BBS-PF. Vekshin reported that the tryptophan fluorescence of BSA could be quenched by various anion, uncharged and cation dyes through energy transfer by resonance and excitation deactivation by the dye and probably by the protein itself which undergoes conformational transition upon sorption of the dye [21]. Here the absorption spectrum of BBS-PF overlaps the spectrum of tryptophan emission (shown in Fig. 1) and the emission of BBS-PF occurs when BSA is excited at 280 nm and the emission intensity increases with the increase of the concentration in BSA (shown in Fig. 4a). Thus, it can be concluded that the energy transfer by resonance from BSA to BBS-PF induces the quenching of BSA. The location of tryptophan residues in proteins has been classified into the following three classes according to their fluorescence parameters such as spectral maximum position (λ_m) and spectral band width ($\Delta\lambda$): class I, one buried in nonpolar regions of the protein (λ_m = 330–332 nm, $\Delta\lambda$ = 48–49 nm); class II, one on the surface which is in limited contract with water that is probably immobilized by bonding at the macromolecular surface (λ_m = 340–342 nm, $\Delta\lambda$ = 53–55 nm); class III, one on the surface which is exposed to water completely (λ_m = 350–353 nm, $\Delta\lambda$ = 59–61 nm) [15]. In this study, the emission peak of tryptophan shows blue-shift from 344 to 336 nm, and the emission spectral band width decreases from 56 to 53 nm when the concentration of BBS-PF increases from 0 to 1.1×10^{-6} M. The fluorescence parameter changes reflect the conformation transitions of BSA from class II to class I. The binding of BBS-PF to BSA could make BSA more folded through ionic or perhaps van der waals forces. A further investigation on the conformation transition should be performed by circular dichroism (CD) spectroscopy, as will be reported later.

Conclusions

In summary, we have investigated the fluorescence of the anionic CPE and BSA and their mutual influence. The fluorescence of CPE BBS-PF can be quenched by BSA at its nanomolar concentrations where BSA induces the aggregation of BBS-PF, yet

the fluorescence increases a little when the concentration increases to micromolar where the van de waals interaction decreases the aggregation. The fluorescence of BSA can also be quenched by the polyelectrolyte BBS-PF, which is attributed to the energy transfer by resonance. BBS-PF could induce the conformational transition of BSA to a more folded state.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant No. 21074010), Beijing Municipal Natural Science Foundation (Grant No. 2112029), Beijing Research Foundation for Excellent Talents (Grant No. 2010D009006000002), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China.

References

1. Feng FD, He F, An LL, Wang S, Li YL, Zhu DB (2008) Fluorescent conjugated polyelectrolytes for biomacromolecule detection. Adv Mater 20:2959–2964
2. Herland A, Inganäs O (2007) Cojugated polymers as optical probes for protein interactions and protein conformations. Macromol Rapid Commun 28:1703–1713
3. Jiang H, Taranekar P, Reynolds JR, Schanze KS (2009) Conjugated polyelectrolytes: synthesis, photophysics, and applications. Angew Chem Int Ed 48:4300–4316
4. Hong Y, Lam JW, Tang BZ (2009) Aggregation-induced emission: phenomenon, mechanism and applications. Chem Commun 4332–4353
5. Chakraborty T, Chakraborty I, Moulik SP, Ghosh S (2009) Physicochemical and conformational studies on BSA-surfactant interaction in aqueous medium. Langmuir 25:3062–3074
6. Gentili PL, Ortica F, Favaro G (2008) Static and dynamic interaction of a naturally occurring photochromic molecule with bovine serum albumin studied by UV-visible absorption and fluorescence spectroscopy. J Phys Chem B 112:16793–167801
7. Ding F, Pan H, Li ZY, Liu F, Sun Y (2009) Spectroscopic studies on the binding of barbital to bovine serum albumin. J Lumin 129:650–655
8. Han XL, Mei P, Liu Y, Xiao Q, Jiang FL, Li R (2009) Binding interaction of quinclorac with bovine serum albumin: a biophysical study. Spectrochim Acta part A 74:781–787
9. Pan XR, Liu RT, Qin PF, Wang L, Zhao XC (2010) Spectroscopic studies on the interaction of acid yellow with bovine serum albumin. J Lumin 130:611–617
10. Wittemann A, Haupt B, Ballauff M (2006) Polyelectrolyte-mediated protein adsorption. Progr Colloid Polym Sci 133:58–64
11. Cooper CL, Dubin PL, Kayitmazer AB, Turksen S (2005) Polyeletrolyte-protein complexes. Curr Opin Colloid Interface Sci 10:52–78
12. Xia YQ, Chen EQ, Liang DH (2010) Recognition of single- and double-stranded oligonucleotides by bovine serum albumin via nonspecific interactions. Biomacromolecules 11:3148–3166
13. Pu KY, Liu B (2010) Fluorescence turn-on responses of anionic and cationic conjugated polymers toward proteins: effect of electrostatic and hydrophobic interactions. J Phys Chem B 114:3077–3084
14. Bao BQ, Yuwen L, Zhan XW, Wang LH (2010) Water-soluble hyperbranched polyelectrolytes with high fluorescence quantum yield: facile synthesis and selective chemosensor for Hg^{+} and Cu^{+} ions. J Polym Sci Polym Chem 48:3431–3439
15. Burstein EA, Vedenkina NS, Ivkova MN (1973) Fluorescence and the location of tryptophan residues in protein molecules. Photochem Photobiol 18:263–279
16. Fan CH, Plaxco KW, Heeger AJ (2002) High-efficiency fluorescence quenching of conjugated polymers by proteins. J Am Chem Soc 124:5642–5643
17. Turro NJ (1978) Modern molecular photochemistry. Benjamin Cummings, Menlo Park
18. Amrutha SR, Jayakannan M (2008) Probing the π-stacking induced molecular aggregation in π-conjugated polymers, oligomers, and their blends of *p*-phenylenevinylenes. J Phys Chem B 112: 1119–1129
19. Kim J (2002) Assemblies of conjugated polymers. Intermolecular and intramolecular effects on the photophysical properties of conjugated polymers. Pure Appl Chem 74:2031–2044

20. Kim IB, Dunkhorst A, Bunz UHF (2005) Nonspecific interactions of a carboxylate-substituted PPE with proteins. A cautionary tale for biosensor applications. Langmuir 21:7985–7989
21. Vekshin NL (1998) Efficiency of energy transfer by resonance in quenching of protein fluorescence by dyes. J Appl Spectrosc 65:304–307