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Polymer 47 (2006) 6496−6500

polymer

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Polymer Communication

π-Stacking of rhodamine B onto water-soluble polymers containing aromatic groups

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Received 16 May 2006; received in revised form 24 July 2006; accepted 24 July 2006

Abstract

Evidences of the π-stacking of rhodamine B onto poly(sodium 4-styrenesulfonate) are given by ^{1}H NMR spectroscopy. As a consequence of these $\pi-\pi$ interactions, changes on the diafiltration and UV−vis absorbance and fluorescence patterns are shown comparing water-soluble polymers containing aromatic rings as poly(sodium 4-styrenesulfonate) and poly(*N*-methacryloyl-5-aminosalicylic acid) with other polyanions that do not contain aromatic rings as poly(sodium vinylsulfonate) and poly(acrylic acid).

Keywords: Rhodamine B; Water-soluble polymers; $\pi-\pi$ Interactions

1. Introduction

Molecular association involving $\pi-\pi$ interactions occurs in many systems containing aromatic groups as nucleic acids, porphyrins, molecular clips, proteins, etc. [1−11]. These interactions present a short-range electrostatic character together with a hydrophobic contribution. They are one of the principal non-covalent forces governing molecular recognition and biomolecular structure. Several effects as a consequence of $\pi-\pi$ interactions in biological systems have been described in recent literature as a change on the hydrogen bonding capacity of DNA bases [9,10] or enzymatic catalysis via a raise of the pK_a of the substrate [11]. However, no such interactions are generally described in the literature for model systems composed of synthetic water-soluble polymers (WSP) and low molecular-weight molecules (LMWM) [12,13].

In the course of our investigations on the interactions between polyanions and LMWM, we have found that aromatic counterions such as triphenyltetrazolium chloride (TTC) and chlorpheniramine maleate (CPM) exhibit some resistance to the interaction cleaving effect of added simple electrolytes, when the polyanions bear aromatic groups [14,15]. As this cleaving effect is due to the shielding of long-range electrostatic forces, short-range interactions are assumed to be important in these systems.

Rhodamine B (RB) [16−18] is an interesting molecule that bears two perpendicular aromatic groups; it is zwitterionic at pH higher than 3.2 and concentration lower than 10^{-5} M, so we can expect that long-range electrostatic interactions are

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doi:10.1016/j.polymer.2006.07.059

minimized at these conditions, allowing the exploration of short-range interactions. On the other hand its spectral-luminescence properties make it useful for such studies. In a recent paper [19] we have observed that the apparent acid–base properties of this molecule change by interaction with poly(sodium 4-styrenesulfonate) (PSS), as well as its luminescence properties. Both a bathochromic shift in absorbance UV–vis spectroscopy and fluorescence quenching were found. Remarkably, the pK_a of RB is increased from 3.2 to approximately 4 by interaction with the polymer.

In this communication, we give evidence of the π-stacking of rhodamine B (RB) on polyanions containing aromatic groups such as PSS and poly(*N*-methacryloyl-5-aminosalicylic acid) (P5HMA). The corresponding results are compared with those obtained with poly(sodium vinylsulfonate) (PVS) and poly(acrylic acid) (PAA), polyanions that do not contain aromatic rings. For this purpose we use separation techniques (diafiltration) and spectroscopic techniques (UV–vis absorbance and fluorescence, and ^1H NMR spectroscopies).

2. Experimental section

2.1. Reagents

Commercially available PSS (Aldrich, synthesized from the *para*-substituted monomer, average Mw 70,000 for NMR studies and 200,000 for diafiltration studies), PVS (Aldrich), PAA (Aldrich), and RB (Sigma) were used. The synthesis of P5HMA was reported elsewhere [20]. The pH was adjusted with NaOH and HNO_3. The structures of the molecules are shown in Fig. 1.

2.2. Equipment

The unit used for diafiltration studies consisted of a filtration cell (Amicon 8010, 10 mL capacity) with a magnetic stirrer, a regenerated cellulose membrane with a MWCO of 10,000 Da (Biomax, 25 mm diameter), a reservoir, a selector, and a pressure source. The pH was controlled with a Quimis Q400M2 pH meter. UV–vis experiments were performed in a Unicam UV 500 spectrophotometer at room temperature and 1 cm of path length. Fluorescence was measured in a Kontron SFM25 spectrophotometer. ^1H NMR measurements were made in a JNM-Lamda500 (JEOL, 500 MHz) and in an AVANCE600 (Bruker, 600 MHz) spectrometers.

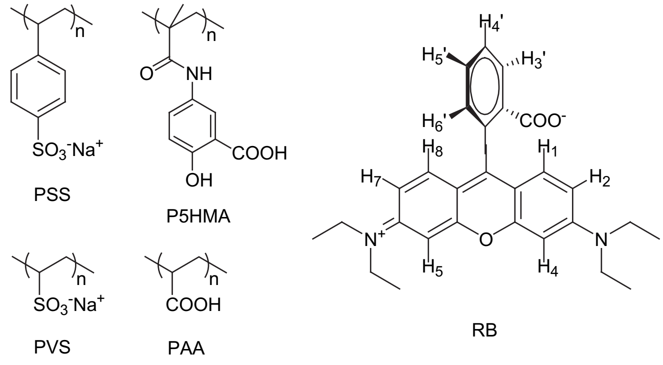

Fig. 1. Molecular structures.

2.3. Procedures

Conventional procedures have been followed. Details for diafiltration procedures may be found elsewhere [14,15,19,21]. Experimental conditions are provided in the figure captions.

3. Results and discussion

We detected some differences in the behavior of RB in the presence of both aromatic polymers and non-aromatic ones. The diafiltration profiles for all the RB–WSP systems show that the interaction of RB with PSS or P5HMA is more intense than with the non-aromatic analogs PVS or PAA (see Fig. 2). The features for diafiltration analyses and interpretation have been described elsewhere [14,21]. Diafiltration profiles are obtained by plotting the logarithm of the concentration of the LMWM in the filtrate ($c_{\mathrm{LMWM}}^{\mathrm{filtrate}}$) versus the filtration factor (F), defined as the ratio between the volume in the filtrate and the constant volume in the diafiltration cell. The rate of filtration of the LMWM given by the slope of the profile gives an idea of the distribution of molecules associated with the WSP or free in the solution, so that they can pass through the membrane pores. It can be seen that the rate of filtration of RB is much lower upon interaction with PSS or P5HMA than with PVS or PAA at pH 4.5. For the latter polymers, the interaction is so weak that the corresponding diafiltration profiles are fairly distinguishable from that of the blank experiment (made in the absence of any WSP); this is due to the minimization of long-range electrostatic interactions by the zwitterionic character of RB at these conditions. On the contrary, short-range interactions are enhanced by the polymeric aromatic rings.

A color change together with fluorescence quenching is directly visible to the eye upon interaction of RB with PSS or P5HMA, but not in the case of the other polymers. As can be seen in Fig. 3, the UV–vis absorbance spectra of aqueous 2.5×10^{-6} M RB at pH 4.5 are shifted as much as 12 nm (measured at λ_{max}) in the presence of excess of PSS ($\lambda_{max} = 566$ nm) and 8 nm in the presence of P5HMA ($\lambda_{max} = 562$ nm). On the contrary, the absorption band is not shifted at all in the presence of excess of PVS or PAA ($\lambda_{max} = 554$ nm). On the other hand, fluorescence quenching of a 2×10^{-6} M RB solution, measured at the maximum emission frequency (580 nm) after excitation at 552 nm, is significantly noticeable in the presence of 10 times PSS or P5HMA, but not in the case of PVS or PAA as can be seen in Fig. 4, where the relative fluorescence intensities are given considering the absorbance at the excitation frequency.

In order to be able to attribute to π–π interactions these differences on the behavior of polyanions bearing aromatic groups and their correspondent non-aromatic analogs, ^1H NMR spectroscopy studies have been done. NMR is a

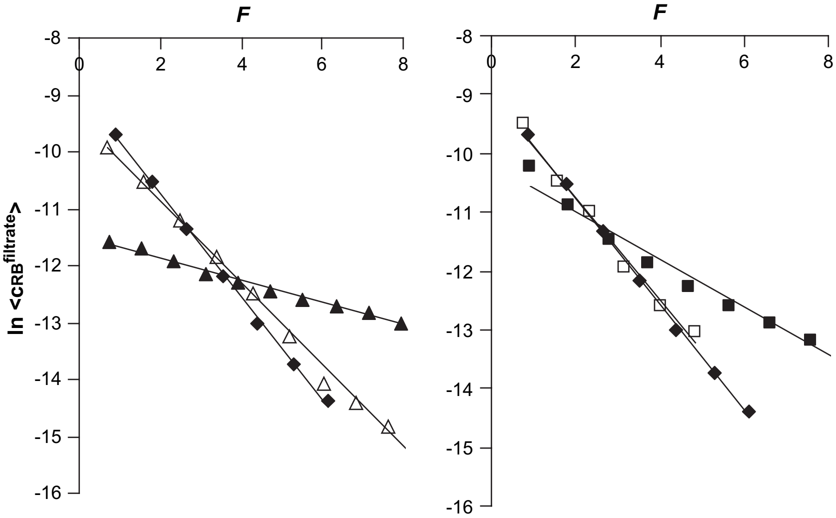

Fig. 2. Diafiltration profiles (data points; respective linear adjustments) of 10^{-4} M RB at pH 4.5 in the absence of any WSP (◆; $y = -0.90x - 8.94$) and in the presence of 2×10^{-4} M of the following WSP: PSS (▲; $y = -0.20x - 11.5$); PVS (△; $y = -0.72x - 9.46$); P5HMA (■; $y = -0.40x - 10.2$); PAA (□; $y = -0.88x - 8.98$).

useful tool to probe the stacking of aromatic rings. The effect of stacking the rings is to place one ring in the shielding cone of the second, resulting in upfield shifts of ^{1}H resonances for the stacked rings. We show here the aromatic region of some interesting spectra (Fig. 5). Fig. 5a corresponds to RB at pHs over (Fig. 5a1) and under (Fig. 5a2) its pK_a, respectively. According to previous works [18] the assignments of the signals to the protons outlined in Fig. 1 are given. Note that protonation of RB produces a downfield shift of H3′, H4′, H5′, and the equivalent H4H5, while H1H8 are shifted upfield. The signals for the RB protons broadened in the presence of the aromatic polymer (see Fig. 5b1 and b2) at pH 7.5 and 3.2, respectively. The aromatic region of the spectrum of PSS alone is shown in Fig. 5d. Although in the presence of PSS RB is more prone to protonate, as evidenced by the change in its pK_a [19] and the downfield shift of the H3′ signal, all the other RB signals are shifted upfield, as well as the polymer band centered at 6.5 ppm. All these changes indicate π-stacking of RB onto PSS. The most affected proton is H6′, whose band undergoes a considerable upfield shift of c.a. 0.6 ppm. For comparison, the spectrum of RB in the presence of PVS at pH 7.5 is also shown in Fig. 5c, with no significant shifts of RB signals. NOESY experiments have been done in order to demonstrate the proximity of the aromatic rings. As can be seen in Fig. 6 NOE effects are observed between PSS and RB aromatic protons at pH 3.2 and 7.5. The chemical shifts of the aliphatic protons of RB are also affected by the stacking of RB onto PSS and the signals are shifted upfield. The corresponding chemical shifts, in ppm, are: a1: 3.43 (CH_2), 1.10 (CH_3); a2: 3.44, 1.10; b1: 3.25, 0.90; b2: 3.26, 0.88.

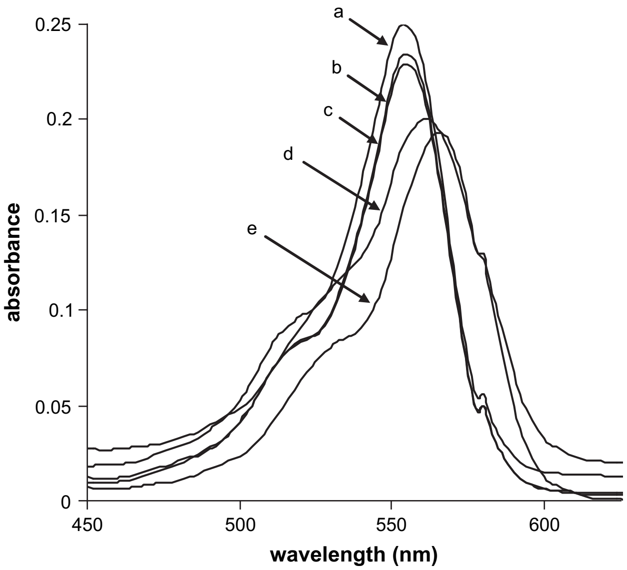

Fig. 3. UV–vis spectra of 2.5×10^{-6} M RB at pH 4.5 in the absence of any WSP (a) and in the presence of 5×10^{-5} M of PAA (b), PVS (c), P5HMA (d), and PSS (e).

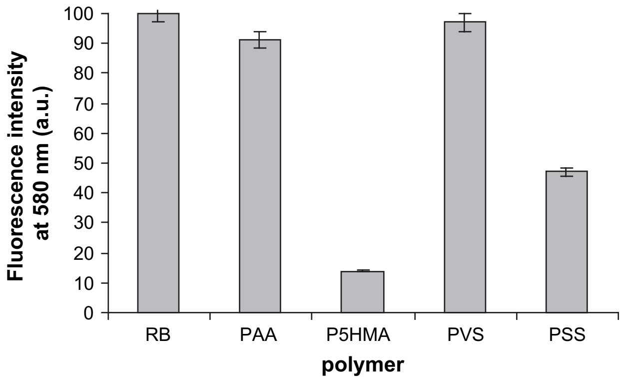

Fig. 4. Fluorescence intensity measured at 580 nm relative to the corresponding emission absorbance at 552 nm of a 2×10^{-6} M RB at pH 4–4.5 in the presence of 2×10^{-5} M of different WSP in arbitrary units.

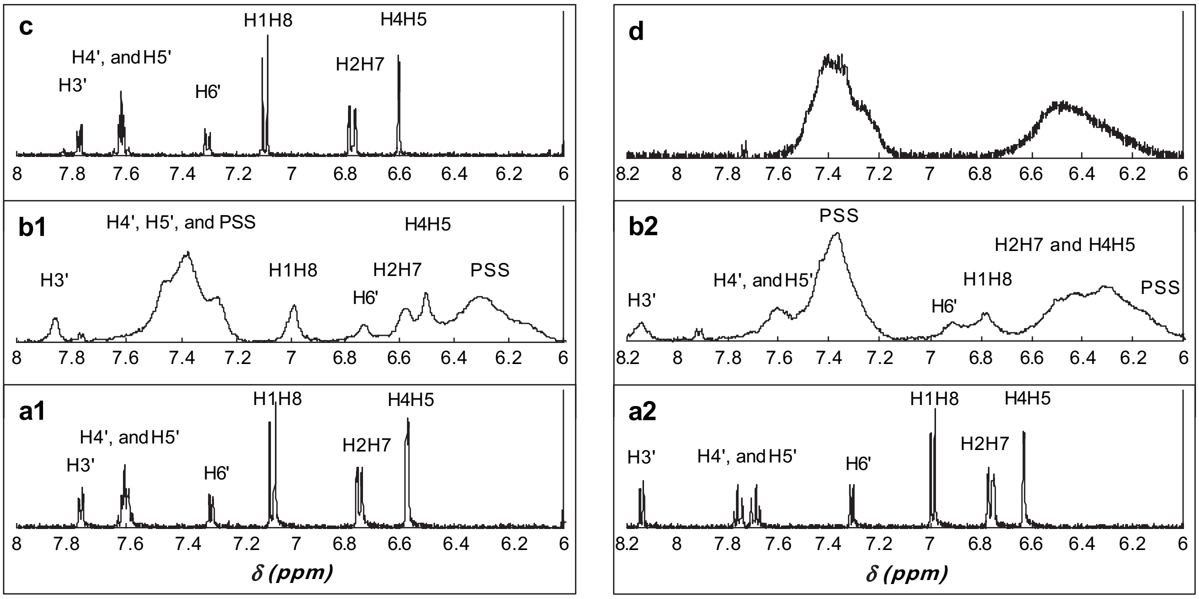

Fig. 5. ^{1}H NMR spectra (500 MHz) in D_2O of RB 10^{-3} M at pH 7.5 (a1), RB 10^{-3} M at pH 2 (a2), RB 10^{-3} M in the presence of PSS 10^{-2} M at pH 7.5 (b1), RB 10^{-3} M in the presence of PSS 10^{-2} M at pH 3.2 (b2), RB 10^{-3} M in the presence of PVS 10^{-2} M at pH 7.5 (c), and PSS 10^{-2} M (d).

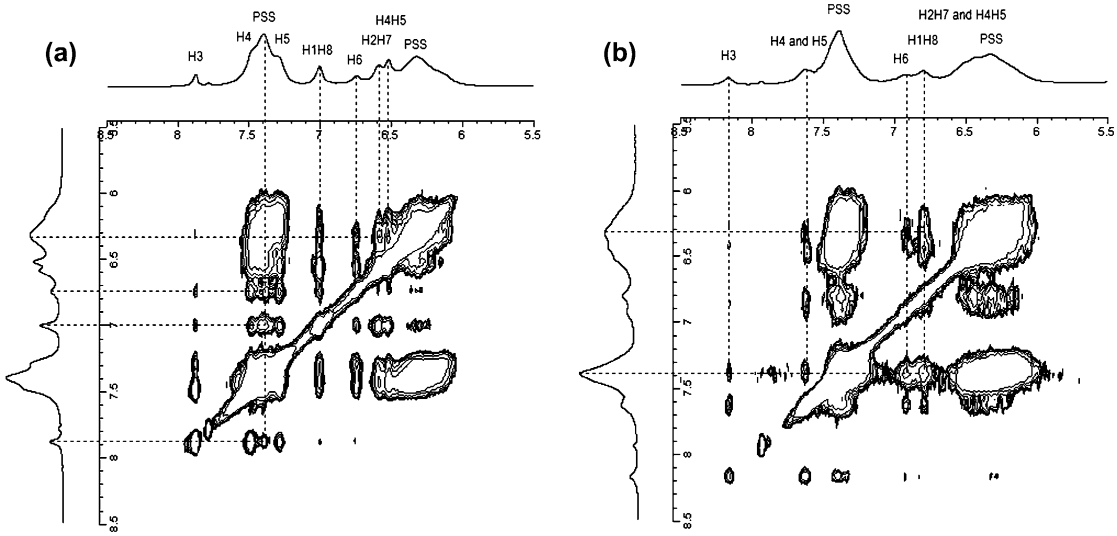

Fig. 6. ^{1}H NMR NOESY spectra (600 MHz) in D_2O of RB 10^{-3} M in the presence of PSS 10^{-2} M at pH 7.5 (a) and pH 3.2 (b).

4. Conclusions

Upon the simple observations shown here we can conclude that π–π interactions take place between RB and negatively charged WSP containing aromatic rings. These interactions produce particular effects that are not observed in other systems dominated by long-range electrostatic interactions, as a change on the acid–base properties of the LMWM or changes on its spectral-luminescence properties. The possibility of modulating both the pK_a of LMWM and their responses to light in laboratory systems by means of supramolecular π–π interactions constitutes an interesting area of study of non-covalent interactions with projections in both applied chemistry and theoretical chemistry.

Acknowledgement

The authors thank Fondecyt (Grant Nos. 1030669 and 1060191) and the 21COE program "Practical Nano-Chemistry" at Waseda University for MEXT, Japan for financial support.

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