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Novel cyanine dyes as fluorescent pH sensors: PET, ICT mechanism or resonance effect?

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Abstract

Cyanine and related polymethine dyes have been widely used as fluorescent and phosphorescent dyes for biological investigations. But the mechanism of this kind of cyanine dye as pH sensor is still not clear. In this paper, two groups of cyanine dyes were designed and synthesized, each including two asymmetry cyanine dyes and one symmetry dye. The UV–vis and fluorescent spectra of compounds **Ia–b** and **IIa–b** were recorded in phosphate buffers with different pH value. It was found that these compounds could be used as a kind of pH sensors with high pK_a value. The calculation on structures or conformations of compounds **Ia–b**, **IIa–b** and **Ic** before and after protonation revealed that the planar conjugations were different between non-protonic and protonic forms. It implied that the change from PET to ICT system was possible in the process of binding between polymethine cyanine dyes and proton.

Keywords: Cyanine dyes; Fluorescent pH sensors; PET mechanism; ICT mechanism; Resonance effect

1. Introduction

Cyanine and related polymethine dyes [1] have been employed in the photographic films, optical recording media, and as fluorescent and phosphorescent dyes for biological investigations [2,3]. The spectral properties of common cyanine dyes are only slightly sensitive to environment changes and they are generally considered as labeling dyes. However, it was reported recently that the heptamethine cyanine dyes with excited-state ICT mechanism could have large stocks shift and much stronger fluorescence [4] and diaminocyanine with photo-induced electron transfer mechanism was NO-sensitive fluorescence probe [5]. It was also reported that dipicolycyanine was a ratiometric fluorescent Zinc ion probe [6]. In 2000, Akkaya E.U.'s group reported a heptamethine cyanine–BAPTA conjugate, which signaled calcium [7]. In that year, Briggs et al. [8] also reported a pentamethine cyanine dye with a pK_a of 7.5, which was fluorescent when protonated, became non-fluorescent upon proton abstraction. They considered that the probe existed in two forms, as either the fluorescent cyanine dye or the complementary non-fluorescent base and suggested that the intense cyanine dye absorption and emission properties were largely due to the resonance effect between the two nitrogen atoms of the two indole rings *via* the conjugated pentamethine bridge, abstraction of a proton from this system destroyed this resonance, and subsequently leaded to the non-fluorescent base form (Fig. 1). But in our research work, we found that for different alkyl substituents on indole-ring carbon-3 of cyanines (Fig. 2), the fluorescent intensities of cyanines were totally different. If the intensity of fluorescence was only affected by the proton abstraction and resonance effect, it should have no obvious changes with different alkyl substituents in carbon-3 of indole-ring. So the mechanism of this kind of cyanine dyes as pH sensors was still not clear in details and need to be studied. In order to study the mechanism of this kind of dyes as pH sensors, two kinds of compounds were designed and synthesized, their energy-minimized conformations and UV–vis and fluorescent spectra of these compounds were also been studied.

2. Results and discussion

Two groups of cyanine dyes were designed and synthesized, each including two asymmetry cyanine dyes (Fig. 2) and one

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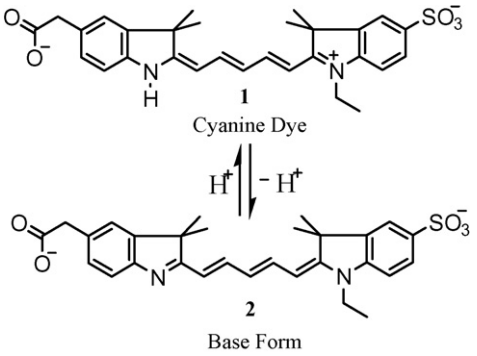


Fig. 1. The cyanine dye-base form of the pH sensitive fluorescent probe.

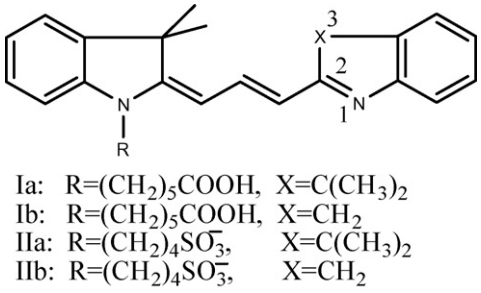
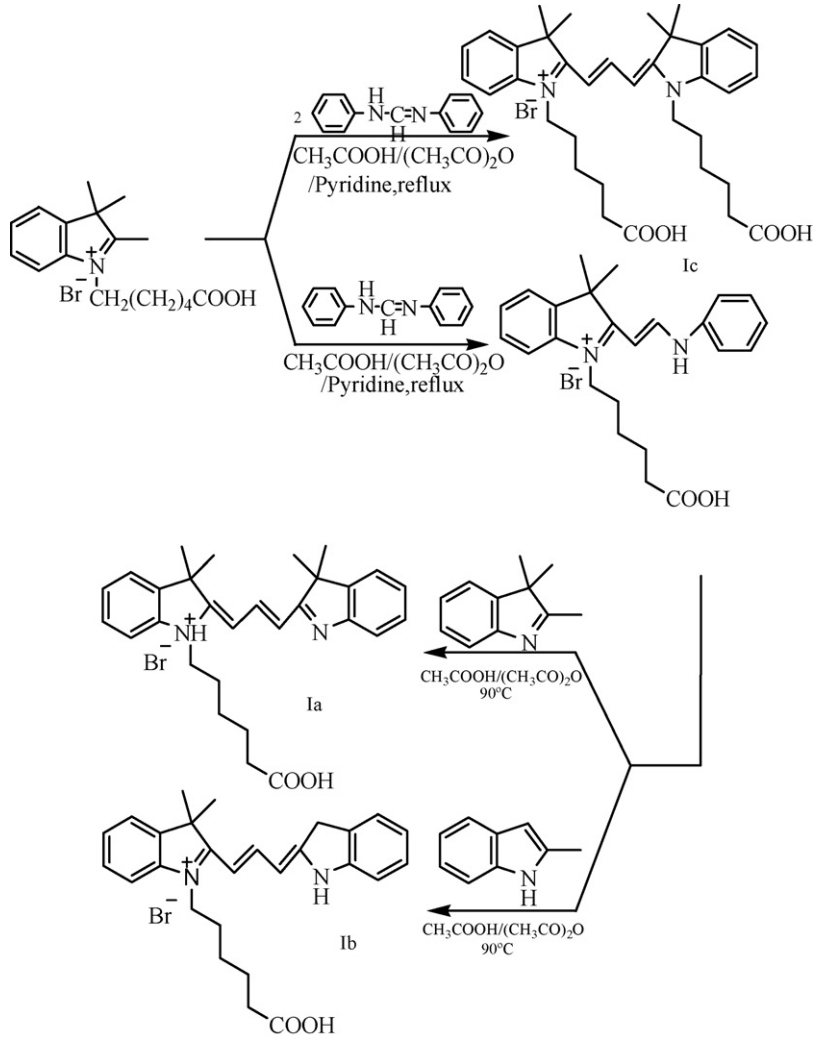


Fig. 2. The structure of asymmetrical cyanine.

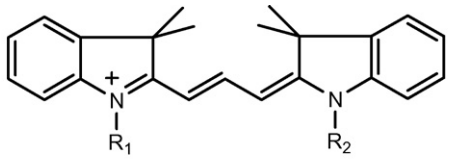
symmetry dye (Fig. 3), their spectra were also recorded and conformations were calculated.

2.1. The synthesis of cyanines

The sensors were synthesized by standard cyanine dye methods [1] (Scheme 1). *N*-Alkylate-2,3,3-trimethyl-3*H*-indol-1-ium and *N*,*N*′-diphenyl-formamidine were dissolved in acetic acid and refluxed for 6 h to form dark red solution. After purified by PTLC, yellow solid of *N*-alkylate-3,3-dimethyl-2-(2-phenylamino-vinyl)-3*H*-indolium was obtained. In the presence of acetic acid, acetic anhydride and pyridine, the yellow solid was condensed with *N*-alkylate-2,3,3-trimethyl-3*H*-indol-1-ium, 2,3,3-trimethyl-3*H*-indole and 2-methyl-1*H*-indole at 90 °C, respectively to form **Ia–c** and **IIa–c** accordingly. The products were purified by PTLC [9].



Scheme 1. Synthetic route of compounds **Ia–c**.



Ic: R_1=$(CH_2)_5COOH$, Br^-, R_2=$(CH_2)_5COOH$
IIc: R_1=$(CH_2)_4SO_3^-$, R_2=$(CH_2)_4SO_3H$

Fig. 3. The structure of symmetrical cyanine.

The fluorescence and UV–vis spectra of compounds **Ia–c** and **IIa–c** were recorded under phosphate buffers with different pH value (Figs. 4 and 5).

2.2. pH-dependent change of the UV–vis spectra

The observation of the UV–vis spectra of probes can be achieved in phosphate buffers of different pH. Only **Ia** and **IIa** demonstrate significant changes as shown in Fig. 4. It could be found that as the buffer solutions become acidic, the characteristic absorption maximum for **Ia** and **IIa** at 460 nm is greatly reduced and a new peak evolves at 548 nm. This phenomenon fits the results of conformation calculation with PC model (the details in the following section on calculation), that is, the chromophore system was changed. The absorption peak of **Ia** and **IIa** in 460 nm was attributed by the chromophore of indole cycle without *N*-alkylate conjugating with the poly-methine linker (non-protonic form), which was no fluorescence emission owing to quenching effect of PET from the other indole cycle with *N*-alkylate, while the peak in 548 nm was attributed by the whole coplanar system after protonation, which emitted strong ICT fluorescence with the increase of pH (Fig. 4).

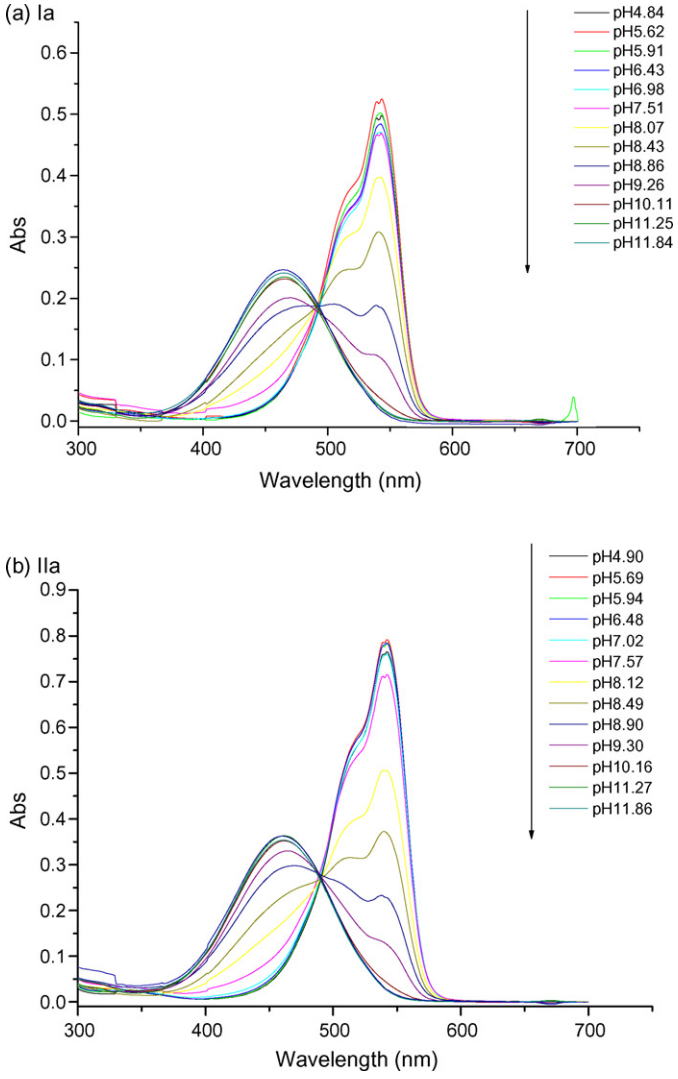


Fig. 4. UV–vis characteristics of **Ia** and **IIa** under different pH.

The above results suggested that for **Ia** and **IIa** there are two conformations in equilibrium with absorption at 460 and 548 nm, respectively, their ratio was just depended on the concentration of protons. But no obvious changes of conformations for **Ib**, **IIb**, **Ic**, and **IIc** were observed in absorption experiment before and after prononation.

2.3. pH-dependent change of the fluorescent properties

The fluorescent properties of these dyes can be found in Fig. 5 and Table 1. Equimolar solutions of the dyes were made up in phosphate buffers over a pH range of 4.84–11.84. The fluorescences of **Ib** and **IIb** at 525 nm are very weak ($\Phi_{methanol}$ = 0.005, 0.003), no intensity changes and obvious emission shifts were observed before and after protonation. The fluorescences of **Ic** and **IIc** at 565 nm are very strong ($\Phi_{methanol}$ = 0.216, 0.153) and no intensity changes and obvious emission shifts were observed in different pH buffers. The fluorescences of **Ia** and **IIa** before protonation are very weak as those of the **Ib** and **IIb** at 525 nm. After protonation, the fluorescences of **Ia** and **IIa** become very strong ($\Phi_{methanol}$ = 0.129, 0.164) and red-shift to 570 nm.

Fig. 6 illustrates the pH response of sensors **Ia** and **IIa** as afunction of I/I_{max} *versus* pH, where I was the measured fluorescent emission at that pH, and I_{max} was the maximum output of the probe. It showed high pK_a values of 8.92 and 8.97 for **Ia** and **IIa**, respectively, so that they might have potential application in basic environment.

Table 1
The spectral characteristics and quantum yields of compounds in different solvents

	Compounds					
	Ia	**Ia**	**Ib**	**IIb**	**Ic**	**IIc**
Methanol						
λ_{max}	550.3	549.5	493.9	495.4	548.7	548.5
$\lambda_{Ex\ max}$	553.0	554.5	496.3	500.3	549.6	548.8
$\lambda_{Em\ max}$	569.4	571.4	528.0	525.1	564.0	565.1
Δ_S	16.4	15.9	34.1	24.8	14.4	16.3
Φ	0.129	0.164	0.005	0.003	0.216	0.153
Water						
λ_{max}	540.3	540.3	490.4	489.5	544.5	543.6
$\lambda_{Ex\ max}$	546.7	547.5	494.1	494.1	546.0	545.3
$\lambda_{Em\ max}$	566.6	567.2	516.1	521.6	560.1	560.1
Δ_S	19.9	19.7	22.0	27.5	13.9	14.7
Φ	0.060	0.060	0.007	0.004	0.132	0.090

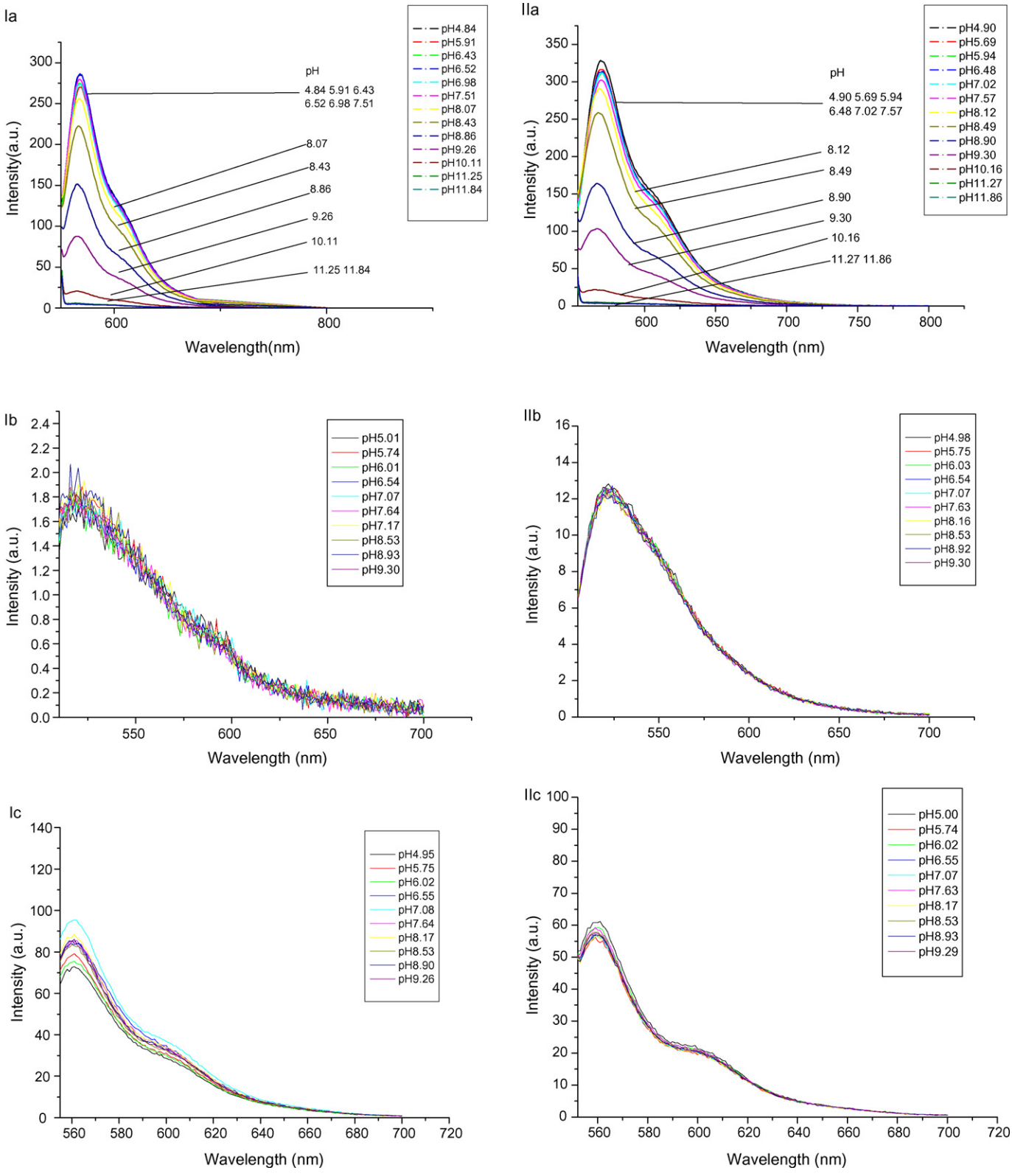


Fig. 5. Fluorescence characteristics of **Ia–c** and **IIa–c** under different pH.

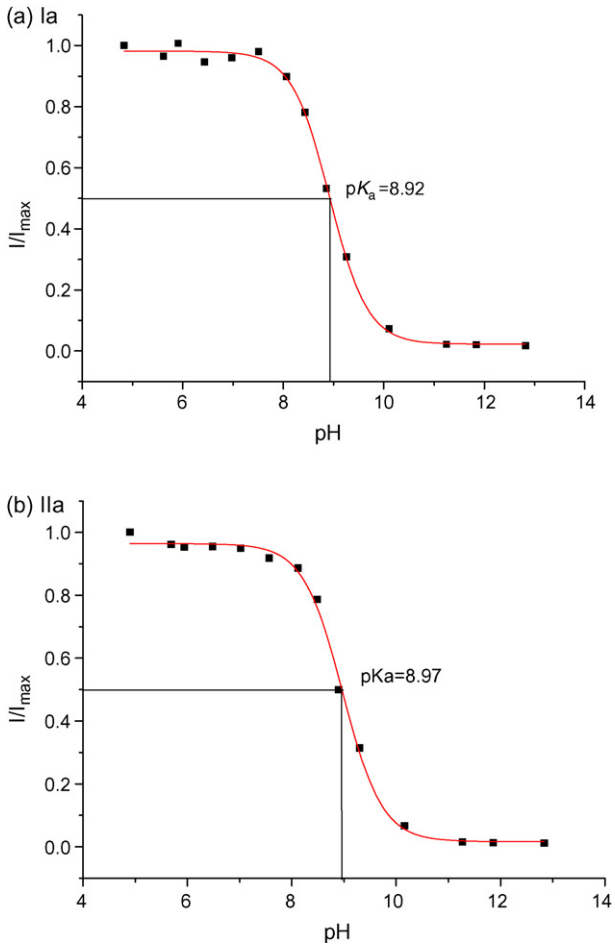


Fig. 6. pH dependence of **Ia** and **IIa** (I/I_{max}) at 295 K in phosphate buffers of differing pH.

2.4. The calculation on structures of cyanine dyes

As we know, there were two binding possibilities (form **1** and form **3**) between the proton and the cyanine dye (as shown in Figs. 1 and 7). We calculated the minimum energies of **1** and **3** using PCMODEL software. The minimum energy of the former is at 234.6 kJ/mol, while that of the latter is at 355.3 kJ/mol, which suggested that the possibility for the compound to be existed in the form **1** was higher than that in form **3**.

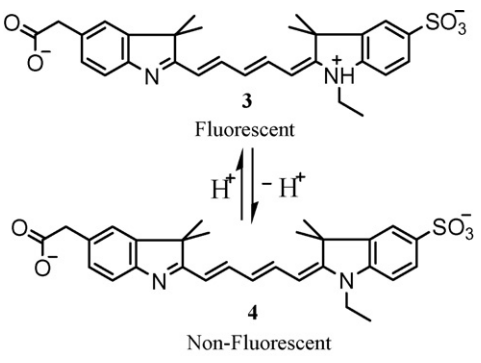


Fig. 7. The fluorescent and non-fluorescent forms of the pH sensitive florescent probe.

Table 2
The dihedral data of compounds in non-protonic and protonic forms

	Compounds			
	Ia	**Ib**	**Ia**	**IIb**
Non-protonic form	12.3	10.1	17.4	7.9
Protonic form	1.3	14.2	3.5	11.0

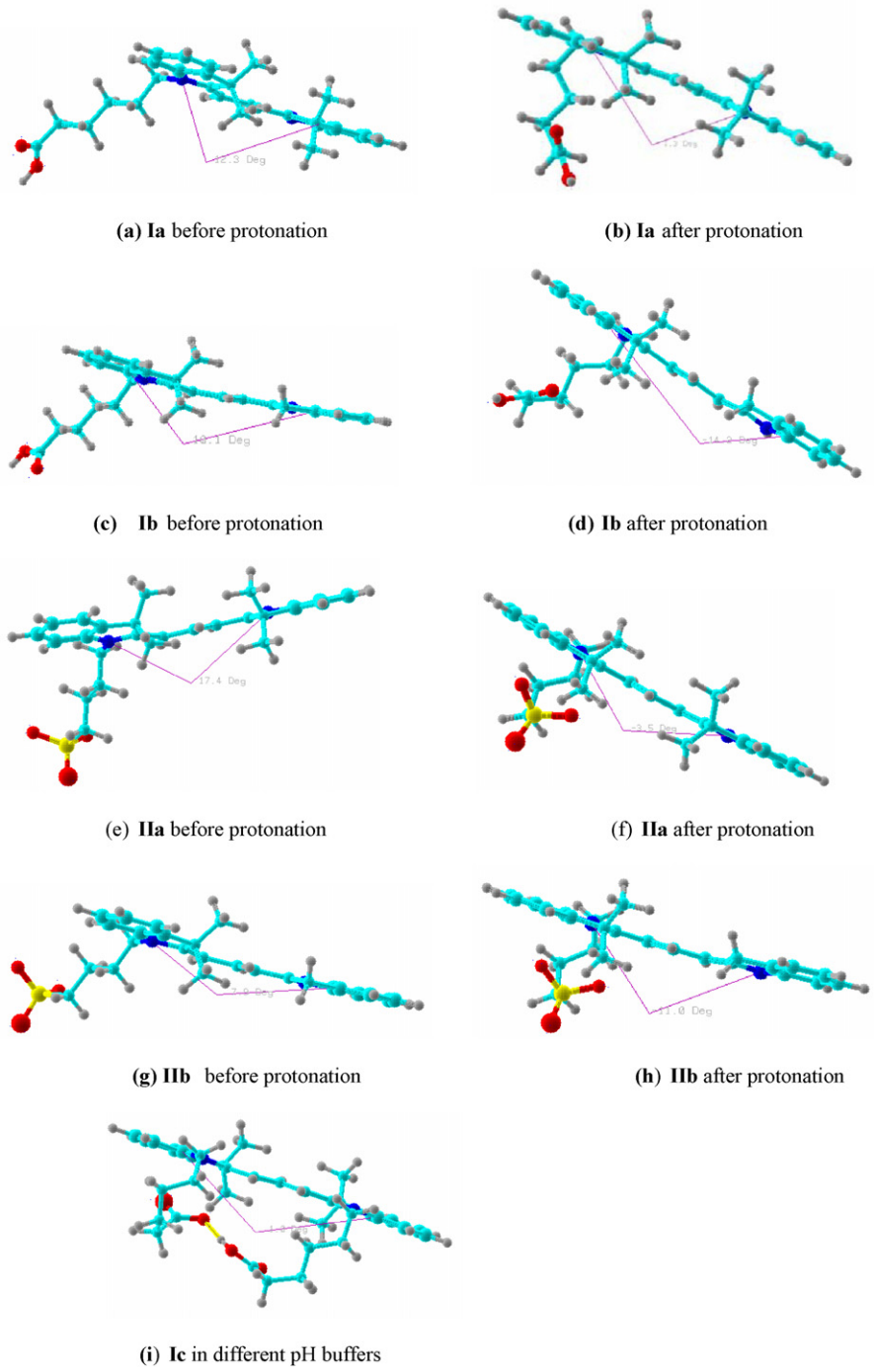
The calculation on the conformations of **Ia** and **IIa** showed that their conjugation areas seems to be separated as two parts and really not in one planar system before protonation, while they became one coplanar system after protonation (the dihedral data were shown in Table 2). However, the calculation on conformations of **Ib** and **IIb** revealed that their conjugation areas were always to be separated as two parts and not in one coplanar system before protonation or after protonation. Therefore, we conjecture the pH sensitive property of this pentamethine cyanine dyes (**Ia** and **IIa**) was related with the change from photo-induced electron transfer (PET) [3] to intramolecualr charge transfer (ICT) process. For most of cyanines, the electronic properties of two nitrogen atoms were not equal and much different, if the two cyclic indole planes were not coplanar, there existed two π electron systems. In this case, one nitrogen with high electronic density will probably serve as the electronic donor and the other with low electronic density as acceptor for PET. That is, the indole cycle containing nitrogen atom with low electronic density and conjugating with the poly-methine linker, might become chromophore, the other indole cycle with high electronic cloudy is an electron donor for PET to quench the fluorescence, which is also a potential receptor for proton (Fig. 8).

For **Ia** and **IIa**, the two indo-cycles became a new coplanar conjugation system after protonation, which induced stronger ICT effect from unprotonated nitrogen to the protonated nitrogen between the two indole moieties, and the original PET process was inhibited, so the fluorescent intensity increased greatly and the wavelengths of absorption and emission were red-shifted.

For **Ib** and **IIb**, their conjugation areas were always to be separated as two parts and not in one coplanar system before protonation or after protonation, therefore, PET process will possibly exist between two indole moieties. The only difference is the direction for PET, that is, the PET would happen before protonation from indole with alkyl group to the other indole, and after protonation from one indole to the protonated indole.

For **Ic** and **IIc**, there always are ICT process in different pH buffers, as their conjugation areas were always the same and coplanar. Therefore, **Ic** and **IIc** always showed strong fluorescence and no intensity changes and wavelength shift were observed.

Obviously, the structure, conformation and planarity of cyanine is depended on its symmetry, steric substituent and protonation. This investigation suggested that the basic structural requirements for cyanine as fluorescent pH sensor included an unsymmetrial cyanine with one non-alkylated N atom and some



(a) Ia before protonation

(b) Ia after protonation

(c) Ib before protonation

(d) Ib after protonation

(e) **IIa** before protonation

(f) **IIa** after protonation

(g) IIb before protonation

(h) IIb after protonation

(i) Ic in different pH buffers

Fig. 8. The energy-minimized conformations of **Ia–b**, **IIa–b** and **Ic** before and after protonation.

steric hindrance from the two substituents' groups of both C3 indo-rings of cyanine.

3. Conclusions

In summary, we suggested that the change from PET to ICT system is responsible for polymethine cyanine dyes' pH sensitivity. Meanwhile we observed a kind of pH sensors with high pK_a value, which is expected to be used as applicable probes for medicinal biology.

4. Experimental section

4.1. Materials and methods

Melting points were taken on a digital melting point apparatus WRS-1 made in Shanghai and it was uncorrected. Infrared spectra were recorded on a Nicolet FT-IR-20SX, mass spectra on a Hitachi M80, ^{1}H NMR on a Bruker AM-300 or AM-500 using TMS as an internal standard. Combustion analysis for elemental composition was done on Italy MOD. 1106 analyzer. Absorption

spectra were measured on Shimadzu UV-265, fluorescence spectra on a Hitachi 850.

4.2. 2,3,3-Trimethyl-3H-indole (**1**) [9]

2,3,3-Trimethyl-3*H*-indole (**1**) was prepared according to the literature [9]. GC–MS—*m*/*z*: 159 (M^+).

4.3. 2-Methylindole (**2**) [10]

2-Methylindole (**2**) was prepared according to the literature [10]. mp: 61–63 °C (lit. mp: 62 °C). GC–MS—*m*/*z*: 131 (M^+).

4.4. 1-(Carboxy-pentyl)-2,3,3-thimethyl-indolium; bromide (**3**) [10]

1-(Carboxy-pentyl)-2,3,3-thimethyl-indolium; bromide was prepared according to the literature [10].

4.5. 1-(δ-Sulfobuty)-2,3,3-thimethylindolium; betaine (**4**) [11]

1-(δ-Sulfobuty)-2,3,3-thimethyl-indolium; betaine (**4**) was prepared according to the literature [12], mp: 240 °C. ^{1}H NMR (500 MHz, D_2O): 7.80–7.60 (m, 4H, 4-H, 5-H, 6-H, 7-H), 4.5 (t, 2H, J = 7.5 Hz, 11-H), 2.95 (t, 2H, J = 7.6 Hz, 8-H), 2.14–2.07 (m, 2H, 10-H), 1.90–1.85 (m, 2H, 9-H), 1.55 (s, 6H, 13-H,14-H).

4.6. N,N′-Diphenylformamaidine (**5**) [12]

N,*N*′-Diphenylformamaidine was prepared according to the literature [12]. Yield: 60%. mp: 151–152 °C (lit. mp: 143 °C). GC–MS—*m*/*z*: 196 (M^+).

4.7. 1-(ε-Carboxy-pentyl)-3,3-dimethyl-2-2-benzen-amino-vinyl-3H-indolium,bromide (**6**)

To the solution of acetic acid (6 ml), compound **3** (0.7 g, 0.002 mol), compound **5** (0.09 g, 0.0022 mol) were added under argon atmosphere. The mixture refluxed 6 h, cooled, concentrated, washed with ether and silica gel purification (R_f 0.65, methanol:acetone 2:1, v/v) to give yellow product 6. ^{1}H NMR (500 MHz, CD_3OD): 8.60 (d, 1H, J = 11.0 Hz, 16-H), 7.30–6.95 (m, 9H, 4-H, 5-H, 6-H, 7-H, 17-H, 18-H, 19-H, 20-H, 21-H), 5.74 (d, 1H, J = 11.0 Hz, 15-H), 3.40 (t, 2H, J = 7.4 Hz, 8-H), 2.08 (t, 2H, J = 7.4 Hz, 12-H), 1.72–1.60 (m, 4H, 9-H, 11-H), 1.40–1.34 (m, 2H, 10-H), 1.09 (s, 6H, 13-H, 14-H). ESI-MS—*m*/*z*: 377.2 [M − Br + H], 399.2 [M − Br + Na].

4.8. 1-(δ-sulfobutyl)-3,3-dimethyl-2-(2-benzenaminovinyl)-3H-indolium; betaine (**7**)

Preparation and purification of this compound was accomplished following the procedure described for 6. Compound **4** (0.6 g, 0.002 mol), compound **5** (0.43 g, 0.0022 mol), silica gel purification (R_f 0.40, methanol:acetone 1:4, v/v) to give yellow product 7. ^{1}H NMR (500 MHz, CD_3OD): 8.59 (d, 1H, J = 11.0 Hz, 15-H), 7.60–7.20 (m, 9H, 4-H, 5-H, 6-H, 7-H, 16-H, 17-H, 18-H, 19-H, 20-H), 6.11 (d, 1H, J = 11.0 Hz, 14-H), 4.13–4.01 (m, 2H, 11-H), 2.95 (t, 2H, J = 7.4 Hz, 8-H), 2.01–1.80 (m, 4H, 9-H, 10-H), 1.69 (s, 6H, 12-H, 13-H).

4.9. 1-(ε-Carboxy-pentyl)-2-{3-[1-(ε-carboxy-pentyl)-3,3-dimethyl-1,3-dihydro-indol-2-ylidene]-propenyl}-3,3-dimethyl-3H-indolium; bromide (**Ic**)

To the solution of acetic acid (5 ml), anhydride acetic acid (1 ml), pyridine (1 ml), compound **3** (0.35 g, 0.001 mol), compound **5** (0.098 g, 0.0005 mol) were added under argon atmosphere. The mixture refluxed 2 h, cooled, concentrated, washed with ether and PTLC purification (R_f 0.50, methanol:acetone 4:1, v/v) to give red product. IR, ν(KBr, cm^{-1}): 3400, 2930, 1570, 1420. ^{1}H NMR (500 MHz, CD_3OD): 8.43 (dd, 1H, $J_1 = J_2$ = 13.4 Hz, 16-H), 7.50–6.72 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), 6.40 (d, 2H, J = 13.4 Hz, 15-H, 15′-H), 3.53–3.48 (m, 4H, 8-H, 8′-H), 2.12–2.07 (m, 4H, 12-H, 12′-H), 1.76–1.29 (m, 12H, 9-H, 10-H, 11-H, 9′-H, 10′-H, 11′-H), 1.20 (s, 12H, 13-H, 14-H, 13′-H, 14′-H). ESI-MS—*m*/*z*: 557.3 [M − Br + H], 579.2 [M − Br + Na].

4.10. 1-(δ-Sulfobutyl)-2-{3-[1-(δ-sulfobutyl)-3,3-dimethyl-1,3-dihydro-indo-l-2-ylidene]-propenyl}-3,3-dimethyl-3H-indolium; betaine (**IIc**)

Preparation and purification of this compound was accomplished following the procedure described for Id. Compound **4** (0.3 g, 0.001 mol), compound **5** (0.098 g, 0.0005 mol), silica gel purification (R_f 0.30, ethyl acetate:methanol 2:1, v/v) to give yellow product **IIc**. IR, ν(KBr, cm^{-1}): 3400, 3000, 1570, 1420. ^{1}H NMR (500 MHz, CD_3OD): 8.46 (dd, 1H, $J_1 = J_2$ = 13.4 Hz, 15-H), 7.44–7.18 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), 6.43 (d, 2H, J = 13.4 Hz, 14-H, 14′-H), 4.10 (m, 4H, 11-H, 11′-H), 2.82 (m, 4H, 8-H, 8′-H), 1.93–1.80 (m, 8H, 9-H, 10-H, 9′-H, 10′-H), 1.67 (s, 12H, 12-H, 13-H, 12′-H, 13′-H). ESI-MS—*m*/*z*: 645.3 [M + 2Na-H], 599.1 [M − H].

4.11. 6-((2E)-3,3-Dimethyl-2-((2E)-3-(3,3-dimethyl-3H-indol-2-yl)allylidene)indolin-1-yl)hexanoic acid (**Ia**)

To the solution of acetic acid (10 ml), anhydride acetic acid (10 ml), compound **6** (0.46 g, 0.001 mol), compound **1** (0.64 g, 0.004 mol) were added under argon atmosphere. The mixture was heated to 90 °C, reacted 22 h, cooled, concentrated, washed with ether and PTLC purification (R_f 0.70, ethyl acetate:methanol:acetic acid 400:150:1, v/v/v) to give red product **Ia**. IR, ν(KBr, cm^{-1}): 3400, 2930, 1570, 1490, 1450, 1360, 1190, 1150. ^{1}H NMR (500 MHz, CD_3OD): 8.20 (dd, 1H, J_1 = 14.1 Hz, J_2 = 12.3 Hz, 16-H), 7.29–6.95 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), 6.08 (d, 1H, J = 14.1 Hz, 15-H), 5.87(d, 1H, J_1 = 12.3 Hz, 10′-H), 3.82–3.74 (m, 2H, 8-H), 2.12 (t, 2H, J_1 = 7.4 Hz, 12-H), 1.98–1.56 (m, 6H, 9-H, 10-H, 11-H), 1.38 (s, 12H, 13-H, 14-H, 8′-H, 9′-H). ESI-MS—*m*/*z*: 443.2 [M + H], 465.5 [M + Na].

*4.12. 6-((2E)-3,3-Dimethyl-2-((2E)-3-(3-methyl-3H-indol-2-yl) allylidene)indolin-1-yl)hexanoic acid (**Ib**)*

Preparation and purification of this compound was accomplished following the procedure described for **Ia**. Compound **6** (0.46 g, 0.001 mol), compound **2** (0.52 g, 0.004 mol), PTLC purification (R_f 0.70, ethyl acetate:methanol:acetic acid 150:75:1, v/v/v) to give yellow product **Ib**. IR, ν(KBr, cm^{-1}): 3400, 2900, 1570, 1430, 1380. ^{1}H NMR(500 MHz, CD_3OD): 8.45 (dd, 1H, $J_1 = J_2 = 15.0$ Hz, 16-H), 8.40 (d, 1H, $J = 15.0$ Hz, 15-H), 8.03–7.25 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), 7.00 (d, 1H, $J = 15.0$ Hz, 8′-H), 3.84 (s, 2H, 3′-H), 3.56–3.47 (t, 2H, 8-H), 2.09 (t, 2H, 12-H), 1.92–1.48 (m, 6H, 9-H, 10-H, 11-H) 1.35 (s, 6H, 16-H, 17-H). ESI-MS—*m/z*: 415.3 [M + H], 437.2 [M + Na].

*4.13. 1-(δ-Sulfobutyl)-2-{3-[3,3-dimethyl-1,3-dihydro-indol-2-ylidene]-propenyl}-3,3-dimethyl-3H-indolium; betaine (**IIa**)*

Preparation and purification of this compound was accomplished following the procedure described for **Ia**. Compound **7** (0.4 g, 0.001 mol), compound **1** (0.64 g, 0.004 mol), silica gel purification (R_f 0.70, ethyl acetate:methanol 7:3, v/v) to give red product **IIa**. IR, ν(KBr, cm^{-1}): 3400, 2930, 1570, 1510, 1450, 1400, 1190, 1040. ^{1}H NMR (500 MHz, CD_3OD): δ8.35 (dd, 1H, $J_1 = 13.2$ Hz, $J_2 = 13.6$ Hz, 15-H), δ7.34–7.08 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), δ6.19 (d, 1H, $J = 13.2$ Hz, 14-H), δ6.12 (d, 1H, $J = 13.6$ Hz, 10′-H), δ4.00 (t, 2H, $J_1 = 8.0$ Hz, 11-H), δ2.80 (t, 2H, $J = 6.8$ Hz, 8-H), δ1.92–1.84 (m, 4H, 9-H, 10-H), δ1.63 (s, 6H, 12-H, 13-H) δ1.42 (s, 6H, 8′-H, 9′-H). ESI-MS—*m/z*: 487.1 [M + Na], 465.3 [M + H], 463.1 [M − H].

*4.14. 1-(δ-Sulfobutyl)-2-{3-[1,3-dihydro-indol-2-ylidene]-propenyl}-3,3-dimethyl-3H-indolium; betaine (**IIb**)*

Preparation and purification of this compound was accomplished following the procedure described for **Ia**. Compound **7** (0.4 g, 0.001 mol), compound **2** (0.52 g, 0.004 mol), silica gel purification (R_f 0.50, ethyl acetate:methanol 4:3, v/v) to give yellow product **IIb**. IR, ν(KBr, cm^{-1}): 3400, 2930, 1570, 1470, 1420, 1340, 1290, 1230, 1210, 1170, 1100, 1030. ^{1}H NMR (500 MHz, CD_3OD): 8.44 (d, 1H, $J = 15$ Hz, 15-H), 8.00–7.24 (m, 8H, 4-H, 5-H, 6-H, 7-H, 4′-H, 5′-H, 6′-H, 7′-H), 7.05 (d, 1H, $J = 15$ Hz, 14-H), 4.45 (s, 2H, 3′-H), 4.40 (t, 2H, $J = 7.7$ Hz, 11-H), 2.81 (t, 2H, $J = 6.4$ Hz, 8-H), 2.08–2.04 (m, 2H, 10-H), 1.96–1.92 (m, 2H, 9-H), 1.76 (s, 6H, 12-H,13-H). ESI-MS—*m/z*: 459.1 [M + Na], 437.1 [M + H], 435.1 [M − H].

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