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Water-soluble poly(*p*-phenylene) incorporating methoxyphenol units: Highly sensitive and selective chemodosimeters for hypochlorite

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ABSTRACT

A series of water-soluble poly(*p*-phenylene)s (PPPs), named N-PPPx (x = 10, 25 and 50), were directly synthesized by Suzuki coupling in aqueous solution. The structures of the polymers were characterized by ^{1}H NMR and elemental analysis. The polymers exhibit similar absorption and emission spectra with three absorption maxima at *ca.* 205, 290 and 350 nm, and emission maximum at 420 nm in phosphate buffer saline (PBS) solution. Upon addition of hypochlorite, N-PPPx shows a decrease of absorption band at *ca.* 350 nm and a fluorescent quenching. Compared to their model compound PMOPP, N-PPPx shows a significantly amplified fluorescent quenching. Moreover, the K_{sv} is decreased with the increasing content of methoxyphenol moieties in N-PPPx. In view of the sensitivity and selectivity, N-PPP10 and N-PPP25 are very promising polymeric fluorescent probes to hypochlorite under the aqueous condition.

1. Introduction

Water-soluble conjugated polymers (CPs) can combine the advantages of conventional polyelectrolytes and signal amplification of CPs, and thus offer unique potential in fluorescent probes [1–4]. Over the past several years, water-soluble CPs have been widely used in biosensors [5–10]. Recently, chemodosimeters have attracted considerable attention, however a few of chemodosimeters based on water-soluble conjugated polymers have been reported [11–15]. For example, Swager's group reported a poly(*p*-phenyleneethynylene) based chemodosimeter for fluoride ions by using fluoride triggered Si-O bond cleavage [11]; Wang's group reported a water-soluble cationic polyfluorene with boronate-protected fluorescein covalently linking to the polymer backbone for the detection of hydrogen peroxide and glucose in serum [14].

As a kind of reactive oxygen species (ROS), hypochlorous acid (HClO) plays a crucial role in our daily lives and host defense against invading pathogens [16,17]. In living organisms, hypochlorous acid is generated by the reaction of hydrogen peroxide with chloride ions under the catalysis of the heme enzyme myeloperoxidase (MPO), which is synthesized and secreted by activated phagocytes [18–21]. However, because of its high reactivity and non-specificity [22], excessive hypochlorous acid can lead to damage of host tissue that is implicated in a wide range of human diseases, such as kidney disease [23,24], atherosclerosis [25–28], and arthritis [29–31]. Unfortunately the detailed pathogenic mechanism is not fully understood, because of the lack of a feasible method for detecting hypochlorous acid.

Recently, a few probes for hypochlorous acid (HClO) have been reported based on its strong oxidation property [32–39]. For example, Nagano's group [32] and Libby's group [33] reported rhodamine- and fluorescein-based fluorescent probes for HClO, respectively, but the fluorescent probes involved in complicated synthesis; Ma's group also developed two fluorescent probes for HClO [34,35], which works in organic co-solvent system. Moreover, these probes are based on small molecule fluorophores, the sensitivity is still needed to be improved. Considering the signal amplification of conjugated polymers, it will be of significant interest to design a fluorescent polymeric probe for HClO.

Very recently, we have reported a dual-signaling chemodosimeter for HClO based on water-soluble *p*-methoxyphenol derivative probe PMOPP [40]. Based on this research, we designed and synthesized a series of water-soluble CPs by introducing *p*-methoxyphenol moieties into the poly(*p*-phenylene) backbone. We anticipated that the oxidation of *p*-methoxyphenol moieties on the copolymer backbone by HClO would induce an intramolecular charge transfer (ICT), and result in the changes of absorption and fluorescence quenching of the copolymer.

2. Experimental part

2.1. Materials

All chemicals were purchased from commercial sources and were used without further purification unless otherwise noted.

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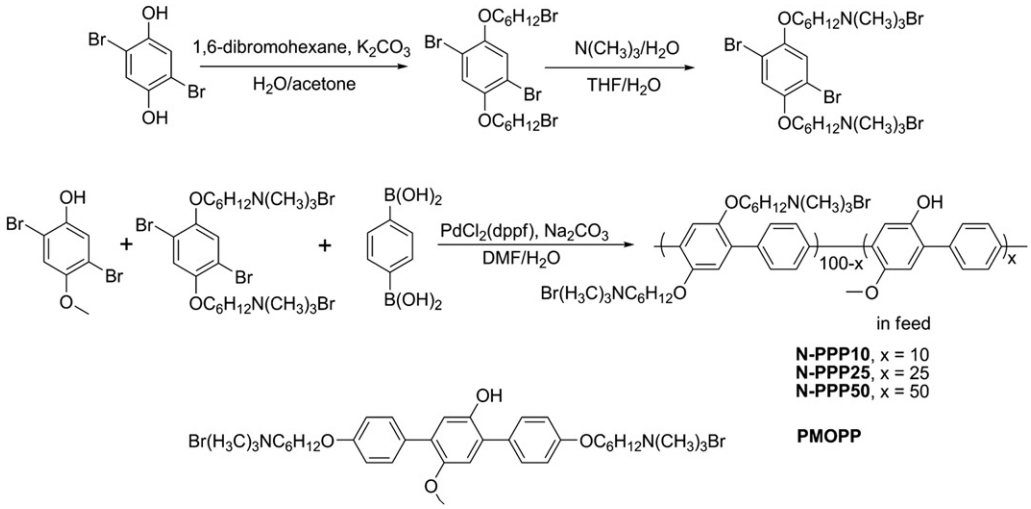


Scheme 1. Synthetic route of the polymers and structure of model compound PMOPP.

2,5-Dibromo-4-methoxyphenol was synthesized according to literature procedures [41]. Dimethylformamide (DMF) was purified by distillation from calcium hydride.

2.2. General methods

^{1}H NMR and ^{13}C NMR spectra were obtained in the indicated solvent with tertramethylsilane as an internal standard on a MECUYRVX300 spectrometer. Mass spectra were measured on a ZAB 3F-HF mass spectrophotometer. Liquid chromatography mass (LC-Mass) spectra were measured on a Waters ZQ-Mass electrospray ionizer (ESI). Elemental analyses of carbon, hydrogen, and nitrogen were performed on a Carlorerba-1106 microanalyzer. Thermogravimetric analysis (TGA) was performed under nitrogen with a NETZSCH STA 449C instrument in the temperature range from 25 to 800 °C at the heating rate of 10 °C min^{-1}. UV–vis absorption measurements were performed on a Shimadzu 2550 spectrophotometer. Fluorescence measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer. All the tests of optical response for ClO^- were carried out after a few seconds of the addition of hypochlorite solution. Testing solutions were prepared by adding 2 or 20 μL of the probe stock solution (5 mM) to 2 mL PBS buffer in a test tube and then adding an appropriate aliquot of each ROS stock solution. The absorption and fluorescence sensing of ROS was run immediately after the ROS were added.

2.3. Synthesis of 1,4-dibromo-2,5-bis(6-bromohexyloxy)benzene

A mixture of 2,5-dibromobenzene-1,4-diol (5.36 g, 20 mmol), 1,6-dibromohexane (12.4 mL, 80 mmol), potassium carbonate (10.00 g, 72 mmol), acetone (10 mL), and water (2.5 mL) was refluxed for 5 h, after which an additional potassium carbonate was added and stirring and reflux continued for 3 h. After filtration, the residue was washed with chloroform and the filtrate was combined. After evaporation of the solvent, excessive 1,6-dibromohexane was removed under reduce pressure, and then the residue was recrystallized from ethanol to afford 1,4-dibromo-2,5-bis(6-bromohexyloxy)benzene as a white solid. Yield: 7.38 g, 62%. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.08 (s, 2H), 3.96 (t, $J = 6.3$ Hz, 4H), 3.43 (t, $J = 6.9$ Hz, 4H), 1.90 (m, 4H), 1.80 (m, 4H), 1.53 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 150.3, 118.7, 111.4, 70.2, 34.1, 32.9, 29.2, 28.1, 25.4. EI-MS: *m/z* 590 (M^+). Anal. Calcd for $C_{18}H_{26}Br_4O_2$: C, 36.40; H, 4.41. Found: C, 36.09; H, 4.03.

2.4. Synthesis of 6,6′-(2,5-dibromo-1,4-phenylene)bis(oxy) bis(N,N,N-trimethylhexan-1-aminium) bromide

To a solution of 1,4-dibromo-2,5-bis(6-bromohexyloxy)benzene (5.94 g, 10 mmol) in tetrahydrofuran (THF) (50 mL) was added trimethylamine aqueous solution (20 mL, 200 mmol). After stirring for 0.5 h, H_2O (10 mL) was added to the mixture. Then additional trimethylamine aqueous (20 mL, 200 mmol) solution was added and the mixture was stirred for 48 h. The solvent was removed by rotary evaporation, and the crude product was recrystallized from ethanol to afford the product as a white solid. Yield: 6.62 g, 93%. ^{1}H NMR (300 MHz, D_2O, ppm): δ 7.26 (s, 2H), 3.96 (t, $J = 6.3$ Hz, 4H), 3.16 (t, $J = 8.4$ Hz, 4H), 2.94 (s, 18H), 1.66 (m, 8H), 1.41 (m, 4H), 1.26 (m, 4H). ^{13}C NMR (75 MHz, D_2O, ppm): δ 151.3, 118.9, 112.2, 70.3, 66.6, 53.8, 34.5, 33.0, 29.3, 28.4, 25.5. ESI-MS: *m/z* 275 ((M^{2+}-2Br)/2). Anal. Calcd for $C_{24}H_{44}Br_4N_2O_2$: C, 40.47; H, 6.23; N, 3.93. Found: C, 40.43; H, 5.74; N, 3.62.

2.5. Synthesis of N-PPP10, N-PPP25 and N-PPP50

General procedure for the copolymerization by Suzuki cross-coupling reaction: To a mixture of 6,6′-(2,5-dibromo-1,4-phenylene)bis(oxy)bis(N,N,N-trimethylhexan-1-aminium)

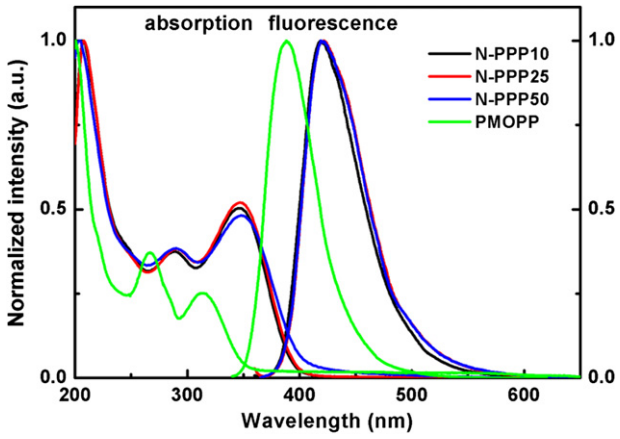


Fig. 1. Normalized UV–vis absorption and fluorescence spectra of the polymers and the model compound PMOPP in PBS solution. Excitation wavelength: N-PPPx, 350 nm; PMOPP, 320 nm.

Table 1
Absorption, emission and Stern-Volmer Constants (K_{sv}) of the N-PPPx and the model compound PMOPP for hypochlorite. Absorption and emission spectra were tested in PBS solution.

Compound	$\lambda_{max,\ abs}$ (nm)	$\lambda_{max,\ em}$ (nm)	K_{sv} (M^{-1})
N-PPP10	208, 289, 347	420	1.35×10^6
N-PPP25	208, 291, 347	420	1.18×10^6
N-PPP50	204, 290, 348	420	1.40×10^5
PMOPP	<200, 267, 314	388	1.00×10^5

bromide, 2,5-dibromo-4-methoxyphenol, 1,4-phenylenediboronic acid, and 3.0 mol% Pd(dppf)Cl_2 was added a degassed mixture of DMF and 0.2 M sodium carbonate aqueous solution (2:5 in volume). The mixture was vigorously stirred at 90 °C for 72 h. After the mixture was cooled down, the solvent was removed under reduce pressure. The crude product was purified by dialysis against Mill-Q water using a 3.5 kDa molecular weight cutoff dialysis membrane for 5 days. After evaporation of the solvent, the resulting polymer was obtained as deep brown powder.

N-PPP10: Yield: 550 mg, 47%. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 8.0 (br), 7.7–7.4 (br, 4H), 7.2–7.1 (br, 2H), 4.04 (br, 4H), 3.7 (br), 3.30 (br, 4H), 3.08 (br, 18H), 1.77 (br, 4H), 1.54 (br, 4H), 1.39 (br, 4H). Anal. Calcd for $(C_{30}H_{48}Br_2N_2O_2)_{0.9}$ $(C_{13}H_{10}O_2)_{0.1}$: C, 58.05; H, 7.61; N, 4.31. Found: C, 59.79; H, 8.71; N, 2.72.

N-PPP25: Yield: 440 mg, 42%. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 8.0 (br), 7.7–7.4 (br, 4H), 7.2–7.1 (br, 2H), 4.00 (br, 4H), 3.7 (br), 3.24 (br, 4H), 3.04 (br, 18H), 1.73 (br, 4H), 1.49 (br, 4H), 1.36 (br, 4H). Anal. Calcd for $(C_{30}H_{48}Br_2N_2O_2)_{0.75}(C_{13}H_{10}O_2)_{0.25}$: C, 59.37; H, 7.45; N, 4.03. Found: C, 61.80; H, 7.94; N, 2.75.

N-PPP50: Yield: 434 mg, 52%. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 8.0 (br), 7.7–7.4 (br, 4H), 7.2–7.1 (br, 2H), 3.99 (br, 4H), 3.7 (br), 3.21 (br, 4H), 3.03 (br, 18H), 1.71 (br, 4H), 1.48 (br, 4H), 1.33 (br, 4H). Anal. Calcd for $(C_{30}H_{48}Br_2N_2O_2)_{0.50}(C_{13}H_{10}O_2)_{0.50}$: C, 62.47; H, 7.07; N, 3.39. Found: C, 64.46; H, 7.38; N, 2.38.

3. Results and discussion

3.1. Synthesis and characterization

The synthetic route of the water-soluble CPs is shown in Scheme 1. The cationic *p*-dibromobenzene derivative was synthesized from their corresponding neutral compounds by quaternization with trimethylamine aqueous solution. The water-soluble CPs were directly prepared through Suzuki coupling reaction of the cationic *p*-dibromobenzene derivative, 2,5-dibromo-4-methoxyphenol, and 1,4-phenylenediboronic acid. The feed ratios of 2,5-dibromo-4-methoxyphenol were 10, 25 and 50 mol%, and the corresponding polymers were named N-PPP10, N-PPP25 and N-PPP50, respectively.

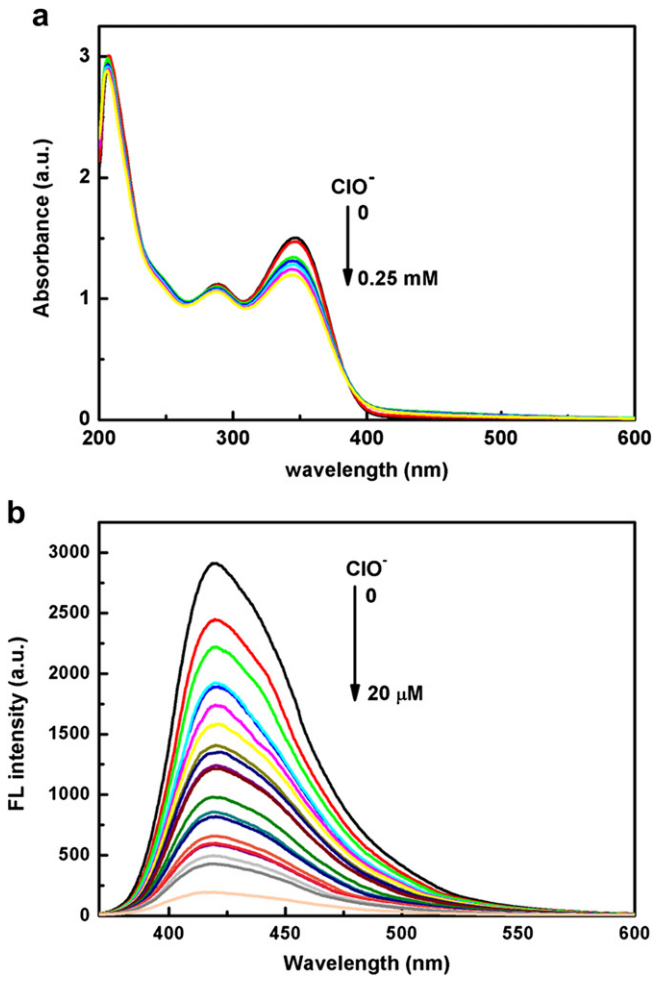


Fig. 2. (a) UV–vis absorption ([N-PPP10] = 50 μM) and (b) fluorescence ([N-PPP10] = 5 μM) spectra of polymer N-PPP10 in PBS buffer (10 mM, pH 7.4) as a function of hypochlorite. The excitation wavelength is 350 nm.

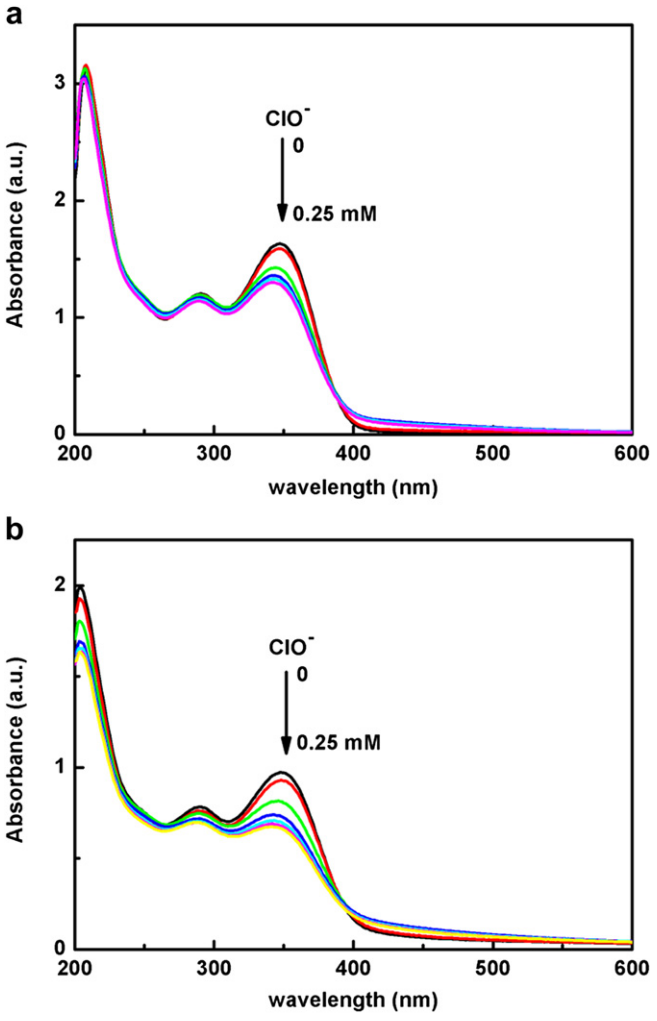


Fig. 3. UV–vis absorption spectra of polymer (a) N-PPP25 and (b) N-PPP50 in PBS buffer (10 mM, pH 7.4) as a function of hypochlorite. [N-PPP25] = [N-PPP50] = 50 μM.

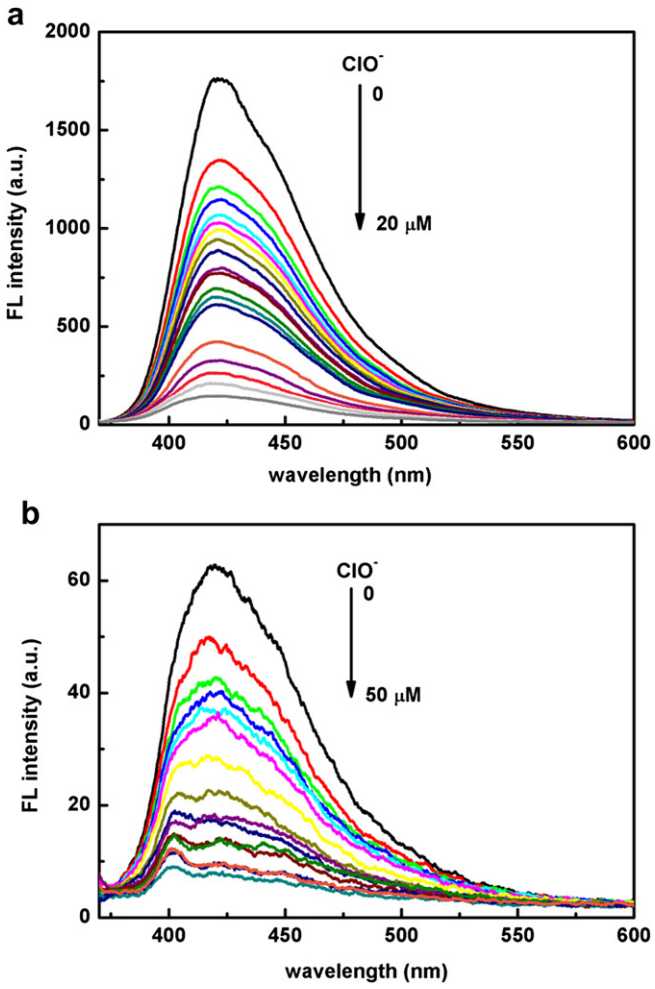


Fig. 4. Fluorescence spectra of polymer (a) N-PPP25 and (b) N-PPP50 in PBS buffer (10 mM, pH 7.4) as a function of hypochlorite. [N-PPP25] = [N-PPP50] = 5 μM. The excitation wavelength is 350 nm.

The structures of the polymers were characterized by ^{1}H NMR and elemental analysis. The *p*-methoxyphenol moieties on the polymer backbone were confirmed by the broad peaks at around 8.0 and 3.7 ppm of ^{1}H NMR spectra. The polymers are soluble in methanol and water with a solubility of 8 mg/mL. In the TGA thermograms of the three polymers (see ESI), a weight loss of 5–10% between 100 and 150 °C was observed for all the polymers, which could be ascribed to the loss of absorbed and bound water [42–44].

3.2. Photophysical properties

The absorption and emission spectra of the polymers and the model compound in 10 mM phosphate buffer saline (PBS) solution with pH of 7.4 are shown in Fig. 1. All water-soluble CPs exhibited almost the same absorption and emission spectra, which are significantly red shifted compared to model compound PMOPP. This should be ascribed to that the polymers have a more extended conjugated length than the model compound PMOPP. The UV–vis absorption spectra of N-PPPx in PBS solution exhibited three maxima at *ca.* 205, 290 and 350 nm (Table 1). All the fluorescent experiments were carried out with an excitation wavelength of 350 nm. The fluorescence spectra of the polymers showed a maximum at *ca.* 420 nm. The fluorescence quantum yield of N-PPP10, N-PPP25 and N-PPP50 in PBS solution was 21%, 26% and 4%, respectively.

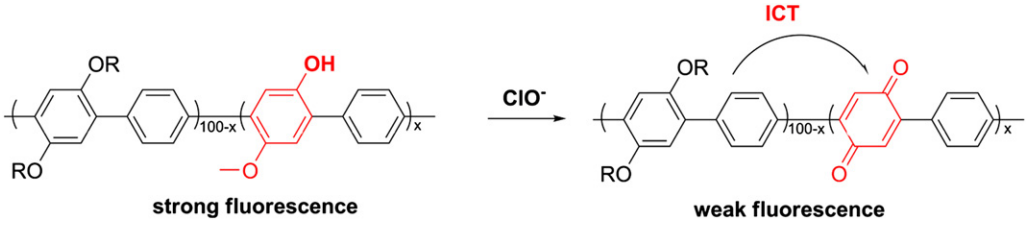
3.3. Optical response for hypochlorite

We first examined the sensing properties of the polymer N-PPP10 to hypochlorite in PBS solution. As shown in Fig. 2, upon addition of hypochlorite from 0 to 0.25 mM, the absorption band at *ca.* 350 nm decreased, whereas the absorption bands at 208 and 290 nm were almost unchanged. In contrast, when 20 μM of hypochlorite was added, the fluorescence intensity was decreased dramatically with a 15-fold fluorescence quenching. Then we investigated the responses of N-PPP25 and N-PPP50 to hypochlorite. Both N-PPP25 and N-PPP50 showed the same absorption decrease at *ca.* 350 nm in the presence of hypochlorite (Fig. 3). Compared to PMOPP, there was no obvious appearance of new absorption band at long wavelength (>400 nm) because the absorptions of the polymers are mainly attributed to the backbone of poly(*p*-phenylene). Meanwhile, as shown in Fig. 4, upon addition of hypochlorite, both N-PPP25 and N-PPP50 showed a fluorescence quenching, though N-PPP50 needed more hypochlorite to quench the fluorescence completely. Similar to the previous mechanism based on the small-molecules, the methoxyphenol moieties of the polymers were oxidized to benzoquinone upon the addition of hypochlorite, which induced an ICT from the polymer backbone to benzoquinone moieties, and thus resulted in a fluorescence quenching (Scheme 2).

The efficiency of fluorescence quenching is quantified through the Stern-Volmer constant (K_{sv}), which is calculated according to the Equation (1).

$$F_0/F = 1 + K_{sv}[\text{quencher}] \quad (1)$$

The K_{sv} of N-PPPx and PMOPP are listed in Table 1. It was found that the K_{sv} decreased with the increasing content of methoxyphenol moieties in the polymers N-PPPx. Correspondingly, the limit of detection of N-PPP10, N-PPP25, N-PPP50 and PMOPP is 0.02 μM, 0.03 μM, 0.2 μM and 0.8 μM, respectively. This can be elucidated from the fact that more methoxyphenol moieties require more hypochlorite to react. In addition, the Stern-Volmer constants of N-PPPx were obviously higher than that of the model compound PMOPP. This indicated that the CPs of N-PPPx amplified the fluorescence quenching due to the facile energy migration along the polymer backbone.



Scheme 2. Mechanism of the reaction of N-PPPx to ClO⁻.

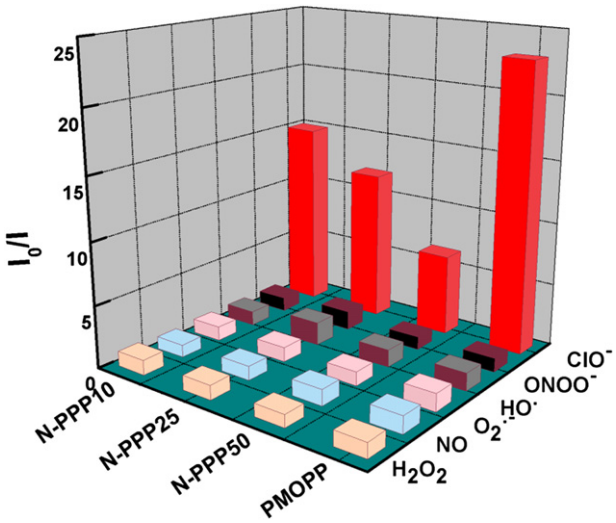


Fig. 5. Fluorescence emission response profiles of the polymers N-PPP10, N-PPP25, N-PPP50 and the model compound PMOPP upon addition of various ROS in PBS. [N-PPP10] = [N-PPP25] = [N-PPP50] = [PMOPP] = 5 μM H_2O_2: 40 μM H_2O_2. NO: 40 μM sodium nitroferricyanide (III) was added and the mixture was stirred at 25 °C for 30 min. $O_2^{\cdot-}$: 40 μM KO_2 was added and the mixture was stirred at 37 °C for 30 min. $^{\bullet}OH$: 40 μM ferrous perchlorate and 80 μM H_2O_2. $ONOO^-$: 40 μM sodium peroxynitrite. ClO^-: 20 μM $Ca(ClO)_2$.

To assess the selectivity, we investigated the sensing properties of N-PPPx to various reactive oxygen species (ROS). Upon addition of the examined ROS, including H_2O_2, $O_2^{\cdot-}$, NO, $HO^{\bullet}$, $ONOO^-$, no significant change of fluorescence spectra was observed (Fig. 5). Compared to N-PPP10, N-PPP25 and PMOPP, N-PPP50 displays a low selectivity, owing to its weak fluorescence intensity and high reactivity. We have to point out that the selectivity experiments were carried out in a high concentration of 40 μM of ClO^-. When the concentration of ClO^- was higher than 10 μM, the fluorescence of the polymers of N-PPPx almost didn't change with the addition of ClO^-. On the other hand, in terms of sensitivity, K_{sv} is calculated in range of low concentration of ClO^-, in which case facile energy migration along the polymer backbone works out. Therefore, though the selectivity of the model compound is higher than those of the polymers, the K_{sv} of PMOPP is lower than those of the polymers (Table 1). In view of the sensitivity and selectivity, the polymer N-PPP10 and N-PPP25 are very promising fluorescent probes to hypochlorite capable of running in the aqueous condition.

4. Conclusion

In conclusion, we have developed a series of water-soluble CPs by incorporating the methoxyphenol moieties into the poly(*p*-phenylene) backbone. The polymers exhibit highly sensitive and selective fluorescent quenching to hypochlorite under the aqueous condition. The content of methoxyphenol moieties in polymer N-PPPx plays a crucial role in the behaviors of fluorescence quenching efficiency. Furthermore, the conjugated polymers show a significantly amplified quenching efficiency compared to their model compound. The results reveal that the water-soluble poly(*p*-phenylene) derivatives have good potential in detecting hypochlorous acid in biological system.

Acknowledgments

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.polymer.2012.03.063.

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