Competition Between Energy Transfer Quenching and Chelation Enhanced Fluorescence in a Cu (II) Coordinated Conjugated Polymer System

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Abstract An investigation of the mechanism of the fluorescence quenching by Cu^{2+} for a conjugated polymer system initially designed as a fluorescence "turn-on" chemosensor based on chelation enhanced fluorescence (CHEF) is described in this paper. Unlike all other metal cations tested, the polymer/Cu^{2+} hybrid system with a 1:1 ratio between the receptor and Cu^{2+} has only weak fluorescence with $\lambda_{max} = 490$ nm and a quantum yield of 0.004 in THF at room temperature. In solvent glasses at 77 K the fluorescence remained quenched suggesting that the quenching mechanism was due to energy transfer between the Cu^{2+} and the conjugated polymer backbone. The energy transfer quenching competes effectively with the electron transfer involved in the CHEF resulting in a more selective chemosensory system.

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Introduction

The design of fluorescent chemosensors spans a wide range of structures from small molecules to large conjugated polymers [1–3]. A continuing challenge is the preparation of systems that have a fluorescence "turn-on" response. Recently, we reported the synthesis of poly[p-(phenyleneethynylene)-*alt*-(thienyleneethynylene)] (PPETE) with a N, N, N'-trimethylethylenediamino receptor loaded on the thienylene ring (tmeda-PPETE) (Fig. 1 *left*) based on a strategy we advanced for a series of fluorescence "turn-on" chemosensors [4]. A chelation-enhanced fluorescence (CHEF) mechanism resulted from coordination of a cation to the redox active amino receptor and subsequent termination of a photoinduced electron transfer (PET) quenching process between the fluorophore and an electron donating receptor. Most cations, including Li^+, Na^+, K^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Ca^{2+} and H^+, were observed to turn on the fluorescence of tmeda-PPETE to varying degrees [4–6]. Interestingly, Cu^{2+} was found to exhibit a noteworthy quenching of the fluorescence in this system [5]. We have made use of this exceptional Cu^{2+} quenching to prepare an organic/inorganic hybrid polymer with fluorescence "turn-on" chemosensory behavior that was sensitive and selective for iron cations [5].

Here we present a study of the fluorescence quenching of tmeda-PPETE by Cu^{2+} in order to determine how the quenching process competes with the CHEF "turn-on" response. The investigation of copper quenching was based on the photophysical properties of the model PPETE, tmeda-PPETE and tmeda-PPETE/Cu^{2+} hybrid system. The structures of the model PPETE, tmeda-PPETE and the proposed structure of tmeda-PPETE/Cu^{2+} are shown in Fig. 1.

Fig. 1 Structures of tmeda-PPETE, model PPETE and proposed structure of tmeda-PPETE
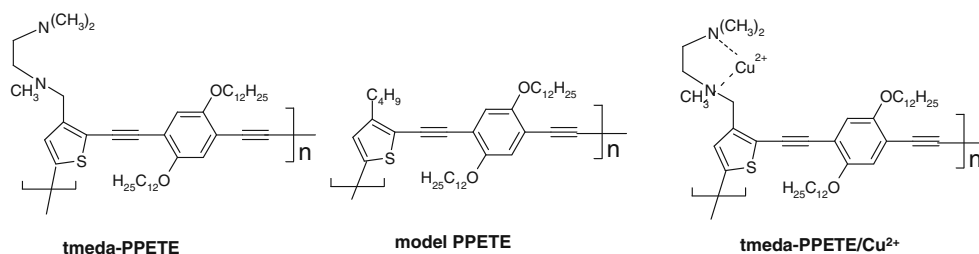
Experimental

In this study, UV-vis spectra were obtained on a Perkin-Elmer Lambda 2S spectrophotometer in room temperature THF solution using 1 cm quartz cuvette cells. Fluorescence spectra were measured on a SLM 48,000s spectrofluorometer using an excitation wavelength of 408 nm. For all optical experiments, the Cu^{2+} solution was prepared by dissolving $CuCl_2$ in water. The polymer solution was prepared by dissolving the polymer in THF. Addition of 100 μL or 200 μL of $2.5 \times 10P^{-4P}$ M cation solutions into the 50 ml polymer solution (5 μM with respect to the repeat unit) was performed for each fluorescence emission measurement. Fluorescence quantum yields were determined in argon degassed anhydrous THF, relative to quinine sulfate in 0.5 M H_2SO_4 solutions with a reference quantum yield of 0.546, using an excitation wavelength of 365 nm [7]. The experiments were repeated three times and the final value of the quantum yields were reported as the average within a deviation of ±0.01. Lifetime data were obtained by time-correlated single photon counting at the University of Pennsylvania as described previously [8]. Low temperature (77 K) fluorescence measurements were completed in THF using an EPR tube inserted into a finger dewar with liquid N_2. For comparison, the room temperature fluorescence measurements for each sample were also recorded on the same sample.

The synthesis of both tmeda-PPETE and model PPETE have been reported previously [4, 6]. The tmeda-PPETE was found to have a number average molecular weight of 3.67×10^4 g/mol with a polydispersity of 2.73 by Gel Permeation Chromotography (GPC). The model PPETE was found to have a number average molecular weight of 6.79×10^4 g/mol with a polydispersity of 2.97. Both polymers have a maximum absorption around 450 nm and an emission maximum around 490 nm. The absorption and emission have been assigned to the same π-π* transition from the conjugated polymer backbone [8, 9].

The quantum yields of tmeda-PPETE, model PPETE and tmeda-PPETE with Cu^{2+} are shown in Table 1. The initial decrease in quantum yield upon addition of the electron donor receptor to the polymer backbone (tmeda-PPETE) compared to the model polymer is consistent with photoinduced electron transfer between the amino receptor and the conjugated polymer backbone. An additional decrease in quantum yields by an order of magnitude for tmeda-PPETE/Cu^{2+} suggested an additional quenching mechanism. This depression in the background fluorescence can be utilized to improve the overall sensitivity of these polymers as fluorescent turn-on sensors [5].

The fluorescence spectra of tmeda-PPETE in THF upon titration of Cu^{2+} are shown in Fig. 2. In order to quantify the change in fluorescence with concentration, Fig. 3 shows the maximum fluorescence intensity change (I/I_0) vs Cu^{2+} concentration (left axis) and the Stern-Volmer plot, I_0/I vs Cu^{2+} concentration (right axis). The maximum fluorescence intensity was quenched by approximately 50% when adding Cu^{2+} at a concentration (500 nM) equal to 10% of the receptor concentration. With continued addition of Cu^{2+} up to 5 μM, the fluorescence was almost totally quenched with less than 2% residual fluorescence. The nonlinear Stern-Volmer relationship between I_0/I and $[Cu^{2+}]$ is very similar to previous studies about conjugated polymer "turn-off" chemsensors based on the PPETE backbone as described [8, 9]. However, those system did not contain an electron donating receptor. Similar Cu^{2+} quenching exception was also observed in some small molecule PET fluorescence "turn-on" chemosensors [10–15]. The quenching mechanism in these cases was attributed to either electron transfer or energy transfer between the fluorophore and the Cu^{2+}. However, assignment of the specific mechanism in the case of the polymer system has not been reported.

There was negligible change in the absorbance spectra when titrating the Cu^{2+} into the tmeda-PPETE solution. The absorbance spectra were very similar to the spectrum for

Table 1 Quantum yields of tmeda-PPETE, model PPETE, and tmeda-PPETE/ Cu^{2+}

Samples	Quantum yields
tmeda-PPETE	0.090
tmeda-PPETE/Cu^{2+} (cation/ receptor: 1/1 mole ratio)	0.004
Model PPETE	0.262

[a] The concentration of polymers was held at 5 μM with respect to the repeat unit

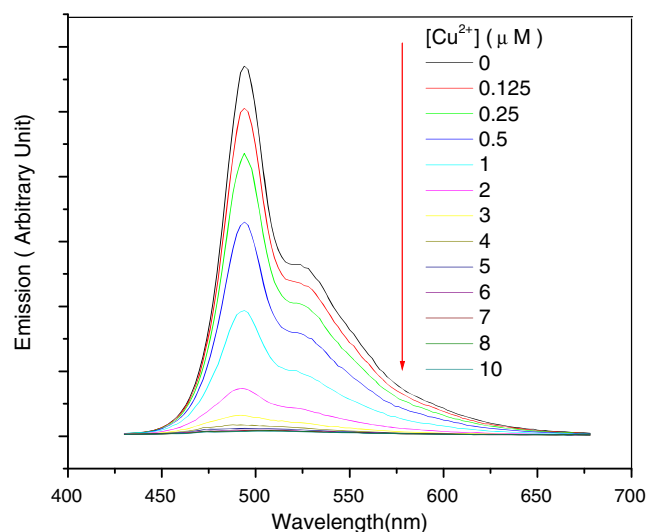**Fig. 2** Fluorescence quenching upon titration of Cu^{2+} aqueous solution. The polymer in THF was fixed 5 μM with respect to receptor units

the polymer backbone in the model PPETE. All the absorbance spectra were provided in the supplementary information to this paper. However, the fluorescence lifetimes were found to be nearly identical for the model, tmeda-PPETE and tmeda-PPETE/Cu^{2+} solution. The emission decays for the fluorescence lifetime measurement were best fit to a biexponential function for all polymers with equal 0.5 ns and 0.2 ns components. These data suggest that fluorescence quenching in this system is a static binding process prior to excitation, rather than dynamic or collisional in nature [16].

The tmeda-PPETE was initially designed as a fluorescence "turn-on" sensor with a chelation enhanced fluorescence (CHEF) upon the binding of cations to the receptor. This assumption was consistent for all cations tested as demonstrated by an increase the fluorescence intensity. The exception of Cu^{2+} quenching could be explained by a different binding mechanism for Cu^{2+} or direct interaction with the polymer backbone. To address this question, several experiments were attempted based on NMR and EPR spectroscopies to show these binding modes. While this data was found to be inconclusive, a titration of Cu^{2+} into a THF solution of model PPETE showed no change in emission intensity or spectral profile. From this result, we conclude that the chelating amino group is required for the observed Cu^{2+} quenching.

Both energy transfer and electron transfer are possible pathways to deactivate the fluorescence in the case of Cu^{2+} and have been discussed in some small molecule systems by Fabbrizzi et al [11, 13]. Low temperature fluorescence experiments in solvent glasses can be used to distinguish between the energy transfer and electron transfer pathways. Electron transfer involves charge separation during the

quenching process. This requires reorientation of the solvent molecules in fluid solution. At liquid nitrogen temperatures, 77 K, the frozen solvent glass would be immobile and electron transfer would be inhibited. In contrast, energy transfer does not involve any charge transfer and would therefore not be limited in a frozen solvent glass.

Parallel emission experiments carried out at room temperature and in frozen solvent glasses at 77 K were studied for the model PPETE, tmeda-PPETE and tmeda-PPETE/Cu^{2+} (Fig. 4). The solutions were prepared to have the same absorbance values (~ 0.15) at the excitation wavelength (408 nm). Here the model PPETE was used as a control for comparing the different fluorescence behaviors of tmeda-PPETE and tmeda-PPETE/Cu^{2+} at room temperature and low temperature.

In contrast to the room temperature results, the fluorescence intensity of the tmeda-PPETE were very close to that of the model PPETE polymers at low temperature. The recovery of the fluorescence for the tmeda-PPETE system is consistent with termination of the photoinduced electron transfer quenching between the amino receptor and polymer backbone when frozen at low temperature. Interestingly the fluorescence of tmeda-PPETE/Cu^{2+} did not reappear in the frozen solution glass. This suggests that the quenching of the polymer fluorescence by Cu^{2+} continues at low temperature. Given that it has previously been shown that energy transfer can continue in frozen glass matrices due to lack of any additional electrostatic work terms as found in electron transfer, we conclude that an energy transfer mechanism is involved in this case [16].

There are three possible energy transfer mechanisms: Trivial, Dexter, and Förster [17, 18]. The trivial mechanism involves emission and reabsorption of a photon and is only significant in very long range systems. The Dexter energy
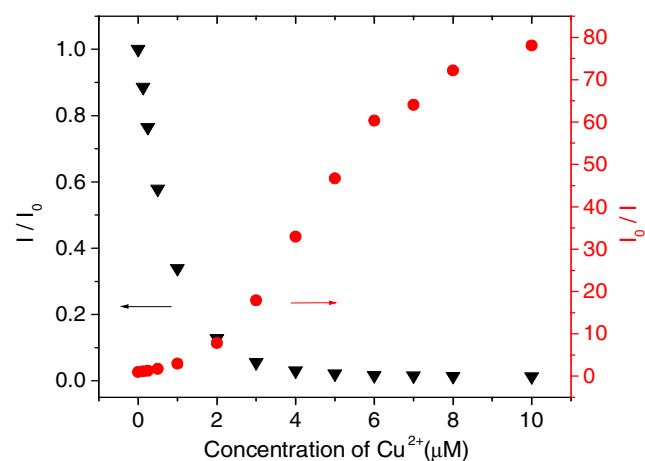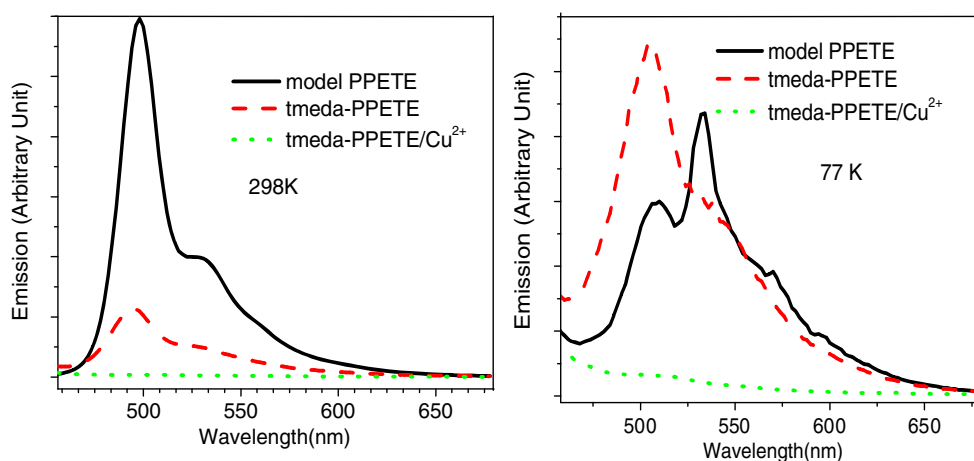

Fig. 3 I/I_0 (left axis) and I_0/I (right axis) versus Cu^{2+} concentration. Concentration of tmeda-PPETE was held at 5 μM with respect to receptor units

Fig. 4 Emission spectra for model-PPETE, tmeda-PPETE and tmeda-PPETE/Cu^{2+} at room temperature (*left*) and low temperature (*right*)

transfer mechanism requires electronic overlap of the orbitals involved. In the small molecule sensors, this mechanism was found to be dominant due to the flexibility of the spacer and close contact between the cation and excited fluorophore [17, 18]. The Förster energy transfer mechanism involves dipole-dipole coupling between the donor and acceptor. It is commonly found in conjugated polymer systems involving long range exchange of excitation energy through space without a path of direct orbital overlap [17, 18]. In this case, overlap of the emission and absorption spectrum is the key factor and there is no dependence on conjugation.

Based on the flexibility and conjugation between the polymer backbone and the receptor, the Dexter energy transfer is most likely in the tmeda-PPETE/Cu^{2+} as shown in Fig. 5. Previous studies with this polymer system have demonstrated fluorescence quenching based on a Dexter mechanism as well [8]. Due to the Jahn-Teller effect, Cu^{2+} with a d^9 electronic configuration is known to have an elongated-octahedral structure that would further suggest the Dexter energy transfer mechanism via a direct orbital overlap between the fluorophore and the cation [10, 11, 13]. It is possible that only Cu^{2+} has sufficient orbital overlap compared to the other ions tested. Further computational studies are necessary to confirm this assignment.

There are several other interesting observations that can be made from the low temperature spectra. Both the model PPETE and tmeda-PPETE emission spectra undergo a red shift at low temperature. The red-shift of model PPETE is from 498 nm to 508 nm and from 530 nm to 534 nm, for

the peak and the shoulder, respectively, while peak of the tmeda-PPETE shifted from 494 nm to 504 nm. This could be attributed to increased rigidity of the polymer backbone at 77 K and an increase in conjugation. This could be useful in the preparation of next generation chemosensors since this should result in improved sensitivity.

Another observation is that the emission spectral profiles of the model PPETE and tmeda-PPETE at 77 K were different from those at room temperature. Another observation is that the emission spectral profiles of the model PPETE and tmeda-PPETE at 77 K were different from those at room temperature. At room temperature, both spectra had similar profile with a peak around 495 nm and a shoulder around 530 nm. This can be attributed to vibrational fine structure on the π-π* electronic transition in the conjugated polymer backbone. At low temperature, the different substituents in each system cause a significant shift in the vibrational structure. In particular, the model polymer shows an enhancement in the second vibrational component suggesting that more distortion in the excited state is present in this system.

Conclusion

The competition between fluorescence quenching and chelation enhanced fluorescence for tmeda-PPETE upon binding Cu^{2+} has been studied. Literature and experimental investigations suggest that the d^9 electronic configuration and Jahn-Teller distorted orbital shape of Cu^{2+} make this

Fig. 5 Dexter (double-electron exchange) energy transfer between an excited fluorophore and Cu^{2+} (modified from reference 11). The Cu^{2+} takes an octahedral orbital shape due to the Jahn Teller effect
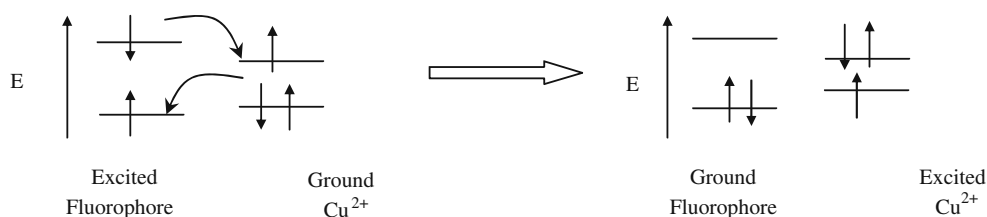
binding unique compared to all other cations studied previously. With Cu^{2+}, fluorescence quenching dominates the interaction, an effect which can be exploited to create a new class of fluorescent sensors [4]. To our knowledge, this is the first time this phenomenon has been found in a conjugated polymer system, though some small molecule fluorescence PET "turn-on" sensors also showed quenched fluorescence effects when binding with Cu^{2+} [10–15]. In addition, the magnitude of the quenching was enhanced compared to small molecule sensors for Cu^{2+} due to energy migration of the exciton along the "molecular wire" backbone [19, 20].

References

1. de Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, Rice TE (1997) Signaling recognition events with fluorescent sensors and switches. Chem Rev 97 (5):1515–1566 doi:10.1021/cr960386p

2. McQuade DT, Pullen AE, Swager TM (2000) Conjugated polymer-based chemical sensors. Chem Rev 100(7):2537–2574 doi:10.1021/cr9801014

3. Thomas SW, Joly GD, Swager TM (2007) Chemical sensors based on amplifying fluorescent conjugated polymers. Chem Rev 107(4):1339–1386 doi:10.1021/cr0501339

4. Fan LJ, Zhang Y, Jones WE (2005) Design and synthesis of fluorescence "Turn-on" chemosensors based on photoinduced electron transfer in conjugated polymers. Macromolecules 38 (7):2844–2849 doi:10.1021/ma047983v

5. Fan LJ, Jones WE (2006) A highly selective and sensitive inorganic/organic hybrid polymer fluorescence "Turn-on" chemosensory system for iron cations. J Am Chem Soc 128(21):6784–6785 doi:10.1021/ja0612697

6. Fan LJ, Jones WE (2006) Studies of photoinduced electron transfer and energy migration in a conjugated polymer system for fluorescence "turn-on" chemosensor applications. J Phys Chem B 110(15):7777–7782 doi:10.1021/jp056381q

7. Pang Y, Li J, Barton TJ (1998) Processible poly[(p-phenyleneethynylene)-alt- (2,5-thienyleneethynylene)]s of high luminescence: their synthesis and physical properties. J Mater Chem 8 (8):1687–1690 doi:10.1039/a802032i

8. Murphy CB, Zhang Y, Troxler T, Ferry V, Martin JJ, Jones WE (2004) Probing Förster and Dexter energy-transfer mechanisms in fluorescent conjugated polymer chemosensors. J Phys Chem B 108(5):1537–1543 doi:10.1021/jp0301406

9. Zhang Y, Murphy CB, Jones WE (2002) Poly[p-(phenyleneethynylene)-alt- (thienyleneethynylene)] polymers with oligopyridine pendant groups: highly sensitive chemosensors for transition metal ions. Macromolecules 35(3):630–636 doi:10.1021/ma011479y

10. Fabbrizzi L, Licchelli M, Pallavicini P (1999) Transition metals as switches. Acc Chem Res 32(10):846–853 doi:10.1021/ar9900131

11. Bergonzi R, Fabbrizzi L, Licchelli M, Mangano C (1998) Molecular switches of fluorescence operating through metal centred redox couples. Coord Chem Rev 170(1):31–46 doi:10.1016/S0010-8545(98)00069-1

12. de Santis G, Fabbrizzi L, Licchelli M, Mangano C, Sacchi D, Sardone N (1997) A fluorescent chemosensor for the copper(II) ion. Inorg Chim Acta 257(1):69–76 doi:10.1016/S0020-1693(96)05452-7

13. Fabbrizzi L, Maurizio L, Piersandro P, Angelo P, Angelo T, Donata S (1996) Fluorescent sensors for transition metals based on electron-transfer and energy-transfer mechanisms. Chem Eur J 2(1):75–82 doi:10.1002/chem.19960020114

14. De Santis G, Fabbrizzi L, Licchelli M, Mangano C, Sacchi D (1995) Redox switching of anthracene fluorescence through the CuII/CuI Couple. Inorg Chem 34(14):3581–3582 doi:10.1021/ic00118a003

15. Bernhardt PV, Flanagan BM, Riley MJ (1999) Metal-centred versus ligand-centred luminescence quenching of a macrocyclic copper(II) complex. J Chem Soc Dalton Trans (20):3579–3584 doi:10.1039/a902881a

16. Murtaza Z, Zipp AP, Worl LA, Graff DK, Jones WE Jr, Meyer TJ (1991) Energy Transfer in the inverted region. J Am Chem Soc 113:5113

17. Lakowicz JR (1999) Principles of Fluorescence Spectroscopy. Kluwer academic/Plenum, New York

18. Guillet J (1985) Polymer Photophysics and Photochemistry. Cambridge University Press, Cambridge, UK.

19. Swager TM (1998) The molecular wire approach to sensory signal amplification. Acc Chem Res 31:201–207 doi:10.1021/ar9600502

20. Zhou Q, Swager TM (1995) Fluorescent chemosensors based on energy migration in conjugated polymers: the molecular wire approach to increased sensitivity. J Am Chem Soc 117:12593–12602 doi:10.1021/ja00155a023