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# Fluorescence Quenching of Two Conjugated Polyelectrolytes by Natural Amino Acids and Hemeproteins

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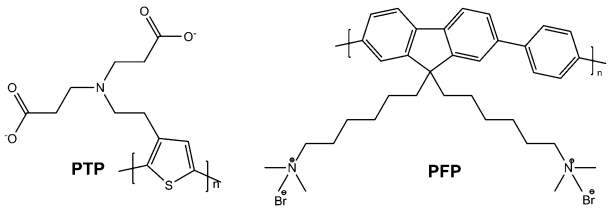
We found that none of twenty natural amino acids quenches the fluorescence of anionic PTP, but phenylalanine, tryptophan, and tyrosine do quench obviously the fluorescence of cationic PFP. The overlaps between the *Q*-band absorption spectrum of myoglobin, cytochrome *c*, and horseradish peroxidase and the fluorescence emission spectrum of PTP indicate that the fluorescence quenching of PTP by hemeproteins is via fluorescence resonance energy transfer, not via photoinduced electron transfer.

In recent years, conjugated polyelectrolytes (CPEs) have been studied extensively as optical sensors for small ions, biomolecules, proteins, and nucleic acids.[1] Comparing with the conventional fluorophores, the unique structure of the CPEs offers many advantages.[1d] First, the amphiphilic structure of CPEs provides a platform to interact with the analytes through either electrostatic or hydrophobic interaction. Second, the delocalized electronic structure of CPEs allows the excitation energy transfer along their whole backbone resulting in amplified signals and improved sensitivity. Three types of sensing mechanisms of CPEs have been suggested as the fluorescence resonance energy transfer, the photoinduced electron transfer, and the analyte-induced aggregation or conformational changes of CPEs.[2] In 1999, Chen et al. reported the first example of a CPE-based biosensor through taking advantage of amplified fluorescence quenching behavior.[3] In 2002, Fan et al. reported the nonspecific interaction of a sulfonated poly(*p*-phenylenevinylene) with cytochrome *c*, myoglobin, and lysozyme.[4] More recently, Liu and co-workers revealed the roles of electrostatic and hydrophobic interactions in the fluorescence response of CPEs toward protein with two newly synthesized cationic and anionic poly(fluorenyleneethynylene-*alt*-benzothiadiazole)s.[5]

Although CPEs have been studied extensively as optical probes to detect the interaction with proteins,[4,6] it is very interesting that there is no report in the literature to address the interaction between CPEs and natural amino acids. It has been reported that the fluorescence of conventional fluorophores could be quenched dramatically by a certain amino acid via photoinduced electron transfer when they are in close van der Waals contact.[7] Therefore, studies of fluorescence quenching of CPEs by natural amino acids would help researchers to further understand the interaction between CPEs and proteins. Herein, we selected two opposite charged CPEs, cationic PFP and anionic PTP (Scheme 1), and studied their fluorescence quenching behaviors by twenty natural amino acids. We identify that phenylalanine, tryptophan, and tyrosine quench obviously the fluorescence of PFP, but none of twenty natural amino acids quenches the fluorescence of PTP.




**Scheme 1.** Chemical structures of PTP and PFP.

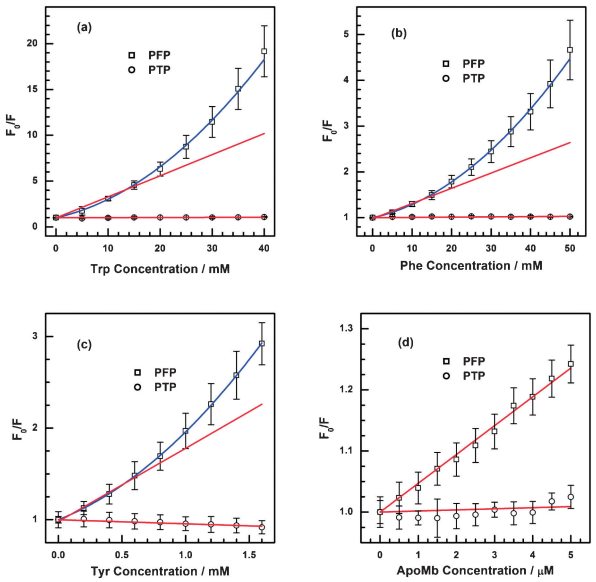



**Figure 1.** Fluorescence intensity Stern–Volmer plots of PTP and PFP in the presence of different quenchers in 100 mM sodium phosphate buffer at pH 7.1. (a) Tryptophan, (b) phenylalanine, (c) tyrosine, and (d) apomyoglobin. Excitation wavelength: 400 nm. The data of the error bar shown in figures are obtained through 5 repetitive measurements.

The water-soluble conjugate polymers, PTP and PFP, were generous gifts of Prof. Shu Wang at ICCAS, and their static absorption and fluorescence spectra are shown in Figure S1.[8] Figure 1 shows the fluorescence intensity Stern–Volmer plots of PFP and PTP in the presence of different quenchers. The data for tyrosine is limited to 1.6 mM of quencher concentration due to its low solubility in aqueous solution. From the data shown in Figure 1, it can be found that phenylalanine, tryptophan, and tyrosine quench dramatically the fluorescence of PFP, but none of them quenches the fluorescence of PTP. With the linear region data of the quencher concentration dependent $F_0/F$ shown in Figures 1a–1c, we find that tryptophan quenches the fluorescence of PFP with a Stern–Volmer constant ($K_{SV}$) value of

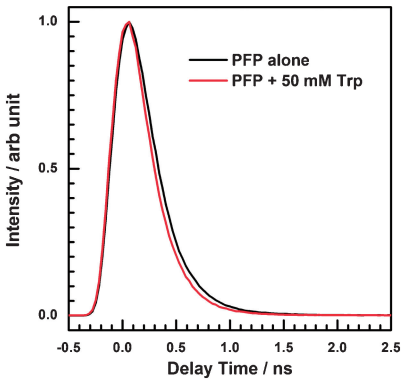



**Figure 2.** Fluorescence decay time profiles of PFP with (red line) and without (black line) 50 mM tryptophan in 100 mM sodium phosphate buffer at pH 7.1 obtained by the time correlated single photon counting measurement. All plots are normalized to 1 at their maxima. Pump: 390 nm, probe: 440 nm.

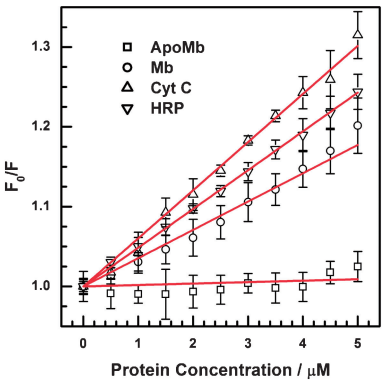



**Figure 3.** Fluorescence intensity Stern–Volmer plots of PTP in the presence of apomyoglobin (ApoMb, □), myoglobin (Mb, ○), cytochrome *c* (Cyt C, △), and horseradish peroxidase (HRP, ▽) in 100 mM sodium phosphate buffer at pH 7.1. Excitation wavelength: 480 nm. The data of the error bar shown in figure are obtained through 5 repetitive measurements.

220 $M^{-1}$, phenylalanine quenches the fluorescence of PFP with a $K_{SV}$ value of 33 $M^{-1}$, and tyrosine quenches the fluorescence of PFP with a $K_{SV}$ value of 800 $M^{-1}$. Clearly, tyrosine and tryptophan are strong fluorescence quenchers of PFP, while phenylalanine is a weak fluorescence quencher of PFP. The nonlinear and upward curvature characters of Stern–Volmer plots shown in Figure 1 generally indicate that the fluorophore is quenched by both static complex formation and dynamic collision with the same quencher.[1d] However, the fluorescence lifetimes of PFP with and without 50 mM tryptophan do not change significantly (both are about 250 ps, Figure 2), indicates that the dynamic collision quenching is not obvious. Thus, the superlinear fluorescence quenching of PFP at higher concentrations of quencher should be attributed to other processes. Bazan, Heeger, and co-workers have applied a "sphere-of-action" quenching model to explain the superlinear quenching of CPEs at higher quencher concentrations.[9] In order to better understand the fluorescence quenching of PFP and PTP by natural amino acids, we further measured the fluorescence quenching behaviors of PFP and PTP with apomyoglobin. The apomyoglobin is made from myoglobin, but it does not have the heme chromophore. The fluorescence intensity Stern–Volmer plots of PFP and PTP in the presence of apomyoglobin were also displayed in Figure 1 (Figure 1d). Clearly, apomyoglobin does not quench significantly the fluorescence of PTP, but it does quench the fluorescence of PFP with a $K_{SV}$ value of $4.90 \times 10^4$ $M^{-1}$.

Since both twenty natural amino acids and apomyoglobin do not quench the fluorescence of the anionic conjugated polymer PTP, we can use PTP as a model fluorophore to study the interaction between an anionic CPE and heme-containing proteins. Figure 3 displays the fluorescence intensity Stern–Volmer plots of PTP in the presence of apomyoglobin, myoglobin, cytochrome *c*, and horseradish peroxidase, respectively. Clearly, all ferric heme-containing proteins quench dramatically the fluorescence of PTP. Myoglobin quenches the fluorescence of PTP with a $K_{SV}$ value of $3.96 \times 10^4$ $M^{-1}$, cytochrome *c* quenches the fluorescence of PTP with a $K_{SV}$ value of $6.27 \times 10^4$ $M^{-1}$, and horseradish peroxidase quenches the fluorescence of PTP with a $K_{SV}$ value of $4.78 \times 10^4$ $M^{-1}$. All these obtained $K_{SV}$ values for PTP in the presence of respective hemeprotein are smaller than that for anionic poly[lithium 5-methoxy-2-(4-sulfobutoxy)-1,4-phenylenevinylene][4] and poly[2,5-bis(3-sulfonatopropoxy)-1,4-phenylene-*alt*-1,4-phenylene].[6b] Fan et al.[4] reported that the $K_{SV}$ value of poly[lithium 5-methoxy-2-(4-sulfobutoxy)-1,4-phenylenevinylene] in the presence of myoglobin is about 2–3 orders of magnitude lower than that with cytochrome *c*. They attributed the superquenching effect in the presence of cytochrome *c* to electron transfer from the excited polymer to $Fe^{3+}$ center in cytochrome *c*.[4] In contrast, Kim et al.[6c] found that both myoglobin and cytochrome *c* were similarly effective as quencher for two carboxylate-substituted poly(*p*-phenyleneethynylene)s. Herein, we also found that all heme-containing proteins, myoglobin, horseradish peroxidase, and cytochrome *c*, have similar $K_{SV}$ values for PTP, which is in agreement well with Kim's observation.[6c] The distinctions to Fan's result[4] could be attributed to the different backbone (thiophene vs. phenylenevinylene) and the different ionic group (carboxylate vs. sulfonate) of CPEs used in these studies.

Three mechanisms, including the fluorescence resonance energy transfer, the photoinduced electron transfer, and the analyte-induced aggregation or conformational changes of CPE, have been used to explain the fluorescence quenching of a CPE by proteins.[2] The absorption spectrum of the respective natural amino acid does not have an overlap with the fluorescence emission spectrum of PFP, and hence the fluorescence resonance energy transfer cannot occur in the fluorescence quenching of PFP by phenylalanine, tryptophan, and tyrosine. Therefore, the photoinduced electron transfer between PFP and respective amino acid should be the dominant process in the fluorescence quenching of PFP by amino acids. In the fluorescence quenching of PTP by myoglobin, ctrochrome *c*, and horseradish peroxidase, we found that the *Q*-band absorption spectrum of the respective hemeprotein overlaps well with the fluorescence emission spectrum of PTP (Figure 4). And the area of *Q*-band absorption spectrum of respective hemeprotein under the fluorescence emission spectrum of PTP increases with the order of myoglobin, horseradish peroxidase and cytochrome *c*.

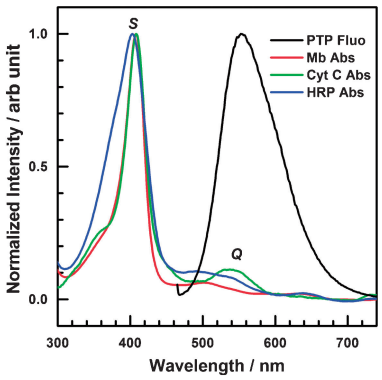



**Figure 4.** The normalized static fluorescence spectrum of PTP (black line) and the normalized static absorption spectrum of myoglobin (Mb, red line), cytochrome *c* (Cyt C, green line), and horseradish peroxidase (HRP, blue line) in 100 mM sodium phosphate buffer at pH 7.1.

Meanwhile, we found that the $K_{SV}$ value of PTP with respective hemeprotein increases with the order of myoglobin, horseradish peroxidase, and cytochrome *c* (Figure 3). Thus, the fluorescence resonance energy transfer between PTP and respective hemeprotein should be the main process in the fluorescence quenching of PTP by hemeproteins. This conclusion is in agreement with Xing's report, where they studied the fluorescence quenching behavior of PTP in the presence of 5,10,15,20-tertrakis[4-(6-*N*,*N*,*N*-trimethylammoniohexyloxy)-phenyl]porphyrin bromide.[10] Our studies show that both natural amino acid and apomyoglobin could quench the fluorescence of PFP. It is no doubt that we would see both the fluorescence resonance energy-transfer quenching and the photoinduced electron-transfer quenching contributions if we study the fluorescence quenching of PFP by myoglobin, horseradish peroxidase, and cytochrome *c*. Unfortunately, we cannot select an appropriate wavelength to excite the PFP alone due to the absorption characters of PFP and respective hemeprotein.

In summary, we investigated the fluorescence quenching behavior of cationic PFP and anionic PTP in the presence of natural amino acids and hemeproteins. We found that the amino acid phenylalanine, tryptophan, and tyrosine quench the fluorescence of cationic PFP via photoinduced electron transfer, respectively. The fluorescence nonquenching behaviors of PTP in the presence of twenty natural amino acids and apomyoglobin offer us a good model to study the fluorescence quenching mechanism of PTP by hemeproteins. The overlaps between the *Q*-band absorption spectrum of myoglobin, cytochrome *c*, and horseradish peroxidase and the fluorescence emission spectrum of PTP indicate that the fluorescence quenching of PTP by hemeproteins is via fluorescence resonance energy transfer, not via photoinduced electron transfer. In light of these findings, the potential interaction between cationic CPEs and natural amino acids should be considered in the interpretation of data that involve quantitative measurements of the fluorescence intensity of a CPE. The femtosecond transient absorption of PTP and PFP is under investigation.

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