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High-Efficiency Fluorescence Quenching of Conjugated Polymers by Proteins

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We report that the well-known electron transfer (ET) protein, cytochrome *c* (cyt *c*), acts as an efficient fluorescence quencher of the conjugated polymer poly[lithium 5-methoxy-2-(4-sulfobutoxy)-1,4-phenylenevinylene] (MBL-PPV, Scheme 1), with K_{sv} excess of 10^8. This study also confirms that efficient fluorescence quenching relies on the formation of a polymer−quencher complex driven by attractive Coulomb interactions.

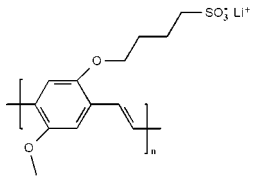
Conjugated polymers have been extensively studied as materials with a unique combination of properties.[1] As a result of these properties, a variety of novel applications are under development, including light-emitting displays, lasers, and solar cells.[2] This class of materials has also found potential utility in the biosensor field. As noted by Swager et al., the fluorescence emission of conjugated polymers can be made to respond to very minute quantities of analytes.[3] As a result, these polymers can serve as the foundation for highly sensitive chemical sensors.[4] The initial work directed toward such sensors, however, was carried out in organic solvents[5] thus greatly limiting their applications to the biosensor field.

Recently, Chen et al. proposed a novel, fluorescent biosensor based on luminsecent polyelectrolytes such as MBL-PPV.[6] They demonstrated that the luminescence of MBL-PPV is readily quenched by the electron acceptor, methyl viologen (MV^{2+}). By tethering MV^{2+} to a ligand that is sequestered by binding to a specific, biorelevant target, they created a novel, sensitive class of biosensors. The efficiency of luminescence quenching is quantified through measurements of the Stern−Volmer constant, K_{sv}:

$$\phi^\circ/\phi = 1 + K_{sv}[\text{quencher}] \qquad (1)$$

where ϕ° and ϕ are the photoluminescence (PL) quantum efficiencies in the absence and presence of the quencher, respectively. Under conditions where all other variables are held constant, the higher the K_{sv}, the lower the concentration of quencher required to achieve PL quenching. The use of a quencher with high K_{sv} in the quencher−tether−ligand unit leads directly to high sensitivity in the biosensor application. Therefore, the development of high-sensitivity biosensors requires fluorophore−quencher pairs with high K_{sv}.[7] These authors showed that MV^{2+} quenches the PL of MBL-PPV with a K_{sv} of 1.7×10^7,[6] a remarkable improvement over the highest $K_{sv} = 65$ previously reported.[4] This large K_{sv} results from a combination of two effects. First, because the polymer and the MV^{2+} quencher are oppositely charged, they form a weak complex. As a result, static quenching via ultrafast photoinduced ET dominates the quenching mechanism.[8] Second, experiments demonstrated that a single quencher will quench hundreds of repeat

Scheme 1



units on the luminescent conjugated chain; to a first approximation, a single MV^{2+} quenches the entire MBL-PPV polymer.[6]

Cyt *c* is a heme-containing protein that plays an essential role in the mitochondrial respiratory chain. The structure and function of cyt *c* have been extensively studied.[9] Cyt *c* is a cationic polyelectrolyte at neutral pH[10] and thus readily forms complexes with anionic polyelectrolytes.[11] Spectroscopic investigations suggested that in the presence of polyanions, the conformation of ferric cyt *c* resembles that observed when cyt *c* complexes with its natural ET partner, cytochrome *c* oxidase.[11a,12] This suggests that ferric cyt *c* not only has an electron-deficient heme, but that the molecular conformation is well-designed for rapid ET through complexation with polyanions.

Cyt *c* is a highly efficient quencher of MBL-PPV fluorescence (Figure 1). From the Stern−Volmer plot (eq 1) we find $K_{sv} = 3.2 \times 10^8$ at pH 7.4. This value is more than an order of magnitude higher than that observed for MBL-PPV/MV^{2+}. A possible quenching mechanism is as follows:

$$\text{MBL-PPV} \xrightarrow{h\nu} \text{MBL-PPV*} \qquad (2a)$$

$$\text{MBL-PPV*} + \text{cyt } c \text{ Fe(III)} \rightarrow \text{MBL-PPV} + \text{cyt } c \text{ Fe(II)} \qquad (2b)$$

where MBL-PPV* stands for the excited state of the polymer, cyt *c* Fe(III) and cyt *c* Fe(II) are the ferric and ferrous state of cyt *c*, respectively. Detailed studies of the mechanism are underway using photoinduced absorption measurements to identify the spectral changes that occur upon photoinduced ET. Consistent with the increased K_{sv}, the quenching is readily detectable in a simple fluorimeter at cyt *c* concentrations as low as 10^{-11} M.

Earlier studies demonstrated that the quenching efficiency of the MBL-PPV/MV^{2+} system is influenced by the charge states of viologen.[13] Therefore, we have investigated the correlation of surface charges on cyt *c* with the efficiency of fluorescence quenching (K_{sv}). We find that K_{sv} changes dramatically in response to pH-induced changes in surface charge[14] (Figure 2): the higher the charge, the more efficiently cyt *c* quenches MBL-PPV fluorescence. Note, however, that the K_{sv} is still very high (2.6×10^6) even at pH 10, conditions under which cyt *c* is slightly negatively charged.[15] As the pH rises above 10 and the protein becomes still more negative,

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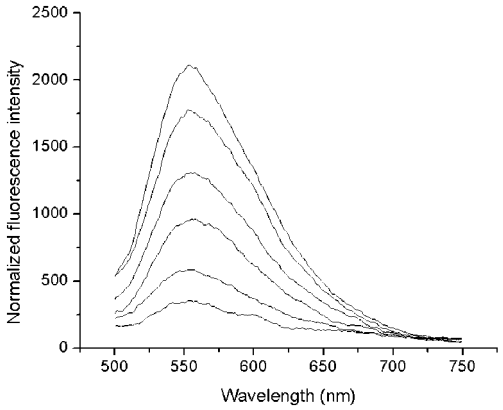

Figure 1. Fluorescence spectra (excited at 488 nm) of 1.0×10^{-6} M MBL-PPV (in monomer repeat units) in a 10 mM potassium phosphate buffer with pH 7.4 and in the presence of a series of concentrations of cytochrome *c*: From top to bottom: 0, 0.25, 1, 2.5, 5, and 9.4 nM.

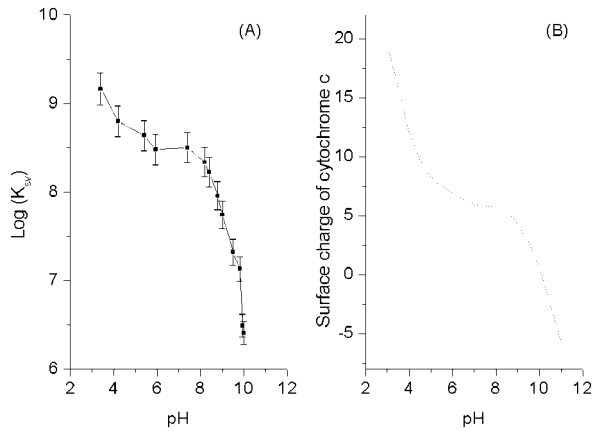

Figure 2. (a) The pH dependence of the K_{sv} of MBL-PPV/cyt *c* as a function of pH. Above pH 10 the K_{sv} drops to below the detection limit ($<10^3$). (b) Plot of surface charge of cytochrome *c* versus pH.[23]

K_{sv} drops below the detection limit of $<10^3$ M. These data imply that while the intrinsic ET property of cyt *c* quenches the fluorescence, the efficiency can be "tuned" over more than 6 orders of magnitude by controlling the protein's charge state.

To further clarify these two effects, we performed experiments on the proteins myoglobin (Mb) and lysozyme.[16] While Mb is a heme protein, extensive in vivo and in vitro studies demonstrate that the ET reactivity of Mb is very low.[17] Consistent with its low ET reactivity, the quenching efficiency of ferric Mb (metMb[18]) is much lower than that of cyt *c*. At the isoelectric point (pI) of Mb (pH 7.4, the pH at which the net charge of Mb is zero) we do not observe quenching even at the highest Mb concentration employed (5.0×10^{-6} M). We estimate that the K_{sv} at this point is at least 3 orders of magnitude lower than that of cyt *c* at its pI (pH 9.6; $K_{sv} \sim 10^7$). At pH 5.0, where Mb has a surface charge of about +7,[19] some quenching is observed: the K_{sv} is $\sim 10^6$, about 2 orders of magnitude less than that of cyt *c* with approximately the same surface charge (pH 7.4, $K_{sv} = 3.2 \times 10^8$). This control experiment suggests that quenching efficiency is dependent on the ET reactivity of the protein.

Lysozyme is highly positively charged at neutral pH, but it does not contain an ET center.[20] Nevertheless, low concentrations of lysozyme modulate the fluorescent MBL-PPV. For example, both lysozyme and cyt *c* reduce the PL by 10% at a concentration of 10^{-10} M. However, the lysozyme quenching saturates at ca. 50% at pH 7.4. In contrast, the fluorescence of MBL-PPV can be almost fully quenched by cyt *c*. This "nonspecific" quenching is also dependent on the charge state of the protein: at pH 9.0 the effect saturates at ca. 20% reduction in emission. These results imply that MBL-PPV fluorescence is sensitive to low concentrations of polycations, probably as a result of polymer aggregation induced by the ion-pair formation[5] and subsequent self-quenching.[6,21]

In conclusion, cyt *c* acts as an efficient quencher of MBL-PPV PL. The remarkably high efficiency, $K_{sv} = 3.2 \times 10^8$ at pH 7.4, is attributed to a combination of ultrafast photoinduced ET[22] between cyt *c* and the luminescent conjugated polymer and the formation of bound complexes (static quenching) between the cationic and anionic polyelectrolytes[8] (the local concentration of cyt *c* arising from complex formation with MBL-PPV enables the short ranged interaction required for ET between the conjugated polymer and the heme). Considering the pivotal role of cyt *c* as an ET relay in vivo and the efficient photoinduced charge separation observed here, our results open opportunities for potentially greatly improved PPV-based biosensors.

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