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Streptavidin sensor and its sensing mechanism based on water-soluble fluorescence conjugated polymer

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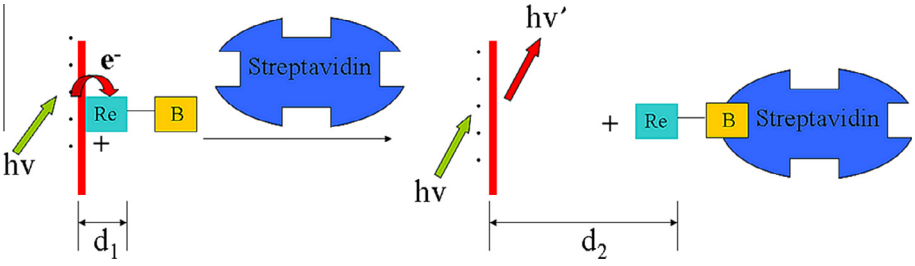
HIGHLIGHTS

- The sensing mechanism was stable whether in non-buffer system or buffer system.
- Re-Biotin probe is superior to BPP^+ probe.
- Strptavidin or avidin can be classified from other proteins by Re-Biotin.
- Strptavidin or avidin can be quantitatively determinated by Re-Biotin.

GRAPHICAL ABSTRACT



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ABSTRACT

Fluorescence quenching effect of water-soluble anionic conjugated polymer (CP) (poly[5-methoxy-2-(3-sulfopoxy)-1,4-phenylenevinylene] (MPS-PPV)) by [Re(N-N)$(CO)_3$(py-CH_2-NH-biotin)](PF_6) [N-N=2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline; py-CH_2-NH-biotin=N-[(4-pyridyl) methyl] biotinamide] (Re-Biotin) and fluorescence recovery in the presence of streptavidin (or avidin) were investigated using Re-Biotin as quencher tether ligand (QTL) probe. Meanwhile, the mechanisms of fluorescence quenching and recovery were discussed to provide new thoughts to design biosensor based on water-soluble CPs. The results indicate that the sensing mechanisms of streptavidin sensor or avidin sensor, using Re-Biotin as QTL probe, are the same and stable, whether in non-buffer system (aqueous solution) or different buffer systems [0.01 $mol \cdot L^{-1}$ phosphate buffered solution (pH = 7.4), 0.1 $mol \cdot L^{-1}$ ammonium carbonate buffered solution (pH = 8.9)]. There exists specific interactions between streptavidin (or avidin) and biotin of Re-Biotin. Fluorescence quenching and recovery processes of MPS-PPV are reversible. Mechanisms of Re-Biotin quenching MPS-PPV fluorescence can be interpreted as strong electrostatic interactions and charge transferences between Re-Biotin and MPS-PPV. Fluorescence recovery mechanisms of Re-Biotin—MPS-PPV system can be interpreted as specific interactions between streptavidin (or avidin) and biotin of Re-Biotin making Re-Biotin far away from MPS-PPV. Avidin or strptavidin as re-Biotin probe can not only be quantitatively determinated, but also be identified.

Introduction

In 1999, a new bioprobe technique (quencher tether ligand, QTL) based on water-soluble fluorescence conjugated polymers (CPs) was establish in non-buffer system by Whitten et al. [1] from the US Department of Energy (DOE), Los Alamos National Laboratory, selecting biotin–avidin as ligand receptor theoretical model, and a high sensitive biosensor detecting avidin was synthesized. Since 2000, biosensor develops rapidly using water-soluble fluorescence CPs as sensing materials. At present, it has become the hotspot of biosensor area. Many scientists had summarized the synthesis, properties and applications of some water-soluble fluorescence CPs in the field of chemical and biological sensor [2–9]. Wang et al. [8,9] had summarized the research progresses of

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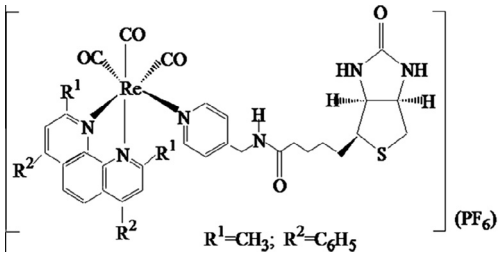
water-soluble fluorescence CPs in DNA, protein detection and sensing, cell and cell fluorescence imaging, construction of biological devices and so on. Furthermore, they forecasted that there must be significant application in high sensitive diagnosis of early stage of serious disease, drug design and individualized therapy.

At present, water-soluble fluorescence CPs have been applied in DNA detection [3,6,8,10–26], DNA and enzymes [27], RNA–proteins [28], proteins [29–33], antibodies of dinitrophenol derivative [34], enzymes and enzyme activities [35–43], cell [44–47] and cell fluorescence imaging [48–54] and so on. Although water-soluble fluorescence CPs have been used as sensitive materials to detect the biomacromolecular sensors above, their sensing mechanisms have not been very clear. Dwight et al. [29] studied the action mechanism among water-soluble anionic CP (poly[5-methoxy-2-(3-sulfopoxy)-1,4-phenylenevinylene] (MPS-PPV)), biotinylated quenching agent Q-B [N-(biotinoyl)-N′-(acetyl 4,4′-pyridylpyridinium iodide)] ethylenediamine (BPP^+)) and avidin in non-buffer system (aqueous solution) and buffer system (ammonium carbonate buffered solution of pH = 8.9). Two sensing models were put forward. In non-buffer system (aqueous solution), there is strong fluorescence quenching of MPS-PPV by Q-B in the absence of avidin, while it can make Q-B far away from MPS-PPV in the presence of avidin, so that the fluorescence of MPS-PPV can recover, which is similar with the QTL model designed by Whitten et al. [1]. While in the ammonium carbonate buffered solution of pH = 8.9, there is weak fluorescence quenching of MPS-PPV by Q-B in the absence of avidin, and the fluorescence quenching will be weaker in the presence of avidin, which is different from the QTL model designed by Whitten et al. Why the action mechanisms among water-soluble anion CP MPS-PPV, biotinylated quenching agent Q-B and avidin are different in non-buffer system (aqueous solution) and buffer system (ammonium carbonate buffered solution of pH = 8.9)? Whether other buffer systems are the same as ammonium carbonate buffer system? What are the action mechanisms among different biotinylated quenching agent Q-B, MPS-PPV and avidin?

Streptavidin (SA) is a kind of protein which has the similar biological property with avidin (A). In practical application, the detection sensitivity of SA is obviously superior to that of A [55]. SA has the same properties and application utility with A. One molecular SA or A can combine with 4 moleculars biotin. Therefore, biotin streptavidin system is constantly used to prepare biosensors just as biotin avidin system (BAS).

The application of MPS-PPV in polylysine [56], NO sensor [57] has been reported, as well as the influence of MPS-PPV fluorescence by various surfactants and metal ions [58]. Then the sensors used for rapidly detecting noble metal Pd^{2+}, Ru^{3+}, Pt^{2+} based on MPS-PPV were prepared [59].

In this article, streptavidin sensor was prepared based on water-soluble CP as sensitive material. In the experiments, biotinylated Re (I) complex—[Re(N-N)$(CO)_3$ (py-CH_2-NH-biotin)](PF_6) [N-N=2, 9-dimethyl-4,7-diphenyl-1,10-phenanthroline; py-CH_2-NH-biotin=N-[(4-pyridyl) methyl] biotinamide] (Re-Biotin, Scheme 1) was used as QTL probe. The experiments were completed according to the fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence recovery characteristic in the presence of streptavidin (or avidin). And the mechanism of fluorescence quenching and recovery was discussed. The results show that the sensing mechanisms of streptavidin sensor and that of avidin sensor are the same and stable, whether in non-buffer system (aqueous solution) or different buffer systems [0.01 mol·L^{-1} phosphate buffered solution (pH = 7.4), 0.1 mol·L^{-1} ammonium carbonate buffered solution (pH = 8.9)].



Scheme 1. Chemical structure of Re-Biotin.

Experimental

Apparatus and reagents

The pH was recorded with a PHS-3C precision pH meter. The fluorescence emission spectra were measured on a LS 55 luminescence spectrometer (Perkin Elmer Co.). MPS-PPV was prepared according to Ref. [60]. Re-Biotin [61] was offered by Dr. Jinrong Luo from City University of Hong Kong. Streptavidin, avidin, bovine serum albumin (BSA) and protamine sulfate (PS) were purchased from Sigama Co. and other reagents were domestic analytical reagents. Buffered solutions were 0.01 mol·L^{-1} phosphate buffered solution (PBS, pH = 7.4) and 0.1 mol·L^{-1} ammonium carbonate buffered solution (pH = 8.9). The Milli-Q water was used as pure water.

Fluorescence spectra measurement

The fluorescence intensity I_0 was measured on a LS 55 luminascence spectrometer (Perkin Elmer Co.). MPS-PPV solutions were added into fluorescence cuvette, and the fluorescence spectra were recorded with excitation at 387 nm and emission at 496 nm. Under the same condition, the fluorescence intensities I were recorded while adding various concentrations of Re-Biotin and the mixture solutions of Re-Biotin and streptavidin (or avidin or other proteins) into MPS-PPV solutions.

Results and discussion

Fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence potentiation of Re-Biotin—MPS-PPV System by streptavidin in phosphate buffered solution (pH = 7.4)

Fig. 1 is the fluorescence spectrum of 3.0×10^{-5} mol·L^{-1} MPS-PPV in the absence and presence of Re-Biotin in 0.01 mol·L^{-1} phosphate buffered solution. With the addition of Re-Biotin and enlargement of its concentration, the fluorescence intensities of the system gradually reduce and the peaks blue shift appear (from 496 nm to 465 nm). It was found that fluorescence of MPS-PPV could be rapidly quenched while trace amounts of Re-Biotin tetrahydrofuran solutions were added to MPS-PPV solution. It indicates that there exist strong electrostatic interactions between Re-Biotin carrying a unit positive charge and anionic polymer MPS-PPV. At the same time, electrons of anionic polymer MPS-PPV feedback to d orbital of Re(I) of Re-Biotin (that is charge transferance). The peaks blue shift may result from scattering peaks shaping from MPS-PPV agglomerating superpose on fluorescence peaks because of electrostatic interactions between Re-Biotin and MPS-PPV. The illustration in Fig. 1 is Stern–Volmer chart of Re-Biotin quenching MPS-PPV. The mechanisms of Re-Biotin quenching MPS-PPV can be interpreted as the strong electrostatic interactions and charge transferance between Re-Biotin and MPS-PPV [1,62,63]. Re-Biotin quenches MPS-PPV fluorescence according to Stern–Volmer equation:

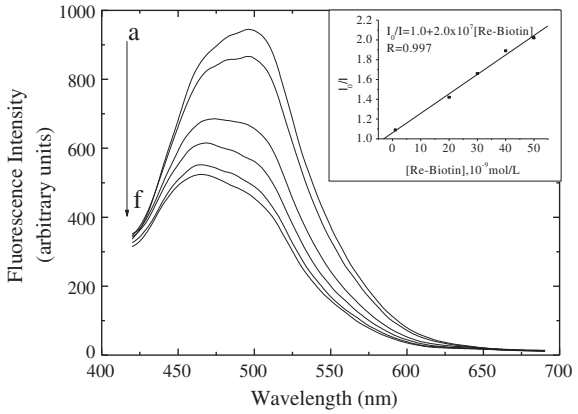


Fig. 1. Fluorescence spectra of MPS-PPV in the present of different quantities of Re-Biotin Concentrations of Re-Biotin from a to f are 0, 1, 20, 30, 40 and 50×10^{-9} - mol·L^{-1}. Experiment condition: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 K, [PBS] = mol·L^{-1} (pH = 7.4).

$$I_0/I = 1 + K_{sv}[\text{Re-Biotin}]$$

where I_0 and I are the fluorescence intensities of MPS-PPV in the absence and present of Re-Biotin, respectively, and [Re-Biotin] is the concentration of Re-Biotin. The value of constant K_{sv} can reflect the sensitivity of fluorescence quenching, and the quenching constant (K_{sv}) of Re-Biotin to MPS-PPV is 2.0×10^7 mol·L^{-1}.

In Fig. 2, curve a is the fluorescence spectral curve of 3.0×10^{-5} mol·L^{-1} MPS-PPV in the presence of 5.0×10^{-8} mol·L^{-1} Re-Biotin in 0.01 mol·L^{-1} phosphate buffered solution (pH = 7.4). With the addition of streptavidin and enlargement of its concentration, fluorescence intensities of the system gradually increase and the peaks red shift appear (from 465 nm to 496 nm). It was discovered that fluorescence intensities of MPS-PPV rapidly recovered in the presence of streptavidin solution. It indicates that the reactions between Re-Biotin and streptavidin are fast and the action forces are strong. Fluorescence intensities gradually increase and peaks red shift are displayed maybe because specificity interactions occur between streptavidin and biotin of Re-Biotin in the presence of streptavidin. Attenuation and disappearance of electrostatic interaction between Re-Biotin and MPS-PPV result into the dispersion and disappearance of coacervated MPS-PPV. In Fig. 1, with enlargement of Re-Biotin concentration, fluorescence intensities of the system gradually reduce and the peaks blue shift occur (from 496 nm to 465 nm). However, with addition of streptavidin and enlarement of its concentration, fluorescence intensities gradually increase and the peaks red shift are displayed (from 465 nm to 496 nm). It indicates that in phosphate buffer system, fluorescence quenching and recovery processes are reversible. So it can be inferred that strong interactions between Re-Biotin and streptavidin are benefit to develop reversible biosensor.

According to experiment method, different concentrations of streptavidin were added into phosphate buffered solution of 3.0×10^{-5} mol·L^{-1} MPS-PPV and 5.0×10^{-8} mol·L^{-1} Re-Biotin to test fluorescence intensity. It was discovered that with enlargement of streptavidin concentration, fluorescence intensities of the system gradually increased, and it would not change (about 85% of original intensity) until streptavidin reached a certain concentration. Plot a figure with concentration ratios of streptavidin and Re-Biotin ([Streptavidin]/[Re-Biotin]) as abscissa and fluorescence intensities as ordinate. As shown in Fig. 3, combination ratio of streptavidin and Re-Biotin is 1:4, which indicates that each streptavidin can combine with 4 Re-Biotins.

The illustration in Fig. 3 is the correlation of relative fluorescence intensity of Re-Biotin—MPS-PPV and concentration of streptavidin in the absence and presence of streptavidin. I_Q and I_R in the figure are fluorescence intensity of Re-Biotin—MPS-PPV in the absence and presence of streptavidin. As shown in the illustration, the linear equation between concentration of streptavidin [Streptavidin] and I_R/I_Q is $I_R/I_Q = 0.8955 + 6.43 \times 10^7$ [Streptavidin] and correlation coefficient is $R = 0.9941$. In the figure, the linear range and detection limit of streptavidin are 1.0×10^{-9}~ 1.2×10^{-9} mol·L^{-1} and 3.0×10^{-10} mol·L^{-1}, repectively. The results indicate that rapidly and sensitively detection of streptavidin in phosphate buffered solution could be realized.

Fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence potentiation of Re-Biotin—MPS-PPV by streptavidin in ammonium carbonated buffered solution (pH = 8.9)

Influence of MPS-PPV fluorescence by Re-Biotin and that of Re-Biotin—MPS-PPV by streptavidin were investigated while using ammonium carbonate buffered solution instead of phosphate buffered solution. As shown in Figs. 4 and 5, fluorescence quenching effect of MPS-PPV ammonium carbonate buffered solution by Re-Biotin and fluorescence potentiation of Re-Biotin—MPS-PPV

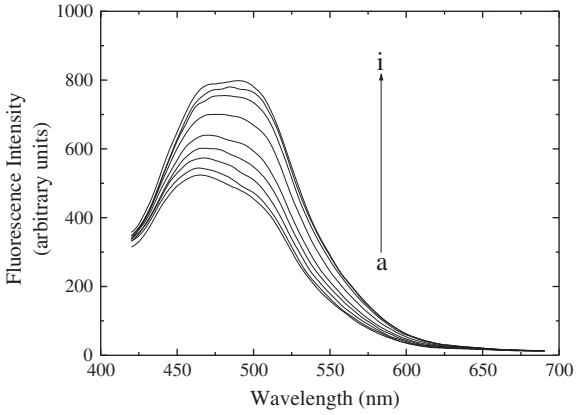


Fig. 2. Recuperative fluorescence spectra of Re-Biotin—MPS-PPV in the presence of streptavidin Concentrations of streptavidin from a to i respectively are: 0, 1.67, 3.34, 5.01, 6.68, 8.35, 10.02, 11.69 and 15.03×10^{-9} mol·L^{-1}. Experiment conditions: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 K, [PBS] = 0.01 mol·L^{-1} (pH = 7.4), [Re-Biotin] = 5.0×10^{-8} mol·L^{-1}.

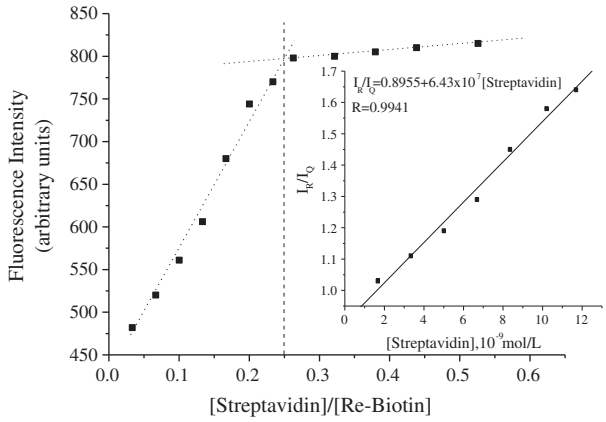


Fig. 3. Relational graph of MPS-PPV fluorescence intensity of different concentration ratio of streptavidin and Re-Biotin related to concentration ratio of streptavidin and Re-Biotin; (insert) relational graph of relative fluorescence intensity of Re-Biotin—MPS-PPV and concentration of streptavidin in the absence and presence of streptavidin. Experiment conditions: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 K, [PBS] = 0.01 mol·L^{-1} (pH = 7.4), [Re-Biotin] = 5.0×10^{-8} mol·L^{-1}.

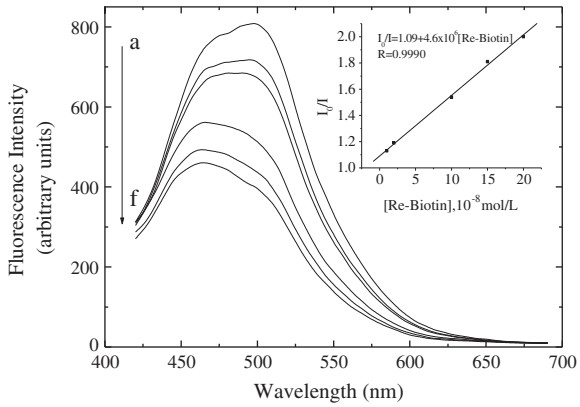


Fig. 4. Fluorescence spectra of MPS-PPV in the presence of different quantities of Re-Biotin concentrations of Re-Biotin from a to f are 0, 1, 2, 10, 15 and 20×10^{-8} mol·L^{-1}. Experiment conditions: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 k, [$(NH_4)_2CO_3$] = 0.1 mol·L^{-1} (pH = 8.9).

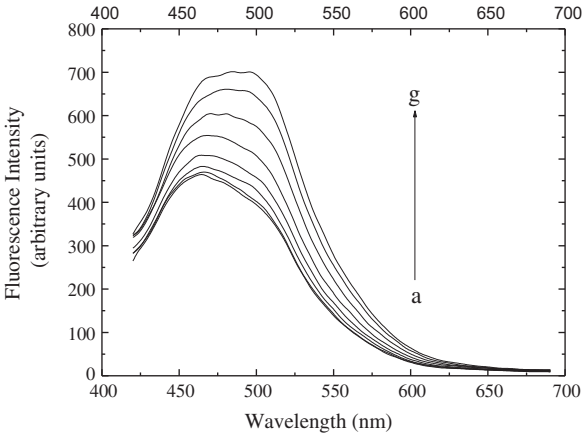


Fig. 5. Recuperative fluorescence spectra of Re-Biotin—MPS-PPV in the presence of streptavidin Concentrations of streptavidin from a to g are 0, 4, 16, 24, 32, 40, 48 and 60×10^{-9} mol·L^{-1} Experiment conditions: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 K, [$(NH_4)_2CO_3$] = 0.1 mol·L^{-1} (pH = 8.9), [Re-Biotin] = 2.0×10^{-7} mol·L^{-1}.

system by streptavidin are similar with that of phosphate buffered system.

In MPS-PPV ammonium carbonate buffered solution, with enlargement of Re-Biotin concentration, fluorescence intensities of the system gradually reduce and the peaks blue shift are displayed (from 496 nm to 465 nm). Quenching constant MPS-PPV by Re-Biotin is $K_{sv} \approx 4.6 \times 10^6$ L·mol^{-1}. From quenching constant K_{sv}, it can be found that efficiency of Re-Biotin quenching MPS-PPV is much lower in ammonium carbonate buffered solution than in phosphate buffered solution. That is to say efficiency of Re-Biotin quenching MPS-PPV in ammonium carbonate buffered solution is 23% of that in phosphate buffered solution. It indicates in Figs. 5 and 6 that in 0.1 mol·L^{-1} Re-Biotin—MPS-PPV ammonium carbonate buffered solution, with enlargement of streptavidin concentration, fluorescence intensities of the system would gradually increase and the peaks would red shift appear (from 465 nm to 496 nm). Fluorescence intensity would not change until streptavidin reaches to a certain concentration. Therefore, in ammonium carbonate buffered solution, fluorescence quenching and recovery processes of MPS-PPV are reversible.

As shown in Fig. 6, in MPS-PPV solution containing higher concentration of Re-Biotin (2.0×10^{-7} mol·L^{-1}), fluorescence would gradually recover in the presence of low concentration of

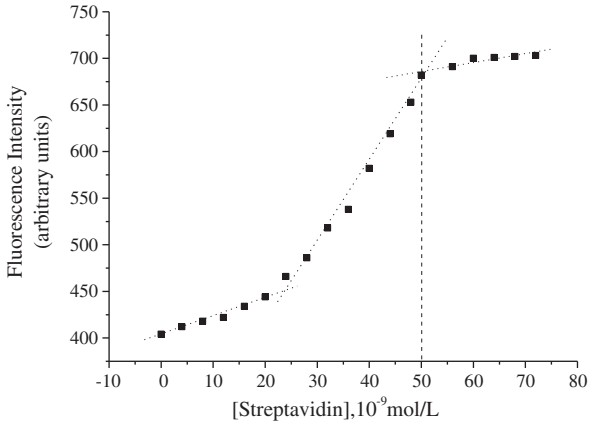


Fig. 6. Relational graph of streptsvidin concentration and fluorescence intensities of different quantities streptavidin in MPS-PPV—Re-Biotin ammonium carbonate solution. Experiment conditions: [MPS-PPV] = 3.0×10^{-5} mol·L^{-1}, T = 298 K, [$(NH_4)_2CO_3$] = 0.1 mol·L^{-1} (pH = 8.9), [Re-Biotin] = 5.0×10^{-7} mol·L^{-1}.

streptavidin. There may exist competitive reactions among MPS-PPV, Re-Biotin and streptavidin. With enlargement of streptavidin concentration, fluorescence recovery increases fast (fluorescence intensities increase obviously). Fluorescence intensities of the whole system would not change until the concentration ratio of streptavidin and Re-Biotin is about 1:4. That is the combination ratio of streptavidin and Re-Biotin is 1:4. It demonstrates that each streptavidin can combine with 4 Re-Biotins.

Fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence potentiation of Re-Biotin—MPS-PPV by streptavidin in aqueous solution

Influence of fluorescence of MPS-PPV by Re-Biotin and that of Re-Biotin—MPS-PPV by streptavidin were investigated while using aqueous solution instead of phosphate buffered solution [59]. The results indicate that fluorescence quenching effect of MPS-PPV by Re-Biotin aqueous solution and fluorescence potentiation of Re-Biotin—MPS-PPV system by streptavidin are similar with that of phosphate buffered system.

In MPS-PPV aqueous solution, with enlargement of Re-Biotin concentration, fluorescence intensities of the system gradually reduce and the peaks show clear blue shift (from 496 nm to 465 nm). Quenching constant of MPS-PPV by Re-Biotin is $K_{sv} \approx 2.9 \times 10^7$ L·mol^{-1}. In Re-Biotin—MPS-PPV aqueous solution, with tenlargement of streptavidin concentration, fluorescence intensities of the system would gradually increase and the peaks would red shift would be observed (from 465 nm to 496 nm). Fluorescence intensities would not change until streptavidin reached to a certain concentration. The results indicate that in aqueous solution, fluorescence quenching and recovery processes of MPS-PPV are reversible.

Specific interactions between Re-Biotin and streptavidin

As shown in Table 1, influences of fluorescence intensity of Re-Biotin—MPS-PPV by different proteins were investigated. It was discovered that in Re-Biotin—MPS-PPV phosphate buffered solution, fluorescence would not recover in the presence of 1.0×10^{-7} mol·L^{-1} bovine serum albumin (BSA) and it would just recover 1.7% when BSA concentration increased to 1.0×10^{-6} mol·L^{-1}. While in the presence of 1.5×10^{-8} mol·L^{-1} streptavidin, fluorescence intensity of the system recovers 34.9%, 84.4% of the original intensity. Protamine sulphate (PS) is a kind of protein

Table 1
Influences of Fluorescence of MPS-PPV by Protein.

Number	Testing system [0.01 mol·L^{-1} PBS (pH = 7.4)]	Fluorescence intensity (I)	Change rate of fluorescence intensity
a	3.0×10^{-5} mol·L^{-1} MPS-PPV	945	
b	*a* + 5.0×10^{-8} mol·L^{-1} Re-Biotin	468	*b* is 49.5% of *a*
c	*b* + 1.5×10^{-8} mol·L^{-1} Streptavidin	798	*c* recovers 84.4% of *a*, recovers 34.9% *c* increases 170.5% to b
d	*b* + 1.0×10^{-7} mol·L^{-1} BSA	468	*d* is 49.5% of *a* *d* and *b* are the same
e	*b* + 1.0×10^{-6} mol·L^{-1} BSA	484	*e* is 51.2% of *a* *e* is 103.4% of *d*
f	*a* + 3.0×10^{-7} mol·L^{-1} PS	699	*f* is 74.0% of *a*
g	*f* + 1.5×10^{-8} mol·L^{-1} Streptavidin	700	*g* is 74.1% of *a*, the same as *f*

carrying positive electron, which can partly quench fluorescence of MPS-PPV. For instance, after adding 3.0×10^{-7} mol·L^{-1} PS into MPS-PPV, fluorescence of MPS-PPV would quench 26%. Fluorescence of the system would not change after adding another 1.5×10^{-8} mol·L^{-1} streptavidin. On the other hand, in Re-Biotin—MPS-PPV system in the presence of the same concentration of streptavidin, fluorescence would recover obviously (recover 34.9%). All above indicate that there exist specific interactions between Re-Biotin and streptavidin.

Interactions between streptavidin and Re-Biotin—MPS-PPV

Influences of fluorescence of Re-Biotin—MPS-PPV system by avidin were investigated while using avidin instead of streptavidin. The results indicate that whether in non-buffer system (aqueous solution) or different buffer systems [0.01 mol·L^{-1} phosphate buffered solution (pH = 7.4), 0.1 mol·L^{-1} ammonium carbonate buffered solution (pH = 8.9)], fluorescence of Re-Biotin—MPS-PPV can reversibly recover in the presence of avidin. Each avidin can combine with 4 Re-Biotins. There still exist specific interactions between avidin and biotin of Re-Biotins.

The mechanism of Interactions among Re-Biotin, MPS-PPV and streptavidin

Fig. 7 is the schematic diagram of interactions among Re-Biotin, MPS-PPV and streptavidin. As shown in Fig. 7, whether in non-buffer system or different buffer systems, there exist specific interactions between streptavidin (or avidin) and biotin of Re-Biotin, and fluorescence quenching and recovery processes of MPS-PPV are reversible. Sensing mechanism of streptavidin sensor using Re-Biotin as QTL probe and that of avidin sensor are the same and stable, which is the same as sensing model designed by Whitten et al. [1]. Mechanism of Re-Biotin quenching MPS-PPV fluorescence can be interpreted as strong electrostatic interactions and charge transferences between Re-Biotin and MPS-PPV. Fluorescence recovery mechanism of Re-Biotin—MPS-PPV system can be interpreted as specific interactions between streptavidin (or avidin) and biotin of Re-Biotin making Re-Biotin far away from MPS-PPV.

However, Dwight et al. [29] have studied interactions among MPS-PPV, QTL probe—Q-B and avidin in non-buffer system (aqueous solution) and buffer system (ammonium carbonate buffered solution of pH = 8.9), and the result was nondeterminacy of its sensing mechanism. Dwight et al. discovered that in non-buffered system (aqueous solution), fluorescence quenching of MPS-PPV by Q-B (BPP$^+$) was strong in the absence of avidin, while MPS-PPV fluorescence would recover in the presence of avidin, which resulted from Q-B (BPP$^+$) away from MPS-PPV made by avidin. But in ammonium carbonate buffered solution of pH = 8.9, fluorescence of BPP$^+$—MPS-PPV system would not recover, on the contrary, fluorescence of the system would continue to be quenched with addition of avidin. The reason may be QTL probe in Ref. [29]—constitute and structure of BPP$^+$ are different from those of Re-Biotin. It indicates that interaction mechanisms among different biotinylated quencher (Q-B), MPS-PPV and avidin (or streptavidin) are different.

All above demonstrate that sensing mechanisms may be different due to different mediums and probes. It also indicates that biotinylated transition metal complex Re-Biotin probe is superior to biotinylated organic cation BPP$^+$ probe. The former has stable sensing mechanism and is suitable for all kinds of mediums. Electrostatic interaction and charge transference between Re-Biotin and MPS-PPV may lead to fluorescence quenching of MPS-PPV. Streptavidin (or avidin) can combine with biotin of Re-Biotin then release MPS-PPV, which results in fluorescence enhancement of the system. The phenomenon may be applied to design biosensor. Therefore, while designing biosensor based on water-soluble fluorescence CPs, constitute, structure and selectivity of polymer fluorescence probe and its specific combination with biomacromolecules should be considered into, which is the key to guarantee its property stability of biosensor. Re is a transition metal element whose outermost layer configuration is $5d^5 6s^2$. Precious metal cations Ru^{3+}, Pd^{2+} and Pt^{2+} have high property to quench MPS-PPV fluorescence. It indicates that complexes corresponding to precious metal cations Ru^{3+}, Pd^{2+} and Pt^{2+} may be designed into QTL probe of excellent properties after biotinylation. And it will provide new thoughts to design high biosensor of stable sensing mechanism based on water-soluble CPs.

Conclusions

Mechanisms of QTL probe—Re-Biotin quenching MPS-PPV fluorescence can be interpreted as the strong electrostatic interactions and charge transferences between Re-Biotin and MPS-PPV.

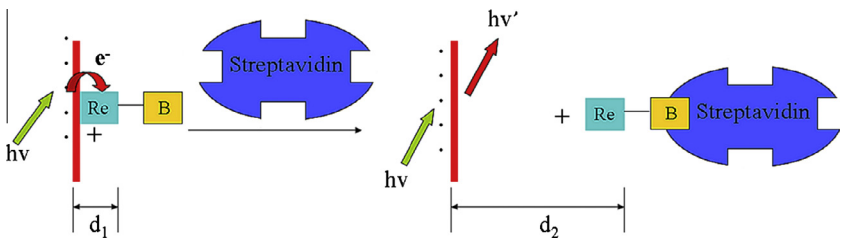


Fig. 7. Schematic diagram of recuperative fluorescence of MPS-PPV quenched by Re-Biotin in the presence of streptavidin.

Fluorescence recovery mechanism of Re-Biotin—MPS-PPV system can be interpreted as Re-Biotin far away from MPS-PPV due to specific interactions between streptavidin (or avidin) and biotin of Re-Biotin. In non-buffer system (aqueous solution) and different buffer systems [0.01 mol·L^{-1} phosphate buffered solution (pH = 7.4), 0.1 mol·L^{-1} ammonium carbonate buffered solution (pH = 8.9)], there exists specific interactions between streptavidin (or avidin) and biotin of Re-Biotin. Fluorescence quenching and recovery processes are reversible. The study demonstrates that in non-buffer system and different buffer systems, sensing mechanisms of streptavidin sensor and avidin sensor using Re-Biotin as QTL probe based on water-soluble CP MPS-PPV are the same and stable. Using Re-Biotin probe, avidin or strptavidin can not only be quantitatively determinated, but also be classified. Sensing mechanisms of biosensors may be different due to different mediums and probes. Biotinylated transition metal complex Re-Biotin probe is superior to biotinylated organic cation BPP^+ probe. Its stable sensing mechanism is suitable for different mediums. Transition metal (especially precious metal) complex QTL probes have significant potential application in designing of biosensor.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.11.058.

References

[1] L. Chen, D.W. McBranch, H.L. Wang, R. Helgeson, F. Wudl, D.G. Whitten, Proc. Natl. Acad. Sci. USA 96 (1999) 12287–12292.
[2] H. Xu, H.P. Wu, C.H. Fan, W.X. Li, Z.Z. Zhang, Sci. Bull. 49 (2004) 1925–1929.
[3] B. Liu, G.C. Bazan, Chem. Mater. 16 (2004) 4467–4476.
[4] S.W. Thomas III, G.D. Joly, T.M. Swager, Chem. Rev. 107 (2007) 1339–1386.
[5] X.F. Liu, L.H. Wang, S.P. Song, C.H. Fan, W. Huang, Prog. Chem. 20 (2008) 1375–1384.
[6] H.A. Ho, A. Najari, M. Leclerc, Acc. Chem. Res. 41 (2008) 168–178.
[7] H. Jiang, P. Taranekar, J.R. Reynolds, K.S. Schanze, Angew. Chem. Int. Ed. 48 (2009) 4300–4316.
[8] X.R. Duan, L.B. Liu, F.D. Feng, S. Wang, Acc. Chem. Res. 43 (2010) 260–270.
[9] X.L. Feng, L.B. Liu, S. Wang, D.B. Zhu, Chem. Soc. Rev. 39 (2010) 2411–2419.
[10] H.A. Ho, M. Boissinot, M.G. Bergeron, G. Corbeil, K. Dore, D. Boudreau, M. Leclerc, Angew. Chem. Int. Ed. 41 (2002) 1548–1551.
[11] B.S. Gaylord, A.J. Heeger, G.C. Bazan, Proc. Natl. Acad. Sci. USA 99 (2002) 10954–10957.
[12] S.A. Kushon, K.D. Ley, K. Bradford, R.M. Jones, D. McBranch, D. Whitten, Langmuir 18 (2002) 7245–7249.
[13] K.P.R. Nilsson, O. Inganäs, Nat. Mater. 2 (2003) 419–424.
[14] B. Liu, S. Wang, G.C. Bazan, A. Mikhailovsky, J. Am. Chem. Soc. 125 (2003) 13306–13307.
[15] A.N. Zelikin, E.S. Trukhanova, D. Putnam, V.A. Lzumrudov, A.A. Litmanovich, J. Am. Chem. Soc. 125 (2003) 13693–13699.
[16] S.A. Kushon, K. Bradford, V. Marin, C. Suhrada, B.A. Armitage, D. Mcbranch, D. Whitten, Langmuir 19 (2003) 6456–6464.
[17] B. Liu, G.C. Bazan, J. Am. Chem. Soc. 126 (2004) 1942–1943.
[18] K. Dore, S. Dubus, H.A. Ho, I. Levesque, M. Brunette, G. Corbeil, M. Boissinot, G. Boivin, M.G. Bergeron, D. Boudreau, M. Leclerc, J. Am. Chem. Soc. 126 (2004) 4240–4244.
[19] S. Wang, B.S. Gaylord, G.C. Bazan, J. Am. Chem. Soc. 126 (2004) 5446–5451.
[20] H.A. Ho, K. Doré, M. Boissinot, M.G. Bergeron, R.M. Tanguay, D. Boudreau, M. Leclerc, J. Am. Chem. Soc. 127 (2005) 12673–12676.
[21] B.S. Gaylord, M.R. Massie, S.C. Feinstein, G.C. Bazan, Proc. Natl. Acad. Sci. USA 102 (2005) 34–39.
[22] B. Liu, G.C. Bazan, Proc. Natl. Acad. Sci. USA 102 (2005) 589–593.
[23] F. He, Y.L. Tang, M.H. Yu, F.D. Feng, L.L. An, H. Sun, S. Wang, Y.L. Li, D.B. Zhu, G.C. Bazan, J. Am. Chem. Soc. 128 (2006) 6764–6765.
[24] C.J. Sun, B.S. Gaylord, J.W. Hong, B. Liu, G.C. Bazan, Nat. Protoc. 2 (2007) 2148–2151.
[25] X.R. Duan, W. Yue, L.B. Liu, Z.P. Li, Y.L. Li, F.C. He, D.B. Zhu, G.Q. Zhou, S. Wang, Nat. Protoc. 4 (2009) 984–991.
[26] J.A. Wigenius, K. Magnusson, P. Björk, O. Andersson, O. Inganäs, Langmuir 26 (2010) 3753–3759.
[27] X.L. Feng, Q.L. Xu, L.B. Liu, S. Wang, Langmuir 25 (2009) 13737–13741.
[28] S. Wang, G.C. Bazan, Adv. Mater. 15 (2003) 1425–1428.
[29] S.J. Dwight, B.S. Gaylord, J.W. Hong, G.C. Bazan, J. Am. Chem. Soc. 126 (2004) 16850–16859.
[30] C.C. You, O.R. Miranda, B. Gider, P.S. Ghosh, I.B. Kim, B. Erdogan, S.A. Krovi, U.H.F. Bunz, V.M. Rotello, Nat. Nanotechnol. 2 (2007) 318–323.
[31] X.R. Duan, L.B. Liu, X.L. Feng, S. Wang, Adv. Mater. 22 (2010) 1602–1606.
[32] K.Y. Pu, J. Shi, L. Wang, L. Cai, G. Wang, B. Liu, Macromolecules 43 (2010) 9690–9697.
[33] D. Zhao, J. Du, Y.G. Chen, X.H. Ji, Z.K. He, W.H. Chan, Macromolecules 41 (2008) 5373–5378.
[34] D. Wang, X. Gong, P.S. Heeger, F. Rininsland, G.C. Bazan, A.J. Heeger, Proc. Natl. Acad. Soc. USA 99 (2002) 49–53.
[35] W. Xia, F. Rininsland, J.S. Wittenburg, X. Shi, K. Achyuthan, D. McBranch, D. Whitten, Assay Drug. Dev. Technol. 2 (2004) 183–192.
[36] M.R. Pinto, K.S. Sehanze, Proc. Natl. Acad. Soc. USA 101 (2004) 7505–7510.
[37] S. Kumaraswamy, T. Bergstedt, X. Shi, F. Rininsland, S. Kushon, W. Xia, K. Ley, K. Achyuthan, D. McBranch, D. Whitten, Proc. Natl. Acad. Soc. USA 101 (2004) 7511–7515.
[38] F.D. Feng, Y.L. Tang, S. Wang, Y.L. Li, D.B. Zhu, Angew. Chem. Int. Ed. 46 (2007) 7882–7886.
[39] F.D. Feng, Y.L. Tang, F. He, M.H. Yu, X.R. Duan, S. Wang, Y.L. Li, D.B. Zhu, Adv. Mater. 19 (2007) 3490–3495.
[40] Y. Liu, K. Ogawa, K.S. Schanze, Anal. Chem. 80 (2008) 150–158.
[41] X.L. Feng, X.R. Duan, L.B. Liu, F.D. Feng, S. Wang, Y.L. Li, D.B. Zhu, Angew. Chem. Int. Ed. 48 (2009) 5316–5321.
[42] Y.Y. Wang, Y. Zhang, B. Liu, Anal. Chem. 82 (2010) 8604–8610.
[43] F. Pu, D. Hu, J.S. Ren, S. Wang, X.G. Qu, Langmuir 26 (2010) 4540–4545.
[44] A. Bajaj, O.R. Miranda, I.B. Kim, R.L. Phillips, D.J. Jerry, U.H.F. Bunz, V.M. Rotello, Proc. Natl. Acad. Soc. USA 106 (2009) 10912–10916.
[45] L.B. Liu, M.H. Yu, X.R. Duan, S. Wang, J. Mater. Chem. 20 (2010) 6942–6947.
[46] F.D. Feng, L.B. Liu, S. Wang, Nat. Protoc. 5 (2010) 1255–1264.
[47] A. Bajaj, O.R. Miranda, R. Phillips, I.B. Kim, D.J. Jerry, U.H.F. Bunz, V.M. Rotello, J. Am. Chem. Soc. 132 (2010) 1018–1022.
[48] I.B. Kim, H. Shin, A.J. Garcia, U.H.F. Bunz, Bioconjugate Chem. 18 (2007) 815–820.
[49] R.L. McRae, R.L. Phillips, I.B. Kim, U.H.F. Bunz, C.J. Fahrni, J. Am. Chem. Soc. 130 (2008) 7851–7853.
[50] C. Wu, B. Bull, C. Szymanski, K. Christensen, J. McNeill, ACS Nano 2 (2008) 2415–2423.
[51] A. Åslund, C.J. Sigurdson, T. Klingstedt, S. Grathwohl, T. Bolmont, D.L. Dickstein, E. Glimsdal, S. Prokop, M. Lindgren, P. Konradsson, D.M. Holtzman, P.R. Hof, F.L. Heppner, S. Gandy, M. Jucker, A. Aguzzi, P. Hammarström, K.P.R. Nilsson, ACS Chem. Biol. 4 (2009) 673–684.
[52] X.L. Feng, Y.L. Tang, X.R. Duan, L.B. Liu, S. Wang, J. Mater. Chem. 20 (2010) 1312–1316.
[53] X.L. Feng, F.T. Lv, L.B. Liu, H.W. Tang, C.F. Xing, Q. Yang, S. Wang, ACS Appl. Mater. Interfaces 2 (2010) 2429–2435.
[54] F. Tang, F. He, H.C. Cheng, L.D. Li, Langmuir 26 (2010) 11774–11778.
[55] H.D. Wang, S. Zhou, Biomedicine marking tracer technique, People's Medical Publishing House, Beijing, China, 1995.
[56] Y.G. Chen, Z.K. He, Chem. J. Chin. Univ. 26 (2005) 1428–1431.
[57] Y.Z. Wang, Y.G. Chen, Z.K. He, S.S. Hu, Chem. J. Chin. Univ. 27 (2006) 43–46.
[58] J. Du, D. Zhao, Y.G. Chen, Z.K. He, Acta Chim. Sinica 64 (2006) 963–967.
[59] Y.G. Chen, D. Zhao, Z.K. He, X.P. Ai, Spectrochim. Acta Part A 66 (2007) 448–452.
[60] S. Shi, F. Wudl, Macromolecules 23 (1990) 2119–2124.
[61] K.K.W. Lo, W.K. Hui, D.C.M. Ng, J. Am. Chem. Soc. 124 (2002) 9344–9345.
[62] D. Wang, J. Wang, D. Moses, G.C. Bazan, A.J. Heeger, Langmuir 17 (2001) 1262–1266.
[63] C.Y. Tan, M.R. Pinto, K.S. Schanze, Chem. Commun. 5 (2002) 446–447.