Different effects of Fe^{2+} and Fe^{3+} on conjugated polymer $PPESO_3$: a novel platform for sensitive assays of hydrogen peroxide and glucose†

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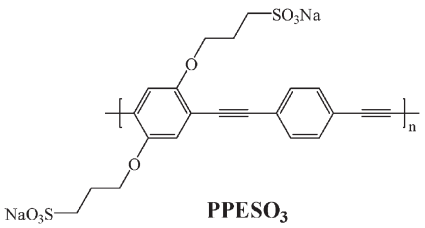
Fe^{2+} and Fe^{3+} as a redox pair showing different effects on a water-soluble conjugated polymer $PPESO_3$ is definitely an interesting and useful phenomenon in view of signal transduction and has been utilized to develop sensitive assays of hydrogen peroxide and glucose.

Conjugated polymers (CPs) are attractive sensing materials for chemical and biological sensors. Compared to small molecule counterparts, the unique π–π* conjugated electronic structure of CPs allows for rapid transfer of excitation along the whole backbone to energy/electron receptors and results in remarkable amplification of optical response. Therefore, in recent years, CP-based bio- and chemo-sensors have attracted increasing attention for the detection of organic molecules, metal ions and bio-molecules.[1] During the past several years, not only have conjugated polymers bearing diverse structures and functionalities been synthesized, but various novel sensing systems have been developed, with the analytes covering a wide range, including metal ions,[2,3] explosives,[4,5] saccharide,[6,7] protein,[8,9] enzyme,[10–13] DNA,[14] RNA,[15,16] *etc*.

Water-soluble conjugated polymers, in particular, have proven advantageous for biological molecules assays. Among these $PPESO_3$, first reported by Schanze *et al*.,[17] is an anionic water-soluble conjugated polymer bearing sulfonate side-chains (shown in Scheme 1) and has been extensively studied in the field of sensors.[6,10–12] Very recently, we also reported a facile synthetic route for its preparation.[18] In order to evaluate the interference of metal ions when using $PPESO_3$ as sensing material, the interactions between $PPESO_3$ and metal ions have been investigated in both methanol and aqueous solutions in detail (see supporting information†). It is very interesting to find that Fe^{2+} and Fe^{3+} show opposite effects on $PPESO_3$. As seen in Fig. 1, 10 μM Fe^{3+} could effectively quench the fluorescence of $PPESO_3$ in aqueous solution, while Fe^{2+} at the same concentration caused nearly no change. The absorption changes when titrating an aqueous solution of $PPESO_3$ with Fe^{3+} and Fe^{2+} are shown in Fig. S3 (supporting information†). Although the exact mechanism still requires more evidence to confirm it, the fact[19] that Fe^{3+} in protein can serve as an electron transfer (ET) center more effectively compared to Fe^{2+} may provide us with some clue for understanding it. Anyhow, it is definitely an attractive phenomenon which might be able to be utilized as a potential signal transducer in bio- and chemo-sensors. For instance, a general sensing platform based on a combination of the redox of Fe^{2+}/Fe^{3+} and the subsequent fluorescence "superquenching" of $PPESO_3$ could be established for the detection of both oxidant and reductant, as well as their related substrates.

Herein, hydrogen peroxide was chosen as a proof-of-concept for the reason that H_2O_2 can easily oxidize Fe^{2+} to Fe^{3+} due to its high oxidation–reduction potential. In addition, there is growing interest in the detection of hydrogen peroxide in many fields. On the one hand, hydrogen peroxide is a widely used oxidant in real life and industry and, therefore, is a common contaminant in air and water. On the other hand, as a vital component in physiological processes, hydrogen peroxide can act as an important transducer in cellular signal transduction and is closely correlated to various human diseases such as cancer[20] and Alzheimer's disease.[21] Furthermore, hydrogen peroxide can be generated in almost all oxidations catalyzed by oxidases;[22] this means that both the enzymes and the enzyme substrates can be conveniently assayed by determining the H_2O_2 produced. Consequently, the development of novel H_2O_2 sensors will be of great significance for environmental protection, clinical diagnostics and biological analysis.[23,24]

The proposed hydrogen peroxide assay is illustrated in Scheme 2. $PPESO_3$ emits strong fluorescence in an aqueous solution containing Fe^{2+} (Situation A). Once H_2O_2 is introduced into the system, Fe^{2+} will be oxidized into Fe^{3+}, and the latter can effectively quench the fluorescence of $PPESO_3$ (Situation B). Accordingly, the quantity of H_2O_2 can easily be determined by measuring the fluorescence reduction of $PPESO_3$.



$PPESO_3$

Scheme 1 Chemical structure of conjugated polymer $PPESO_3$.

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† Electronic supplementary information (ESI) available: Interactions between $PPESO_3$ and metal ions; absorption spectra when titrating $PPESO_3$ with Fe^{3+} and Fe^{2+}; Stern–Volmer plot for both $PPESO_3$ and $PESO_3$; effects of temperature. See DOI: 10.1039/b808586b

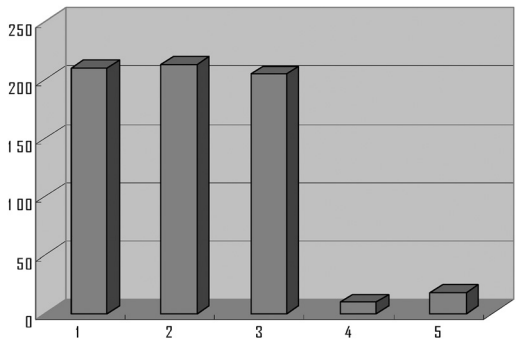


Fig. 1 Fluorescence intensity changes of $PPESO_3$ at 530 nm before (1) and after addition of Fe^{2+} (2), H_2O_2 (3), Fe^{3+} (4) and "Fe^{2+} + H_2O_2" (5), respectively. [$PPESO_3$] = 1 μM, [Fe^{2+}] = [H_2O_2] = [Fe^{3+}] = 10 μM.

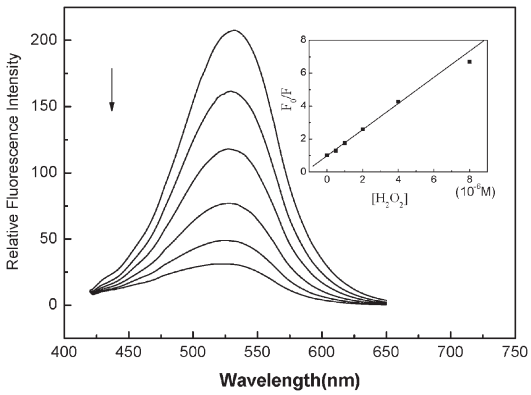
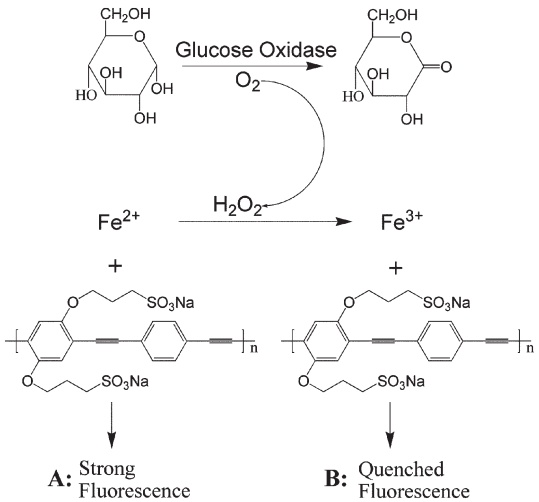


Fig. 2 Fluorescence quenching upon adding H_2O_2 to a solution of $PPESO_3$ (1 μM), Fe^{2+} (10 μM) and H^+ (20 μM). From top to bottom, the concentrations of H_2O_2 are 0, 0.5, 1, 2, 4, 8 μM, respectively.



Scheme 2 Schematic illustration of the hydrogen peroxide and glucose assays.

Fig. 1 gives a comparison of fluorescence intensity changes when adding Fe^{2+}, H_2O_2, Fe^{3+} and "Fe^{2+} + H_2O_2" to an aqueous solution of $PPESO_3$, respectively. As illustrated in this figure, neither Fe^{2+} nor H_2O_2 can quench the fluorescence of $PPESO_3$. However, after addition of 10 μM H_2O_2 to the solution of $PPESO_3$ and Fe^{2+} (10 μM), the fluorescence intensity was dramatically decreased to a state comparable to that caused by Fe^{3+}, which to a large extent corroborated our sensing scheme. Fig. 2 shows the fluorescence quenching upon titrating a solution containing $PPESO_3$ (1 μM), Fe^{2+} (10 μM) and H^+ (20 μM)[25] with H_2O_2. It was noticed that a good linear correlation could be obtained between the fluorescence quenching of $PPESO_3$ and the concentration of hydrogen peroxide in a range of 0–4 μM (Fig. 2, insert), giving a detection limit of 2.1×10^{-7} M. It is believed that the high sensitivity of the proposed hydrogen peroxide assay is mainly derived from the amplified fluorescence quenching of conjugated polymers. In previous reports, Swager *et al.* have ascribed the reason to a collective response of multiple fluorophores by means of energy or electron migration.[1d,26,27] Besides, according to Schanze and Schwartz,[28] the aggregation of conjugated polyelectrolytes in aqueous solution can also play an important role in the effective quenching, where the inter-chain exciton migration is efficient. Meanwhile, the electrostatic interaction between cationic metal ions and anionic conjugated polymer in this case can help to improve the quenching efficiency as well.[29] When compared to its small molecule counterpart $PESO_3$,[17a] the Stern–Volmer constant K_{SV} of $PPESO_3$ caused by Fe^{3+} is greatly increased by more than 100 fold (see supporting information†). Consequently, by utilizing the fluorescence "superquenching" of conjugated polymer $PPESO_3$, the H_2O_2 sensor we developed intrinsically characterizes high sensitivity, and it will certainly benefit the sensitive analysis of bio-molecules based on this H_2O_2 probe.

In order to demonstrate the potential of the CP-based H_2O_2 sensor in extended bioanalytical applications, a sensitive assay of glucose has also been developed. As seen in Scheme 2, in the presence of glucose oxidase (GOD), glucose can be specifically oxidized into gluconolactone and release an equivalent hydrogen peroxide. Immediately, the produced H_2O_2 can oxidize Fe^{2+} into Fe^{3+} and this leads to strong fluorescence quenching of $PPESO_3$, which can be directly correlated to the quantification of glucose. Using an aqueous solution containing $PPESO_3$ (1 μM), Fe^{2+} (10 μM), GOD (10 U) and H^+ (20 μM),[25] a fluorescence titration experiment with glucose was carried out at 30 °C. In these circumstances, no evident fluorescence variation of $PPESO_3$ caused by GOD was observed. Moreover, although the activity of GOD decreases slightly at this pH (≈4.7),[30] the content of 10 active units is enough to completely catalyze the oxidation of 5 μM glucose in 5 min. More importantly, the presence of H^+ can prevent hydrogen peroxide from decomposing and greatly improve the stability and repeatability of the proposed glucose assay. Fig. 3 shows the fluorescence response of the above-mentioned system upon titration, indicating that successive addition of glucose resulted in remarkable fluorescence quenching of $PPESO_3$. Similarly, the fluorescence quenching of $PPESO_3$ *versus* glucose concentration in the range of 0–5 μM exhibits good linearity as well (Fig. 3, insert), providing a detection limit of 4.3×10^{-7} M. Note that this newly developed glucose sensor should be able to work in a wide range of temperature, including room temperature (see supporting information†).

In summary, the phenomenon of Fe^{2+} and Fe^{3+} showing different effects on conjugated polymer $PPESO_3$ is very interesting and useful in view of signal transduction, which can serve as a general sensing platform for both oxidant and

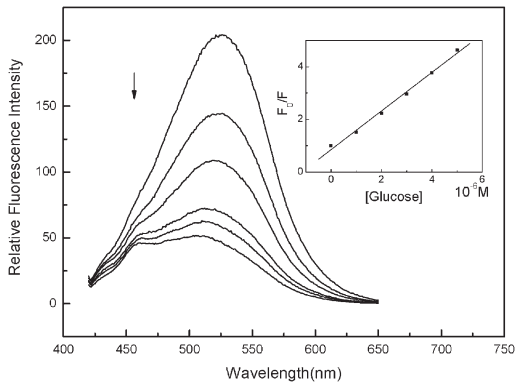


Fig. 3 Fluorescence quenching of a solution containing $PPESO_3$ (1 μM), Fe^{2+} (10 μM), GOD (10 U) and H^+ (20 μM) titrated with glucose at 30 °C. From top to bottom, the concentrations of glucose are 0, 1, 2, 3, 4, 5 μM, respectively.

reductant. Based on these findings, sensors for detecting hydrogen peroxide and glucose have been demonstrated, which can determine H_2O_2/glucose in a rather simple and sensitive manner. It is also noteworthy that H_2O_2 can be released in almost all oxidations catalyzed by oxidases, which suggests that this newly proposed H_2O_2 probe can be readily extended to sense other oxidases and their specific substrates.

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