Conjugated Polymer-Based Hybrid Materials for Turn-On Detection of CO₂ in Plant Photosynthesis

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ABSTRACT: Detection of carbon dioxide (CO₂) is of fundamental importance in diverse applications ranging from environmental analysis to agricultural production. In this work, a hybrid probe based on guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polythiophene (PTP) has been developed for the turn on detection of CO₂ with low background signal, taking advantage of the efficient fluorescence quenching of the tight aggregate of G-OF/PTP. In the presence of CO₂, the electrostatic repulsion between G-OF and PTP can be effectively enhanced through protonation of the side chains, leading to the disaggregation and thus the “turn-on” fluorescence. The strategy allows for the light-up visible detection of CO₂ with high sensitivity. Importantly, this system is capable of sensitively monitoring the concentration changes of CO₂ in the process of the photosynthesis, which represents a concept to monitor the photosynthesis based on water-soluble conjugated polymers.

Carbon dioxide (CO₂) is a greenhouse gas contributing immeasurably to the global climate change and acts as an asphyxiant with the maximal acceptable concentration defined as 3.0%.1−3 CO₂ also plays an essential role in plant photosynthesis. A total of 90−95% of the dry weight of plants derives from the assimilation of CO₂ during photosynthesis.4,5 In this context, monitoring the photosynthesis of vegetation is not only crucial for raising the productivity of agriculture but also important for the better fixation of CO₂. Therefore, the detection of CO₂, in particular during the process of photosynthesis, is of great significance for global warming monitoring,6,7 medical diagnosis,8,9 and agricultural production.10 Optical CO₂ detection methods have exhibited their advantages including convenience, low cost, and fastness, over the traditional analytical methodologies such as near-infrared spectroscopy,11 gas chromatography/mass spectrometry,12,13 and electrochemical techniques.14 For example, several effective fluorescent probes based on aggregate-induced emission (AIE) have been recently developed for monitoring CO₂.15−19 Yoon and coauthors realized colorimetric and fluorescent CO₂ detection by applying polydiacetylene in aqueous solution and in the solid state.20 However, most of the established approaches have relatively high background fluorescence signal or require additional accessory molecules.20 Moreover, to the best of our knowledge, very few examples of optical sensors have been reported to detect the changes of CO₂ in the process of photosynthesis, which is highly desirable.

Water-soluble conjugated polyelectrolytes are characterized by their delocalized π-electronic backbones and extraordinary light-harvesting capacities, exhibiting a signal amplification effect.21−24 In the past few years, water-soluble conjugated polymers have been widely employed as an efficient platform for the detection of small molecules25−27 and biomolecules such as DNA,28−30 protein,31,32 and enzymes,33,34 with specificity and high sensitivity. They have been further developed into promising materials for broad biological applications in disease diagnosis,35 drug screening and delivery,36,37 cell imaging,38 and antimicrobial susceptibility testing.39−41 Inspired by these studies, we illustrate here a fluorescent, visible and “turn-on” detection system for CO₂ in dissolved and gaseous states as well as in plant photosynthesis, by hybridizing the guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polymer poly(3-(2′-N,N-dipropanoic acid-ethylamino)-2,5-thiophene) (PTP). As illustrated in Scheme 1, PTP is designed to be functionalized with one amino group and two carboxylic groups in each repeat unit, while G-OF has guanidinium groups in the side chains. In the absence of CO₂, the guanidinium group of G-OF is partly protonated and the polymer exhibits positive charges, interacting with anionic PTP by tight electrostatic and π−π...
Scheme 1. (a) Charge Changes of G-OF and PTP upon Bubbling with CO2 and (b) Schematic Representation of G-OF/PTP Hybrid Probe for CO2 Detection in Plant Photosynthesis

"The charge changes of G-OF and PTP according to the different pK values of each side chain, the guanidinium, tertiary amine and carboxyl groups with pK values of 13.6, 9.54, and 2.2, respectively. The fluorescence of both polymers is quenched upon the formation of the tight aggregate of G-OF/PTP but gets efficiently recovered in the presence of CO2.

** Experimental Section

Materials and Measurements. G-OF and PTP were synthesized referring to the procedure reported in the literature. All chemicals were purchased from Acros, Aladdin, or Alfa Aesar and used as received if not specially stated. All organic solvent were purchased from Tianjin Guangfu Ltd. CO2, N2, and O2 were certified food grade (99.99% purity) and obtained from Tianjin Lianbo Ltd. All solutions were prepared with precooling Milli-Q water at 4 °C.

** Results and Discussion

PTP and G-OF were synthesized referring to the reported procedures. The detection of CO2 by the G-OF/PTP hybrid probe is based on the efficient fluorescence quenching of both polymers. G-OF emits bright blue light in aqueous solution with the fluorescence spectrum maximized at 420 nm, while PTP emits yellow fluorescence with a maximum at 560 nm. The fluorescence of G-OF was gradually quenched as the plant photosynthesis is consuming the CO2. Furthermore, the CO2 detection can be visualized directly in view of color changes of G-OF/PTP in aqueous medium under UV light.
The Stern–Volmer constant ($K_{SV}$) value was deduced to be $1.38 \times 10^7 \text{ M}^{-1}$ from the linear Stern–Volmer plot with low concentrations of PTP (0–25.0 nM) (Figure 1b), indicating the superquenching of G-OF by PTP. The intense electrostatic and $\pi-\pi$ stacking interactions between G-OF and PTP were attributed to drive the formation of the tight aggregation of G-OF/PTP complex and to induce the superquenching behavior.

The sensitivity of the assembled G-OF/PTP complex to CO$_2$ was first examined in the solution state, by bubbling CO$_2$ into the aqueous solution of G-OF in the presence of PTP and the results are summarized in Figure 2. As shown in Figure 2a, the fluorescence intensity of G-OF in the presence of PTP with and without CO$_2$ was found to induce very little effect on the emission spectra of G-OF in the absence of PTP (Figure 2d), while the emission of PTP was decreased with the addition of CO$_2$ (see Figure S3 in the Supporting Information). This is consistent with the working mechanism proposed in Scheme 1, where the net charges of PTP is decreased and the dominating interchain $\pi-\pi$ stacking leads to the formation of aggregates and results in the self-quenching of PTP, with the increasing amount of CO$_2$. The $\zeta$ potentials provide further evidence for the interactions between G-OF and PTP in the presence and absence of CO$_2$ (Figure 2e). $\zeta$ potentials of G-OF and PTP became more positive upon reaction with CO$_2$ because the guanidinium groups of G-OF and the amino and carboxylate groups of PTP became completely protonated. Additionally, the dynamic light scattering (DLS) measurements were also conducted. As shown in Figure 2f, the average hydrodynamic radius of G-OF/PTP aggregates is 842.2 nm, which is much larger than that in the presence of CO$_2$ (104.1 nm). This is a direct indication that the G-OF/PTP aggregates were separated due to the electrostatic repulsion between G-OF and PTP. In order to further study the mechanism of our system, the fluorescence recovery of the hybrid probe G-OF/PTP with other gases in water was measured. As illustrated in Figure S4 in the Supporting Information, no fluorescence recovery was produced by N$_2$ or O$_2$ indicating little interference from other gases in atmosphere. Moreover, the interference of SO$_2$ was checked as well. As illustrated in Figure S5, the emission of G-OF/PTP cannot be recovered in the presence of SO$_2$, possibly resulting from the total quenching of G-OF by SO$_2$ which is different from that in the presence of CO$_2$. Therefore, these results demonstrate a fluorescent, visible, and turn-on detection for CO$_2$ with high sensitivity and selectivity.

Taking the practicality into consideration, CO$_2$ is always present in the atmosphere and acts as an asphyxiant. Therefore, the fluorescence spectra of the G-OF/PTP hybrid probe before and after bubbling with CO$_2$/N$_2$ mixtures with different ratios were examined. As illustrated in Figure 3, the emission was recovered gradually with the increasing ratio of CO$_2$ in a series of CO$_2$/N$_2$ mixtures and the detected minimum ratio of CO$_2$ was 1.0% (Figure 3b). In practical terms, the maximal acceptable concentration was defined under 3.0%. Furthermore, the hybrid probe G-OF/PTP was also applied for solid-state...
sensing of CO₂. G-OF/PTP was sprayed onto a substrate of the cellulose ester film, and a dark blue to bright blue color change was observed under UV light upon exposure to CO₂ atmosphere (see Figure S6 in the Supporting Information). Therefore, our probe system offers a CO₂ detection approach allows for the assessment of the asphyxial risk.

Furthermore, the G-OF/PTP-based detection for CO₂ in gaseous phase was studied in confined spaces. As shown in Figure 4a, the detection solution of G-OF in water was placed in a confined chamber with CO₂ atmosphere (1.0 atm) for 12 h, and then the emission spectra of the G-OF/PTP hybrid probe was measured. Figure 4b exhibits that the gaseous CO₂ induces the significant recovery of emission through the absorption to the detection solution. Additionally, the different volume ratios of CO₂/N₂ mixtures in confined spaces were also detected, and the detected minimum ratio of CO₂ was 1.0% as well (see Figure S7 in the Supporting Information). In this way, the G-OF/PTP-based system allows for the detection of the gaseous CO₂ in confined spaces, which is extremely important for the workers in closed spaces to avoid the risk of asphyxia.

Finally, the CO₂ was monitored in the process of photosynthesis. It is well-known that plant photosynthesis converts CO₂ into carbohydrate by assimilation of sunlight and H₂O. Here, we employed Zea mays, a C₄-photosynthetic plant with high photosynthetic rate, as a model system to study the response of the G-OF/PTP-based probe to monitor the CO₂ changes in plant photosynthesis. As exhibited in Figure 4c, Zea mays was placed in the confined chamber with light and CO₂, and the fluorescence intensity of the G-OF/PTP hybrid probe was decreased gradually with the increase of the illumination time (Figure 4d), indicating the assimilation of CO₂ by photosynthesis. Therefore, our CO₂ detection strategy can be applied to monitor CO₂ changes in plant photosynthesis.

**CONCLUSIONS**

In summary, we have designed a hybrid probe comprising guanidinium-pendent oligofluorene (G-OF) and water-soluble conjugated polythiophene derivative (PTP) for sensing CO₂ with very low background signal. This detection strategy takes advantage of the superquenching property of G-OF by PTP in the tight aggregates of G-OF/PTP, and the CO₂ controlled aggregation induces the turn-on signal of fluorescence. The new, simple, and light-up visible CO₂ assay system has several unique characteristics. First, the G-OF/PTP-based hybrid probe realizes the quantitative detection of CO₂ in both gaseous and dissolved phase. Second, the strategy can be applied for assessment of asphyxial risk in confined spaces. Furthermore, our CO₂ optical detection system enables the monitoring of CO₂ in plant photosynthesis. Therefore, the G-OF/PTP-based turn-on approach for CO₂ detection provides meaningful applications in asphyxia diagnosis and monitoring plant photosynthesis.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b01489.

Comparison of the strategies of "pre-mixing" (CO₂ was first mixed with G-OF) and "post-mixing" (CO₂ was first mixed with PTP); the fluorescence recovery of the hybrid probe G-OF/PTP with other gases; and detection of CO₂ by composite film and additional spectral figures (PDF).

**AUTHOR INFORMATION**

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**Author Contributions**

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We are grateful for the financial supports from the National Natural Science Foundation of China (Grant No. 21574037), the “100 Talents” Program of Hebei Province, China (Grant No. E2014100004), the Program for Excellent Innovative Talents in Universities of Hebei Province, China (Grant No. BJ20140111), the Natural Science Foundation of Hebei Province (Grant No. B2015202330), the Tianjin Natural Science Foundation (Grant No. 15JCYBJC17500), and The Netherlands Organization for Scientific Research (NWO) with the Veni Grant (Grant No. 680-47-437). The authors acknowledge Prof. Shu Wang (Institute of Chemistry, Chinese Academy of Sciences) for valuable suggestions on this work.

**REFERENCES**

However, when G-OF was shown in Figure S1 (Supporting Information), the
pre-mixing (CO2 was first mixed with PTP and then CO2 was first mixed with G-OF) and
post-mixing (CO2 was first mixed with PTP) were compared. As shown in Figure S1 (Supporting Information), the fluorescence can be recovered by CO2 whether CO2 was premixed with G-OF or PTP. However, when G-OF was first mixed with PTP and then CO2 was introduced, no subsequent emission recovery was produced (see Figure S2 in the Supporting Information). Therefore, in our approach, the G-OF was first premixed with CO2 for 5.0 min at 4 °C and then PTP was added to the solution.

(68) Yuan, H.; Xing, C.; Xu, J. unpublished results.
(69) To demonstrate the interactions involved for the CO2 sensing, the strategies of “pre-mixing” (CO2 was first mixed with G-OF) and “post-mixing” (CO2 was first mixed with PTP) were compared. As shown in Figure S1 (Supporting Information), the fluorescence can be recovered by CO2 whether CO2 was premixed with G-OF or PTP. However, when G-OF was first mixed with PTP and then CO2 was introduced, no subsequent emission recovery was produced (see Figure S2 in the Supporting Information). Therefore, in our approach, the G-OF was first premixed with CO2 for 5.0 min at 4 °C and then PTP was added to the solution.