LETTER

A New Approach to Fluorescence "Turn-On" Sensing of α-Amino Acids

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ABSTRACT For the first time, a conjugated fluorescent polymer was utilized to probe α-amino acids, sensitively and selectively, through a new approach. First the strong fluorescence of the prepared polyfluorene (**P1**) was quenched by trace copper ions, and then the quenched fluorescence was recovered upon the addition of α-amino acids, making **P1** a new and sensitive biosensor toward α-amino acids. The experimental results demonstrated that the α-amino acid selective nature of **P1** over other analytes was relatively good. Thus, the work reported here might open up a new avenue for developing new biosensors.

KEYWORDS: polyfluorene • biosensor • α-amino acids • indirect strategy

Fluorescence sensing of target guests via changes in the fluorescent signals has attracted growing interest because of their high sensitivity and potential applications in chemistry and biology. Especially, conjugated polymer-based fluorescent (CPF) chemosensors attracted much attention because the "molecular wire effect" in conjugated polymers usually greatly enhanced the sensitivity of the polymer-based chemosensors because of the enhanced electronic communication among them (1, 2). As to the biosensing applications, the use of CPF has undergone enormous growth in recent years, and many biomolecules could be detected, such as adenosine triphosphate, sugars, proteins, and DNA (1, 2a). However, it seems that there were no reports concerned with the CPF chemosensors for α-amino acids, although they are key constituents of proteins. Actually, other α-amino acid fluorescent chemosensors, rather than CPF ones, were still scare, especially those of the fluorescence "turn-on" type (1). This might be due to the difficulty in designing fluorescent chemosensors, which could give out fluorescent signals while forming complexes directly with α-amino acids. Thus, in contrast to the normal method for the development of fluorescent chemosensors, we wondered if it was possible to design new ones through an indirect approach. With this idea in mind, we checked the properties of α-amino acids and found that α-amino acids could form stable complexes with some metal ions, i.e., copper ions (Chart S1 in the Supporting Information) (1). Generally, copper ions could quench the strong fluorescence of some conjugated fluorescent polymers efficiently (1); thus, it is reasonable to think that the quenched fluorescence might recover upon the addition of α-amino acids to the complex of the copper ions and CPF because they might snatch the copper ions from the polymers (Chart 1). Herein, we present such an example.

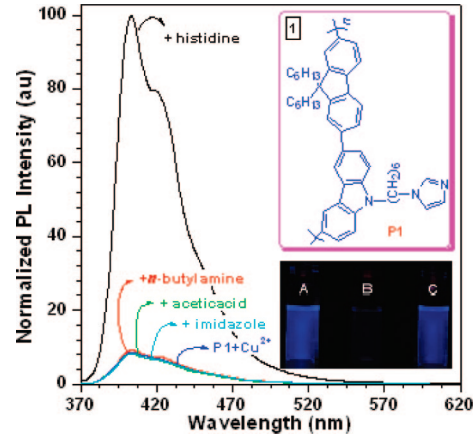
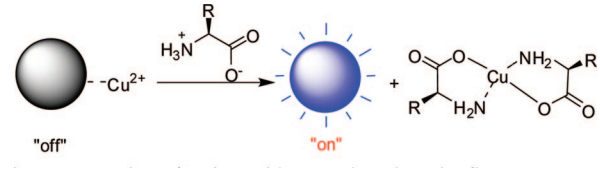


FIGURE 1. Fluorescence emission spectra of P1 + Cu^{2+}, before and after the addition of His (1×10^{-5} M), *n*-butylamine (1×10^{-5} M), acetic acid (1×10^{-5} M), and imidazole (1×10^{-5} M), respectively. The polymer concentration is 5.0×10^{-6} mol/L, while that of Cu^{2+} is 4.0×10^{-6} mol/L. Excitation wavelength (nm): 355. Inset 1: structure of P1. Inset: photographs of solutions of P1 (A) and the complex of P1 and copper ions before (B) and after (C) the addition of His, taken under UV illumination.

Chart 1. Schematic Representation of an Amino Acid Sensor Based on the Fluorescence "Turn-On" of the Complex of a Luminophore and Copper Ion



As shown in the inset 1 of Figure 1, we designed a new polyfluorene (**P1**, the detailed synthesis of which is presented in the Supporting Information) to realize the above idea because polyfluorenes exhibited strong fluorescence with high quantum yields. Imidazole moieties were linked to the backbone of **P1**, to act as the acceptor groups to trap the copper ions. Really, the strong fluorescence of **P1** (5.0

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$\times 10^{-6}$ mol/L) could be quenched efficiently by trace copper ions (4.0×10^{-6} mol/L; Figure 1). Then, we tried to add some α-amino acids, for example, glycine (Gly), to the complex of **P1** and copper ions; *immediately* the quenched fluorescence of **P1** turned on even at a concentration of Gly as low as 0.33×10^{-5} mol/L (0.24 ppm) and a ratio of signal/noise (S/N) higher than 3 (Figure S8 in the Supporting Information), and the intensity increased rapidly while the concentration of Gly increased. The fluorescent intensity could recover to about 85% of the original one of **P1**, when the concentration of Gly was 1.0×10^{-4} mol/L. To confirm that the dramatic change of the fluorescent intensity was really caused by Gly and to exclude the possible influence of the water added, a controlled experiment was conducted: different amounts of water were added to the solution of the **P1**/Cu^{2+} complex. As shown in Figure S9 in the Supporting Information, no obvious disturbance was observed even though the amount of added water was as large as 30 μL, much larger than that added in all of the experiments of the detection of α-amino acids and other analytes. Thus, the obtained results confirmed that α-amino acids really could be probed by the indirect approach. To check whether the method is a common one for all of the α-amino acids or just a special case for Gly, we repeated the experiment by utilizing other α-amino acids (their structures are shown in Chart S2 in the Supporting Information) instead of Gly. As shown in Figure S10–S31 in the Supporting Information, all of the other α-amino acids could be detected sensitively. Among them, the detection of histidine (His) demonstrated even higher sensitivity (Figure S24 in the Supporting Information); the fluorescent intensity of **P1** could recover to about 96% at a concentration as low as 3.0×10^{-5} mol/L (4.66 ppm) (Figure 1). As shown in the photographs of the solutions of **P1**, the **P1**/Cu^{2+} complex before/after the addition of His, under UV illumination (inset of Figure 1), the difference was apparent and could be observed visually. Thus, by using the indirect method reported here, α-amino acids could be detected with the aid of a UV lamp.

It was reasonable that the detection sensitivity of His was higher than those of other α-amino acids because the imidazole moieties in it (Chart S2 in the Supporting Information) might help His snatch the copper ions from the imidazole moieties in **P1**. However, another question was raised: could imidazole itself snatch the copper ions, making some influence for the detection of α-amino acids? From this point, also, to evaluate the amino acid selective nature of **P1**, the influence of other analytes, including imidazole, *n*-butylamine, acetic acid, and histamine, was investigated. As shown in Figures 1 and S32–S35 in the Supporting Information, these analytes nearly gave no disturbance to the selective sensing of amino acids except phosphate. Thus, the selectivity of **P1** for amino acids over other related analytes was relatively high.

Considering that there were generally three isomers of amino acids, α-, β-, and γ-amino acids, we studied whether **P1** could give different responses to them. Accordingly, α-, β-, and γ-aminobutyric acids (ABAs; Chart S3 in the Supporting Information) were used to evaluate the selectivity of **P1** toward α-amino acids. As shown in Figure 2, while the addition of α-ABA turned on the strong fluorescence of **P1**, the **P1**/Cu^{2+} complex only emitted weak fluorescence upon the addition of β- and γ-ABAs, respectively. The difference could also be observed visually (inset of Figure 2): under UV illumination, α-ABA-containing bottle C emitted strong luminescence, while nearly no light could be seen in bottles D and E, to which β- and γ-ABAs were added, respectively. The main reason might be that the complexes of α-amino acids and copper ions were more stable with the formed five-membered rings than those from β- and γ-amino acids. These results demonstrated that **P1** could be considered as a good selective chemosensor for α-amino acids. Thus, combined with the high sensitivity, the example of **P1** realized our thought of developing new chemosensors through an indirect approach. Also, according to the measured fluorescent spectra, the binding constants for the polymer/copper or amino/copper interactions were calculated (Table S1 in the Supporting Information), and the data were in accordance with the obtained results.

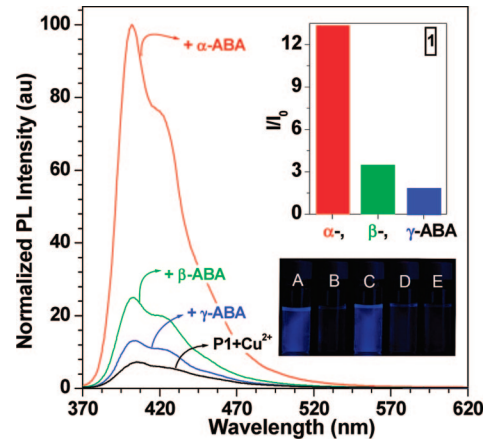

FIGURE 2. Fluorescence emission spectra of P1 + Cu^{2+}, before and after the addition of α-, β-, and γ-ABA (1×10^{-5} M), respectively. Inset 1: fluorescence emission response profiles of P1 + Cu^{2+} upon the addition of ABAs. The polymer concentration is 5.0×10^{-6} mol/L, while that of Cu^{2+} is 4.0×10^{-6} mol/L. Excitation wavelength (nm): 355. Inset: photographs of solutions of P1 (A) and the complex of P1 and copper ions before (B) and after the addition of α- (C), β- (D), and γ- (E) ABAs, taken under UV illumination.

In conclusion, we have successfully developed a new biosensor to fluorescence turn-on sensing of α-amino acids based on an indirect approach. The preliminary results demonstrated the following:

1. For the first time, conjugated fluorescent polymers were utilized to probe α-amino acids sensitively and selectively in a few seconds, with the detection limit of ~0.2 ppm, through an indirect approach. This perhaps is a novel idea to develop new α-amino acid chemosensors. Also, it was believed that many other reported good cation chemosensors could be found to be "novel" good α-amino acid chemosensors.

2. Many other biomolecules could form stable complexes with metal ions selectively, thus, the indirect approach reported here might open up a new avenue for the development of new sensitive and selective biosensors. Further

studies, especially on water-soluble biosensors, are still underway in our laboratory.

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Supporting Information Available: Details of the experimental procedure, structural characterization data, and spectra; fluorescence emission spectra; structures of general α-amino acids and α-, β-, and γ-ABAs; and a reaction equation. This material is available free of charge via the Internet at http://pubs.acs.org.

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