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Proceeding Paper

Amino Acid-Functionalized Polyfluorenes: A Class of Ultra-Sensitive Fluorescent Sensors Favoring $Cr_2O_7^{2-}$ †

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Abstract: Conjugated polymers (CPs) are an intriguing material with which to build fluorescent $Cr_2O_7^{2-}$ sensors with excellent sensitivity, but they often lack specific recognition groups. In this study, several typical amino acids with N and O atom-identifying groups were incorporated into fluorene, and then six polyfluorene derivatives were synthesized using electrochemical polymerization. Compared to other cations and anions, all of these amino acid-functionalized polyfluorenes have good selectivity towards $Cr_2O_7^{2-}$ and enable ultra-trace responses with detection thresholds at pM or even fM level.

Keywords: $Cr_2O_7^{2-}$; conjugated polymer; polyfluorene; amino acid; fluorescent sensor

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1. Introduction

The development of modern industry has caused a large amount of heavy metals to be released, causing transitional accumulation of heavy metals in the soil and expanding the area of contamination, thereby affecting the growth of crops and lowering the quality of products. Cadmium ion is more frequently acknowledged as an internationally recognized carcinogen than other heavy metal ions [1]. For example, once its content exceeds the standard in water (existing in the form of $Cr_2O_7^{2-}$), it will be enriched in crops and passed onto humans through the food chain, which will cause various diseases [2,3]. Therefore, it is necessary to detect $Cr_2O_7^{2-}$ quickly, easily, and accurately.

Among the various methods for the detection of $Cr_2O_7^{2-}$, fluorescence analysis has attracted significant attention due to its high sensitivity, simplicity, and rich output signal [4]. Conjugated polymers (CPs) have high molar absorption coefficients and strong light absorption abilities, and are good light energy harvesters [5]. Moreover, these molecules have a unique "molecular line effect" that can amplify the fluorescence response signal hundreds or thousands of times, which makes them stand out in the fields of biomonitoring and environmental analysis [6,7]. Unfortunately, there have been few reports on the specific identification of $Cr_2O_7^{2-}$ relying on CPs-based fluorescent materials.

Fluorene is a classical blue luminescent polymer material that not only has a high fluorescence quantum yield and good optical stability, but can also further improve its photoelectric performance by introducing amino acid groups containing N and O atoms in the side chain [8,9]. Amino acids containing N and O atoms are characterized by high ion-binding ability, good selectivity, and a strong pH response, which give them certain advantages in the development of efficient and sensitive ion sensors [10,11]. Therefore, our group proposed a design strategy, selecting fluorene as the fluorescence group and introducing amino acid moieties at the C9 position as recognition groups. Then, six polyfluorene fluorescent derivatives were successfully fabricated via electrochemical polymerization to construct fluorescence sensors for $Cr_2O_7^{2-}$ detection [2,3,10,11].

2. Materials and Methods

Six short chains modified with N and O atoms as recognition groups were introduced into fluorene to obtain six monomers (Fmoc-Arg-OH, Fmoc-Glu-OH, Fmoc-GABA-OH, Fmoc-Osu, Fmoc-Ala-OH and Fmoc-Thr-OH) before electrochemical polymerization in the boron trifluoride ethyl ether (BFEE) system (Figure 1). Considering that six monomers cannot be electrochemically polymerized in common organic solvents such as tetrahydrofuran (THF), N,N-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) containing supporting electrolytes, an attempt was made to add trifluoroacetic acid (TFA) into BFEE as the electrolyte solution. On one hand, BFEE, as a Lewis acid, has a catalytic effect on the electropolymerization of aromatic compounds [2,3,6,11]. On the other hand, the addition of TFA to BFEE leads to the formation of complexes between the monomer and TFA, which reduces the resonance of the aromatic ring and improves the stability of the radical ion while increasing the electrical conductivity [2,3,10,11].

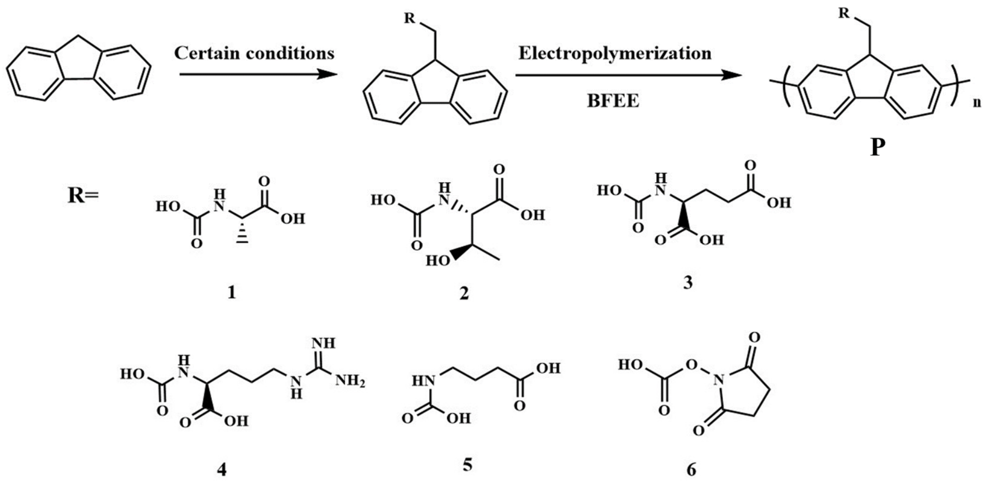

Figure 1. Electrochemical polymerization of six fluorenyl derivatives. (1, Fmoc-Ala-OH; 2, Fmoc-Thr-OH; 3, Fmoc-Arg-OH; 4, Fmoc-Glu-OH; 5, Fmoc-GABA-OH; 6, Fmoc-Osu).

3. Results and Discussion

3.1. Selectivity of Polyfluorene Derivatives towards $Cr_2O_7^{2-}$

To explore the recognition capability of $Cr_2O_7^{2-}$, selectivity experiments with six polyfluorenes for common anions and cations were carried out. It has been experimentally shown that only $Cr_2O_7^{2-}$ could quench the fluorescence of six polymers, which indicated these molecules could specifically recognize $Cr_2O_7^{2-}$. It is speculated that the N and O atoms may interact with $Cr_2O_7^{2-}$, which caused the aggregation of the fluorene skeleton and resulted in its fluorescence quenching [2,3,10,11]. All the polymers also exhibited good selectivity towards $Cr_2O_7^{2-}$, which was not interfered with by other anions and cations. Besides introducing N and O atoms for chelating $Cr_2O_7^{2-}$, another reason for using amino acid groups was to exclude the interference of natural amino acids in agricultural detection. Such good anti-interference ability is shown in Figures 2 and 3.

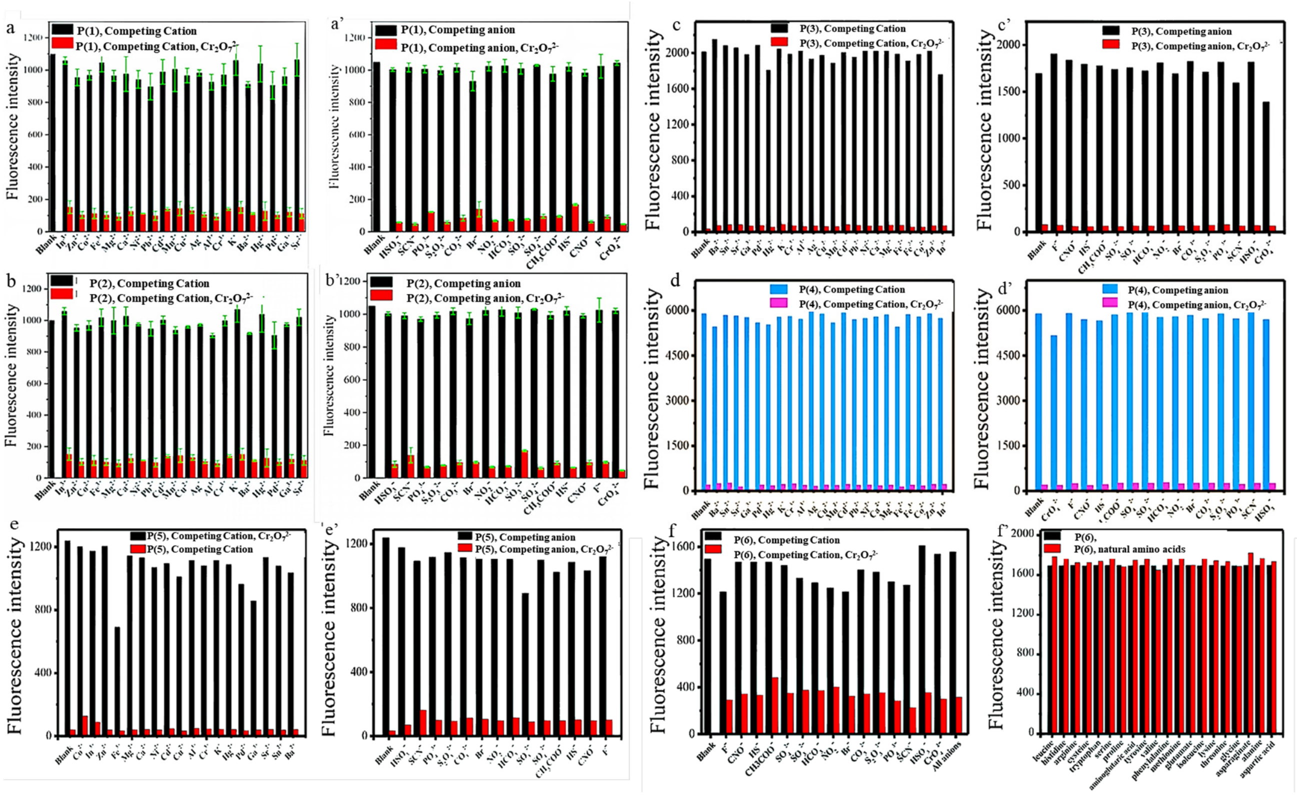

Figure 2. Fluorescence response of P (1) (**a**,**a′**), P (2) (**b**,**b′**), P (3) (**c**,**c′**), P (4) (**d**,**d′**), P (5) (**e**,**e′**) and P (6) (**f**,**f′**) in the mixed DMSO/PBS (1:1000, *v*/*v*) containing various ions. The black and blue bars represent the addition of the competing ions to a solution of six polymers. The red and pink bars represent the change of the emission that occurs upon the subsequent addition of $Cr_2O_7^{2-}$ to the above solution [2,3,10,11].

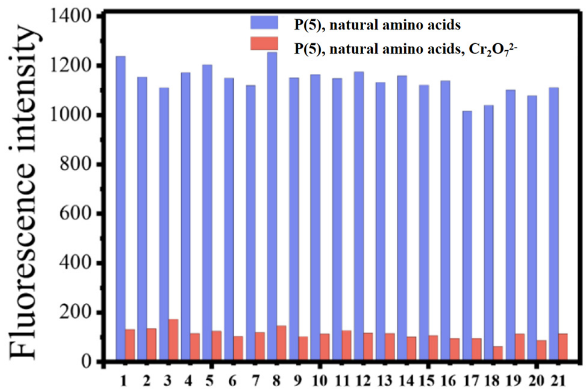

Figure 3. Fluorescence intensity of P(5) upon the addition of 0.1 mM $Cr_2O_7^{2-}$ to DMSO-PBS (*v*/*v* = 1:500) solution containing 0.1 mM natural amino acids. The abscissa is from left to right: 1. Blank, 2. Cysteine, 3. Arginine, 4. Histidine, 5. Isoleucine, 6. Asparagine, 7. Glycine, 8. Alanine, 9. Proline, 10. Glutamine, 11. Serine, 12. Aspartic acid, 13. Valine, 14. Lysine, 15. Glutamic acid, 16. Threonine, 17. Tyrosine, 18. Methionine, 19. Leucine, 20. Phenylalanine, 21. Tryptophan. (Ex = 334 nm).

3.2. Sensitivity Test of Fluorene and Polyfluorene Derivatives towards $Cr_2O_7^{2-}$

According to the linear relationship tests between the fluorescence intensity of fluorenes and polyfluorene derivatives in DMSO-EtOH solutions and the analyzed $Cr_2O_7^{2-}$ concentrations, we further explored their sensitivity, and the linear relationship between fluorescence intensity and $Cr_2O_7^{2-}$ concentration was studied. As shown in Table 1, monomers 1 and 2 have sensitivity to $Cr_2O_7^{2-}$ at the nM level, while the other four monomers only achieve μM. When they were prepared as polymers, their detection sensitivity greatly improved, reaching up to pM and even fM (Table 1), which verifies that the molecular wire effect of polymers can greatly improve the sensitivity of the detection of $Cr_2O_7^{2-}$, which further indicates that this type of amino acid-functionalized polyfluorene fluorescence material has the ability to carry out ultra-trace detection of $Cr_2O_7^{2-}$ [2,3,10,11].

Table 1. Limit of detection (LOD) for monomers and polymers.

Monomers	**1**	**2**	**3**	**4**	**5**	**6**
LOD	0.11 nM	0.27 nM	3.3 μM	16.6 μM	1.67 μM	83.3 μM
Polymers	P1	P2	P3	P4	P5	P6
LOD	1.98 fM	3.72 fM	16.67 pM	3.33 μM	16.6 fM	8.3 fM

4. Conclusions

In sum, this paper summarizes a design strategy that involves the construction of a series of amino acid-functionalized polyfluorenes as fluorescent $Cr_2O_7^{2-}$ sensors with high selectivity and sensitivity. Thanks to the side-chain groups decorated with N and O atoms, the obtained six monomers and their polymers were not interfered with by common cations, anions, or natural amino acids, and could achieve specific recognition of $Cr_2O_7^{2-}$. In comparison, the polymers showed a much higher sensitivity to LODs at the pM and fM levels. This work provides an effective method for the design of high-efficiency fluorescent sensors for $Cr_2O_7^{2-}$ even in complex environmental systems.

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