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Cationic conjugated polyelectrolyte-based sensitive fluorescence assay for adenosinetriphosphate and alkaline phosphatase

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ABSTRACT

The cationic water-soluble poly(diethynylfluorene-*co*-diethynylcarbazole-*co*-diethynylbenzothiadiazole) (**P1–P3**) were synthesized and used for adenosine triphosphate (ATP) detection. The incorporation of diethynylbenzothiadiazole (DBT) into the polymer backbone offers dual emissive polyelectrolytes with both blue and red emission bands in the dilute solution resulting from incomplete intramolecular energy transfer. In the solution of **P2**, addition of ATP can quench the emission of fluorene-*co*-carbazole units of **P2** more efficiently than that of DBT segments, which causes the fluorescent color change from blue to red. The asymmetric quenching of the blue and red emission bands in the presence of ATP might become a possible method for a real-time ATP detection. In addition, when ATP is cleaved into fragments by enzyme alkaline phosphatase (ALP), the blue emission of **P2** can be recovered. Thus, it is possible to assay the enzyme activity by triggering the change in color and intensity of the emission of the solution of **P2**.

1. Introduction

Adenosine triphosphate (ATP) is a very important sensing analyte because it plays several important roles in cell biology, mainly as the universal energy currency in living cells [1] and a signaling molecule to coordinate responses to energy status, in part by modulating ion channels [2] and activating signaling cascades [3]. Therefore, the detection and quantification of ATP by ATP-selective receptors or sensors is essential in biochemistry and clinic diagnosis, and thus receives considerable attention. For example, by virtue of the sensitive conformation-dependent absorption properties of the polythiophene (PT) backbone, some groups have developed colorimetric assays for ATP sensing using water-soluble PT derivatives due to their amplified fluorescence signals and high sensitivity in comparison to their small molecule counterparts [4–6]. On the other hand, enzyme alkaline phosphatase (ALP) is known to have the ability to remove the 5′-phosphate groups from ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) to convert each of these species into adenosine [7]. ALP is not only one of the most commonly used enzymes in gene assays and immunoassays, but also the target in routine clinical analysis because of its involvement in hepatobiliary and bone disorder [8–10]. Currently, several optical techniques to assay ALP have been employed, such as chemiluminescence, [11] colorimetric [12] and fluorometric methods [13].

Conjugated polymers (CPs) containing benzothiadiazole (BT) chromophore units have been utilized as fluorescence probes to monitor biomacromolecule [14–16]. Addition of opposite charged substrate into the solution of BT-containing CPs induces energy transfer process and triggers a fluorescence color variation from blue to green, which is a result of the increase in the interchain contacts of BT-containing CPs by the formation of an electrostatic complex and aggregation. The low-energy emission of BT indicates that it could be incorporated into high energy CPs to allow intramolecular energy transfer [14]. Although some BT based conjugated polymers have been synthesized, there is no report on water-soluble BT-containing CPs and their applications for ATP detection.

We have recently developed a sensitive fluorescent probe based on **CPFC** (**P0,** Scheme 1) to detect DNA [17]. The interchain interactions of **CPFC** lead to the formation of tight aggregates, which results in fluorescence quenching due to π-stacking between the main chains of the conjugated polymers. Keeping the strategies mentioned above in mind, we further designed new CPs containing diethynylbenzothiadiazole (DBT) units, namely, the cationic poly[(9,9-bis{6′-[(*N*,*N*-diethyl)-*N*-methylammonium]hexyl}-2,7-diethynylfluorene)-*co*-9-[6′-(*N*,*N*-diethylamino)-*N*-methylammonium]hexyl]-9H-3,6-diethynylcarbazole]-*co*-diethynylbenzothiadiazole] triiodide

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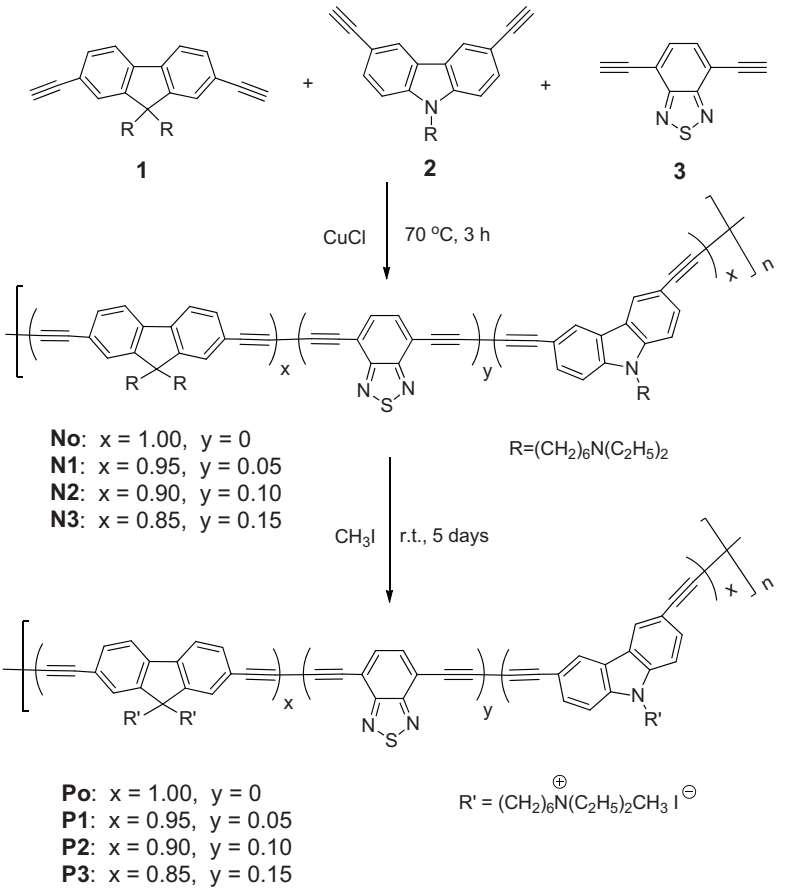

Scheme 1. Synthetic routes of **P0–P3**.

(**P1**–**P3**, Scheme 1) as fluorescent probes for the detection of ATP. Moreover, the complex of **P2**/ATP by electrostatic interactions is utilized as probe for sensitive fluorescence assays for ALP, where the products are ATP fragments and the free chains of **P2**.

2. Experimental

2.1. Materials and physical measurements

The ATP was purchased from Bio Basic Inc. The ADP and AMP were purchased from Amresco. The calf intestinal ALP was purchased from Promega. The fluorescence measurements were recorded on a Hitachi F-4500 spectrophotometer equipped with a Xenon lamp excitation source. All fluorescence spectra were measured at an excitation wavelength of 390 nm. The water was purified using a Millipore filtration system.

2.2. Synthetic process of the polymers *P1–P3*

P1–**P3** were prepared according to the procedure in our recent publication [17]. A typical procedure for the synthesis of precursor polymer **N2** was described here:

To a dried round-bottomed flask attached an inlet and outlet for oxygen, a solution of CuCl (0.02 mmol, 2 mg), *N*,*N*,*N*,*N*-tetramethylethylene diamine (TMEDA) (0.14 mmol, 16 mg) in anhydrous 1,2-dichlorobenzene (4 mL) was heated at 70 °C. After 15 min, a solution of monomer **1** (33 mg, 0.063 mmol), monomer **2** (25 mg, 0.063 mmol) and monomer **3** (1.2 mg, 0.0063 mmol) in 1,2-dichlorobenzene (2 mL) was added. The mixture was stirred at 70 °C for 3 h and precipitated into methanol (2 mL). The polymer precipitate was collected and washed with methanol, and then dried under vacuum at room temperature to yield **N2** (35 mg, 61.4%) as orange solids. ^{1}H NMR (400 MHz, CDCl3, δ, ppm): 0.58 (s, br, 4H), 0.97–0.99 (m, 18H), 1.07–1.40 (m, 18H), 1.88–1.97 (m, 6H), 2.28–2.29 (m, 4H), 2.34–2.38 (m, 2H), 2.46–2.51 (m, 12H), 4.30 (s, br, 2H), 7.37–7.39 (m, 2H), 7.51–7.55 (m, 4H), 7.64–7.69 (m, 4H), 8.28–8.29 (m, 2H). M_w: 6457; M_w/M_n: 1.23 (GPC). IR (cm^{-1}): 2206, 2132(C≡C–C≡C).

2.3. Synthesis of *P2*

To a solution of **N2** (40 mg) in 10 mL THF, 0.5 mL CH_3I was added. The solution was stirred at room temperature for 5 days. The polymer precipitate was collected and washed with THF, and then dried under vacuum at room temperature to yield **P2** (45 mg, 84.9%) as orange solids. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 0.49 (br, 4H), 1.09–2.09 (m, 34H), 2.84–2.88 (m, 8H), 3.01–3.13 (m, 6H), 3.20–3.26 (m, 12H), 3.31 (s, 9H), 4.47 (br, 2H), 7.67 (m, 2H), 7.78–7.81 (m, 6H), 7.99 (m, 2H), 8.59 (m, 2H).

2.4. ATP detection

To a solution of **P2** ([RU] = 5.0×10^{-6} M, where RU is defined as the repeating unit, for example, 1 mol RU of **P2** includes 0.1 mol of DBT units and 0.45 mol of 2,7-diethynylfluorene and 0.45 mol of 3,6-diethynylcarbazole units) in TEA buffer (0.01 M, pH 7.5) containing 50% DMF was added ATP ([ATP] = 1.0×10^{-7} to 2.0×10^{-4} M) at room temperature and the fluorescence spectra were measured with an excitation wavelength of 390 nm. The assay procedures for ADP and AMP are the same as that for ATP assay, except for using ADP and AMP ([ADP] = [AMP] = 7.0×10^{-7} to 2.0×10^{-4} M) instead of ATP.