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Signal-amplification and real-time fluorescence anisotropy detection of apyrase by carbon nanoparticle☆

Jinhua Liu [a,b,d,*], Jing Yu [a,b], Jianrong Chen [a,b,**], Ronghua Yang [c], Kaimin Shih [d,**]

[a] College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, People's Republic of China
[b] College of Geography and Environmental Science, Zhejiang Normal University, Jinhua 321004, People's Republic of China
[c] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, People's Republic of China
[d] Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong SAR, People's Republic of China

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ABSTRACT

Carbon nanomaterial combined with aptamer has been developed as an efficient bioanalytical method in sensor design. Herein, depending on carbon nanoparticle (cCNP)-enhanced fluorescence anisotropy (FA), a novel aptamer-based sensor (aptasensor) enabling signal-amplification and real-time detection of apyrase is reported. The foundation of our sensor design based on ATP-aptamer(P) can be adsorbed on the surface of cCNPs, resulting in the increase of FA due to the mass of cCNPs, and P–ATP complex has weak binding ability to cCNPs with minimal change of FA. Apyrase, being an integral membrane protein, can hydrolyze ATP and make P–ATP complex disassemble, and thus lead to the increasing of FA. Therefore, this approach is demonstrated to be a novel candidate for the detection of apyrase, with high sensitivity and selectivity. The linear dynamic range for the concentrations of apyrase is between 0.1 and 0.5 U/μL along with a detection limit of 0.05 U/μL. Furthermore, these results indicated that our design is a flexible and sensitive method for biomolecule analysis, which makes it promising for practical biomolecule analyses.

1. Introduction

Apyrases are integral membrane glycoproteins that, in the presence of millimolar concentrations of divalent cations, hydrolyze nucleoside triphosphates to ADP and phosphate with a very high specific activity [1,2]. The members of this family of apyrase include Na^+–K^+ATPase, ecto-ATPase, vacuolar ATPase, P-ATPases and so on [3–9]. Taking Na^+–K^+ATPase as an example, it is the only one capable of transporting one type of cation (extracellular K^+) in exchange for another (cytoplasmic Na^+). The Na^+–K^+ATPase is ubiquitously expressed, and its function is critical for the control of osmotic cell balance, cell pH, and the resting membrane potential of most tissues. In addition, the enzyme plays a primary role in driving secondary Na-coupled transport systems and the reabsorption of salt and water in many epithelia [10]. Therefore, it is very important to study apyrase. Despite the class, structure and function of apyrase have been intensely studied at present, however, little has been reported on detection of apyrase.

Fluorescence anisotropy (FA), as a novel technique for the routine analysis [11–14], provides information on molecular orientation, mobility, and interaction processes based on the change in molecular weight of the fluorophore [15]. Recently, a few strategies based on nanomaterials-enhanced FA for detection have been reported. The FA is enhanced for detection by covalent modification DNA on Au nanoparticles (AuNPs) or silicon nanoparticles (SiNPs) surface, when the fluorophore binds with nanoparticles, FA values will increase as molecular weight of nanoparticles [16,17]. These methods, however, require complicated material preparation or covalent labeling of the nanoparticles. Currently, graphene oxide (GO) for signal-amplification FA detection of small molecule and metal ion have been reported [18,19], which solve the above insufficience successfully by using carbon materials. Our group recent work has reported noncovalent assembly of carbon nanoparticles and aptamer for detection of thrombin and DNA methylation [20,21]. However, all these methods of detection combination carbon nanoparticle with aptamer are based on the fluorescence spectra-technology, the sample fluorescence fluctuation and photobleaching hamper the further application in biological analysis and detection [22].

Herein, we develop a signal-amplification FA sensor for apyrase detection using cCNPs, on the basis of the difference in interaction between cCNPs binding ssDNA and ssDNA-target. The foundation of our sensor design (Scheme 1) is based on the following: (1)Dye labeled single-stranded DNA (ssDNA) can be adsorbed on the cCNP surface,

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* Correspondence to: Jinhua Liu, College of Chemistry and Life Science, College of Geography and Environmental, Science, Zhejiang Normal University, Jinhua 321004, People's Republic of China. Tel./fax: +86 579 82282269.
** Corresponding authors.
E-mail addresses: liujh@zjnu.cn (J. Liu), cjr@zjnu.cn (J. Chen), kshih@hku.hk (K. Shih).

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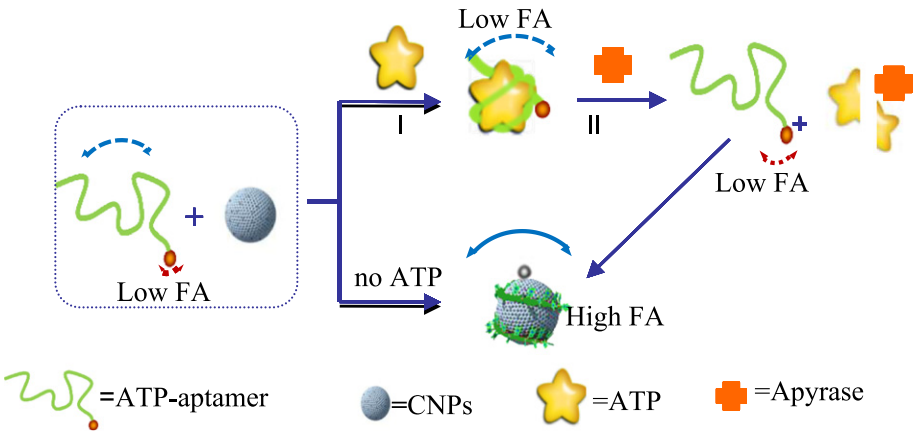

Scheme 1. A schematic (not to scale) illustrating the cCNP-based amplification fluorescent anisotropy detection apyrase.

resulting in the increase of FA because of the mass of cCNPs. (2) ssDNA-target complex has weak binding ability to cCNPs, which can be explained by the fact that the target has higher binding affinity to ssDNA than cCNPs due to the different structures between single stranded aptamer and aptamer-target complexes. Thus, we utilize cCNP-enhanced FA as a new sensor for high sensitivity and selectivity detection of apyrase. The linear dynamic ranges for the FA detection of apyrase, extending from 0.1 to 0.5 U/μL were observed, along with the detection limits of 0.05 U/μL. The present approach has the potential for real time monitoring of apyrase in complicated biological environments.

2. Experimental

2.1. Chemical and apparatus

All of the DNA synthesis reagents were purchased from Glen Research. All olgionucleotides with different sequences were synthesized using an ABI3400 DNA/RNA synthesizer and dissolved in highly pure water (sterile Millipore water, 18.3 MΩ) as stock solution. Fluorescein CPG was used for the synthesis of fluorescent oligonucleotides. Fluorescence measurements and fluorescence anisotropy measurements were performed using a Photon Technology International (U.S.A.). The microstructures of the cCNPs were examined using a JEOL JSM-6700F scanning electron microscope (SEM) (Japan). A multimode AFM (SPI3800N-SPA400, Seiko Instrument, Japan) having a piezoscanner with a maximum scan range of 100 μm × 100 μm × 5 μm. Dynamic light scatter (DLS) experiments were carried out in a Malvern Nano-ZS system equipped with a He–Ne laser working at 633 nm to examine the hydrodynamic diameter (number-weighted mean diameter).

ATP aptamer was synthesized by Shanghai Biotech (Shanghai, China) and labeled at end with FAM dye. DNA sequence of ATP aptamer was P: (5′-FAM-ACCTGGGGGAGTATTGCGGAGG AAGGT-3′). ATP, BSA and IgG were bought from Sigma (U.S.A.). KCl, $MgCl_2$, $CaCl_2$, HCl, HNO_3 and Tris(Hydroxymethyl)aminomethane were of analytical grade and purchased from Changsha Mingrui Chemical Instrument Co., Ltd., China.

2.2. Fluorescence anisotropy

FA is a technique that can provide useful information about the rotational motion of a fluorophore through measuring the polarization of the emitted fluorescence. The technique is a proven method for studying molecular mobility and has enabled the use of fluorescence anisotropy in the study of binding interactions, microviscosities of micellar systems, biological membranes, nucleic acids, proteins, and living cells [23,24]. Steady-state fluorescence anisotropy measurements are carried out by exciting the sample with vertically plane-polarized light. The parallel and perpendicular components of the fluorescence emission are measured using polarizers oriented with vertical and horizontal orientation, respectively. The observed fluorescence anisotropy (r) can then be calculated by Eq. (1),

$$r = \frac{I_{II} - I_{\perp}}{I_{II} + 2I_{\perp}} \quad (1)$$

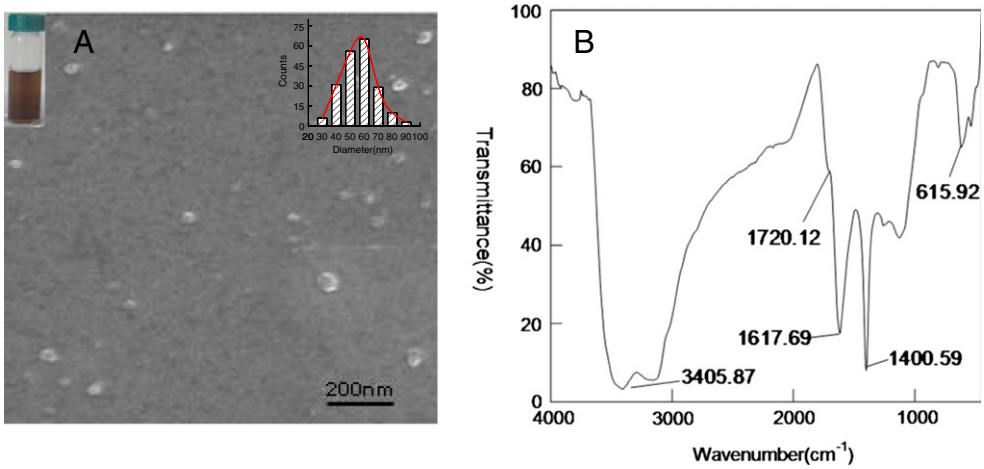

Fig. 1. Characterization of CNPs: (A) SEM images; (B) FT-IR spectroscopy.