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In-capillary self-assembly and proteolytic cleavage of polyhistidine peptide capped quantum dots

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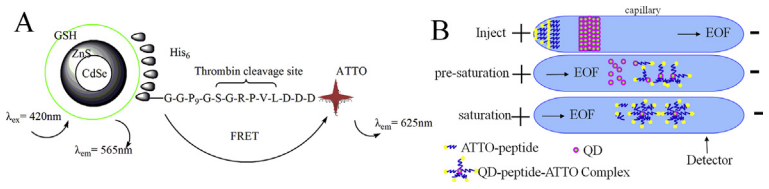
HIGHLIGHTS

- We examined the self-assembly QDs with H6-ATTO inside a capillary.
- We prove CE-FL to be a powerful method to resolve QDs-H6-ATTO complex.
- We achieve chromatographic separation of QDs-H6-ATTO complex.
- We discovered a novel strategy for the online detection of thrombin.
- This technique integrated "injection, mixing, reaction, separation and detection".

GRAPHICAL ABSTRACT



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ABSTRACT

A new method using fluorescence coupled capillary electrophoresis (CE-FL) for monitoring self-assembly and proteolytic cleavage of hexahistidine peptide capped quantum dots (QDs) inside a capillary has been developed in this report. QDs and the ATTO 590-labeled hexahistidine peptide (H6-ATTO) were injected into a capillary, sequentially. Their self-assembly inside the capillary was driven by a metal-affinity force which yielded a new fluorescence signal due to Förster resonance energy transfer (FRET). The highly efficient separation of fluorescent complexes and the FRET process were analyzed using CE-FL. The self-assembly of QDs and biomolecules was found to effectively take place inside the capillary. The kinetics of the assembly was monitored by CE-FL, and the approach was extended to the study of proteolytic cleavage of surface conjugated peptides. Being the first in-depth analysis of in-capillary nanoparticle −biomolecule assembly, the novel approach reported here provides inspiration to the development of QD-based FRET probes for biomedical applications.

1. Introduction

As an alternative to conventional fluorophores, quantum dots (QDs) offer several attractive features, including high photobleaching threshold, good chemical stability, relatively narrow and symmetric luminescence bands, readily tunable spectral properties and a rather large detectable optical signal [1]. Owning

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excellent characteristics, QDs-based bioprobes have been extensively used in cell imaging [2,3], biomolecule detection and bioanalysis [4,5].

QD bioconjugation is the key step in preparation of bioprobes and has been widely used in recent years [6]. Many methods are used for QD bioconjugation such as covalent [7,8], electrostatic adsorption [9,10] and metal-affinity methods [11,12]. Amongst them, noncovalent binding of polyhistidine peptides to metals is a simple method to modify QDs with high affinity. Using this approach, various biosensors have been developed by the peptide-QDs self-assembly based on FRET [13,14]. With the rapid development of QDs bioconjugation, there is an urgent need to expand to new analytical methods to elucidate QD–biomolecule conjugation. Until now, the cuvette-based fluorescent technique has been the best to measure QDs-peptide interactions. In fact, this technique has a simple method of measuring the sample's average fluorescence without distinguishing each entity in the mixture, individually. For example, the use of designed peptide substrates conjugated to QDs can detect the activity of various proteases [15–17] including caspase-1 [15], thrombin [16], chymotrypsin [17], and collagenase [17]. It was also demonstrated that QD–peptide assemblies could serve as surrogate substrates in a simple homogeneous assay for protein kinase activity [15]. In a recent report, a hexahistidine-appended synthetic starter peptide was ligated to the appropriately functionalized target biomolecule of interest using chemoselective ligation, and was then ratiometrically self-assembled to QDs to use for the detection of caspase-3 and elastase's activity [18]. However, QDs are heterogeneous nanoparticles and their heterogeneity usually becomes worse after bioconjugation. The current cuvette-based fluorescent method fails to measure the heterogeneity information. Moreover, it is difficult for this technique to probe the interaction-induced changes upon binding.

Over the past two decades, capillary electrophoresis (CE) expanded widely due to its high performance, high speed, low sample consumption and high separation resolution, etc. It has also been recognized as a powerful tool in the research of QDs conjugation [19–21]. Compared to the cuvette-based fluorescent method, CE-FL technique has many advantages. 1) The CE signal is dependent, not easily influenced and has a relatively large signal range; 2) CE can detect individual components, which is appropriate for detection in the complex biological solutions; 3) Its ability to conduct multi-channel detection is particularly suitable for FRET analysis, excluding the influence of other fluorescent molecules present in the solution. All these difficulties have been overcome in CE-FL method. Despite the potential advantages of CE-FL, only a few applications were reported. For example, CE-FL was demonstrated as a new way of monitoring the activation of caspase-3 during cell apoptosis [22]. CE-FL was also applied for sensitive bioanalysis using QD-based FRET sensors with low analysis uncertainty and high FRET efficiency [21].

Therefore, combining CE-FL with FRET, we could provide a new method for FRET research. We utilized CE successfully to detect FRET based on QDs and found that the FRET efficiency measured in CE improved to a certain extent of the conventional fluorescence measurement [21]. Our group also attempted to combine CE with FRET, to measure the fast self-assembly kinetics in solution. We designed a four-arm polyhistidine peptide dendrimer (PHPD) and observed a 50-fold increase in its affinity for CdSe/ZnS QDs [23]. However, these studies were carried out in solution; QDs and biological molecules first self-assembled in solution and the analysis was then completed after reaching the equilibrium.

It is a great advantage to combine capillary electrophoresis with FRET, as in complex biological systems (e.g., serum, blood), biomolecules interact with each other in the flowing bio-fluid. In other words, biomolecules associate and dissociate with each other in a very short time scale, forming a dynamic equilibrium. Hence, it is a great challenge to study the dynamic interaction between QDs and biomolecules. Herein, we presented a strategy for probing QDs and H6-ATTO self-assembly inside the capillary. The influence of molar ratio, volume injection and interval time on self-assembly were investigated by CE-FL. Additionally, we discovered a novel strategy for the online detection of thrombin by following the change of FRET signals inside the capillary, which showed the way to a fast detection of enzyme inside cells.

2. Experimental

2.1. Materials and instruments

2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Fmoc-protected amino acids, Rink Amide-MBHA resin and N-hydroxybenzotriazole (HOBt) were obtained from GL Biochem Ltd (Shanghai, China). Glutathione (GSH) and HPLC-grade acetonitrile were purchased from Adamas-Beta Co. Ltd. (Adamas-Beta, Shanghai, China). Triisopropylsilane (TIS) was purchased from Sigma–Aldrich Co. Ltd. (Milwaukee, WI, USA). ATTO 590 was bought from Baoji Juguang Biochem., LTD. N,N-dimethylformamide (DMF), N,N-Diisopropylethylamine (DIPEA), ethanedithiol (EDT) and trifluoroacetic acid (TFA) were from Meryer Technologies Co. Ltd. (Shenzhen, China). Oil-soluble CdSe/ZnS QDs-605 nm was purchased from JiaYuan Quantum Dots Co. Ltd. (Wuhan, China). All other chemicals and materials were of analytical grade. Ultrapure water ($\geq$18.2 MΩ) purified by Milli-Q system (Millipore, Bedford, MA, USA) was used for preparation of all solutions. The electrophoresis buffers were filtered through a 0.22 μm filter before use. Thrombin (Mw $\approx$ 37 kDa, specific activity = 10,000 U mg^{-1}, 1 U will cleave $\geq$ 90% of a test GST-fusion proteins in 1 × PBS at 22 °C for 16 h) was from GE healthcare UK Limited (Little Chalfont, United Kingdom). Capillary electrophoresis analyses with fluorescence detection were carried out on a home-built system, consisting of a high voltage supply (0–30 kV) (Shanghai Nuclear Research Institute, Shanghai, China), fused-silica capillaries with an inner diameter (ID) of 75 μm (Yongnian Optical Fibre Factory, Hebei, China) and an inverted IX71 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a 100-W mercury lamp, an excitation filter (BP 420 ± 20 nm), a dichromatic mirror (DM 455) and a fiber optic spectrometer QE65000 (Ocean Optics, Dunedin, FL, USA) attached to the side port. The hydrodynamic diameter (HD) and ζ-potential of QDs and QD-peptide-ATTO were determined by a Nano ZS90 (Malvern, UK) according to a dynamic light scattering (DLS) technique at 25 °C.

2.2. Peptide synthesis and purification

ATTO 590-D_3LVPRGSG$P_9G_2H_6$ (H6-ATTO) was synthesized by solid phase peptide synthesis technique based on the 9-fluorenylmethoxycarbonyl (Fmoc) chemistry using Rink Amide-ChemMatrix® resin, and a 5-fold excess of amino acid activated by one equivalent of 1:1 HBTU/HOBt in DMF. Each coupling lasted 30 min. The Fmoc group was deprotected by piperidine/DMF (20%, v/v) during 30 min. The labeling was then achieved by reacting the amino group of the lysine with a 5-fold excess of ATTO 590 free acid in the presence of DIPEA activated by 1:1 EDC/HOBt in DMF. H6-ATTO was cleaved from the resin by a cleavage cocktail of TFA, EDT, water, and TIS (94:2.5:2.5:1, v/v) for 2 h at room temperature. It was then precipitated in ice-cold diethyl ether, pelleted by centrifugation, dissolved in water, purified by semi-preparative reversed-phase HPLC, and lyophilized. Analytical reversed-phase HPLC was performed on a Vydac (218TP54) RP-HPLC column