Talanta 159 (2016) 47–54

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Aptamer and PNIPAAm co-conjugated nanoparticles regulate activity of enzyme with different temperature

Jiemiao Yu [a,b,c], Liangrong Yang [b,*], Xiangfeng Liang [a], Tingting Dong [b,c], Hongnan Qu [a,b,c], Meng Rong [b,c], Huizhou Liu [b,c,**]

[a] Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China
[b] Key Laboratory of Green Process and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form
22 May 2016
Accepted 29 May 2016
Available online 30 May 2016

Keywords:
Temperature responsive
Gold nanoparticles
Aptamer
Thrombin
Regulation
Activity

ABSTRACT

In this paper, we described a temperature responsive nano-system that can regulate activity of enzyme with different temperature. Temperature responsive polymer poly(N-isopropylacrylamide) (PNIPAAm), with low critical solution temperature of 32 °C, was synthesized with thiol modification. PNIPAAm and thrombin aptamer were co-functionalized on the surface of gold nanoparticles for effective regulation of thrombin activity with different temperature. On the one hand, we studied the thermal responsive properties of this inhibitor via UV–visible spectroscopy. On the other hand, we investigated the regulation of thrombin activity by this platform with different temperature. The PNIPAAm chains could extend and shrink with different temperature, which suggested that PNIPAAm on the surface of gold nanoparticles could regulate interaction between thrombin and aptamer according to temperature changing. At 25 °C, PNIPAAm was hydrophilic extended state, which blocked the interaction between thrombin and aptamer on the surface of gold nanoparticles, therefore thrombin activity had no change. On the contrary, at 37 °C, PNIPAAm transformed from hydrophilic extended state to hydrophobic shrank state, allowing the aptamer to capture thrombin, inhibiting the activity of thrombin. More interestingly, this regulation was reverse to normal condition, where 37 °C was always the optimum reaction temperature for most of human enzymes. This system we prepared was opposite, which was capable of inhibiting the thrombin activity at 37 °C. Furthermore, this was the first report of regulation of thrombin activity using this temperature responsive platform.

1. Introduction

Aptamers are artificial single strand DNA or RNA that possess high affinity and special recognition towards molecular targets ranging from small molecules and bio-macromolecules to even cells and tissues, which are selected in vitro from large combinatorial pools [1,2]. Compared to traditional recognition element antibody, aptamers have a great of unique features, such as minimal immunogenicity, synthesis convenience, ease of isolation and modification, and structural stability and flexibility, which make them have wildly potential applications in biosensor [3–6], separation technology [7–10] and drug delivery [11–14] etc.

Thrombin has two aptamers, including 15-base-long aptamer (named APT15) (kD = 100 nM), and the 29-base-long aptamer (named APT29) (kD = 0.5 nM), which bind to different sites of thrombin, respectively [1,15]. The thrombin contains two binding sites called Exosite 1 and Exosite 2. Exosite 1 is required for thrombin binding to several thrombin substrates (fibrinogen, thrombin receptor, heparin cofactor II) and ligand thrombomodulin. Exosite 2 is involved in the binding of heparin, glycoprotein 1b and prothrombin fragment 2 [18,19]. Dysfunction of thrombin is associated with numbers of diseases such as thrombosis, hemophilia, atherosclerosis, inflammation, even cancer [18,19,22,23]. Because the thrombin aptamers have strong specificity and high affinity with thrombin, which means thrombin aptamers can be used as an ideal alternative candidate for the anticoagulants [3–6]. Recently, many inhibitors based on aptamers have been developed, for example, Chang et al. have developed aptamers-conjugated gold nanoparticles to regulate the activity of thrombin. In these inhibitors, aptamers are used as affinity ligand to recognize thrombin and the gold nanoparticles are employed as solid matrix to support aptamers, which can enhance the inhibition of

* Corresponding author.
** Corresponding author at: Key Laboratory of Green Process and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China.
E-mail addresses: lryang@ipe.ac.cn (L. Yang), hzliu@ipe.ac.cn (H. Liu).

http://dx.doi.org/10.1016/j.talanta.2016.05.077
0039-9140/

aptamers via affinity and steric hindrance [24–27]. Light-responsive inhibitor also has been developed by Tan's group, which is able to regulate its inhibition to thrombin via different wavelength light irradiation [28]. This light-responsive system comprises thrombin aptamer (APT15), polyethylene glycol linker, and azobenzene-modified complementary sequence. UV light induces a trans-to-cis isomerization of the azobenzene that destabilizes the duplex of aptamer and azobenzene-modified complementary sequence, resulting in thrombin binding to aptamer sequence and losing its enzyme activity. Visible light irradiation results in DNA duplex rehybridization and thrombin is released with enzyme function recovered. However, so far, interestingly, temperature responsive inhibitor towards enzyme has been reported rarely. Hoffman's group has reported that using stimuli-responsive polymer to regulate the interaction between protein and ligand, which conjugated the polymer to a genetically engineered site on a protein [29]. This method can control efficiently the interaction between protein and ligand, however it needs complicated modification process.

In this paper, we developed a new inhibitor that was capable of modulating activity of thrombin in response to different environment temperature. The optimum reaction temperature of enzymes in human body is 37 °C, which mean if reaction temperature is higher or lower than 37 °C, activity of enzyme will be reduced. Thrombin is a key enzyme in coagulation cascade, and the temperature near plaques is higher than the normal body temperature [30,31]. Here, we developed this inhibitor that was able to reduce the activity of thrombin at 37 °C, while increase its activity at 25 °C, which was different to the normal. This inhibitor contained three parts: (1) aptamer as affinity ligand and inhibition element; (2) temperature responsive polymer poly(N-isopropylacrylamide) (PNIPAAm) as environment response element, whose lower critical solution temperature (LCST) is about 32 °C [32]; and (3) gold nanoparticles (AuNPs) as solid matrix for aptamer and polymer. At T(25 °C) < LCST, the polymer chains on the surface of AuNPs were hydrophilic and extended, which blocked the interaction between aptamer and thrombin, leaving thrombin in a free state and activating its function. On the contrary, at T(37 °C) > LCST, the polymer chains were hydrophobic and underwent state transition from extended to curled, allowing the aptamers to expose and interact with thrombin, inhibiting the activity of thrombin. Pioneering works of this platform were performed by Maye et al. who had investigated their thermal aggregation properties and application as a nanocarrier for drug delivery [33,34]. However, this system also can be applied to regulate activity of enzyme, and this application has not been reported so far.

2. Experiment section

2.1. Materials

The synthetic thiol-aptamer (APT15), fluorescence labelled complementary sequence (named comAPT15) were obtained from Sangon Biotech (Shanghai, China) Co., Ltd. The sequences of these oligonucleotides are as follows:

APT15: SH-$(CH_2)_6$-5′-TTTTTTTTTTGGTTGGTGTGGTTGG-3′,

comAPT15: FAM-5′-CCAACCACACCAACC-3′,

Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot H_2O$, 99.99%), sodium citrate, n-isopropyl acrylamide, 1ethyl3(3dimethyllaminopropyl)carbodiiehydrochlide (EDCI), 1-hydroxybenzotriazole (HOBT), 3-mercaptopropionic acid (MPA) and sodium chloride were from Sinopharm Chemical Reagent Co., Ltd (SCRC, China). The α-thrombin, fibrinogen (Bovine), tris-(2-carboxy-ethyl) phosphine hydrochloride (TCEP) and were purchased from Sigma-Aldrich (USA). Thrombin Chromogenic Substrate (S-2238) was purchased from Adhoc-tech (China). Other reagents were of analytical grade. Deionized water (18.2 MΩ cm) was used to prepare the aqueous solutions. The UV–visible spectrometer (HITACHI U-4100, Japan), Fluorescence spectrophotometer (HITACHI F-7000, Japan) were used to monitor the spectrum. The FITR spectra was obtained from BRUKER TENSOR 27 and dynamic light scattering (DLS) data was obtained from LLS spectrometer (ALV/CGS-3) equipped with a multi-τ digital time correlator (LSE-5004). Transmission electron microscopy (TEM) images were obtained on a HITACHI H-7650 electron microscope (Japan) operating at an accelerating voltage of 80 kV.

2.2. Functionalization of AuNPs with SH-aptamer and SH-PNIPAAm (named AuNP-APT/PNI and AuNP-PNI)

AuNPs were modified with SH-aptamer and SH-PNIPAAm according to a documented procedure with slight modification [34]. Briefly, the 5′-thiol-modified aptamers (2 OD, 200 μL) were incubated with TCEP (5 μL, 100 mM) in 50 mM acetate buffer (pH 5.2) for 1 h. Then, the solution of aptamer was added to the aforementioned citrate-capped AuNPs (5 ml) solution accompanied with shaking for 1 min. After incubation for at least 16 h at room temperature, the SH-PNIPAAm (final concentration 500 μM) was added into the solution and incubated for another 12 h at room temperature. Then 500 mM Tris-acetate (pH 8.2) buffer was added dropwise to vial with gentle hand shaking, and the final tris-acetate buffer concentration was 5 mM. In the subsequent steps, the NaCl concentration was gradually raised to 0.4 M in increments of 0.05–0.1 M by adding buffer solution of 5 M NaCl. After each addition of NaCl, the solution was incubated for 1 h and finally incubated overnight at 0.4 M NaCl at room temperature. The aptamer and PNIPAAm co-functionalized AuNPs samples were washed 3 times by repeated centrifugation and redispersion with 0.01% SDS solution and then dissolved in binding buffer (34 mM Tris–HCl, 150 mM NaCl, 8.5 mM KCl, 1.7 mM $MgCl_2$, 1.7 mM $CaCl_2$, (v/v) glycerol, pH 7.4) before use. The modification of AuNPs with PNIPAAm was performed as above except adding aptamer and NaCl. These two modified AuNPs were named as AuNP-APT/PNI and AuNP-PNI, respectively.

2.3. Determination of the number of aptamers bound at the AuNPs

The average number of aptamer bound per gold nanoparticle was determined using comAPT15 which can hybridize with 15-base thrombin aptamer in binding buffer. The comAPT15 was modified with the fluorescent dye molecule FAM. The sample was incubated at 37 °C with complementary APT15 (comAPT15) for 24 h. Following hybridization, the solution was centrifuged, and the supernatant containing excess comAPT15 was collected. The concentration of excess comAPT15 was found by comparing the fluorescence intensity of the samples to a concentration calibration curve prepared for comAPT15.

2.4. Real-time kinetics of coagulation based on different temperature

To investigate the real-time kinetics of anticoagulation activity by this temperature responsive inhibitor (AuNP-APT/PNI) with different temperature, a simple experiment was designed based on the mixture of sample of thrombin and fibrinogen substrate in binding buffer as previously reported with slight modification [28]. The soluble fibrinogen can become non-fluidic and tends to change the transmittance of light by fibrin aggregation produced by thrombin catalytic reaction. First, 500 μL (5 nM) AuNP-APT/PNI was incubated with 50 nM concentrations of thrombin under different temperature 25 °C and 37 °C for 30 min. Then, 300 μL binding buffer and 200 μL fibrinogen (10 mg/ml) were added and