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Novel Rolling Circle Amplification and DNA Origami-Based DNA Belt-Involved Signal Amplification Assay for Highly Sensitive Detection of Prostate-Specific Antigen (PSA)

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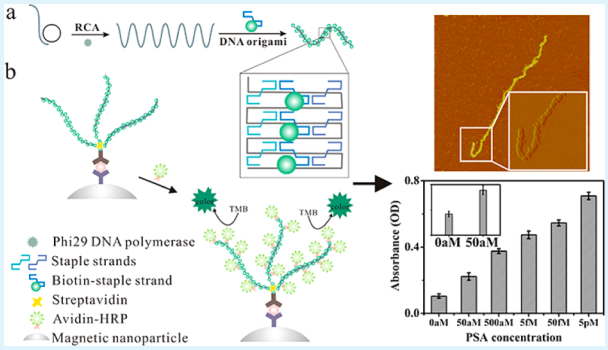
Supporting Information

ABSTRACT: Prostate-specific antigen (PSA) is one of the most important biomarkers for the early diagnosis and prognosis of prostate cancer. Although many efforts have been made to achieve significant progress for the detection of PSA, challenges including relative low sensitivity, complicated operation, sophisticated instruments, and high cost remain unsolved. Here, we have developed a strategy combining rolling circle amplification (RCA)-based DNA belts and magnetic bead-based enzyme-linked immunosorbent assay (ELISA) for the highly sensitive and specific detection of PSA. At first, a 96-base circular DNA template was designed and prepared for the following RCA. Single stranded DNA (ssDNA) products from RCA were used as scaffold strand for DNA origami, which was hybridized with three staple strands of DNA. The resulting DNA belts were conjugated with multiple enzymes for signal amplification and then employed to magnetic bead based ELISA for PSA detection. Through our strategy, as low as 50 aM of PSA can be detected with excellent specificity.



KEYWORDS: *prostate-specific antigen (PSA), rolling circle amplification (RCA), DNA origami, DNA nanostructures, signal amplification*

INTRODUCTION

Prostate cancer is the most common malignancy and the second leading cause of cancer mortality among the male population in many developed countries.[1] And cancer diagnostics at an early stage is considered the best way to control and improve mortality rate from prostate cancer.[2] PSA, an androgen-regulated serine protease, is the most important serum biomarker available for the preoperative diagnosis and screening of prostate cancer. Sensitive detection of PSA has become the key point for early diagnosis and prognosis of prostate cancer.[3−6] Conventional immunosensors, such as ELISA, despite being the most popular techniques that have been used in clinical diagnosis for detection of many cancer biomarkers, still have some drawbacks, including a relatively poor (about 0.1 ng/mL) clinical limit of detection (LOD).[7,8] Meanwhile, the quantitative detection of PSA has been developed by using various detection signals methods based on optics, electrochemistry, fluorescence, radioactivity, colorimetry, Raman spectroscopy, quartz crystal micro balance, and piezoelectric cantilever.[9−15] However, although PSA in biological specimens can be easily detected and identified even at very low levels in these impressive developments, certain challenges still needed to be addressed such as requiring sophisticated instruments, having high cost and complex operation steps, being confined to the laboratory, and being unavailable to commercialization. Here, we demonstrated a new strategy to achieve ultrahigh sensitivity for PSA detection by combining RCA and DNA origami techniques.

DNA nanotechnology has attracted intense research interest and shown great promise in various fields as its high controllability and precision.[16−29] However, the necessity of using hundreds of staple strands, complicated scaffold design, and time-consuming annealing protocols pose significant problems for the applications of DNA nanotechnology.[30]

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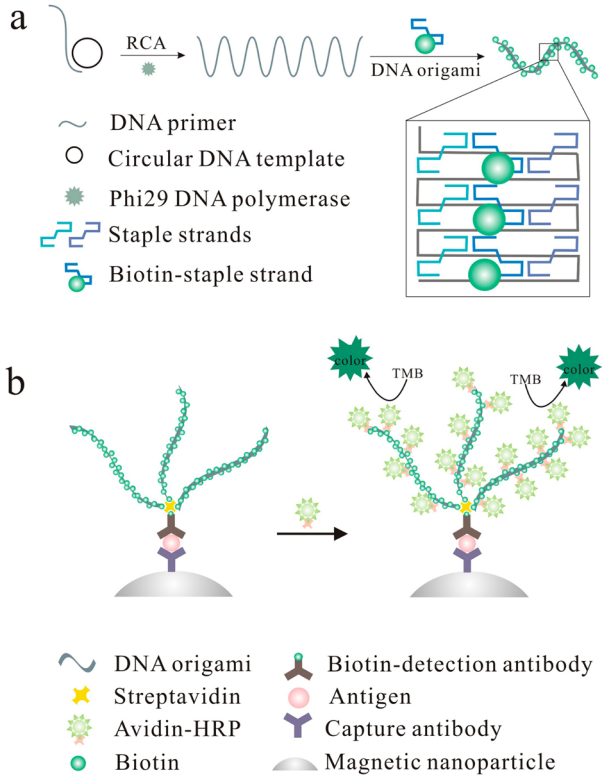
And, only a limited number of studies have been published on DNA origami-based DNA nanostructures for the biodetection.[31,32] Our strategy is to design an ultrahigh sensitive detection for PSA by integrating RCA with DNA origami techniques, which exhibits a significantly enhanced detection performance over previously reported DNA origami-based biosensors. Usually, M13 bacteriophage genomic DNA is used as the long scaffold ssDNA in DNA origami, which is difficult to prepare. In contrast to this conventional scaffold strand, RCA-based ssDNA containing hundreds of short periodic sequences can be easily prepared through the isothermal and highly efficient RCA reaction.[33−37]

RESULTS AND DISCUSSION

We first fabricated DNA nanostructures by using RCA, in which 96-base circular DNA template was employed. After amplification with phi29 polymerase, ssDNA containing 96-base periodic units can be obtained. We then folded the long RCA-based ssDNA scaffold into DNA belts with only three staple strands. Theoretically, the width of the DNA belts would be 16 nm. Biotin recognition sites are designed to be incorporated into the DNA belts in the folding process for the signal amplification via the avidin-HRP conjugation (Scheme 1a). At last, we employed the DNA belts as a novel signal amplification, which were combined to the magnetic bead-based ELISA strategy for improved PSA detection (Scheme 1b).

Scheme 1. (a) Schematic Illustration of the Fabrication of the DNA Belt-Based on Rolling Circle Amplification and DNA Origami; (b) Schematic Illustration of a Novel Signal Amplification Assay Applying DNA Belts into Magnetic Bead-Based ELISA Strategy for the Highly Sensitive and Specific Detection of PSA



After we completed the RCA reaction and DNA origami, we obtained DNA belts that dispersed in the mixture of remaining free staple strands, enzymes, and dNTPs. The free biotin-staple strands would competitive bind to avidin-HRPs when they were employed to the magnetic beads based ELISA strategy without further purification, which could decrease the efficiency of amplification.

Given that, separation of the DNA belts from their crude reaction mixtures is important. Here, we employed electrophoresis (1% agarose gel) to purify the DNA belts. We employed biotin-staple strands as an indicator, which presented a band with relatively high mobility (lane S, Figure 1). Before

Figure 1. Image of 1% agarose gel electrophoretic analysis of origami products. Gel stained with Gelred was imaged under an ultraviolet lamp. Lane O, origami products; lane A, origami products after purification; lane S, biotin-staple strand.

purification, a single clear band of high mobility relative to that of lane S was observed and assigned to the free biotin-staple strands remaining in the crude reaction mixtures. In addition, discrete band of different mobility was observed, suggesting the formation of high-order DNA nanostructures (lane O, Figure 1). After purification, we observed a discrete band similar to lane O close to the sample well, but no band of high mobility was observed at the corresponding position with lane S and lane O, which indicated successful isolation of free staple strands (lane A, Figure 1).

Next, we characterized the RCA products and the resulting DNA belts by atomic force microscope (AFM). After RCA, long, linear ssDNA were produced, but some of them may stick together and condense into globular structures as the flexibility of the sugarphosphate back bones and the electrostatic attraction between each other (Figure S1a in the Supporting Information). However, under the condition of high temperature during DNA origami, globular structures were changed to be dispersed, and long RCA-based scaffolds were then rearranged. Three staple strands were designed to hybridize with long scaffold ssDNA completely; as a result, DNA belts

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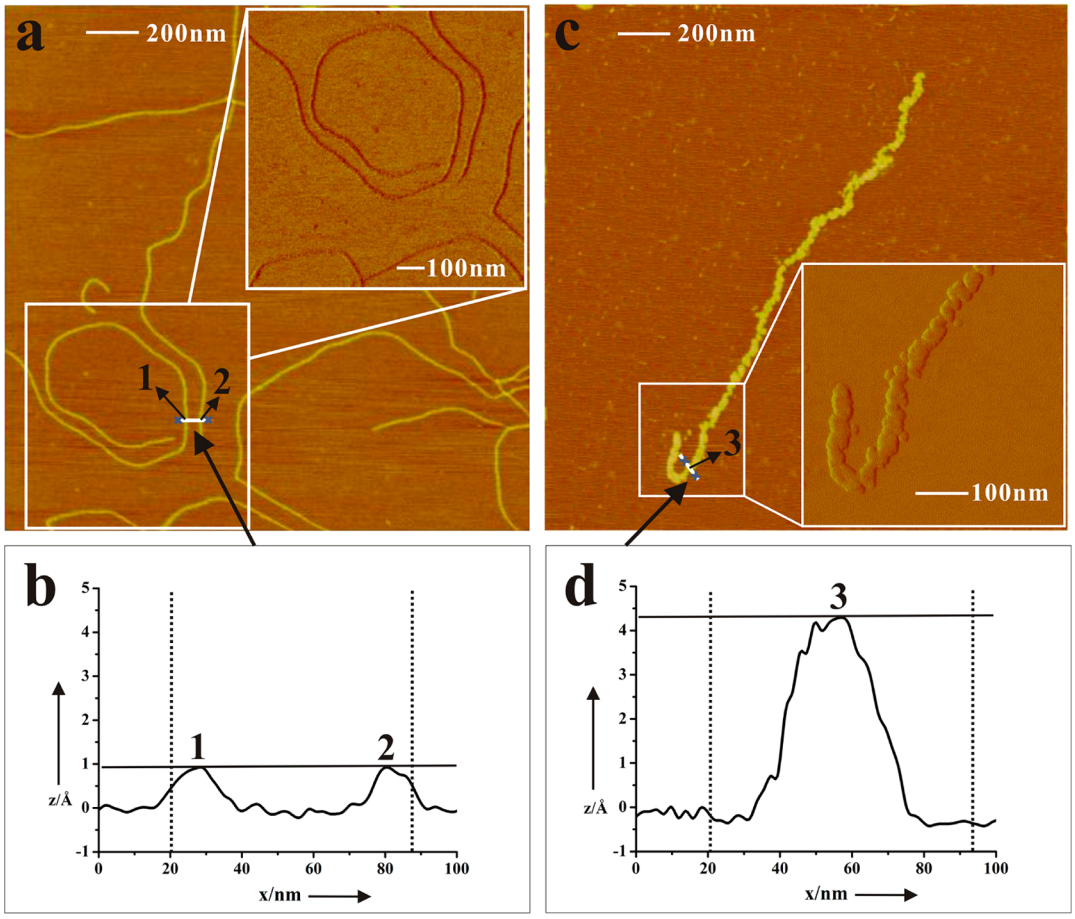

Figure 2. (a) AFM images of the DNA belts fabricated by folding RCA-based scaffolds ssDNA with a zoom in image; black arrow, two marked positions (position 1 and position 2). (b) Height and width of the two positions marked in image a. (c) AFM images of DNA belt/avidin-HRP complexes with a zoom in image; black arrow, position 3 was marked. (d) Height of the position 3, indicating the increase in the height. Height scale: 6 nm.

were then fabricated, which were observed by AFM (Figure 2a). Two different positions (1, 2) of DNA belts were marked at random in Figure 2a, and then measured, which indicated the height of the DNA belts after folding. Long scaffold ssDNA has a height of about 0.5 nm (Data not shown), which was consistent with previous reports.[34,35] But after DNA origami, the heights of DNA belt at the corresponding two positions marked in Figure 2a were approximately 1 nm (Figure 2b). The height increase indicated the successful occurrence of DNA origami with these long ssDNA. What's more, the width of DNA belts was uniform (~16 nm), which is consistent with the theoretical prediction (Figure 2b).

Subsequently, we characterized the biological capabilities of the DNA belts by incubating them with avidin-HRP (av-HRP). AFM images (Figure 2c) clearly demonstrated obviously increase in the height of such protein-decorated DNA belts, comparing with nonfunctionalized DNA belts (Figure 2a). The resultant DNA belt/av-HRP complexes was of 4.3 nm according to position 3 marked in Figure 2c and its corresponding analysis in Figure 2d, which was consistent with the combination of ~1 nm thickness of DNA belts and ~3 nm of av-HRP (see Figure S2 in the Supporting Information). Our results confirmed the successful formation of DNA belts and conjugation of av-HRPs. More details of AFM imaging, such as zoom-in of all AFM pictures can be found in the Supporting Information (Figure S1).

We next investigated the signal amplification of RCA-based DNA belts by applying them into the magnetic beads based ELISA strategy. Conventional corresponding ELISA strategy with no DNA belts was carried out as the control experiments. We have ever previously developed on-nanoparticle rolling circle amplification (nanoRCA), and the nanoRCA-based immunoassay strategy could provide high sensitivity and a broad detection range of proteins in biological fluids.[37,38] Note that we attributed such relatively high detection sensitivity to the urea treatment, which was employed to disrupt DNA–DNA interactions, unfold entangled long ssDNA and expose biotin recognition sites. DNA belts used in this study were expected to significantly enhance the sensitivity directly without the using of urea treatment, owing to more programmable and organized biotin binding sites exposed to av-HRP. Indeed, using a same concentration of PSA (5 nM), the DNA belts based assay produced a significant high signal in practice. In contrast, the control group exhibited negligible signal comparing with blank background (Figure 3).

To further confirm the signal amplification of RCA-based DNA belts, we detected serially diluted PSA ranging from 50 aM to 5 pM under optimized condition determined above. As shown in Figure 4, DNA belt-ased signal amplification exhibited a concentration-dependent change in absorbance (Figure 4). With DNA belt amplification, as low as 50 aM PSA could be easily distinguished from the blank control (3 SD standards,

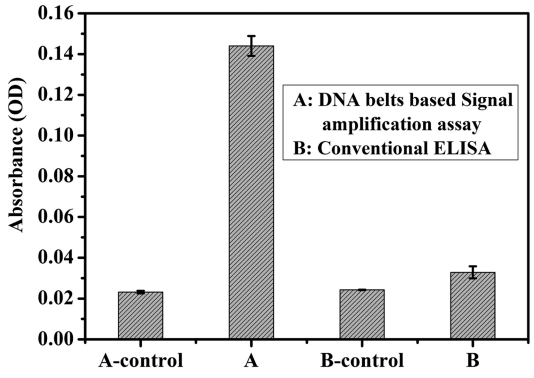

Figure 3. Comparison between sensitivity of DNA belts based signal amplification assay and conventional corresponding magnetic beads-based ELISA with a same PSA concentration (5 nM). A-control and B-control: Groups with no PSA antigen used were carried out as blank control.

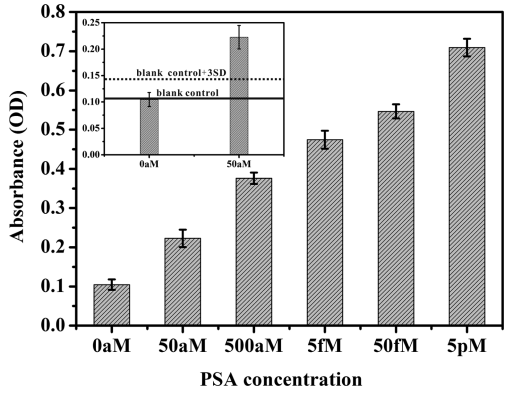

Figure 4. Concentration−response for PSA detection with DNA belt-based signal amplification assay. The concentration of PSA was 0 aM (blank control), 50 aM, 500 aM, 5 fM, 50 fM, and 5 pM, respectively. Inset: DNA belt-based signal amplification assay for blank control and 50 aM PSA. The black solid line stands for the blank control and the black dotted line for the threshold (blank control +3 SD).

inset in Figure 4). This sensitivity is much higher than those DNA-based detection platform reported previously. The high sensitivity can be attributed to the following factors. First, RCA shows strong ability of DNA amplification under isothermal conditions, and periodic unit-contained large DNA fragments were used as scaffold strands. In addition, more well-defined and precisely controlled biotin recognition sites were provided by DNA origami technique. So the high sensitivity can be achieved.

At last, we tested the detection specificity of the DNA belt-based method. It was found that a range of noncognate proteins with concentrations of 10 nM (about 1×10^8-fold higher than PSA of 500 aM) led to signals indistinguishable from the blank background (Figure 5).

Actually, several DNA origami like nanoribbons were designed and prepared in Ouyang's work, which showed superior features as a type of promising drug delivery nanocarriers.[30] Herein this work, we focused on a new application of DNA origami for the signal amplification assay and its performances when it was used to detect cancer biomarker. In the past decade, many signal amplification methods had been integrated into conventional detective methods.[3,6,33,39] Among these methods, immunoassays with polymerase chain reaction (immuno-PCR),[39] immunoassay

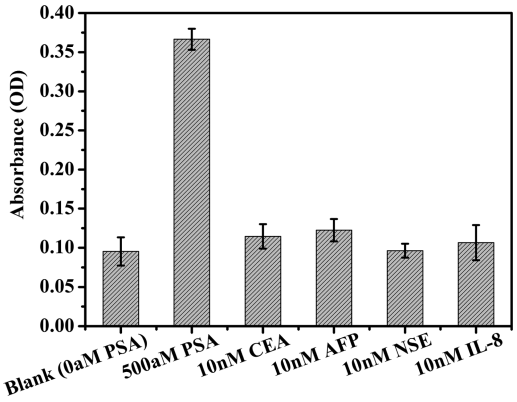

Figure 5. Specificity of the DNA belt-based signal amplification assay for 0 aM PSA (blank control), 500 aM PSA (positive control), 10 nM other proteins, CEA/AFP/NSE/IL-8 (negative controls). AFP = α-fetoprotein, NSE = neuron-specific enolase.

with rolling circle amplification (immunoRCA),[33] and a nanoparticle-based biobar-code approach are widely used.[3,4] As reported previously, immunoRCA can detect as low as fM levels of PSA carried out in microspot and microarray formats. Additionally, coupled with PCR, the biobar−code approach provides a detection sensitivity of 30 aM PSA in a 10 μL sample. However, as the drawbacks such as low ratio of DNA identification sequence to detection antibody (Ab), slow target-binding kinetics, and complicated process of those methods, a DNA origami-based signal amplification assay is expected to be developed, which has not received much attention until now. In this work, we combined RCA-based DNA belts with magnetic bead-based ELISA to achieve the sensitivity of 50 aM PSA, which is comparable to the nanoparticle-based biobar-code approach and 2 orders of magnitude greater than immuno-PCR and immunoRCA methods. Besides, except high sensitivity, RCA-based DNA belt-based signal amplification offered several advantages over other detection methods. First, complex conjugation chemistries that are required to link the Ab and DNA markers is no longer required; Second, the isothermal condition is ready to be realized that eliminate the need of heat-annealing process in PCR process. Third, the operation is simple. We do not need the complicated DNA release and collection steps that are required in biobar code method. And last, the DNA belts used in this study is more cost-effective than other origami-based nanostructures owing to RCA-based scaffold strands and the limited number of staple strands. Note that despite the advantages and potential applications, the DNA belt-based signal amplification assay still remains to be improved in several aspects. First, the purification efficiency of DNA belts should be improved; In addition, nanomaterials, for example, Au nanoparticles (AuNPs) might be used further for loading primer DNA to increase and assemble more recognition sites.

CONCLUSIONS

In summary, we have successfully developed a novel DNA belt-based signal amplification combining RCA with DNA origami for the highly sensitive detection of PSA. The method takes advantages of the high amplification efficiency of RCA and high controllability of DNA origami, leading to a detection limit of 50 aM with high specificity. This method demonstrates several prominent advantages: First, more sensitive over current methods for the cancer biomarker detection; Moreover,

simplify reaction process avoiding complicated operation steps and expensive sophisticated instruments. In addition, this is an innovative attempt to apply the programmable nanostructures basing DNA origami into the area of biodetection and early stage diagnosis of cancer. Finally, considering these merits, we anticipate that our new signal amplification strategy will find numerous applications in detecting a variety of biological related markers and proteomic studies.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, AFM images, and DNA sequences can be found. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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