Nucleic Acids Research, 2005, Vol. 33, No. 6 ***e64***
doi:10.1093/nar/gni063

Proximity extension of circular DNA aptamers with real-time protein detection

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Received January 23, 2005; Revised and Accepted March 16, 2005

ABSTRACT

Multivalent circular aptamers or 'captamers' have recently been introduced through the merger of aptameric recognition functions with the basic principles of DNA nanotechnology. Aptamers have strong utility as protein-binding motifs for diagnostic applications, where their ease of discovery, thermal stability and low cost make them ideal components for incorporation into targeted protein assays. Here we report upon a property specific to circular DNA aptamers: their intrinsic compatibility with a highly sensitive protein detection method termed the 'proximity extension' assay. The circular DNA architecture facilitates the integration of multiple functional elements into a single molecule: aptameric target recognition, nucleic acid hybridization specificity and rolling circle amplification. Successful exploitation of these properties is demonstrated for the molecular analysis of thrombin, with the assay delivering a detection limit nearly three orders of magnitude below the dissociation constants of the two contributing aptamer–thrombin interactions. Real-time signal amplification and detection under isothermal conditions points towards potential clinical applications, with both fluorescent and bioelectronic methods of detection achieved. This application elaborates the pleiotropic properties of circular DNA aptamers beyond the stability, potency and multitargeting characteristics described earlier.

INTRODUCTION

The use of DNA as a nanoscale scaffold has attracted much attention for the construction of objects such as templated nanowires (1), self-assembling 2D and 3D arrays (2–5) and molecular machines (6–8). The exceptional utility of DNA for this purpose is determined by its capacity to act both in specific recognition and as a structural element. Moreover, the ease of synthesis, modification and manipulation of DNA enhances its attractiveness as a building block for nanostructures. The benefits achievable by exploiting these qualities are well appreciated in the diagnostic arena, where the unique recognition and structural properties of nucleic acid aptamers (9–12) have been incorporated into molecular sensors in which analyte detection is accomplished through conformational changes that give rise to measurable signals (13–19). In principle, the use of nucleic acid components in sensors can provide additional benefits through access to enzyme-mediated signal amplification methodologies. Rolling circle amplification (RCA) (20–22), in which circular DNA molecules serve as templates for polymerase-mediated isothermal amplification reactions, is one such example that combines the topological and functional elements of nucleic acids. The RCA technique has been used in the detection of point mutations and in multiplexed protein microarray analysis (23).

Seeking to merge aptameric recognition activity with nanoscale engineering, we have recently introduced a class of circular DNA aptamers ('captamers'), in which multiple aptameric motifs are organized around the vertices of duplex, three- and four-way junction architectures (24). These scaffolds provide a framework for spatial orientation, allowing multiple binding activities to be combined into single molecules, their circularity imparting both enhanced thermal stability and exonuclease resistance. Here we elaborate upon the properties of these molecules to demonstrate a highly sensitive protein detection system that simultaneously relies upon distinct functional elements incorporated into the modular circular architecture. For this implementation, the structural properties of aptameric target recognition are combined with the specificity of DNA hybridization and an isothermal RCA strategy that exploits the circularity of the template aptamer.

The value of integrating multiple functionalities into single molecules can be appreciated from the early precedent of

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immuno-PCR, where antibody–oligonucleotide hybrids combined protein-binding functions with PCR-based nucleic acid amplification for signal generation (25). More recently, the immuno-PCR concept has been elegantly extended by Landegren and co-workers (26,27), who merged aptameric specificity with ligation and PCR amplification to produce a 'proximity ligation' assay for ultrasensitive protein detection. In proximity ligation, which provides the inspiration for this work, concomitant binding of two different aptamers to a protein target raises their effective local concentrations to a point where nucleic acid tail sequences can be efficiently joined by ligation before downstream PCR amplification and detection. The present proximity extension reaction similarly utilizes a change in local concentration of aptamer binding motifs to signal the presence of the target protein. In this case, the utility of modular circularized aptamers allows for protein detection to be observed in real-time using a one-step reaction without the need for thermocycling. The methodology developed here is applied to the detection of thrombin, a critical enzyme in the blood coagulation cascade.

Table 1. Linear aptamer and precursor sequences

Oligo	Sequence
LT-4	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**GCAC**-3′
LT-5	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**TGCAC**-3′
LT-6	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**CTGCAC**-3′
LT-7	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**GCTGCA**-3′
LT-10	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**TGGGCTGCAC**-3′
LT-13	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**TCCTGGGCTGCAC**-3′
LT-6-s	5′-GCACTGGTTGGTGAGGTTGGGTGCT(24)**CTGCAC**-3′
LT-6-m	5′-GCACTGGTTGGTGAGGTTGGGTGCT(36)**CTGCAC**-3′
LT-6-l	5′-GCACTGGTTGGTGAGGTTGGGTGCT(60)**CTGCAC**-3′
LT-6-LNA	5′-GCACTGGTTGGTGAGGTTGGGTGCT(48)**CTGCAC**-3′
LA-1	5′-pAATTCGAGTCCGTGGTAGGGCAGGTTGGGGTGACTCG-3′
LL-4	5′-pAATTGGTCTTTTTT**GTGC**TTTTTAGACC-3′
LL-5	5′-pAATTGGTCTTTTTT**GTGCA**TTTTTAGACC-3′
LL-6	5′-pAATTGGTCTTTTTT**GTGCAG**TTTTTAGACC-3′
LL-7	5′-pAATTGGTCTTTTTT**GTGCAGC**TTTTTAGACC-3′
LL-10	5′-pAATTGGTCTTTTTT**GTGCAGCCCA**TTTTTAGACC-3′
LL-13	5′-pAATTGGTCTTTTTT**GTGCAGCCCAGGA**TTTTTAGACC-3′
EC-2	5′-TTTTTAGACCAATTCG-SH-3′

Aptameric motifs are underlined; hybridization tip sequences are shown in bold. T(48) indicates a run of 48 sequential T residues. The underlined bold residue at the penultimate position of LT-6-LNA is an LNA moiety. The SH moiety of EC-2 is a 3′-terminal thiol.

MATERIALS AND METHODS

Reagents

Unless otherwise stated, reagents were obtained from Sigma–Aldrich, Ajax Chemicals or Bio-Rad and used without further purification. All common buffers were prepared according to standard recipes. Unmodified deoxyribonucleoside triphosphates (dNTPs) were purchased from Promega. Biospin 6 gel filtration columns were purchased from Bio-Rad. Solutions were prepared with Milli-Q deionized water and the pH adjusted by addition of HCl or NaOH where necessary.

Oligonucleotides and circular aptamer construction

Linear aptamer constructs against thrombin exosite I, designated 'LT' in Table 1, were based upon the canonical thrombin DNA aptamer motif (28). To provide additional structural stability to the aptameric head, 4 nt of complementary sequence were added to both the 5′ and 3′ ends of the consensus motif, closing the aptamer into a hairpin-loop structure (29). An additional 48 nt poly(dT) tail was added to the 3′ end of this structure, providing the necessary length to bridge the gap to the second aptamer when both are simultaneously bound to thrombin. A poly(dT) sequence was used to ensure minimal secondary structure and low non-specific binding. A 'hybridization tip', shown in bold in Table 1, was added to the distal 3′ end of the poly(dT) tail. The sequence of this hybridization tip was designed to be complementary to the central sequence within the unstructured loop domain of a compatible circular aptamer. Variant constructs containing 4–13 nt of tip complementarity were subsequently named LT-4 to LT-13 (Table 1). A number of additional variants were designed with changes to the bridge length (LT-6-s, LT-6-m and LT-6-l) or with a 3′-exonuclease-resistant locked nucleic acid (LNA) tail (30) (LT-6-LNA, the penultimate LNA residue is underlined in bold in Table 1).

Circular aptamer constructs against thrombin exosite II were based upon a second previously selected motif (31). Circularity is known to provide stability against the predominant exonuclease activity present in biological fluids and provides a means to engineer multiple active motifs into a single molecule built around simple scaffold architectures (24). Construction of circular aptamers for proximity extension was achieved through ligation of two hairpin precursor modules containing complementary overhangs, one carrying the aptamer motif (LA-1 in Table 1), and the other carrying an unstructured loop (the 'LL' series in Table 1). This method permits the exchange of component hairpins, allowing the production of a large number of variants from a smaller number of compatible modules. Hairpin sequences were designed so that ligation of two identical modules produced a homodimer containing a duplex region with a restriction enzyme (RE) recognition site that could be cleaved, whereas ligation of the two different modules did not produce an RE recognition site and thus could not be cleaved. This provided a mechanism for selecting the correct circular heterodimeric product, as cleaved homodimers (and any residual linear modules) could be enzymatically digested with exonuclease III (24).

In general, aptamer oligonucleotides intended for circularization were purchased from Geneworks, Sigma–Genosys, Oswel, Eurogentec or Genset Pacific with 5′-phosphate groups to allow for subsequent ligation, and purified by gel filtration chromatography. The two hairpin modules for ligation were added in an equimolar ratio, typically at a concentration of 100 μM. Constructs for ligation were heated to 95°C in T4 DNA ligase buffer (40 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8) and slow-cooled to room temperature before reaction. T4 DNA ligase was added at 10 U/μg oligonucleotide and samples incubated overnight at 37°C. EcoR I and Mfe I restriction enzymes were included in the ligation reaction and/or added after ligation to ensure that unwanted homodimeric products were relinearized and subsequently digested (24). Following a further incubation at 75°C for 10 min, products were exchanged into the appropriate buffer (Bio-Rad Micro Biospin-6 columns) and any remaining uncircularized oligonucleotides digested with exonuclease III. Reaction mixtures were purified by phenol–chloroform

extraction and ethanol precipitation before quantitation by UV spectrophotometry (Varian Cary100 Bio). Correct construct production was established by analysis following 12% native and denaturing PAGE.

Variant circular aptamers carrying a range of unstructured loops with 4–13 nt motifs complementary to the 3′ tail tips of the linear aptamer constructs were named CT-4 to CT-13 according to the identity of their 'LL' series precursors (Table 1).

Proximity extension assay

General conditions for proximity extension-mediated RCA were as follows: reaction volumes (10 μl) containing 40 nM each aptamer construct, 250 μM dNTPs (Promega), 0.5 mg/ml BSA (Sigma) and human α-thrombin (Sigma) in 1× reaction buffer were initiated by the addition of 4 U of Bst DNA polymerase (New England Biolabs). Following incubation at 37°C for the desired period, the reaction was stopped by heating to 80°C for 20 min.

Real-time fluorescence measurements

For real-time observation of proximity extension-mediated RCA, the fluorescent dye Sybr Green I (Sigma) was added to the reaction mixture (40 000× dilution) and the reactions monitored using a RotorGene 3000 real-time PCR instrument (Corbett Research). Samples were held at 37°C for the length of the assay and fluorescence monitored in the green channel. Alternative isothermal reactions were performed in a 384-well black microtitre plate and read with a BMG POLARstar fluorescence plate reader.

Oligonucleotide self-assembled monolayer (SAM) construction

Disposable 2 × 4 gold electrode arrays (ECIS Cultureware, Applied Biophysics) were used as the substrate for the formation of oligonucleotide SAMs. These arrays consist of 250 μm gold film electrodes delineated with an insulating film and mounted on a polycarbonate backing, with an eight-well polystyrene top assembly. Electrodes were cleaned by extensive cyclic voltammetry (CV) sweeping between −350 and +650 mV in hybridization buffer (1× PBS buffer supplemented with 100 mM NaCl and 1 mM $MgCl_2$). EC-2 thiolated capture oligonucleotide (Sigma) (Table 1) was incubated with 100 mM DTT in 100 mM ammonium acetate buffer (pH 8.3) at 55°C for 4 h, then desalted twice using Micro Biospin-30 gel filtration columns (Bio-Rad) immediately before use. To form the SAMs, 200 μl of 1 μM thiolated oligonucleotide in hybridization buffer was incubated overnight in the electrode array wells. After washing, wells were incubated with 0.7 μM mercaptohexanol in hybridization buffer for 2 h. Wells were further rinsed and used immediately for proximity extension RCA product capture analysis.

Electrochemical measurements

Electrochemical measurements were performed at room temperature using a BAS 100B electrochemical analyser (Bioanalytical Systems). A conventional three-electrode system consisting of the DNA-modified working electrode, platinum flag auxiliary electrode and a Ag/AgCl reference electrode (E_{ref} = 206 mV versus standard hydrogen electrode; Bioanalytical Systems) was used to carry out CV at a sweep rate of 100 mV s^{-1}. Osteryoung square wave voltammetry (OSWV) was performed with the following parameters: pulse amplitude 25 mV; step 4 mV; and frequency 10 Hz. Synthesis of vinyl-Fc-dUTP is described elsewhere (32). CV and OSWV of DNA SAMs were carried out in phosphate-buffered saline supplemented with 100 mM NaCl and 1 mM $MgCl_2$. All solutions were degassed with argon for 20 min before data acquisition.

RESULTS

DNA aptamer structures and verification of RCA reaction

To construct the DNA components required to couple aptamer recognition with RCA, an aptamer against the heparin exosite II of human thrombin (31) and an unstructured template loop were first combined into a circular dumbbell architecture (24) (Figure 1A). To provide an aptamer capable of performing as a primer against this circular template, a second linear aptamer against thrombin exosite I (28) was engineered with a 3′ tail (Figure 1B). The tail of this linear aptamer was sufficiently long to bridge the distance to the circular aptamer only when both are bound to a target molecule. Upon target binding, the tail of the linear aptamer is then able to hybridize to the complementary unstructured loop of the circular aptamer, thus forming a primer for polymerase-mediated extension and isothermal RCA, converting the circular aptamer from a functional binding element into a structural template (Figure 1C). This RCA reaction should generate a product strand composed of the linear aptamer sequence at the 5′ end, followed by many concatenated sequences complementary to the circular aptamer template. The anticipated high molecular weight of this product was confirmed by the extremely low mobility of purified reaction samples following denaturing gel electrophoresis (Figure 1D, lane 2). Because the circular aptamer construct that acts as the template for RCA contains complementary sequences that form a central duplex, it is expected that the concatenated reaction product will contain corresponding regions of complementarity, generating multiple equivalent duplex structures in the nascent product. As these duplex regions can be designed to contain an RE recognition site, the identity of the RCA product and hence the mechanism of activity can be confirmed by the presence of correctly sized fragments upon restriction digestion of assay products (Figure 1D, lane 3).

Aptamer–primer optimization

The capacity of the proximity extension reaction to specifically quantify a target molecule relies upon the use of only a short region of complementarity between the 3′ end of the linear aptamer, acting as a primer for RCA, and the unstructured loop of the circular aptamer, acting as a template. With a relatively poor dissociation constant K_d for a short primer–loop interaction, the two constructs should not hybridize significantly at normal assay concentrations. However, binding of both aptamers to a target, such as thrombin, should significantly raise their local concentrations (26,27) and hence hybridization potential, resulting in priming of RCA extension. To examine the dependence of amplification upon

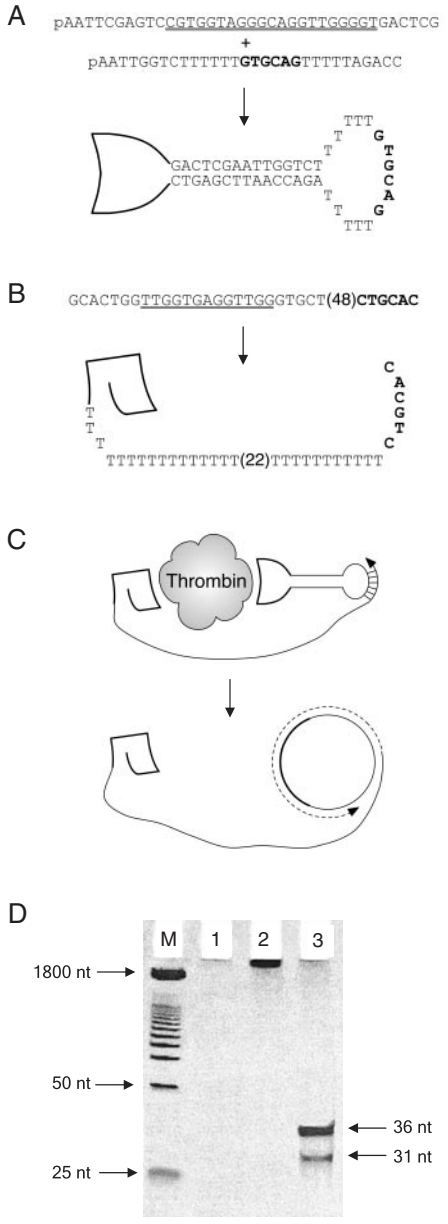

Figure 1. Aptamers for the detection of human thrombin by proximity extension. (**A**) Formation of the circular dumbbell CT-6 aptamer bearing a thrombin exosite II motif (underlined) plus an unstructured template loop (bold). (**B**) Linear LT-6 aptamer bearing a thrombin exosite I motif (underlined) plus a long tail with a complementary 3′-terminus (bold). Poly(dT) stretches of 22 and 48 nt in length are shown in parentheses. (**C**) Simultaneous binding of aptamers to thrombin primes DNA polymerase-mediated RCA. (**D**) Polyacrylamide gel (12%) with markers (M) followed by proximity-extension-mediated RCA products generated in the presence of (1) 0 pM thrombin, (2) 4000 pM thrombin and (3) 4000 pM thrombin with subsequent Taq$^{\alpha}$ I restriction enzyme digestion. No product is generated in the absence of thrombin, while high molecular weight products are observed when thrombin is present. These products can be digested using Taq$^{\alpha}$ I restriction enzyme into fragments displaying the expected sizes (36 and 31 nt for the 67 nt LT-6 template).

the degree of primer–loop complementarity, several linear aptamers with 48 nt poly(dT) bridges carrying 4–13 bp of terminal complementarity (constructs LT-4 to LT-13 in Table 1) were tested for their ability to initiate RCA in the presence and absence of thrombin. RCA was monitored during incubation at 37°C by fluorescence detection with a real-time PCR instrument. Examination of RCA reaction rates indicated that 6 bp of complementarity provided the best differentiation between samples with and without thrombin (Figure 2). Seven base pairs complementarity provided a similar discrimination, but at higher background levels (Figure 2). To determine the optimal distance span for linear constructs, a range of poly(dT)-containing tail lengths was also evaluated (oligonucleotides LT-6, -s, -m and -l in Table 1). In comparison with the standard 48-poly(dT) tail, the use of a 24 nt poly(dT) stretch resulted in a complete loss of proximity-extension-mediated RCA under standard assay conditions. A 36 nt poly(dT) tail produced measurable RCA at a reduced rate, and the 60 nt tail variant displayed no significant rate differences to the standard length 48-poly(dT) aptamers (data not shown). Also, the use of a linear aptamer with an LNA residue at the $n-1$ position relative to the 3′ end of the construct (LT-6-LNA) did not significantly alter the kinetic properties of the assay. Based on these comparative data, the linear construct LT-6 bearing a native CTGCAC 3′-terminus at the end of a 48 nt poly(dT) tail (Figure 1A) was employed for all further experiments.

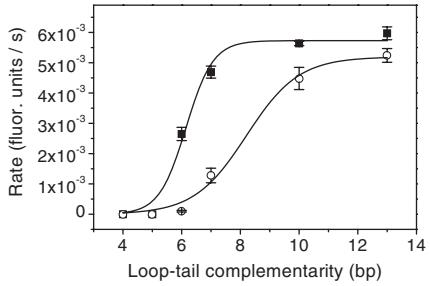

Figure 2. Proximity extension assay performance with different tail–loop hybridization lengths. Reactions were performed with 40 nM aptamer concentrations in the absence (open circles) or presence (black squares) of 2 nM thrombin.

Characteristics of real-time thrombin detection with CT-6/LT-6 aptamers

Using the conditions determined above, a range of thrombin concentrations was tested to examine the reaction characteristics and limit of detection. Maximal reaction rates were calculated from 30 min timecourses (Figure 3A), with results from four separate experiments indicating good linearity between 10 pM and 5 nM thrombin (Figure 3B). A limit of detection of 30 pM was determined from three times the standard deviation of the baseline rate. This value is almost three orders of magnitude lower than the reported K_d values for the individual aptamer motifs (28,31), an excellent improvement in sensitivity over single binding or two-component 'sandwich' methods (33–36) and corresponds well to physiological levels of thrombin during the initiation phase of clotting, which range from low nanomolar down to picomolar concentrations (37). The concentration of BSA was varied over three orders of magnitude (0.05–50 mg/ml) without any appreciable change in the limit of thrombin detection, indicating good assay robustness. The inability of trypsin, a serine protease closely related to thrombin, to initiate proximity-extension-mediated RCA at concentrations up to 100 nM provides further verification of the reaction specificity (Figure 4).

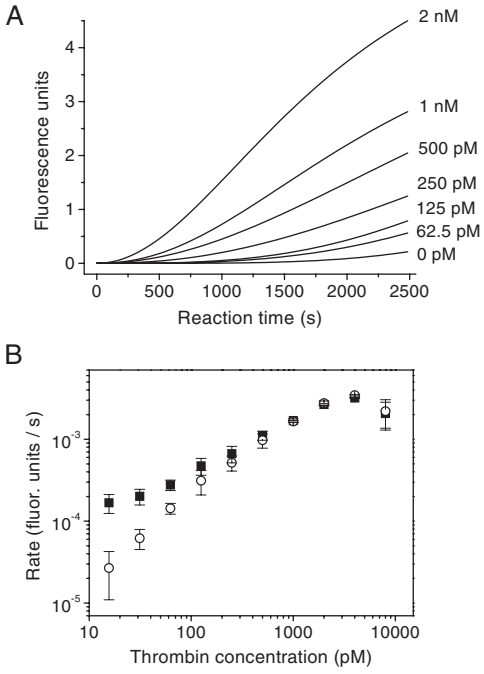

Figure 3. Characteristics of the thrombin-dependent proximity extension-mediated RCA reaction. (**A**) Real-time fluorescence traces of proximity extension-mediated RCA over a range of thrombin concentrations. (**B**) Dependence of maximal RCA rate upon thrombin concentration. Uncorrected rates for 16 pM – 8 nM thrombin (black squares) and background-corrected rates (open circles). The background 0 pM thrombin rate is $1.04 \pm 2.3 \times 10^{-4}$ arbitrary fluorescence units per second.

Bioelectronic detection

To examine the compatibility of the proximity extension reaction with electrochemical detection, reactions were performed with the electrochemically labelled nucleoside triphosphate 'electrotide' vinyl-Fc-dUTP (32,38,39) (Figure 5A) doped into the nucleotide mixture at a level of 35%. Small diameter (250 μm) gold electrode SAM capture arrays (Applied Biophysics) were produced with a capture oligonucleotide complementary to the proximity extension RCA product. To ensure that the long concatenated products generated by RCA could be converted into a fragmented form more efficiently captured by the SAM, the central duplex of the CT-6 dumbbell was engineered to contain a palindromic Taq$^{\alpha}$ I restriction enzyme recognition site, and Taq$^{\alpha}$ I was added to the reaction mixture. Following proximity extension reactions in the presence of a range of thrombin concentrations, RCA products were incubated with the SAM electrodes, washed and analyzed by OSWV. Electrochemical peaks were observed at the expected potential and correlated well to thrombin concentration (Figure 5B).

DISCUSSION

Multivalent circular DNA aptamers

We have previously described the improved thermal and nucleolytic stability of multivalent circular DNA aptamers ('captamers') over linear counterparts, along with the improved potency that accompanies these stability improvements and the enhanced activity that can be obtained by exploiting

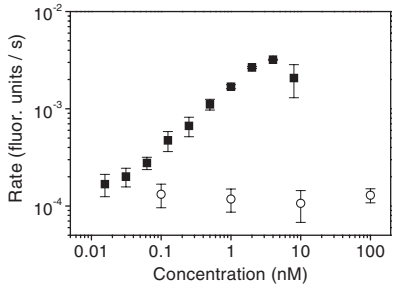

Figure 4. Proximity extension reaction specificity. Proximity extension rates in the presence of thrombin (black squares) and trypsin (open circles). Concentrations between 100 pM and 100 nM were examined in triplicate. For all concentrations of trypsin, the maximal rate of extension over 40 min was not significantly different from that observed in the absence of either thrombin or trypsin.

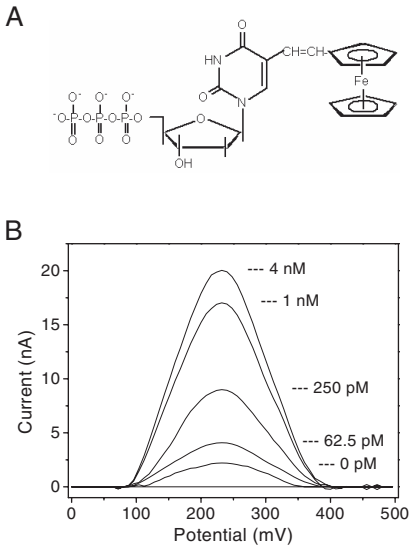

Figure 5. Electrochemical detection of thrombin-dependent proximity-extension-mediated RCA. (**A**) Electroactive nucleoside triphosphate vinyl-Fc-dUTP used in proximity extension assays. (**B**) OSWV of DNA SAMs following capture of electroactive proximity extension RCA products at a range of thrombin concentrations. The baseline is a scan of an SAM constructed with a capture oligonucleotide not complementary to RCA products generated at 4 nM thrombin.

multivalency (24). These properties are particularly attractive for therapeutic applications. In this study, we have demonstrated the utility of this simple circular nanoscale DNA architecture for a diagnostic application. The modular nature of these structures allows for multiple elements to be combined into single molecules, generating features that can be exploited to achieve significant functional benefits. The novel 'proximity extension' assay described here relies upon several such features to achieve target-dependent signalling: aptameric recognition, primer hybridization to a template 'head' and RCA of the circular structure. This reaction significantly extends the detection capabilities of captamers beyond simple fluorophore or reporter labelling strategies.

Performance of the proximity extension reaction

The sensitivity of the proximity extension method is notable, achieving a low pM detection limit from two binding

interactions that individually operate in the nM regime. The linear detection range lies between the sensitivity of an ordinary sandwich assay, which is determined by the K_d of the second signalling component, and the extreme sensitivity of the PCR-amplified proximity ligation assay (26,27). While the dissociation constants of analyte-specific reagents generally set the sensitivity boundary for diagnostic methods, the presence of many targets at lower concentrations makes the development of tools that can operate below these limits highly desirable.

Dual binding assays display a characteristic decline in signal output at high target concentrations owing to an increase in 1:1 binding. This phenomenon has been described for the proximity ligation assay (27) and is evident here in Figures 3B and 4. High target concentrations should therefore be diluted appropriately to locate the positive linear response region or a small dilution series undertaken. The ability of proximity extension to determine target concentration in a one-step homogenous assay, generating results in real-time and without the need for thermocycling fulfils many of the practical requirements for a point-of-care testing method. Selectivity in the presence of an overwhelming concentration of non-specific protein is excellent, due in large part to the simultaneous requirement for two target binding events plus a hybridization.

In considering potential alternative signal transduction methods for the proximity extension assay, electrochemical detection methods have gained much attention for their low cost, high sensitivity and compatibility with direct electronic readout (40). To more tightly couple nucleic acid chemistry with bioelectronics, a range of electroactive nucleoside triphosphates ('electrotides') for polymerase-mediated incorporation has recently been introduced (38,41–44). Electroactive reaction products have been detected in capillary electrophoresis (45) and following capture on oligonucleotide SAM electrodes (32,39). Here, the conversion of the proximity extension assay to a bioelectronic detection platform resulted in the retention of a level of RCA product sensitivity similar to that of the fluorescent methodology.

Use of aptamers in dual simultaneous binding assays

While a number of aptamer-based diagnostic assays utilize a single target recognition molecule (46), it is well appreciated that a strict requirement for two separate binding events provides greater stringency for assay specificity (47). This may be especially useful for applications examining complex biological mixtures in which the presence of cationic and other aptamer-binding components can affect the performance of intrinsically polyanionic aptamers. While aptamer selections against small protein targets have historically produced species that bind to a single epitope, there is no a priori reason why selections against two or more distinct epitopes cannot be performed, especially with chemically modified aptamers (48–50). Several groups are working on this issue, and early results look to be encouraging (51). In any case, the proximity-extension-mediated RCA methodology is also suitable for larger multivalent targets, such as cells and viruses, for which multiple aptamer generation is already straightforward (52–54). Furthermore, the addition of an LNA or other modification to the linear aptamer terminus (30) can make the assay compatible with exonuclease-rich physiological fluids, such as serum.

CONCLUSION

The ability to multitask is a particularly useful attribute of multivalent circular aptamers. The proximity extension reaction, following the further development of polyclonal aptamer selection procedures, is a candidate for the kind of robust, selective, sensitive, real-time protein assay required for the further development of personalized medicine. Several alternative aptamer assay formats are maturing towards widespread application.

ACKNOWLEDGEMENTS

The authors thank Kate Odenthal for assistance with electrode preparation and Andrew Szentirmay for real-time thermocycler access. This work was supported in part by ARC grant LP0347371. Funding to pay the Open Access publication charges for this article was provided by a UNSW Faculty Research Grant.

Conflict of interest statement. None declared.

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