Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supplementary Information

Diagnosing viruses by the rolling circle amplified synthesis of DNAzymes

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Experimental Section

Materials: Oligonucleotides **1**, **2** and **4** (Genosys, Sigma), the deoxynucleotide solution mixture, dNTPs; T4 polynucleotide kinase, and Quick Ligation™ Kit (New England BioLabs, Inc.), M13mp18(+) STRAND DNA and polymerase Klenow fragment exo- (Amersham Biosciences Corp), hemin (Frontier Scientific, Inc.) were used without any further purification. A hemin stock solution was prepared in DMSO and stored in the dark at -20° C. The single-stranded calf thymus DNA, luminol, and H_2O_2 were purchased from Sigma.

Circular DNA template preparation: The circular DNA template was prepared as follows: First, the linear DNA (5'–GATCCTAACCCAACCCGCCCTACCCAAAACCCAACCCGCCCTACCCAAAA CCCAACCCGCCCTACCCAACCACAC–3'), 6×10^{-6} M, was phosphorylated using T4 polynucleotide kinase, 0.4 units/μl, the ligation template (5'-TTAGGATCGTGTGGTT-3'), 3.6×10^{-5} M, in the Quick Ligation™ Kit buffer, at 37° C for 30 min. The synthesis was completed by the Quick Ligation™ Kit, using the manufacturer-supplied protocol. The enzymes were denatured by heating at 90° C for 10 min. The ligated circular DNA was then purified and separated from the ligation template by urea, 8 M, using a centricon filtration device (10,000 cutoff, Millipore Inc.).

RCA assay: In all systems fixed concentrations of the hairpin **4**, 2×10^{-7} M and the circular DNA **2**, 2×10^{-8} M were employed. Polymerase Klenow exo-, 0.4 units/μl and dNTPs, 0.2 mM were included. The RCA process was performed in a buffer solution consisting of 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT and 50 μg/ml BSA.

The conditions for the absorbance/chemiluminescence studies are as follows: Colorimetric measurement: The experiment was performed in a solution consisting of the products; hemin, 4×10^{-7} M; H_2O_2, 4.4×10^{-5} M; $ABTS^{2-}$, 1.82×10^{-4} M in a buffer solution consisting of 25 mM HEPES, 20 mM KCl and 200 mM NaCl, pH 7.4, 25° C. Absorbance changes were followed at 415 nm to characterize the rate of the oxidation of $ABTS^{2-}$. Chemiluminescence measurements: Light emission experiments were performed using a photon counting spectrometer (Edinburgh Instruments, FLS 920) equipped with a cooled photomultiplier detection system, connected to a computer (F900 v.6.3 software). Measurements were made in a cuvette that included a buffer solution consisting of 25 mM HEPES, 20 mM KCl and 200 mM NaCl, pH 9.0, which included the product; hemin, 1×10^{-9} M; 0.5 mM luminol and 30 mM H_2O_2. The light emission was monitored at λ_{em}=420 nm.