Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Ultrasensitive Detection of DNA by the PCR-Induced Generation of DNAzymes: The DNAzyme Primer Approach

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Experimental Section

Materials. Oligonucleotides **1** and **2** (Integrated DNA Technologies, Inc.), Deoxynucleotide solution mixture, dNTPs, and 50 bp DNA Ladder (New England BioLabs, Inc.), M13mp18(+) STRAND DNA (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, H_2O_2, Sybr Green II, JumpStart KlenTaq LA DNA Polymerase, 10 × PCR buffer, and all other compounds were purchased from Sigma.

PCR Assay. DNA replication was performed in a solution consisting of **1**, 2×10^{-7} M; **2**, 2×10^{-7} M; Different concentrations of the M13 phage DNA, 0.2 mM dNTP mixture and the JumpStart KlenTaq LA DNA Polymerase 0.06 u/μl in a 10 × PCR buffer.
Conditions for the Thermal Cycles. Denaturation 30s, 94° C; Annealing 30s, 55° C; Polymerization 30s, 68° C.

Colorimetric Measurement Assay. The experiment was performed in a solution consisting of the PCR product; 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 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl and 1mM dithiothreitol, pH 7.9, 25° C. Absorbance changes at 415 nm. were followed to characterize the rate of 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). Before the sample analysis, a background run without sample was performed. All spectral results were corrected with respect to the background, and the resulting chemiluminescence was integrated. 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 hemin, 1×10^{-9} M, 0.5 mM luminol and 30 mM H_2O_2. The light emission intensity was integrated between 410 to 430 nm.