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Magnetic bead-based label-free chemiluminescence detection of telomeres

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For the first time we report on the detection of telomeres by coupling of the label-free guanine CL detection route with an efficient magnetic isolation of the hybrid.

Nucleic acid hybridization is a basic method in molecular biology and provides new possibilities in various biomedically and biotechnologically oriented fields. Various techniques have been employed extensively for the detection of specific DNA sequences by specific hybridization, for example, radioactive substances, biotin, digoxigenin, enzyme, chemiluminescence (CL) and fluorescent dyes have all become popular reagents for labelling.[1–3] In addition, duplex-specific redox indicators or enzyme tags have also been used for the electrochemical detection of DNA hybridization.[4,5] However, there are potential advantages, in terms of simplicity and speed, for detecting the hybridization step directly without using such labels. For example, Wang *et al.*[6] recently reported label-free gene-sensing schemes based on the intrinsic electroactivity of DNA. These methods have relied primarily on monitoring changes in the guanine oxidation process accrued from the hybridization event.[6–8] In this article we report for the first time the detection of telomeres by the coupling of the label-free guanine CL detection route with an efficient magnetic isolation of the hybrid.

Telomeres are specific DNA structures at the ends of chromosomes and consist of TTAGGG repeat-units in vertebrate. Telomeres protect chromosomes from the loss of DNA, end-to-end fusion and other potential errors. Due to the crucial role of the extreme ends of telomeres, we addressed the task of detecting single-stranded telomeric sequences by a simple new approach. The present assay format (illustrated in Fig. 1) involves: (a) capture of biotinylated capture probes on streptavidin-coated magnetic spheres; (b) the hybridization event and magnetic removal of non-hybridized oligonucleotides; (c) direct detection of the target guanine CL on the bead surface. Compared with previously reported label-free electrochemical detection,[6–8] our method is much simpler. Unlike the label-free electrochemical method, alkaline treatment was not needed for the release and denaturation of the hybrid from the spheres before CL detection.

Briefly, 100 μl of the streptavidin-coated microspheres (Polysciences Inc.) were transferred into the well of a microplate. The microspheres were washed with 100 μl buffer A (20 mM TRIS–HCl, pH 8.0, 0.5 M NaCl) and resuspended in 100 μl buffer A. Then, 5 μl of the biotinylated oligonucleotide probe were added and incubated for 30 min at room temperature with gentle mixing. The bead-captured probes were then separated, and washed three times with 100 μl buffer A. Next, the bead-captured probes were resuspended in 100 μl pre-hybridization solution (KPL formamide hybridization buffer, Kirkegaard & Perry Laboratories, Catalog No. 50-86-10, plus 200 μg ml^{-1} denatured fish sperm DNA), kept at 42 °C for 30 min. The desired amount of the telomere target was added into the wells and the hybridization reaction usually proceeded for 60 min at 42 °C. The hybrid-conjugated beads were then washed twice with 100 μl 2 × SSPE (sodium chloride, sodium phosphate, and EDTA) buffer containing 0.1% SDS (sodium dodecyl sulfate), twice with 100 μl 2 × SSPE buffer, and then once with distilled water. Then hybrid-conjugated beads were transferred into 12 × 75 mm tubes, 10 μl of TPA (n-Pr_4N)–H_3PO_4 buffer (pH 8.5) were added and the tubes were placed in the luminescence reader. Then 100 μl of 30 mM 3,4,5-trimethoxyphenylglyoxal (TMPG) in DMF was injected and the CL signal was displayed.

TMPG reacted with the guanine moiety of DNA at rt, and provided specifically the chemiluminescent derivatives as described previously.[9] As shown in Fig. 2, even on the surface of magnetic beads, guanine base in the telomeres rapidly reacted with TMPG, similar to those in the solution phase. After the addition of TMPG in DMF, a maximum CL signal was reached after less than 10 s and then decreased quickly.

The amounts of magnetic beads and biotinylated capture probes have a profound effect upon the sensitivity of the CL label-free protocol. The hybridization guanine signal firstly

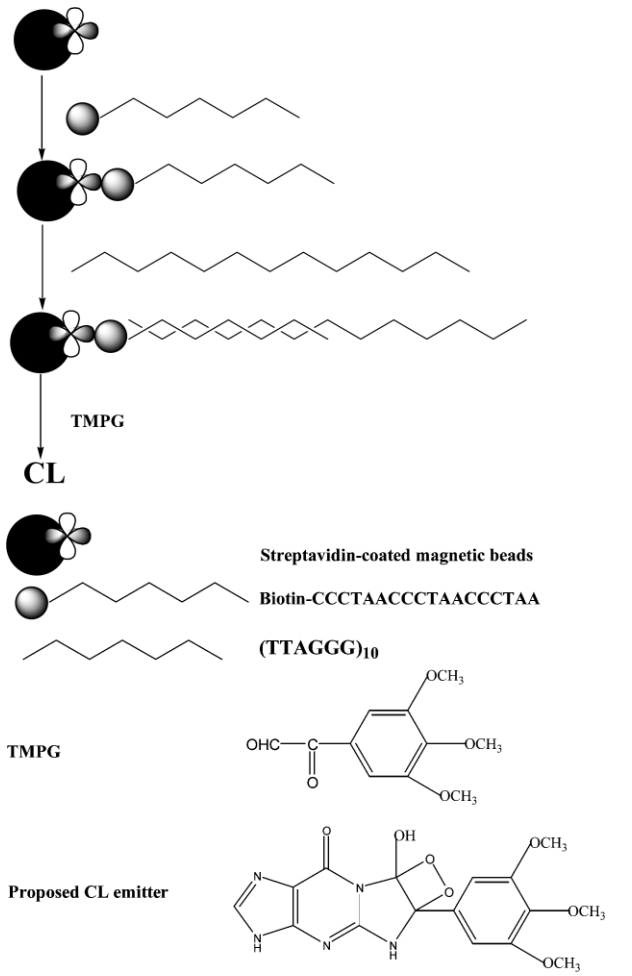

Fig. 1 Schematic representation of the analytical protocol: (a) introduction of the streptavidin-coated beads; (b) magnetic capture of the biotinylated probe; (c) hybridization event; (d) detection of hybrid DNA on the beads using TMPG CL.

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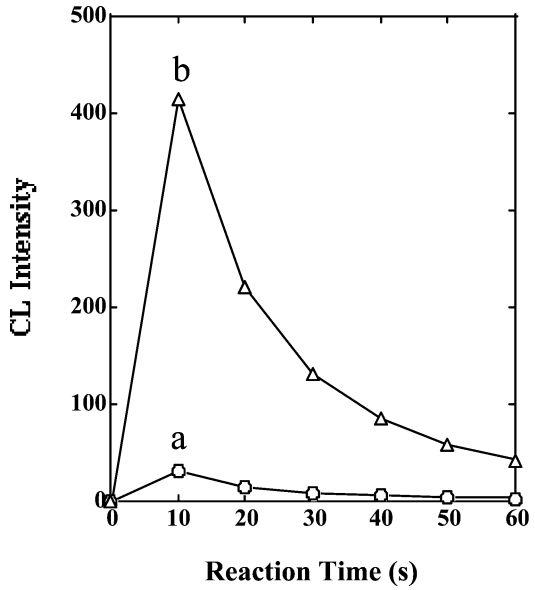

Fig. 2 Kinetic profiles for the reaction between TMPG and guanine bases in the telomeres: (a) on the surface of magnetic beads after hybridization; (b) in the solution phase without hybridization and magnetic beads.

increases with an increase of magnetic beads, and levels off above 300 μg; subsequent work employed 500 μg beads. At a concentration of more than 160 pmole biotinylated capture probes, higher amounts of telomeres could be detected whereas the detection limit decreased. Furthermore, the effect of the hybridization time upon the CL signal was examined by using 500 μg beads and 160 pmole biotinylated capture probes. It was found that a hybridization time of 90 min leads to a decrease of the CL signal, and thus a hybridization time of 60 min was selected for the following experiments. Other parameters affecting the CL signal were also examined and optimized, for example, 10 μl of TPA–H_3PO_4 buffer (pH 8.5) and 100 μl of 30 mM TMPG in DMF were chosen as the optimum concentration in the following experiments.

Besides, negligible signals were observed for a large excess of non-complementary and mismatched oligonucleotides. Other potential interferences, such as RNA or bovine albumin, can also be eliminated by the efficient separation of the magnetic bead capture.

Under the proposed experimental conditions, using a batch method, a calibration graph (Fig. 3) in the telomere concentration range of 0–80 pmole showed a linear correlation (r^2 = 0.994) represented by $I = 0.693C + 3.629$, where I is the maximum intensity and C is the concentration of telomeres. At a concentration of more than 80 pmole, the CL intensity decreased and deviated from the calibration curve. A series of six measurements with 40 pmole of telomeres was used for

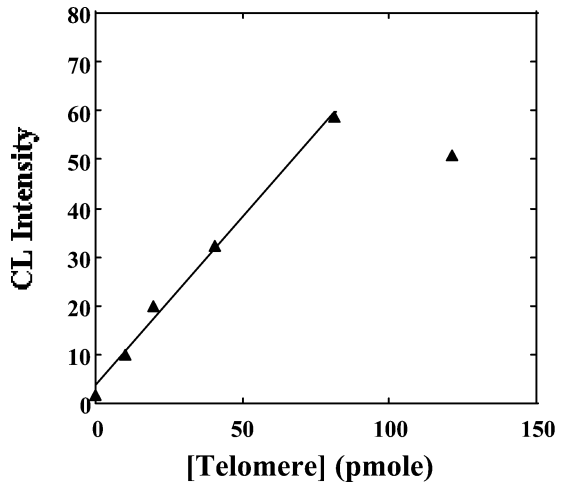

Fig. 3 Calibration curve (reaction conditions: 100 μL of magnetic beads, 164 pmole biotinylated capture probes).

estimating the precision of the calibration curve. This series yielded a relative standard deviation of 4.8%.

In conclusion, we have described a novel route for amplifying the label-free detection of telomeres based on the CL measurement of guanine bases in nucleic acids. In particular, the efficient magnetic separation has been extremely useful for discriminating against unwanted constituents, including a large excess of mismatched and non-complementary oligonucleotides, RNA and proteins. Such coupling of magnetic hybridization surfaces with label-free CL detection eliminates reporter molecule labelling, and offers great promise for centralized and decentralized genetic testing. Further improvements, particularly towards the detection of point mutations, are expected by using this new protocol with peptide nucleic acid (PNA) probes.

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