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Dendrimer-like polymeric DNAs as chemiluminescence probes for amplified detection of telomere DNA on a solid-phase membrane†

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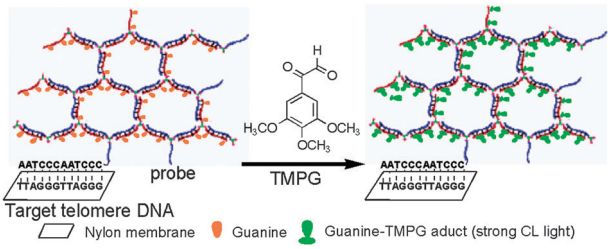
For the first time, an amplified chemiluminescence (CL) detection of the telomere DNA spotted on a nylon membrane is described here, based on the direct hybridization with the CL probe of dendrimer-like polymeric DNAs possessing a large number of guanine moieties. This probe was synthesized by sense and antisense hybridization between Y-shaped DNAs and then could hybridize with the target DNA.

A telomere consists of a simple repeating sequence of six bases $(TTAGGG)_n$, with a length of 5000–15 000 bp, which is located at the end of chromosomes and is synthesized by telomerase.[1] The telomeric repeat has multiple functional roles in cell proliferation, cell cycle regulation and cell division.[2] Its presence is essential for the protection of chromosomes from fusion, recombination and degradation.[3] The telomeric repeat of DNA at the chromosomal end is shortened by 33–120 base pairs with each cell division. The gradual shortening of the telomeric repeats causes genome instability, aging and tumor genesis.[4] Telomerase, a unique ribonucleic acid protein, synthesizes the telomeric repeat at the chromosomal end, which increases the growth of human cancer cells.[1] Therefore, telomere DNA has been used in cancer therapeutics. Conventional telomere DNA detection methods predominantly rely on polymerase chain reaction (PCR) to achieve telomere sequence segments in detectable quantity.[5] Recently, there has been interest in using a novel set of labeling detection methods. In the labeling methods, the target telomere DNA was hybridized with modified complementary DNA as a probe such as ^{32}P,[6] fluorescence[7] and biotin-labeled oligonucleotides.[8] To obviate the complexities of the labeling, several devised DNA detection methods have been reported such as magnetic beads,[9] surface plasmon resonance,[10] and sandwich assays.[11]

We report here the facile synthesis of dendrimer-like polymeric DNAs (YY-DNAs) containing a large number of guanine moieties and their application as polymeric chemiluminescence (CL) probes for the sensitive detection of the telomere DNA on a solid-phase membrane (Scheme 1). The synthesized probe of YY-DNAs can hybridize with the telomere DNA on the nylon membrane and then the resulting assembly is detected by CL reaction with 3,4,5-trimethoxyphenylglyoxal (TMPG).[12–14] The probe of YY-DNAs could be readily synthesized as shown in Scheme 2 by hybridization between sense Y-shaped single-stranded (ss) DNA (Y-DNA) and its antisense Y-shaped ssDNA (Y-cDNA) containing Y-shaped branches as cross-linkers. Sense and antisense ssDNAs of the telomere with a thiol group at the 5′-end were reacted with a synthesized trimeric cross-linker, tris(2-maleimidoethyl)amine (TMEA) (see Scheme S1 and Fig. S1–S3 in ESI†), after reduction and deprotection of the thiol group at the 5′-end with dithiothreitol (DTT), in order to yield Y-DNA and Y-cDNA, respectively. The resulting products contained sense and antisense Y-shaped trimeric, dimeric and monomeric DNAs which were separated on 20% PAGE (Fig. 1A). The sense Y-DNA is composed of 31% trimers, 46% dimers and 23% monomers, while the antisense Y-cDNA is composed of 28% trimers, 50% dimers and 22% monomers.



Scheme 1 Schematic protocol for the CL detection of telomere DNA by means of the direct hybridization with YY-DNAs and TMPG-CL reaction.

Y-DNA and Y-cDNA were carefully designed to have two complete complementary sequences, so that equal amounts of the sense and antisense DNA species in a phosphate buffered saline (1× PBS) solution (pH 7.4) could hybridize with each other to produce the YY-DNAs (Scheme 2). The probe of YY-DNAs was analyzed using 20% PAGE, as shown in Fig. 1B. In addition, bright-field optical microscopy and dynamic light scattering (DLS) measurements

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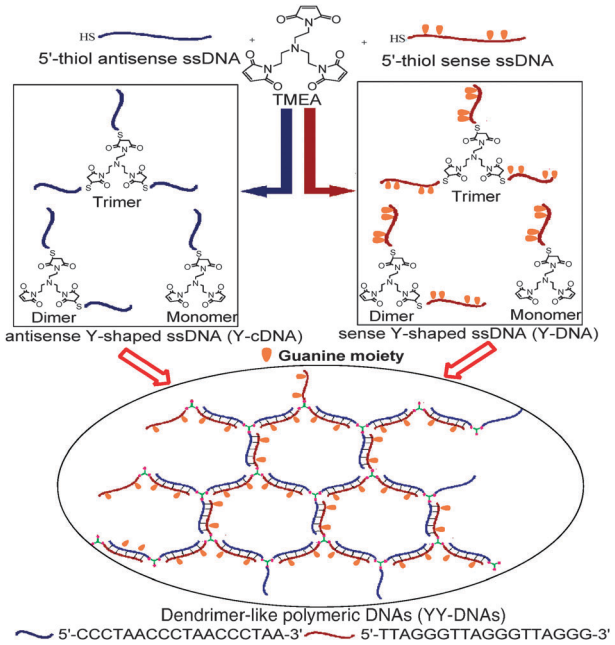

Scheme 2 Synthetic pathway for the preparation of YY-DNAs.

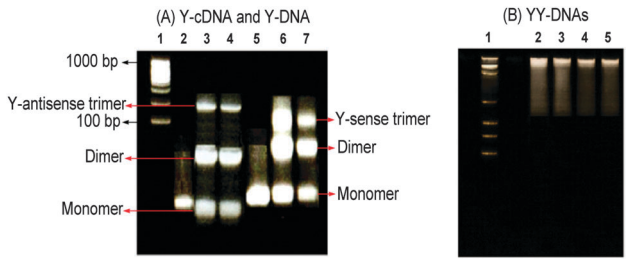

Fig. 1 PAGE analysis of the products of (A) Y-cDNA, Y-DNA and (B) YY-DNAs. (A) Lane 1, 100–1000 bp DNA marker; lane 2, cDNA (362 pmol) containing a 5′-thiol group without TMEA; lanes 3 and 4, Y-cDNA of 362 and 181 pmol with TMEA; lane 5, DNA (342 pmol) containing a 5′-thiol group without TMEA; lanes 6 and 7, Y-DNA of 342 and 171 pmol with TMEA, respectively. (B) Lane 1, 100–1000 bp DNA marker; lanes 2, 3, 4 and 5, YY-DNAs of 1.25, 1.0, 0.92 and 0.625 pmol, respectively. The gel was stained with the fluorescent SYBR-Gold dye.

were used to examine the morphology and size of the YY-DNAs (see Fig. S4 in ESI†). The YY-DNAs showed nearly spherical morphology with an average diameter of 5.2 ± 1.2 μm. During the formation of the self-assembly, the dimeric DNA might extend the chain length and Y-shaped trimeric DNA might serve as a cross-linker in the DNA dendrimer network. In this study, we employed 18-base sticky ends for extra stability of dendrimer-like DNAs. The lengths of the DNA duplex in the YY-DNA dendrimer were designed to make the final dendrimer a three-dimensional structure rather than a two-dimensional one. A normal DNA duplex rotates one full turn in about 10.5 base pairs.[15] In the design of dendrimer-like DNAs, each DNA duplex contains 18 base pairs and thus after self-assembly, this design might lead to a three-dimensional dendritic structure and keep the sticky ends distributed in the outermost layer of generation.

Previously, three-dimensional DNAs such as dendrimer-like DNAs[16] have been synthesized by other researchers. These syntheses were divided into ligation and chemical methods. In the former method, dendrimer-like DNAs were synthesized by ligation of the sticky ends of at least 2 Y-shaped DNA molecules which consisted of three oligonucleotide components that were complementary to each other in such a way that each arm of the Y-DNA molecule possessed a sticky end able to ligate. These Y-shaped DNAs served as both monomers and cross-linkers for self-assembly, and led to a large scale and three-dimensional structure.[16] In the latter method, dendrimer-like DNA molecules were synthesized using the solid-phase phosphoramidite chemistry by frequent coupling of trebling synthons on a glass support in a DNA synthesizer[17] or by self-assembly of two complementary tris-oligonucleotidyls.[18] Therefore, our synthesized Y-shaped DNA and YY-DNAs were different from the conventional ones.

In order to examine the efficiency of the synthesized probe of YY-DNAs, the CL-imaging detection of Y-DNA, Y-cDNA and YY-DNAs for comparison with a commercially available 5′-biotin-labeled antisense ssDNA (biotin-cDNA) was carried out by immobilizing different amounts of them onto nylon membranes (Fig. 2). Each molecule of Y-DNA and YY-DNAs contains at least 9 moieties of guanine, while the Y-cDNA molecule does not contain guanine moieties. Therefore, Y-DNA and YY-DNAs could be detected by TMPG reaction because the TMPG reagent produces CL signals from the guanine moieties in the DNA molecule. The TMPG reagent specifically and quickly reacted with the guanine moieties in the DNA assembly to form unstable derivatives for the generation of intensive CL light[12–14] (see Scheme S2 in ESI†). The biotin-cDNA was detected by the CL reaction with an avidin–horseradish peroxidase (HRP) probe and luminol. We found that the CL signals were proportional to the amounts of Y-DNA and YY-DNAs spotted onto the nylon membrane (Fig. 2C). However, the calibration curve of the biotin-cDNA showed a logarithmic correlation in the concentration range of 2.9–46 pmol. Our YY-DNA probe provided approximately 35 times higher CL signals than that of Y-DNA and 6 times than that

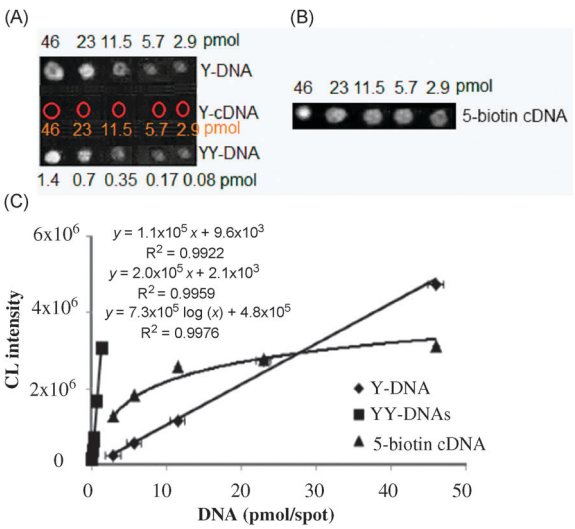

Fig. 2 (A) CL image of Y-DNA, Y-cDNA and YY-DNAs on a nylon membrane by the TMPG-CL detection. (B) CL image of biotin-cDNA on a nylon membrane by CL reaction with an avidin–HRP probe and luminol. (C) Calibration graphs (n = 3 each) of Y-DNA (2.9–46 pmol) (◆), YY-DNAs (0.08–1.4 pmol) (■) and biotin-cDNA (2.9–4.6 pmol) (▲).

of the biotin-cDNA. The lower detection limit of the YY-DNAs was approximately 15 amol (6 ng) at a signal-to-noise ratio of 3 on the membrane. The results indicate that the YY-DNAs possess a large number of guanine moieties and thus can be used as sensitive probes for the sensitive detection of telomere DNA.

The advantage of our strategy to amplify the CL signal from the telomere DNA is the facile synthesis of a spherical dendrimer-like polymeric DNA probe, which has the possibility for the direct hybridization with the target DNA without the use of specially labeled DNA as a reporter. In addition, this synthesized probe has many hybridization sticky ends in the outermost layer that increased the hybridization efficiency. It was reported that avidin-coated polystyrene microspheres for biological binding of a large amount of guanine-rich biotin-DNAs[19] or elongated linear guanine-rich DNAs[20] were detected by the CL reaction with TMPG. Therefore, our strategy for the sensitive detection of telomere DNA based on the amplification of the CL signal with TMPG was different from those in the previous reports.

For the optimized detection of the assembly of the YY-DNAs and the target telomere DNA on a nylon membrane, several parameters were investigated including blocking time, hybridization time and its temperature on a nylon membrane (see Fig. S5 in ESI†). First, the effect of the blocking time using Church's buffer on the nylon membrane spotted with telomere DNA (1.0, 5.0, 10 pmol) was examined. The CL intensity of the telomere DNA was increased with the blocking time in the range of 0.5–2 h at 42 °C. However, the blocking time of over 2 h is ineffective on the CL. This result indicated that nonspecific sites on the membrane were sufficiently blocked after 2 h. The optimum hybridization time of the spotted telomere DNA (1.0–10 pmol) with the probe of YY-DNAs in a hybridization buffer (1× SSC buffer containing 1% SDS) at 42 °C was 6–16 h, indicating the maximum emission of the CL intensity. Thus, the synthesized guanine-containing polymeric probe of YY-DNAs was employed for the sensitive detection of the target telomere DNA adsorbed on the nylon membrane by the direct hybridization, and then the resulting assembly was directly reacted with the TMPG reagent for the CL detection.

As shown in Fig. 3, the CL signals increased upon increasing the amounts of the target telomere DNA spotted on the membrane.

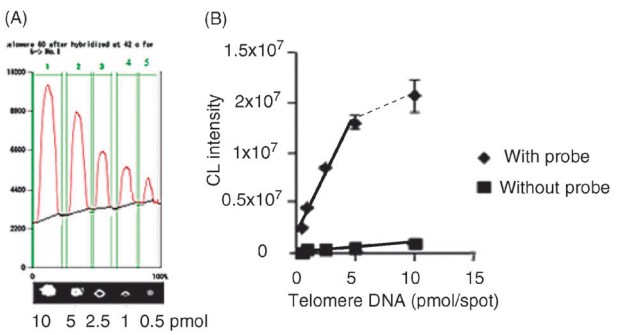

Fig. 3 (A) CL-imaging detection of telomere DNA (0.5–10 pmol) by the direct hybridization with the synthesized probe of YY-DNAs followed by TMPG reaction. (B) Calibration graph of the DNA in the range of 0.5–10 pmol per spot on the nylon membrane.

The CL intensity was proportional up to 5 pmol of the telomere DNA and its calibration graph in the concentration range of 0.5–5 pmol showed a linear correlation (R^2 = 0.9818) represented by $y = 2 \times 10^6 x + 2 \times 10^5$, where y is the CL intensity and x is the amount of the telomere DNA. The lower detection limit of the telomere DNA at a signal-to-noise (S/N) ratio of 3 was found to be approximately 50 fmol (0.9 ng) on the membrane. The sensitivity of CL detection was approximately 18 times higher than that without this probe.

In conclusion, we demonstrated a facile strategy to prepare and utilize a dendrimer-like polymeric probe of YY-DNAs containing a large number of guanine moieties, which was self-assembled *via* sense and antisense hybridization between Y-DNA and Y-cDNA. The CL detection of the telomere DNA spotted on the membrane was successfully performed by means of the direct hybridization with YY-DNAs followed by the TMPG reaction. Its lower detection limit of the telomere DNA was approximately 5 times more sensitive than that with the CL detection using a biotin–avidin–HRP system (see Fig. S6 in ESI†). Its application for the determination of telomere DNA in biological specimens such as HeLa cell lysate on a nylon membrane is in progress, since the membrane is cheap and can be easily handled for high-throughput detection of many samples.

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