Synthesis of long Poly(dG)·Poly(dC) DNA using enzymatic reaction†

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Non-defect Poly(dG)·Poly(dC) of 500 bp (170 nm) has been synthesized by using enzymatic reactions and was characterized by its UV spectrum, showing that conjugated π-electrons between base pairs are spread over the DNA molecule suggesting the absence of structural defects.

Guanine rich strands exist at the end of a chromosome and is denoted a telomeric structure. It is suggested that this structure is associated with cellular aging, since this structure controls the number of cell divisions. Up to now, in order to investigate telomeric structures, DNA with a G- and C-rich strand, or a repeated sequence of ATTTT has been synthesized up to 1000 bp with telomerase, the enzyme which elongates the telomere sequence in DNA. However, homopolymer double stranded DNA has not been synthesized with more than 1000 bp. Particularly, it is difficult to synthesize homopolymer Poly(dG) which consists of only guanine in the same strand of more than 100 bp, since a guanine higher-order structure (guanine quadruplex) is formed in the synthesis.

We already succeeded in synthesizing Poly(dA)-Poly(dT) of a length of more than 1000 base pairs with no structural defects using a multi-step enzymatic reaction. We then applied the same technique to synthesize Poly(dG)-Poly(dC). In this case, however, we failed to synthesize long-chain Poly(dG)-Poly(dC) because single chain Poly(dG) formed a higher-order structure between and in the molecules. Hae III is an enzyme that recognizes the base sequence 5'-CCGG-3' and selectively cuts the guanine–cytosine bond. Again, we applied S1 nuclease reaction. Finally, we added 3 μl of T4 DNA ligase (40 U μl⁻¹) to the DNA solution and allowed reaction at 11 °C for 3 days. In order to prevent the deactivation of the enzyme during the reaction, we added 2 μl of T4 DNA ligase (40 U μl⁻¹) every 24 h. This reaction produces guanine-guanine (cytosine–cytosine) 1 homo-bonding as well as cytosine–guanine 3 (guanine–cytosine 4) hetero-bonding. Again, we applied Sma I and Hae III to cut the guanine–cytosine and cytosine–guanine bonds so that only the homopolymer Poly(dG)-Poly(dC) I would remain. For this reaction we added 3 μl of Sma I (12 U μl⁻¹) and 3 μl of Hae III (10 U μl⁻¹) every 24 h. After the reaction we separated, purified and collected Poly(dG)-Poly(dC) 2 using HPLC.

We then applied the DNA joining enzyme, T4 DNA Ligase, to the purified 50-base Poly(dG)-Poly(dC) 2 to activate the ligation and synthesized a long-chain DNA.

Next, the 50-base Poly(dG)-Poly(dC) 2 was dissolved in 34 μl of ultrapure water. We added 7 μl of TA buffer (× 10), 5 μl of ATP (adenosine 5'-triphosphate) (1 mM) and 2 μl of T4 DNA ligase (40 U μl⁻¹) to the DNA solution and allowed reaction at 11 °C for 3 days. In order to prevent the deactivation of the enzyme during the reaction, we added 2 μl of T4 DNA ligase (40 U μl⁻¹) every 24 h. This reaction produces guanine-guanine (cytosine–cytosine) 1 homo-bonding as well as cytosine–guanine 3 (guanine–cytosine 4) hetero-bonding. Again, we applied Sma I and Hae III to cut the guanine–cytosine and cytosine–guanine bonds so that only the homopolymer Poly(dG)-Poly(dC) 1 would remain. For this reaction we added 3 μl of Sma I (12 U μl⁻¹) and 3 μl of Hae III (10 U μl⁻¹) and left them at 30 °C for 3 days. Deactivation of the enzyme was prevented by adding 3 μl of Sma I (12 U μl⁻¹) and 3 μl of Hae III (10 U μl⁻¹) every 24 h during the reaction. Finally, we applied S1 nuclease to the Poly(dG)-Poly(dC) 1 to remove the single chain species expected to exist in the DNA. S1 nuclease recognizes nick and single-chain regions (structural defects) in DNA and selectively cuts more than 90% of these efficiently. For the reaction we added 3 μl of S1 nuclease (160 U μl⁻¹) and 8 μl of S1 nuclease reaction buffer (× 10) to 70 μl of the reaction solution and allowed reaction at 37 °C for 15 min. We then purified and collected the DNA by phenol extraction and ethanol precipitation. We also separated DNA by polyacrylamide gel electrophoresis and...
The spectrum of this DNA differed from that of the left-handed helical structure and of the guanine quadruplex. Furthermore, in STM lane electrophoresis (Fig. 1). One band was confirmed around 500 bp in synthesized 500 bp Poly(dG)-Poly(dC). We concluded that there were no nicks or structural defects in the parts of the commercial DNA. Therefore the synthesized DNA has a double helix structure with no defects.

Finally, the UV spectrum of the synthesized 500 bp Poly(dG)-Poly(dC) of length up to 500 bp (170 nm) by use of enzymatic reactions. In future work we will evaluate the electronic properties of Poly(dG)-Poly(dC) without structural defects synthesized in this study, and aim at applying it as a novel electronic material.

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Notes and references
11 TA buffer (× 10): Tris-Acetate (33 mM, pH = 7.9), KOAc (66 mM), MgOAc (10 mM), Dithiothreitol (0.5 mM).
12 HPLC equipment and column are AKTA explorer (Amersham Biosciences) and anion-exchange column (TSKgel DNA-NPR TOSHO), respectively. We used two types of eluates: buffer A: Tris-HCl (20 mM, pH = 9.0), and buffer B: Tris-HCl (20 mM, pH = 9.0), NaCl (1 M). The separation, purification and collection of 50-base Poly(dG)-Poly(dC) 2 was carried out under the following conditions. First, buffer B was flowed for 3 min at a level of 25% (A at 75%). Then the proportion was constantly increased from 25 to 70% in 33 min (1.35% min−1). In the next 10 s the buffer B was rapidly increased to 100%. Finally we kept flowing buffer B for 8 min and cleaned out the inside of the column.