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Synthesis of long Poly(dA)·Poly(dT) DNA without structural defects using enzymatic reaction; Tailored ligated Poly(dA)·Poly(dT)†

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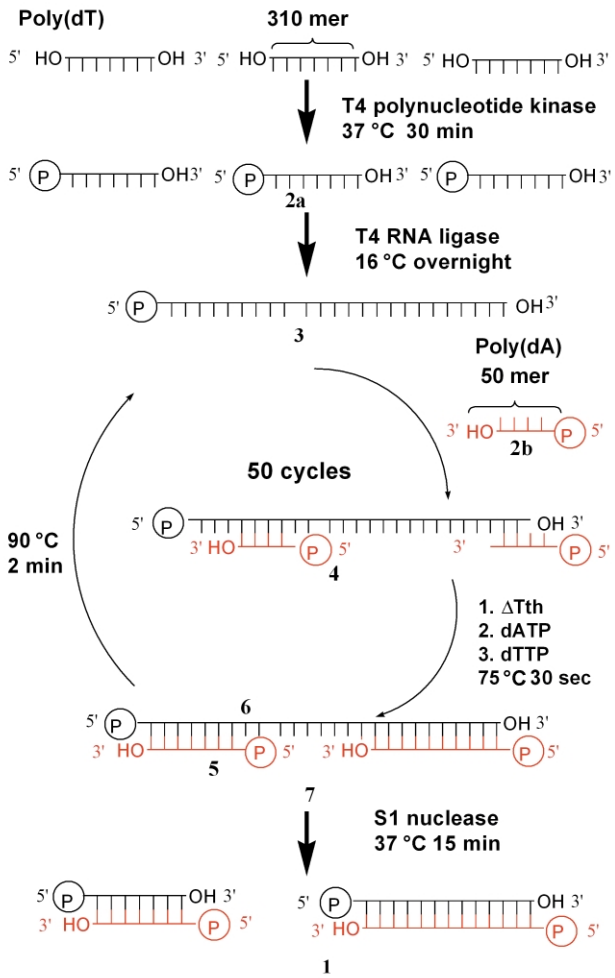
Poly(dA)·Poly(dT) molecules up to 1000 base pairs (bp) have been synthesized using enzymatic reaction, and characterization by STM observation shows that the DNA has no defects in the duplex structure.

A clarification of the electronic conductive phenomenon of DNA molecules is presently one of the most important subjects in the fields of life- and nanoscience, as it would provide opportunities to elucidate not only the mechanisms of gene damage and mutation but also the ability to fabricate molecular electronic devices based on DNA. Although numerous studies on the electrical properties of DNA[1–7] molecules have been undertaken, the conduction mechanism still remains subject to controversy. The reason seems to lie in the fact that the conductivity strongly depends on sequences and circumstances such as pH, buffer, and counter cations. Since the simplest DNA molecules, Poly(dA)·Poly(dT) and Poly(dG)·Poly(dC), are suitable for the investigation of electrical properties, their conductivity has been researched using a conducting-probe atomic-force microscope and nanoelectrode.[7] However, their DNA molecules invariably have both single- and double-strand parts due to the incomplete hybridization (so called structural defects), which lead to electron and polaron localizations. Furthermore, the complete length of Poly(dA)·Poly(dT) and Poly(dG)·Poly(dC) duplex DNA has been below 50 bp (17 nm).

In this communication, we produce long Poly(dA)·Poly(dT) without structural defects (so called Tailored ligated Poly(dA)·Poly(dT)) by using enzymatic reactions, and characterize the molecular structure of the DNA using scanning tunneling microscopy (STM).

Poly(dA) of 310 mer and Poly(dT) of 50 mer, which lack the phosphate at the 5′-end of DNA were used as starting materials. Polynucleotide kinase, T4 RNA ligase, S1 nuclease and Δ*Tth* DNA polymerase were used as reaction enzymes. Single-strand DNAs, and ATP,[8] dATP[8] and dTTP[8] were purchased from Amersham Pharmacia Biotech. Polynucleotide kinase, T4 RNA ligase and S1 nuclease were purchased from Takara Bio Inc., and Δ*Tth* DNA polymerase were purchased from Toyobo Co., Ltd.

The synthesis of long Poly(dA)·Poly(dT) was achieved as shown below in Scheme 1. Kination was first performed for synthetic oligonucleotide Poly(dT) at 37 °C for 30 min by mixing 39 μl of 25.3 μM Poly(dT) with 5 μl of 1 μM ATP, 5 μl of enzyme reaction buffer and 3 μl of T4 polynucleotide kinase (10 U μl^{-1}),[9] which phosphorylated the 5′-end of DNA. The reaction mixture was heated at 80 °C for 15 min to inactivate the enzyme, to produce **2a**. In similar way, kination **2b** was performed for synthetic oligonucleotide Poly(dA) (39 μl, 1.97 μM). The solution of **2a** (52 μl) was allowed to react with T4 RNA ligase and that single-strand DNA was ligated in ATP (9 μl, 1 mM) and BSA buffer[10] (7 μl) solutions at 16 °C overnight, followed by heating at 80 °C for 15 min to inactivate the enzyme. RNA ligase is a bacteriophage T4 enzyme that is capable of covalently joining single-stranded RNA or DNA molecules containing 5′-phosphate and a 3′-hydroxy terminal. These steps produced a provide **3** (*ca.* 1000 bases). The produced **3** was annealed with phosphorylated Poly(dA) (**2b**) as shown in Scheme 1. We have found the optimised **2b**/**3** ratio to be 2/3. A produced hetero duplex DNA that has single-stranded



Scheme 1 Reaction steps **3** and **4** are a cyclic reaction. The number of base molecules on **5** (Poly(dT)) and **6** (Poly(dA)) increase as the reaction steps increase. **7** is consistent with **5** and **6** after 50 reaction steps.

† Electronic supplementary information (ESI) available: circular dichroism (CD) spectrum of tailored Poly(dA)·Poly(dT) in the presence of 20mM Na^+ at 25 °C. See http://www.rsc.org/suppdata/cc/b2/b205821a/

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parts on the structure is shown as **4**. The polymerisation reaction was performed by using Δ*Tth* DNA polymerase. Δ*Tth* DNA polymerase is a thermostable DNA polymerase that lacks an exonuclease activity. Hetero duplex product (24 μl) was mixed with 1 μl of Δ*Tth* DNA polymerase (5 U μl^{-1}), with 5 μl of a reaction buffer provided by the company, 10 μl of dTTP (1 mM) and 10 μl of dATP (1 mM). The polymerase chain reaction consisted of (1) 75 °C for 30 seconds and (2) 90 °C for 2 min, repeated for 50 cycles. The number of base molecules on **5** (Poly(dT)) and **6** (Poly(dA)) increase as the reaction cycles increase. The PCR product **7** was incubated with S1 nuclease (3 μl, 160 U ml^{-1}) and buffer (5 μl) at 37 °C for 15 min. Unpaired parts were degraded with S1 nuclease resulting in complete double-stranded DNA. Recovery of the DNA was immediately performed by using phenol–chloroform extraction and ethanol precipitation, respectively. Product **1** is defined as DNA of 1000 bp.

The length of synthesized Poly(dA)·Poly(dT) was evaluated using electrophoresis[11] (0.9% agarose gel). A smear band of between 500 and 10000 bp appeared in lane 3 before adding S1 nuclease, as shown in Fig 1. Addition of S1 nuclease made the wide band disappear, and two bands of 1000 and 2000 bp appeared as shown in lane 4. This shows that single-strand parts on the DNA before treatment by S1 nuclease were selectively degraded, and that the obtained DNA has a completely duplex structure.[12,13]

Structures of a Poly(dA)·Poly(dT) DNA synthesized by the usual PCR technique and **1** were characterized using STM (UNISOKU, Japan). STM images of the former indicate that the DNA consists of single-strand and double-strand parts, their average width being of approximately 1.0 and 3.0 nm, respectively (Fig. 2(a) and (c)). Since a single-strand DNA is easily bent, many folded structures are observed in the single-strand part, whereas the double-strand part mainly has a linear structure. In the case of synthesized Poly(dA)·Poly(dT), most parts are observed to be unfolded but linear in structure as

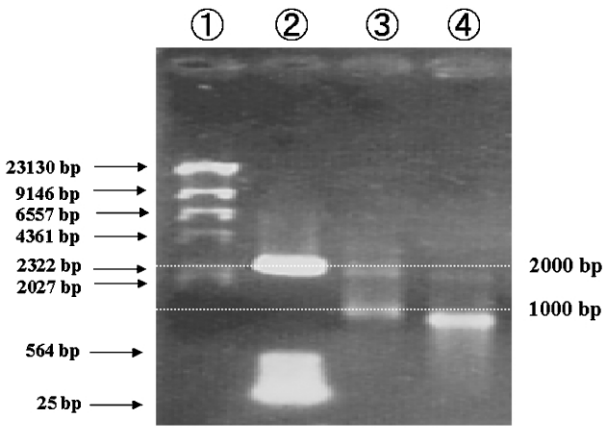

Fig. 1 Agarose gel electrophoresis of synthesized Poly(dA)·Poly(dT). Lane 1 is λ/Hind III (Takara Bio Inc.) and 2 is a 25 bp ladder (Takara Bio Inc.). Lane 3 is a band of synthesized Poly(dA)·Poly(dT) before adding S1 nuclease. Lane 4 is a band of synthesized Poly(dA)·Poly(dT) after adding S1 nuclease.

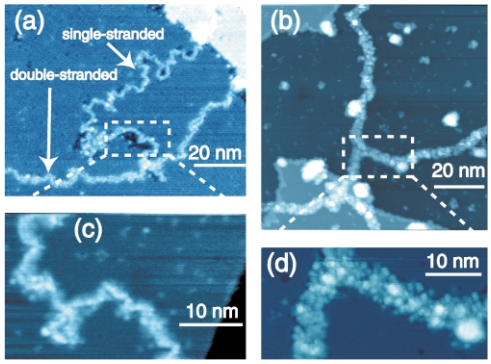

Fig. 2 STM images of (a) Poly(dA)·Poly(dT) synthesized by the usual PCR technique and (b) **1** deposited on clean Cu (111) substrates using a pulse injection method. (c) and (d) show magnified images of the regions indicated by white rectangular frames. STM images are taken at bias voltages and tunneling currents of (a) +5 V, 1 pA; (b) +1 V, 1 pA; (c) +3.5 V, 5 pA; and (d) +2 V, 5 pA; respectively.

shown in Fig. 2(b) and (d). The average width of this DNA is about 3.0 nm, which supports the claim that the DNA molecules have no structural defects.

In summary, a Poly(dA)·Poly(dT) of 1000 bp has been produced, and STM observations show that the DNA has no structural defects. The long length and complete duplex structure (Tailored ligated Poly(dA)·Poly(dT)) will facilitate the clarification of the electronic structure of DNA. The study on electrical conductivity of the DNA in the nanoscale is currently in progress, as is the synthesis of long Poly(dG)·Poly(dC) without structural defects.

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8 ATP: adenosine 5′-triphosphate, dATP: deoxyriboadenosine 5′-triphosphate, dTTP: deoxyribothymidine 5′-triphosphate.
9 One unit (U) is defined as the amount of enzyme activity.
10 BSA buffer: bovine serum albumin aqueous solution (0.1%).
11 In this electrophoresis, 0.9% Agarose gel, λ/Hind III, and a 25 bp ladder as the DNA marker were used. These reagents were purchased from Takara Bio Inc.
12 We used UV/Vis spectroscopy to measure the synthesized Poly-(dA)·Poly(dT). The peak top of the DNA was found to be 253 nm.
13 The circular dichroism (CD) specrtrum revealed that the type of the DNA was B-form. A CD spectrum of the DNA is given in the ESI†.