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Sequence-specifically platinum metal deposition on enzymatically synthesized DNA block copolymer†

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Platinum metal was sequence-specifically deposited on the DNA block copolymer synthesized by the Klenow fragment of *E. coli* DNA polymerase I (3′–5′ exonuclease deficient).

Due to their long linear structure and the high molecular recognition between not only complementary bases, but also DNA and proteins and other small molecules, DNA molecules have attracted considerable attention as a potential template for the fabrication of nanowires used in future electronic circuits through methods such as doping to DNA,[1] polymerization of conducting polymers on DNA,[2–4] reduction of metal ions pre-adsorbed to DNA,[5–7] photoreduction,[8] directed metallization of specifically labelled DNA with aldehyde groups[9] and electroless plating.[10,11] In particular, DNA-based metal deposition has potential application to the fabrication of thin conductive nanowires (below 10 nm). In general, metal deposition occurs randomly as the metal ions and metal seeds for the electroless plating are not sequence-specifically bound to DNA. Cisplatin is a sequence-selective metal complex that preferentially binds to the dGpG of DNA rather than other sequences.[12,13] Since a free platinum complex binds to a Pt atom already covalently bound to DNA in the process of reduction, platinum complex bound to DNA can act as preferential nucleation sites.[14] We have reported that, after reduction, cisplatin can act as a metal seed for the preparation of Ag nanowires on a Lambda DNA template by electroless plating.[11] Through precise control of cisplatin reduction, a platinum nanowire is expected to be fabricated only on the continuous guanine sequences of DNA. If metal complexes containing different types of metal with affinities to a variety of base sequences are used for the metal deposition method on sequence-designed DNA, programmable deposition of different metals can be achieved depending on the base sequence. In order to demonstrate such base sequence programmable nanofabrication, we synthesized a sequence-designed DNA, diblock copolymer-type DNA (poly(dG)(poly(dC)-poly[d(AT)]) since block copolymers have been used as a template for fabrication of programmable deposition of nanoparticle.[15]

DNA elongation *via* DNA polymerase reaction is a useful tool for the preparation of this type of DNA molecule. DNA polymerase is an enzyme associated with biological processes such as DNA replication, transcription, and repair.[15] In addition, DNA polymerase is known as a catalyst for DNA polymerization, which can provide high molecular weight double-stranded polymers used to prime template-primer oligonucleotides, the sequences of which are repetitive (continuous, alternative or triplet repeat), *via* slippage of the strands composing the template-primer, in a process known as the strand slippage model.[16–20] For example, Kotlyar *et al.* have shown that the Klenow fragment (KF^-) of *E. coli* DNA polymerase I (3′–5′ exonuclease deficient, KF^-) provides DNA molecules of a defined length and narrow size distribution.[17] Herein, we applied this reaction to the synthesis of diblock copolymer-type DNA molecules (DNA block copolymers) composed of poly(dG)·poly(dC) (GC-part) and poly-[d(AT)] (AT-part). When the DNA block copolymers are reacted with cisplatin, we expect that platinum metal will be deposited on only the GC-part of the DNA block copolymer. Fig. 1 shows a schematic illustration of preparation of the DNA block copolymer (a) and guanine-selective metallization by the reduction of cisplatin (b). In order to synthesize the DNA block copolymers, a block copolymer-type template-primer (block template-primer) composed of three kinds of oligonucleotides, dG20, dC10 and dC10(AT)10, was employed. As it has one 3′-end and two single-strand break (nick) positions, the block template-primer is expected to be extended at these positions by KF^-. In this report, the extension of DNA homopolymers, poly(dG)(poly(dC) and poly[d(AT)], from two template-primers, dG10·dC10 and d(AT)10, and the DNA block copolymer from a block template-primer, were confirmed by gel electrophoresis. Sequence-specific platinum metal deposition on the

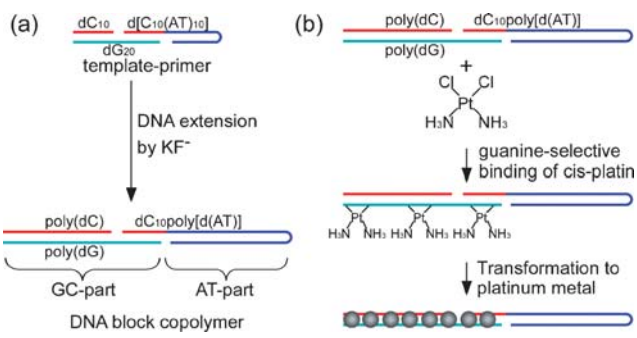

Fig. 1 (a) Schematic illustration of DNA block copolymer preparation by KF^-. (b) Sequence-selective binding of cisplatin to DNA and reduction to platinum metal.

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synthesized DNA block copolymers was confirmed by atomic force microscopy (AFM).

The dG10·dC10 template-primer provided monodispersed products, poly(dG)·poly(dC), and the initial extension was proportional to reaction time (36 bp min^{-1}) (Fig. 2). On the other hand, the products of d(AT)10 were polydispersed and the extension rate (7 bp min^{-1}) was slower than that of dG10·dC10. The alternative-sequenced template-primers may form various duplex structures, for example, intramolecular duplexes. Since the stability of 3′-ends depends on the duplex structures, the formation of the various template-primers induces polydispersity in the synthesized DNA. In addition, the slower extension rate of d(AT)10 may have been caused by differences in the base pair opening times of the duplex ends.[21,22] We observed that the extension rate was fastest for dG10·dC10 and decreased in the following order: dG10·dC10 > d(AT)10 > dA20·dT20 > d(GC)5 (data not shown).

The extension of the block template-primer was also investigated by gel electrophoresis (Fig. 3). Compared with the template-primer (Fig. 3, lane 1), higher weight molecular products corresponding to the extension of the poly[d(AT)] part (AT-part) were obtained using a reaction mixture containing dATP and dTTP, after reaction for 1 h (Fig. 3, Lane 2). The obtained molecular length (about 500 bp) agreed with the length calculated from the extension rate of d(AT)10 template-primers (504 bp) described above, indicating that the presence of the dG·dC duplex in the template-primer did not interrupt

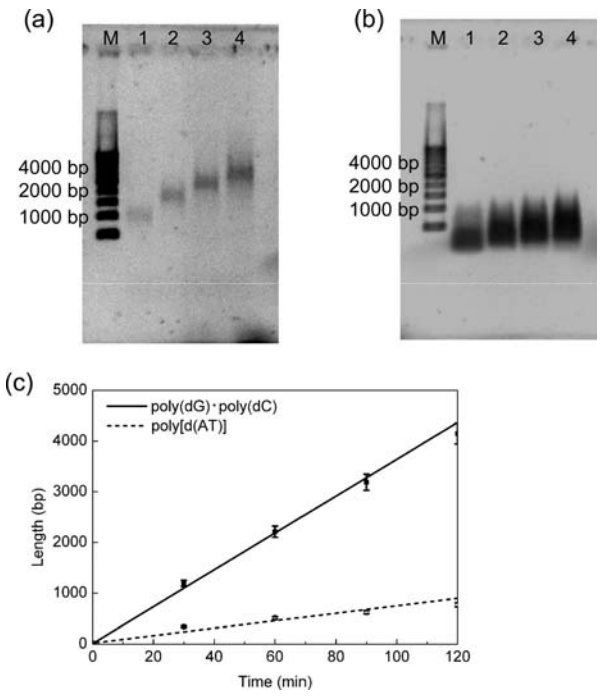

Fig. 2 Time course of poly(dG)·poly(dC) and poly[d(AT)] synthesis. 0.5% agarose gel electrophoresis of synthesized (a) poly(dG)·poly(dC) and (b) poly[d(AT)]. Aliquots of the reaction solutions containing (a) 1 mM dGTP and dCTP or (b) 1 mM dATP and dTTP were taken each 30 min up to 120 min. Lane M shows the marker bands of the 500 bp ladder. Lanes 1–4 represent synthesis for 30, 60, 90 and 120 min, respectively. (c) Dependence of polymer length on reaction time. Each plot was the average molecular weight of three times experiments at the each time.

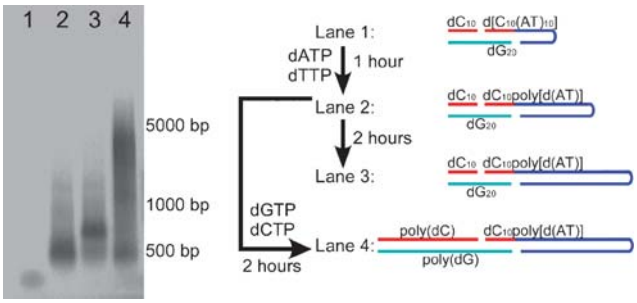

Fig. 3 Electrophoretic results for the synthesized DNA block copolymers. Lane 1: block template-primer, lane 2: reaction mixture containing 0.1 μM block template-primer, 0.1 mM dATP and dTTP, incubated for 1 h at 20 °C, lane 3: lane 2 reaction mixture incubated for an additional 2 h, lane 4: lane 2 reaction mixture incubated for an additional 2 h with the addition of 0.1 mM dGTP and 0.1 mM dCTP.

the extension of the AT-part. In addition, since no band was observed for the block template-primers, all of the block template-primers are thought to have been extended by KF^{-}. After the extension of the AT-part, the reaction solution was reacted for a further 2 h with and without the addition of dGTP and dCTP (Fig. 3, lanes 4 and 3, respectively). The smear band in lane 4, the molecular length of which was from 500 bp to 5000 bp, was of a higher molecular weight than the band in lane 3, indicating that the GC-part was extended. Although the extension of the template-primer dG10·dC10 was monodisperse, band smearing, which indicated discordant extension, was observed in the GC extension reaction of the block template-primer. This was caused by the constraint of strand slippage by the stable stacking of G–C pairs at nick positions hindering the uniform extension of the GC-parts. The results indicated successful DNA block copolymer synthesis by the DNA polymerase.

For the identification of the synthesized DNA block copolymer, we sequence-selectively deposited platinum metal on the DNA block copolymer. Cisplatin is a platinum compound that selectively binds to the N^7 atom of each of the two adjacent purine bases, d(GpG) and d(ApG), to form intrastrand platinum chelates (that comprise 65 and 25% of the total platinum bound to the DNA, respectively). The cisplatin selectively bound to the GC-part in the DNA block copolymer was reduced to platinum metal by a reducing reagent. Fig. 4 shows an AFM image and height histogram of the platinum deposited DNA block copolymer stretched and immobilized on a mica substrate by the LB method.[11,23,24] We produced the height histograms from cross-sectional images of the AFM image shown in Fig. 4(a). The straight lines correspond to stretched single DNA molecules. The height of segments A and B of the line ranged from 1.1 to 2.3 nm (average 1.43 nm) and 0.3 to 0.9 nm (average 0.70 nm), respectively (Fig. 4(d)). The height of segment B was similar to that of naked double-stranded DNA immobilized in the same way (approx. 0.50 nm).[23] The length of segment B (1.5 μm) accorded well with the length of the AT-part estimated from the extension rate of d(AT)10 template-primers. These results indicate that segment B corresponded to the AT-part in the DNA block copolymer. On the other hand, the height of segment A was higher than that of the naked double-stranded DNA, suggesting that platinum metal had been deposited. Although there is a possibility that the height

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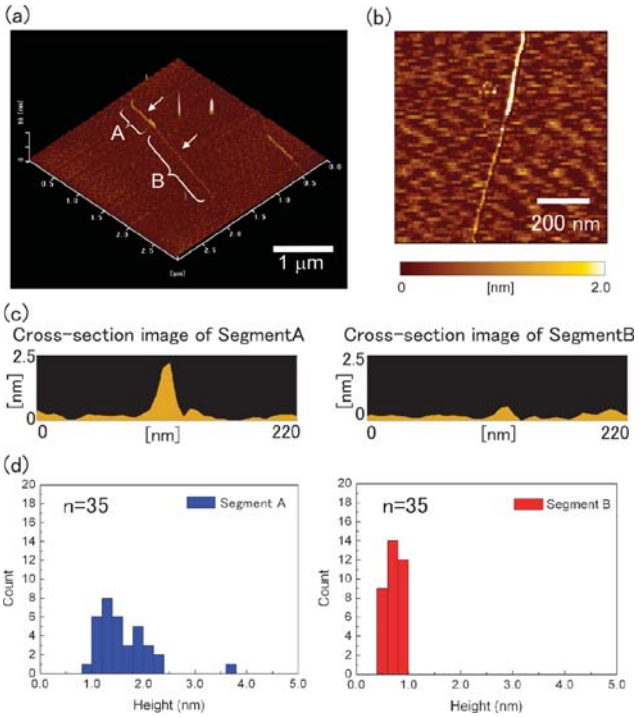

Fig. 4 (a) AFM image of platinum metal bound to the DNA block copolymer. A solution containing 1 mM dATP and 1 mM dTTP was reacted for 6 h. After extension of the AT-part, the solution was reacted at a concentration of 1 mM dGTP and 1 mM dCTP for a further 2 h. (b) The image zoomed in the boundary of segments A and B. (c) Cross-sectional images of segments A and B indicated by the arrows on (a). The cross-sectional image of segment A is 2.3 nm in height and 20 nm in width. The cross-sectional image of segment B is 0.3 nm in height and 20 nm in width. (d) Height histograms of segments A and B. The height of segment A is 1.1–2.3 nm (average 1.43 nm) and that of segment B is 0.3–0.9 nm (average 0.70 nm).

difference is caused by formation of a G-quadruplex, AFM measurement of nonmodified poly(dG)·poly(dC) indicated that it formed a homogeneous linear structure and the height is similar to that of the naked double-stranded DNA (Fig. S1, ESI†).[25] The increment of the height was observed after reduction of cisplatin bound to poly(dG)·poly(dC) and the height is similar to that of platinum clusters on deposited on Lambda DNA molecules.[11] Therefore, the height increment after the reduction of cisplatin bound to the DNA block copolymers indicated the deposition of a platinum cluster on the DNA. The length of segment A (500 nm) was within the range estimated from electrophoretic results (Fig. 2). Some AFM images indicate that the GC-part of the DNA block copolymers varied in length (Fig. S2, ESI†). The stretches of DNA ending in tails which appears significantly higher than the rest is caused by folding of DNA ending in the process of the DNA transferring onto a substrate.[23] Accordingly, segment A could be identified as the GC-part in the DNA block copolymer. This indicated the successful enzymatic synthesis of the DNA block copolymer and its sequence-selective modification of 1–2 nm platinum clusters. Since cisplatin was mixed with the DNA block copolymer solution at 250 eq. relative to DNA base pairs, this stoichiometry was consistent with the deposition of 1–2 nm platinum clusters (number of Pt atoms is about 50–300). The diameter of deposited platinum clusters depended on the concentration of reducing agent (data not shown).

In conclusion, the enzymatic synthesis of a DNA block copolymer composed of a poly(dG)·poly(dC) part and a poly-[d(AT)] part from block copolymer-type template-primers was achieved. We confirmed the sequence-selective platinum metal deposition on the GC-part of the DNA block copolymers by gel electrophoresis and AFM. DNA extension using modified dNTPs, such as ligand-chelating metal ions, will contribute to the fabrication of functional nanowires;[26] for example, hetero-nanowires composed of a metal and a semiconducting material.

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