Title

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Metallization of double-stranded DNA triggered by bound galactose-modified naphthalene diimide

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Graphical Abstract
ABSTRACT

Naphthalene diimide (NDI) derivatives bearing galactose moieties through different spacers, NDI-DS1 and NDI-DS2, were synthesized by the click reaction of the acetylene derivatives of NDI with galactose azide. They bound to double-stranded DNA with threading intercalation, as confirmed by the Topoisomerase I assay and circular dichroism spectroscopy. The binding affinities of these ligands were of the order of $10^5 \, \text{M}^{-1}$ with several times higher affinity for double-stranded DNA than for single-stranded DNA. The silver mirror reaction on the double-stranded DNA bound to these ligands afforded silver nanowires that were converted to gold nanowires. In the atomic force microscopic measurements, the increased height of DNA areas on mica plate observed in the case of double-stranded DNA after NDI-DS2 treatment and subsequently silver mirror reaction, whereas the increased height of DNA areas didn’t observe in the case of single-stranded DNA after same treatment.

Keywords: Naphthalene diimide, Galactose, Silver mirror reaction, Nanowire, Double stranded DNA
1. Introduction

Recently, further densified integration has been required in semi conductive device; many nanodevices have been construction for this purpose. Although top-down and bottom-up approaches are used to fabricate nanodevices, the latter approach is mainly used for molecular wires using carbon nanotubes [1], conducting polymers [2], and metal nanowires [3]. However, it is difficult to arrange these materials in a desired manner. DNA can be assumed to be a linear polymer with a diameter of 2 nm, and a branched wire can be formed by creating a DNA sequence. Furthermore, DNA can be replicated using molecular biological approaches. Therefore, many studies have focused on the nanowires of DNA [4]. As a part of these studies, designed structures have been investigated using DNA complementarity, pioneered by Seeman [5], and developed as DNA origami by Rothemund [6]. This technique is not only used to construct two-dimensional structures such as honeycombs [7] and tiles [8], but also three-dimensional structures such as cubic structures [9]. Although the DNA structure is not stable and exhibits poor conductivity, structured DNA has been utilized as the template for metallization [10–12]. DNA template metallization has been carried out by three methods: metallization of metal ions concentrated on DNA through electrostatic or coordinate interactions [10], metallization of metal nanoparticles introduced on DNA [11], and metallization of metal ions through modified DNA by reductive function [12].

Herein, we synthesized threading intercalators bearing galactose moieties as the reducing sugar to introduce a reductive functionality to DNA indirectly, affording DNA template metal nanowires. Figure 1 shows the concept of DNA template metallization using naphthalene diimides (NDIs) bearing galactose moieties, NDI-DS1 and NDI-DS2, with different linker chain lengths. NDI acts as a threading intercalator, forming a stable complex with
double-stranded DNA bearing many intercalators at every second base pair under saturated binding conditions [13].

After the silver mirror reaction of this complex, the silver ions were reduced by the galactose moieties of the ligands bound to double-stranded DNA, and silver metal was precipitated along the entire length of the DNA.

In this paper, we report the synthesis and binding affinity of NDI-DS1 and NDI-DS2 with DNA and the preparation of nanowires using DNA coated with NDI-DS1 or NDI-DS2. This metallization occurred only on the double-stranded region of DNA, indicating its preference of double-stranded DNA over single-stranded DNA.

2. Experimental

2.1 Materials

2-Morpholinoethanesulfonic acid monohydrate (MES) and ethylenediamine-\(N,N,N',N'\)-tetraacetic acid tetrasodium salt tetrahydrate (EDTA) were purchased from Dojindo (Kumamoto, Japan). M13mp18 single-stranded DNA (Virion DNA), pBR322, Topoisomerase I, and EcoRI were purchased from Takara Bio (Shiga, Japan). Calf thymus DNA (CT-DNA) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used after fragmentation [14]. Oligonucleotides, 5′-GCACGTGC-3′ and 5′-GCTCGGCA-3′ were custom synthesized by Genenet (Fukuoka, Japan). Self-complementary 5′-GCACGTGC-3′ was used as the ds-ODN after annealing, and 5′-GCTCGGCA-3′ was used the ss-ODN. The reagents were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

2.2. Synthesis

NDI-DS1 and NDI-DS2 were synthesized by the route shown in Fig. 2.
2.2.1. Synthesis of 6-azido-6-deoxy-1,2:3,4-diisopropylidene-D-galactose

Azido galactose was synthesized as follows according to a procedure described previously [15]. 2,2'-Azodi(2-methylbutyronitrile) (DIAD, 3.7 g, 14 mmol) and triphenylphosphine (Ph₃P, 3.8 g, 14 mmol) were added to a solution of 1,2:3,4-diisopropylidene-D-galactose (3.7 g, 14 mmol) in 30 mL tetrahydrofuran at 0 °C and stirred for 15 min. After the addition of diphenylphosphoryl azide (DPPA, 3.0 mL, 14 mmol), the reaction mixture was stirred overnight. Furthermore, DIAD (2.9 g, 149 mmol) and PPh₃ (3.8 g, 148 mmol) were added and stirred for 15 min; next, DPPA (3.08 mL, 148 mmol) was added and stirred for two days. The reaction mixture was chromatographed, and the fraction at Rf = 0.39 (developing solvent: CH₂Cl₂) was collected to afford 2.2 g (56% yield) of azido galactose as colorless viscous oil after evaporation under reduced pressure. ¹³C-NMR (100 MHz, D₂O); δ ppm 24.40, 24.85, 25.90, 25.99, 50.65, 66.97, 70.37, 70.78, 71.14, 96.32, 108.76, 109.60.

2.2.2. Synthesis of 6-azido-6-deoxy-D-galactose

The deprotected azido galactose was synthesized as follows according to a procedure described previously [16]. 6-Azido-6-deoxy-1,2:3,4-diisopropylidene-D-galactose (0.50 g, 1.75 mmol) was stirred in 2.1 mL of 80% acetic acid at 70 °C for 16 h. After concentrating the solvent under reduced pressure, 10 mL of water was added to the residue and extracted with 10 mL of CH₂Cl₂ four times. The aqueous phase was evaporated to afford 0.12 g (33% yield) of the deprotected azido galactose as a brown viscous oil. ¹³C-NMR (100 MHz, D₂O) δ ppm 52.50, 52.64, 70.23, 70.39, 70.70, 71.00, 71.40, 73.53, 74.80, 75.04, 94.22, 98.70.
2.2.3. Synthesis of NDI-DS1

A mixture of \( t\)-BuOH/H\(_2\)O (2:1, 3.0 mL) was added to \( N,N'\)-bis[[3-(3-propargyl)piperazin-1-yl]propyl]naphthalene-1,4,5,8-tetracarboxylic acid diimide (NDI-1, 0.16 g, 0.10 mmol) [17], 6-azido-6-deoxy-D-galactose (0.12 g, 0.58 mmol), 1.0 M CuSO\(_4\) (40 μL), 1.0 M sodium ascorbate (40 μL), and tris[(1-benzyl-1\textsubscript{H}-1,2,3-triazol-4-yl)methyl]amine (TBTA, 11 mg, 2.0 mmol) and stirred for 48 h at room temperature. NDI-DS1 was obtained as a yellow viscous oil and purified as follows: A peak at a retention time of 20 min was collected by reverse-phase HPLC using an Inertsil ODS-3 column (inner diameter, 5 μm; size, 4.6 × 250 mm\(^2\); GL Science Inc., Tokyo, Japan) in a gradient mode at a flow rate of 1.0 mL min\(^{-1}\), where the MeOH concentration was changed linearly from 20% to 100% in water containing 0.1% trifluoroacetic acid over 40 min at 40 °C and subsequently freeze-dried. MALDI-TOF-MS (matrix: 2,5-dihydroxybenzoic acid (DHBA)): m/z [M + H] = 1205.16 (theoretical value: C\(_{56}\)H\(_{78}\)N\(_{14}\)O\(_{16}\) + H\(^+\) = 1204.31), \textsuperscript{1}H-NMR (500 MHz, D\(_2\)O) δ ppm 2.14 (4.3, t, \( J = 7.5\) Hz), 2.50 (4.2, t, \( J = 7.0\) Hz), 3.05–3.14 (15.2, m), 3.39 (5.2, t, \( J = 4.0\) Hz), 3.50–3.80 (8.6, m), 3.86 (1.1, d, \( J = 2.0\) Hz), 3.95 (0.68, d, \( J = 4.0\) Hz), 4.14 (3.9, t, \( J = 7.0\) Hz), 4.31 (0.7, d, \( J = 4.0\) Hz), 4.48 (1.1, d, \( J = 2.0\) Hz), 4.5–4.65 (3.8, m), 5.06 (0.72, d, \( J = 4.0\) Hz), 7.80 (2.0, s), 8.47 (4.0, s).

2.2.4. Synthesis of NDI-DS2

A mixture of \( t\)-BuOH/H\(_2\)O (2:1, 3.0 mL) was added to \( N,N'\)-bis[3-(3-propargyl)methylaminopropyl]naphthalene-1,4,5,8-tetracarboxylic acid diimide (0.03 g, 0.062
mmol) [18], 6-azido-6-deoxy-D-galactose (25.4 mg, 0.12 mmol), 1.0 M CuSO₄ (25 μL), 1 M sodium ascorbate (25 μL), and TBTA (0.66 mg, 1.24 μmol) and stirred for 21 h at room temperature. NDI-DS2 was obtained as a yellow viscous oil and purified as follows: A peak at a retention time of 20 min was collected by reverse-phase HPLC using an Inertsil ODS-3 column (inner diameter, 5 μm; size, 4.6 × 250 mm², GL Science Inc., Tokyo, Japan) in a gradient mode at a flow rate of 1.0 mL min⁻¹, where the concentration of MeOH was changed linearly from 10% to 100% in water containing 0.1% trifluoroacetic acid over 30 min at 40 °C and subsequently freeze-dried. MALDI-TOF-MS (matrix: DHBA): m/z [M + H]⁺ = 896.36 (theoretical value: C₅₆H₇₈N₁₄O₁₆ + H⁺ = 896.0),

¹H-NMR (400 MHz, CDCl₃, TMS) δ ppm 2.81 (6.2, s), 3.24 (1.6, m), 3.37 (1.9, m), 3.47 (1.0, d, J = 3.0 Hz), 3.49 (1.0, d, J = 3.0 Hz), 3.67 (1.5, d, J = 2.0 Hz), 3.90 (0.91, d, J = 5.0 Hz), 3.97 (1.1, d, J = 5.0 Hz), 4.12 (4.11, m), 4.24 (1.7, m), 4.44–4.71 (5.1, m), 5.02 (0.7, s), 8.16 (s, 1.0), 8.51 (3.37, s).

2.3. Apparatus

The mass spectra (MS) were recorded using a Voyager™ Linear-SA (PerSeptive Biosystems, Foster City, CA) by the time-of-flight mode with α-cyano-4-hydroxycinnamic acid as the matrix. The ¹H and ¹³C NMR spectra were recorded using a Jeol JNM-A500 spectrometer operating at 400 and 100 MHz for ¹H and ¹³C, respectively, with tetramethylsilane (TMS) as the internal standard. The circular dichroism (CD) spectra were recorded in the 220–550 nm range using a Jasco J820 spectropolarimeter (Jasco Inc., Tokyo, Japan) under the following conditions: response, 2 s; sensitivity, 100 mdeg; speed, 20 nm min⁻¹; resolution, 0.1 nm; band width, 2.0 nm; cumulated number, four times; measuring temperature, 25 °C. The electronic absorption spectra were recorded using a Hitachi
3300 spectrophotometer equipped with an SPR 10 temperature controller under the following conditions: slit width, 5 nm; speed, 600 nm min\(^{-1}\). The kinetic experiments were performed using an SF-61 DX2 double-mixing stopped-flow system (Hi-Tech Scientific Inc., UK) equipped with a temperature controller, Lauda RE206. The single-wavelength kinetic records of absorbance vs. time were recorded. The atomic force microscopy (AFM) images were recorded using a PicoSPM instrument (Molecular Imaging Inc.). The transmission electron microscope (TEM) and energy dispersive X-ray spectroscopy (EDS) measurements were recorded using a H-9000NAR microscope (Hitachi).

### 2.4. CD spectra measurements

The CD spectra of 0.08 mM-bp CT-DNA were measured in the presence of 0, 3.3, 10, 20, or 32 μM NDI-DS1 or NDI-DS2 under 10 mM MES (pH 6.15) and 1 mM EDTA containing 100 mM NaCl.

### 2.5. Topoisomerase I assay

The Topoisomerase I assay was carried out according to a procedure described previously [19]. A mixture of 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5.0 mM MgCl\(_2\), 5.0 mM DTT, 5.0 mM spermidine, 0.01% BSA, 0.5 μg/μL pBR322, and 0.25 U/μL Topoisomerase I was incubated in the presence of varied amounts of NDI-DS1 or NDI-DS2 (0–20 μM) at 37 °C for 2 h. After the addition of 100 μL 1x TE buffer (pH 7.0), 2.0 μL of 10% sodium dodecyl sulfate (SDS), and 0.5 μL of 20 ng/μL proteinase K, the mixture was incubated at 37 °C for 15 min. A mixture (100 μL) of phenol/chloroform (1:1) was added, vortexed, centrifuged, and the aqueous phase was
collected. This operation was repeated for five times. The aqueous solution was washed with 100 μL chloroform/isoamyl alcohol (24:1) for five times. The obtained solution (12μl) containing 1X loading buffer was applied to sample comb of 1.0% agarose gel and electrophiles using Mupid-exU (Advance Co. Ltd.) with 18 V for 3.5 h. After the electrophoresis, the gel was stained with GelStar to photograph using a transilluminator.

2.6. Scatchard analysis using UV–visible measurement

The UV–visible spectra of 5 μM NDI-DS1 or NDI-DS2 in 10 mM MES (pH 6.15) and 1 mM EDTA containing 100 mM NaCl were measured after adding the required amount of CT-DNA and waiting for 1 min at 25 °C. The Scatchard analysis was conducted by analyzing the change in the spectra at 384 nm, the wavelength where the absorption of NDI derivatives is the largest, and is expressed by the following equation (1) [13]:

\[
\frac{r}{c} = K \left(1 - n \frac{r}{C}\right) \left(1 - (n - 1) \frac{r}{C}\right)^{n-1}
\]

(1)

where \( r \) (saturation fraction), \( C \) (the free NDI-DS concentration), \( n \) (sitting number), and \( K \) are the amount of bound ligand per DNA base pair, unbound ligand, number of DNA base pairs covered by ligand, and binding constant, respectively.

2.7. Kinetics analysis using stopped-flow method

The kinetic analysis was carried out according to a procedure described previously [13]. The single absorbance at 384 nm vs. time was recorded in 10 mM MES buffer (pH 6.15) containing 1 mM EDTA and 0.10 M NaCl at 30 °C. In the case where CT-DNA was present in a five-fold excess (12.5, 17.5, 25, 37.5, or 50 μM-bp) over ligand (2.5,
3.5, 5, 7.5, or 10 μM), the association rate constants of NDI-DS1 or NDI-DS2 with CT-DNA were obtained by fitting the exponential data of absorption change to the equation, \( A_1 \exp (k_1t) + A_2 \exp (k_2t) \), where \( A \) and \( k \) are the fractional amplitudes and rate constants, respectively, for a two-exponential fit. The intrinsic second-order association rate constant (\( k_a \)) and dissociation rate constant (\( k_d \)) were obtained from the slope of the plot of apparent association rate constant (\( k_{app} \)) against the CT-DNA concentration according to the equation, \( k_{app} = k_a[\text{DNA}] + k_d \).

The rate constant \( k_d \) for the dissociation of the ligand from CT-DNA was determined by SDS-driven dissociation measurements. Two types of solutions (1% SDS and CT-DNA–ligand complex) were mixed instantaneously using a piston, and the change in the absorption spectrum was measured soon after the mixing. Thus, when the CT-DNA–ligand complex was mixed with an SDS solution, free ligands were incorporated into the SDS micelles. Because this process is diffusion-controlled, the entire absorption change represents the \( k_d \)-dependent process, and therefore, fitting of the kinetic trace by the two-exponential model provides the \( k_d \) values.

### 2.8. Silver mirror reaction of NDI-DS1, NDI-DS2, and glucose

The silver mirror reaction was carried out according to a procedure described previously [12]. Solution A was prepared as follows: \( \text{AgNO}_3 \) (0.17 g, 1.0 mmol) was dissolved in Milli-Q water (1.0 mL) and added to 12% \( \text{NH}_4\text{OH} \) until the appearance of a brown precipitate, followed by the disappearance of the precipitate. Three solutions, 1–3, were prepared as 11 mM NDI-DS1, NDI-DS2, and a glucose-containing 0.26 M \( \text{NaOH} \). Solution A (2 μL) was spotted on three places on a glass plate, and solutions 1–3 (23 μL) were mixed with the spot of solution A. These spots were carefully observed.
The silver mirror reaction mixture was prepared by adding 0.1 M AgNO₃ in NH₄OH at pH 10.5. The Au reaction mixture was prepared as follows: 100 µL KSCN (60 mg/mL) and 100 µL KAuCl₄ (23 mg/mL) were mixed, centrifuged at 4,000g and 25 °C for 1 min, the supernatant was removed, 800 µL phosphate buffer (pH 5.5) was added, 100 µL hydroquinone (5.5 mg/mL) was added, and delivered to the plate as drops. The solution (50 µL) containing linearized M13 DNA with EcoRI digestion (0.16 ng/µL) or λDNA (0.001 ng/µL) in 10 mM HEPES (pH 6.15) and 10 mM MgCl₂ in the presence of 0.4 or 2.3 μM NDI-DS1 or NDI-DS2 was placed on a cleaved mica plate and kept for 30 min. After washing with Milli-Q (50 µL) water for four times, the plate was dried in a desiccator overnight. The silver mirror reaction mixture (0.1 M, 50 µL) was added on this plate, kept in a dark room for 5 h, and the plate was washed with 50 µL of Milli-Q water for four times (total, 200 µL). The Au reaction mixture (50 µL) was also added to the plate, kept for 30 s, and dried in a dark room overnight. The AFM measurement was carried out in a tapping mode and a scan rate of 2 GHz.

An elastic carbon-support membrane immobilized Au100 mesh (Okenshoji Co., Ltd, Tokyo, Japan) was used in the TEM measurements. A mixture of 1.5 nM NDI-DS and 0.001 ng/µL M13 was placed on the membrane and dried overnight in a desiccator. After 50 µL silver mirror reaction mixture was placed on the membrane and kept for 5 h, the membrane was washed with 5 µL Milli-Q water two times. TEM measurements were carried out after drying the samples overnight in a desiccator.
3. Results and discussion

3.1. Interaction mode of NDI-DS1 or NDI-DS2 with double-stranded DNA

The absorbance maximum of 5 μM NDI-DS1 in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 100 mM NaCl at 384 nm shows a hypochromic effect (57%) and red shift (2 nm) upon the addition of CT-DNA (Fig. 2A). The solution of 80 μM CT-DNA in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 100 mM NaCl showed a negative-induced CD band at 383 nm upon the addition of NDI-DS1 (Fig. 2B). The Topoisomerase I assay carried out by adding 1–20 μM NDI-DS1 to 0.5 μg pBR322 shows the unwinding of supercoiled plasmid DNA by the AFM (Fig. 11S). The viscometric titration of NDI-DS1 with [poly(dA-dT)]_2 shows the intercalation binding of NDI-DS1 for double-stranded DNA (Fig. 12S). All the data obtained here show that NDI-DS1 binds to double-stranded DNA via an intercalation mode, particularly threading one expected with its chemical structure [13]. Because similar characteristics were observed in the case of NDI-DS2 in these experiments (Figs. 7S and 13S), NDI-DS2 also binds to the double-stranded DNA via a threading intercalation mode.

3.2. Binding analysis of NDI-DS1 and NDI-DS2 with double-stranded DNA

The absorption change in 5 μM NDI-DS1 or NDI-DS2 in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 100 mM NaCl at 384 nm upon the addition of CT-DNA (Figs. 2A and 7S) was monitored, and the binding constant and binding-site size were estimated by fitting the theoretical curve (Fig. 8S, Table 1) [13]. The binding constants of NDI-DS1 and NDI-DS2 were $3.5 \times 10^5$ M$^{-1}$ and $6.8 \times 10^5$ M$^{-1}$, respectively, with a similar binding-site size of 2.3, consistent with disubstituted NDI derivatives [13]. The binding-site size of ~2 is based on
the nearest-neighbor excluded-site model [13], and these ligands can bind to double-stranded DNA at every two base pairs. The binding preference of NDI-DS1 was evaluated by measuring the binding constants for AT and GC DNA using SPR experiments (Fig. 9S and Table 1S); we found an approximately three times higher affinity of NDI-DS1 for GC DNA than for AT DNA.

The association or dissociation rate constants of NDI-DS1 and NDI-DS2 for CT-DNA were estimated using a stopped-flow spectrophotometer by the fitting the time traces of absorbance at 383 nm (Fig. 10S). The SDS-driven dissociation rate constants of NDI-DS1 or NDI-DS2 for CT-DNA were measured by mixing the SDS solution with the NDI-DS1 or NDI-DS2 solution containing CT-DNA. The association rate constants of NDI-DS1 and NDI-DS2 with CT-DNA were $1.2 \times 10^4$ M$^{-1}$ s$^{-1}$ and $4.7 \times 10^4$ M$^{-1}$ s$^{-1}$ with dissociation rate constants of 0.13 s$^{-1}$ and 0.21 s$^{-1}$, respectively (Table 1). NDI-DS1 and NDI-DS2 exhibited a relatively strong binding affinity of the order of $10^5$ M$^{-1}$ for double-stranded DNA with slow dissociation rate constants, similar to that expect for a threading intercalator. In a close comparison of these ligands, NDI-DS2 shows a two-times higher binding affinity than that for NDI-DS1, because of its faster association rate constant than that for NDI-DS1.

To estimate the preference of ligands for double-stranded DNA over single-stranded DNA, the binding affinity of these ligands was estimated using 8-meric oligonucleotides, ds-ODN or ss-ODN. ds-ODN was a self-complementary oligonucleotide and used after annealing. The spectrophotometric titration of these ligands with ds-ODN or ss-ODN and their Benesi–Hildebrand plot analysis afforded their binding affinity as $10^5$ M$^{-1}$ order of nK. NDI-DS1 or NDI-DS2 showed two- or five-times higher affinity for ds-ODN than that for ss-ODN, respectively.
3.3. Silver mirror reaction of NDI-DS1 or NDI-DS2

First, we checked whether NDI-DS1 and NDI-DS2 can reduce silver as described by the following equation:

\[ R-CHO + 2[Ag(NH_3)_2]^+ + OH^- \rightarrow R-COOH + 2Ag + 4NH_3 + H_2O \]

Twenty mL of 0.1 M Ag(NH₃)₂, prepared by the addition of NH₃ to 0.1 M AgNO₃, was placed on four spots on a glass plate, and a solution of 10.5 mM glucose, NDI-1, NDI-DS1, or NDI-DS2 containing 0.26 M NaOH was placed on these spots, where NDI-1 is naphthalene diimide without galactose moieties as the negative control. Silver precipitation was observed in the case of glucose and NDI-DS1, indicating that NDI-DS1 and NDI-DS2 can participate in the silver mirror reaction.

Second, the DNA–NDI-DS1 complex was observed by AFM. Figs. 4A and 4B shows the AFM image of linearized M13 DNA in the absence or presence of NDI-DS1. The DNA lengths, estimated by Image J, changed from 2013 ± 134 nm to 074 ± 218 nm after NDI-DS1 binding. The average increase in the chain length of DNA by NDI-DS1 binding was 17 nm. This behavior is consistent with the DNA length extending on intercalation and unwinding of the intercalator, as monitored by AFM [20]. The silver mirror reaction was carried out for the complex, M13–NDI-DS1, on the plate for 5 h. Nanoparticles of 20–50 nm size were observed along the entire length of DNA, as shown in Figs. 4C and 4D. Because the TEM measurement showed that these particles were composed of silver, we can conclude that we successfully prepared DNA template silver nanowires (Fig. 5). The
The height of M13 DNA increased from 0.6 nm to 2.0 nm after the silver mirror reaction. The silver atoms may have originated from the galactose core of NDI-DS1 bound to DNA. Because silver ion has a diameter of 0.25 nm, 4–5 atoms of silver accumulated in this case. The height of DNA did not change at a longer reaction time of 18 h. nanoparticles unconnected along DNA was observed in Fig. 4C and 4D. Because NDI-DS1 has slight preference for the GC-rich sequence as shown in Table 1, the heterogeneity of the nanoparticles on DNA should arise from the heterogeneity of NDI-DS1 binding on the DNA sequence.

Furthermore, the reduction of gold ion was conducted on the silver nanowires on DNA using λDNA with a longer length because shorter M13 DNA easily peeled off after the metallization by silver. Chloroaauric acid and KSCN were used to reduce gold on the silver nanowires of DNA after the hydroquinone treatment according to the following equation:

\[
\text{AuCl}_4^- + 4 \text{SCN}^- \rightarrow \text{Au(SCN)}_2^- + (\text{SCN})_2 + 4\text{Cl}^-
\]

The silver nanowires became covered with gold after the reduction of \(\text{Au(SCN)}_2^-\) near the silver nanoparticles. Subsequently, nanoparticles with a height of ~23 nm were observed after a 30 s treatment, as shown in Figs. 4E and 4F. A 60-s treatment afforded a DNA with a height of 46 nm, indicating that the size of nanowires can be controlled by varying the reaction time. The scattering of nanoparticles are based on the nonspecific adsorption of NDI-DS1 on the plate through solver nanoparticles. This arises from the poor washing of the plate because of the easy removable property of silver-metallized DNA. The formation of gold nanoparticles was not observed for the
untreated DNA complex with NDI-DS1 or DNA alone (Fig. 14S). These results indicate that the DNA nanowires were formed by the complexation of NDI-DS1 with double-stranded DNA.

Fig. 5 shows the results of TEM (A and B) and EDS (C) measurements of gold nanowires prepared by the silver mirror reaction of M13 bound to NDI-DS1. Nanoparticles of ~20 nm were observed, as shown in Figs. 5A and 5B, and these nanowires consisted of silver, as identified by the EDS measurements (Fig. 5C). Fig. 5A shows branching structure along the length of DNA, and this may have resulted from the remaining NDI-DS1 near the DNA. Such a branching structure has been reported in the case of DNA template Pd nanowires by Richter [21].

Finally, silver metallization using NDI-DS2 was carried out in the case of single-stranded M13 DNA (ssDNA) and double-stranded M13 DNA (dsDNA) (Fig. 6A). The AFM images of ssDNA and dsDNA showed heights of ~0.25 nm and 0.5 nm, respectively. These values are reasonable because single and double-stranded DNAs were observed in the AFM image. The AFM image of the mixture between 2.0 ng/μL dsDNA and 6.7 μM NDI-DS2 (DNA-bp: NDI-DS = 1:1) showed the unwinding of circular M13 plasmid DNA, whereas the mixture between 2.0 ng/μL ssDNA and 6.7 μM NDI-DS2 (DNA-b: NDI-DS=1:1) showed an aggregated complex. The height of these complexes for dsDNA and ssDNA were 0.4 nm and 0.25 nm, respectively. After the silver mirror reaction, an increased height of 0.9 nm was observed in the case of dsDNA, whereas no height change was observed in the case of ssDNA (0.4 nm) (Fig. 6B). This result indicates that silver metallization occurred in the case of double-stranded DNA bound to NDI-DS2 effectively.

When comparing the metallization using NDI-DS1 and NDI-DS2, silver nanoparticles of ~1 nm were observed in the case of NDI-DS2, smaller than that observed in the case of NDI-DS1 (2 nm). This result indicates that
NDI-DS2 utilized the metallization of double-stranded DNA specifically. The difference in the height of nanowires can be attributed to difference in the linker length of NDI-DS1 and NDI-DS2: A shorter linker chain length of NDI-DS2 located galactose moieties in the groove of double-stranded DNA, whereas the galactose of NDI-DS1 stepped out from the DNA groove.

4. Conclusion

NDI-DS1 and NDI-DS2 bearing galactose moieties were synthesized as the metallization reagents for double-stranded DNA by the reduction of silver ions. These ligands bound to double-stranded DNA with threading intercalation to cover the galactose moieties with DNA, thus affording silver or gold nanowires using DNA as the template. Compared to other previous reports on DNA template metallization, this system achieved the selective metallization of double-stranded DNA. This is useful for the synthesis of metal nanowires bearing single-stranded DNA as the sticky ends for further applications, which should assemble the obtained nanowires through hybridization with their complimentary strand.

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References


Table 1. Binding parameters of NDI-DS1 and NDI-DS2 with CT-DNA, ds-ODN, or ss-ODN.

<table>
<thead>
<tr>
<th>DNA</th>
<th>NDI-DS1</th>
<th>NDI-DS2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CT-DNA</strong></td>
<td>$K(n)$</td>
<td>$3.5 \times 10^5$ (2.3) M$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k_a$</td>
<td>$1.2 \times 10^4$ M$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$k_d$</td>
<td>$0.13$ s$^{-1}$</td>
</tr>
<tr>
<td><strong>ss-ODN</strong></td>
<td>$nK$</td>
<td>$1.5 \times 10^5$ M$^{-1}$</td>
</tr>
<tr>
<td><strong>ds-ODN</strong></td>
<td>$nK$</td>
<td>$3.3 \times 10^5$ M$^{-1}$</td>
</tr>
</tbody>
</table>

a: Scatchard plot, b: Benesi–Hildebrand plot
Fig. 1. Chemical structures of NDI-DS1 and NDI-DS2 and DNA metallization based on their non-covalent interaction with double stranded DNA.
Fig. 2. Synthetic route of NDI-DS1 and NDI-DS.
Fig. 3. (A) Absorption change of 5.0 μM NDI-DS1 upon addition of CT-DNA (B) Circular dichromic spectra change of 80 μM CT-DNA upon addition of NDI-DS1 in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 100 mM NaCl.
Fig. 4. Atomic force microscopic (AFM) images of linearized M13 DNA. (A) 0.16 ng/μL M13, (B) 0.16 ng/μL M13 and 400 nM NDI-DS1, (C) 0.16 ng/μL M13 and 400 nM NDI-DS1 after treated with 0.1M AgNO₃ and NH₃, (D) Extended image of the section in (C), (E) 0.50 ng/μL λDNA and 2250 nM NDI-DS1 after treated with 0.1M AgNO₃ and NH₃ and subsequently treated with 60 mg/mL KSCN and 23 mg/mL KAuCl₄ in phosphate buffer (pH 5.5), (F) Extended image of the section in (E).
Fig. 5. (A) Transmission electron microscopic (TEM) image of the complex between 0.001 ng/μL M13 and 1.5 nM NDI-DS1 on the Au plate after treated with 0.1 M AgNO₃ containing NH₃, (B) Extended image of the section in (A) and (C) Energy-dispersive X-ray spectroscopic (EDS) measurement of the particle site of (A).
Fig. 6 (A) AFM imaging of double and single stranded DNA before and after treated with NDI-DS2 and subsequently silver metallization and (B) DNA heights in each steps.