Forming two-dimensional structure of DNA-functionalized Au nanoparticles via lipid diffusion in supported lipid bilayers

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Abstract

Two-dimensional (2D) forming of DNA-functionalized gold nanoparticles (DNA-AuNPs) has been studied. To confine the DNA-AuNPs in 2D space, we utilized the strong affinity between DNA molecules and supported lipid bilayers (SLBs). DNA-AuNPs adsorbed onto cationic SLB consisting of Dimethyl dioctadecyl ammonium bromide (DDAB) on mica substrate with high affinity. Owing to the high mobility of lipid molecules, 2D diffusion and transportation of DNA-AuNPs on the SLB was achieved. Using AFM imaging, we confirmed a selective adsorption of DNA-AuNPs onto cationic lipid bilayer patches on mica surface, and then we

confirmed a drastic formation change of DNA-AuNPs on the SLB with annealing. We observed that the DNA-AuNPs assembled into a densely-packed single-layered 2D superstructure through DNA hybridization.

Keywords

A1. Self-assembly; B1. Supported lipid bilayer; B1. Nanoparticle; B1. DNA; B1. Lipid;
A3. Superstructure

1. Introduction

Nanoparticles (NPs) have specific chemical and physical properties different from bulk materials [1, 2, 3]. When they are organized into well-defined clusters/superstructures, their collective properties depend on their particle size, interparticle distances and formations [4, 5]. The well-organized NP superstructures have potential applications in the fields of catalytic chemistry [6, 7], electronics [8, 9], photonics [1, 10] and bionics [11, 12]. To realize these devices, it is necessary to develop a method for programmable assembly of NPs into one-, two- or three-dimensional (3D) arrays with nano-meter precision at will.

Recently DNA-guided nanofabrication, which is one of bottom-up approaches, has attracted much attention because it offers programmability in the arrangement of nanocomponents, such as nanoparticles, through base sequence design. DNA molecules have been used as engineering tools to assemble nanoparticles as follows: DNA single

strands, which are attached on a nanoparticle, work as sequence-specific linkers to selectively bind another nanoparticle through DNA hybridization. Actually, many types of 3D crystals have already been assembled using DNA functionalized nanoparticles (DNA-NPs) [13, 14, 15]. In this case, DNA single strands play roles such as interatomic bonds in these crystals. Thus all of the NPs bind each other via DNA single strands in a crystal and it is possible to control the particle formation by changing length and interactions (i.e. sequences) of the DNA molecules and diameters of the NPs.

In terms of practical use and combination with the top-down technologies, two-dimensional (2D) programmable assembly of nanoparticles is crucially important. However, it is more challenging to apply the above method, DNA-guided crystallization, to the 2D assembly in that the isotropic inter-particle bonds are required for 2D-confined growing of the DNA-NP assembly. Therefore, to control/confine the assembling direction of DNA-NPs, which is negatively charged, we figured out to use the surface electrostatic adsorption and the 2D laterally-confined diffusion of DNA-NPs on a positively charged, fluid substrate. If the lateral diffusion of DNA-NPs were realized in the adsorbed state on a substrate, they would bind each other laterally through DNA hybridization. Accordingly, to implement the laterally-confined diffusion of DNA-NPs, we used cationic supported lipid bilayer (SLB) as a fluid substrate.

SLB is a lipid bilayer deposited on a substrate and is often used as a cell membrane model system in vitro [16, 17]. The 2D fluidity of SLB is considered to be due to the thin layer of water resides between the lower lipid layer and the underlying substrate surface [18, 19]. Previous studies revealed that the 2D diffusion of lipid molecules in SLB enhanced by increasing temperature [20, 21, 22]. It is also reported that the

diffusivity of DNA molecules adsorbed on the cationic SLB increased in proportion to that of lipid molecules in the SLB with increasing temperature [23]. Furthermore, the interaction between several types of lipids and DNA molecules has already been investigated. Although the structural model of lipid-DNA complex still remains controversial, mainly two kinds of interactions have been confirmed. One is electrostatic interaction between lipid head group and DNA backbone phosphate and the other is hydrophobic one between lipid aliphatic tail and DNA base. In particular, a strong affinity between cationic lipid head group and DNA backbone phosphate has been confirmed [24, 25], and their binding still exists under high temperature while it becomes slightly weaker [26].

We assumed that if it is possible to utilize lipid molecules in SLB as carriers to transport DNA-NPs, laterally-confined diffusion of DNA-NPs will be achieved. Fig. 1 shows schematic drawings of the process of forming 2D NP superstructure using lipid lateral diffusion and DNA hybridization. Based on this concept shown in Fig. 1, we investigated the effect of the SLB on 2D assembly of DNA-NPs.

2. Materials and Methods

2.1 Formation of Supported Lipid Bilayer

Dimethyl dioctadecyl ammonium bromide (DDAB) (Avanti Polar Lipids) was suspended and stored in chloroform (HPLC grade) in a freezer (-20 °C) until use. SLB were formed on freshly cleaved mica (Nihon Shoji Co., Ltd.) using the vesicle fusion method [27, 28, 29]. The prepared SLB sample was rinsed by exchanging the suspension with 10 mM HEPES buffer (pH 7.4) solution several times. The sample

surface was not exposed to the air during the preparation.

2.2 Preparation of DNA functionalized Au nanoparticles (DNA-AuNPs)

12 nm Au NPs was purchased from Nanopartz. DNA was purchased from Nihon Gene Research Laboratories. As shown in Fig. 2, we prepared a pair of DNA-AuNPs (designated as DNA-AuNP A and DNA-AuNP B), which were functionalized with a pair of complementary thiolated-DNA sequences (sequence A: 5'HS-T15-GTAAGCTGTA3', sequence B: 5'HS-T15-TACAGCTTAC3'). DNA was first de-protected by the addition of 0.1 M dithiothreitol (DTT) for at least 1 hour on ice. The de-protected DNA solutions were purified using desalting NAP-5 columns (Sephadex G-25, Amersham Biosciences). Purified DNA solution was added to AuNPs solution. Borate buffer was added to the mixture of AuNPs and DNA. The solution was stirred for 2 hours before the addition of NaCl (0.05 M). The salt concentration was then increased gradually from 0.05 to 0.3 M NaCl and stirred for 30 minutes. The excess DNA was removed from the solutions by centrifugation for 1 hour at $5433 \times g$ three times. This conjugation method is similar to the protocol in the paper, which yields around 60 DNA per AuNP [30]. The concentration of DNA-AuNP was determined by UV-vis spectroscopy. The concentration of DNA-AuNP A and DNA-AuNP B were 1.9×10^{-7} and 2.2×10^{-7} M respectively.

2.3 Assembly of DNA-AuNPs on supported lipid bilayer

The solution of DNA-AuNPs A was deposited on SLB and left to stand for 60 minutes in order to adsorb to the SLB. After that the sample was rinsed with HEPES

buffer solution to remove extra DNA-AuNPs A and the solution of DNA-AuNPs B was deposited. After 60 minutes, the sample was rinsed with HEPES buffer solution again. This operation has been done at room temperature. After that, temperature of the system was increased at 60 °C. Then the composition of buffer solution was changed for 10 mM HEPES, 1 mM NaCl by depositing NaCl solution to the HEPES buffer to promote the hybridization of DNA and the sample was annealed at 60 °C for 60 minutes. The temperature of the system was controlled by a home-built temperature stage consisting of a Peltier element and a Pt resistance thermometer (Netsudenshi Kogyo). Finally, the sample was cooled slowly in electric hot-water pot. The cooling rate was estimated about 0.02 °C/min. The sample surface was not exposed to the air during the process.

2.4 Atomic force microscopy

AFM images were captured in ScanAsyst mode in aqueous solution at room temperature by a MultiMode 8 atomic force microscope equipped with a Nanoscope VIII controller from Bruker. V-shaped Scan Asyst Fluid+ cantilever with a spring constant of 0.7 N/m was used. The images were analyzed using the AFM software NanoScope version 1.40.

3. Result and Discussion

DNA-AuNP assembly was first tried on SLB consisting of the mixture of cationic and neutral lipids, which is often used for the assembly of bio-molecules [31]. The DNA-AuNPs were deposited on SLB which was composed of DDAB/DOPC

(1,2-dioleoyl-sn-glycero-3-phosphatidylcholine) mixture (weight ratio of DDAB:DOPC=30:70), however, they hardly adsorbed on the SLB. It was concerned that the surface charge of the SLB was not enough to adsorb the DNA-AuNPs, because of the neutral DOPC lipids. Therefore we prepared SLB consisting of only DDAB. Fig. 3(a) shows that an AFM image of the SLB on mica. SLB patches with sizes ranging from dozens of nanometers to several micrometers were observed. The coverage of the mica surface with SLB was about 70% according to the image analysis of Fig. 3(a). The height of the SLB was about 3.5 nm, as measured by cross-sectional profile corresponding to the red line in Fig. 3(a).

After observation of the SLBs on mica, two types of DNA-AuNPs, A and B, were deposited on them. Even though the sample was rinsed with buffer several times after deposition, a lot of DNA-AuNPs were observed on the surface (Fig. 4(a)). The close-up image clearly shows the small patches under DNA-AuNPs (Fig. 4(b)). By indicating the edges of the patches with yellow dashed lines, we confirmed the DNA-AuNPs are always found on the patches, not on the bare mica. According to the cross-sectional profile, corresponding to the blue line in Fig. 4(b), the height of a DNA-AuNP and the patch underlaid beneath the DNA-AuNP were 24 nm and 3.5 nm respectively. The height of the patch corresponds to the SLB shown in Fig. 3(b). These results strongly suggest that the DNA-AuNPs adsorbed preferentially onto cationic SLBs with high affinity than on bare mica (Fig. 4(d)). Thus, DNA molecules can be considered to work as anchors to immobilize DNA-AuNPs on SLB constructed by cationic lipids. However, despite adsorbing of many DNA-AuNPs, 2D array of them was hardly observed at this point.

After heating and slow cooling, the morphology changed dramatically and densely-packed single-layered 2D arrays of DNA-AuNPs were observed (Fig. 5(a)). From the cross-sectional profile corresponding to green line, the height of the array was about 28 nm (Fig. 5(b)). This value was almost the same as the total height of adsorbed DNA-AuNP including underlaid SLB. This means that these assembled arrays were monolayers of DNA-AuNPs. In addition, the DNA-AuNPs still adsorbed on the SLB even after annealing. If the DNA-AuNPs had dissociated from the SLB and diffused in the solution with heat, they would easily assembled into 3D arrays during cooling. We thoroughly analyzed the assembled arrays extensively by AFM, however, 3D DNA-AuNP cluster or multi-layered array was hardly observed. Moreover, within a few micro-scale capture it looks the number of DNA-AuNPs on the SLBs increased enough to cover the SLBs fully and make them invisible. This fact suggests the transportation of DNA-AuNPs from other areas: in other words, the micro-scale 2D diffusion of DNA-AuNPs beyond the sizes of SLB patches. Previous researches on the thermal expansion of SLB demonstrated a drastic increase of surface area of SLB in the vicinity of the gel-liquid crystalline transition temperature [32, 33]. Another research on the diffusion of DNA molecules on SLB demonstrated that the diffusivity of underlying lipid molecules strongly affected that of DNA molecules on the SLB, suggesting DNA molecules on SLB were transported by the underlying lipid molecules [23]. Moreover, the gel-liquid crystalline transition of DDAB has been reported to occur in the vicinity of 55 °C [34]. In our experiment, therefore, it is conceivable that DDAB SLB patches changed to liquid state, expanded and fused each other during annealing and slow cooling at the higher temperature above 55 °C, then DNA-AuNPs might diffuse rapidly

on larger, fused, liquid-state SLB and formed large 2D array. After formation of the 2D arrays, the large SLB might remain during cooling and hide under the large 2D array. From above results, we can also consider that DNA-AuNP binds to SLB due to the DNA-lipid affinity [24, 25], the binding between DNA-AuNP and SLB should be stronger than the interparticle connection between DNA-AuNPs over the range of annealing temperature, and then the DNA-AuNPs diffused laterally and assembled into 2D array. Thus our result suggests that the lipid diffusion was enhanced at high temperature and DNA-AuNPs were transported by high-mobility lipid molecules and then bind each other laterally through DNA hybridization during slow cooling.

4. Conclusion

2D DNA-AuNP arrays were assembled using cationic DDAB SLBs. We confirmed the DNA-AuNPs adsorbed selectively on the DDAB SLBs with strong affinity. After heat treatment and slow cooling, the DNA-AuNPs on the SLBs bound each other through DNA hybridization, and then formed densely-packed single-layered 2D arrays. Hardly-observed 3D DNA-AuNP cluster with thorough AFM analysis suggests that 2D laterally-confined diffusion and assembly of DNA-AuNPs. We demonstrated the possibility of 2D DNA-NP assembly without creating any complex mechanism on nanoparticles to get their own anisotropies.

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Figure Captions

Fig. 1. Schematic drawings of DNA-NP assembly on SLB. The lipid molecules, which attach to DNA-NPs, are indicated by green to distinguish from others. Firstly, adsorption of DNA-NPs onto the SLB (left); secondly, lateral transportation of DNA-NPs by using lipid molecules which attach to DNA-NPs (middle); and finally, connection of DNA-NPs through DNA hybridization and assembly of 2D superstructure (right)

Fig. 2. Schematic drawings of DNA-functionalized nanoparticles. (a) Schematic of DNA-functionalized nanoparticles. (b) A DNA linkage between nanoparticle A and B. Single interparticle linkage is shown for clarity, not to scale. Ten-base recognition sequences are indicated by blue and red lines for A and B respectively. bp: base pair. b: base. s: thiol termination of DNA.

Fig. 3. (a) An AFM image of SLBs on a mica substrate. (b) Cross-sectional line-profile along red line in image (a).

Fig. 4. AFM images of DNA-AuNPs on SLBs. (a) An AFM image for the scan area of $3 \times 3 \mu\text{m}^2$ and (b) $1 \times 1 \mu\text{m}^2$. (c) Cross-sectional line-profile along blue line in image (b). (d) Schematic drawing of DNA-AuNPs absorbed onto SLBs.

Fig. 5. AFM analysis of DNA-AuNP arrays on SLBs. (a) An AFM image of DNA-AuNP arrays on SLBs formed after slow cooling. (b) Cross-sectional line-profile along green line in image (a).

Figure 1

Figure 2

Figure 3

(a)

(b)

Figure 4

Figure 5