Nanostructures via DNA scaffold metallization

Nanostructures via DNA Scaffold Metallization

Ning Chen,[1] Anatoly Zinchenko,[1] Damien Baigl,[2] Olga Pyshkina,[3] Vladimir Sergeyev,[3] Kazunaka Endo,[4] Kenichi Yoshikawa[1]

[1]Physics Department, Graduate School of Science, Kyoto University, Kitashirakawa Oiwake-cho, Kyoto, 606-8502 Japan
[2] École Normale Supérieure, Département de Chimie, UMR CNRS 8640 24, rue Lhomond, F-75231 Paris Cedex 05, France
[3] Polymer Department, Moscow State University, Vorob'yovy Gory, Moscow 119899, Russia
[4] Chemistry Department, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

Abstract. The critical role of polymers in process of noble metals nanostructures formation is well known, however, the use of DNA chain template in this process is yet largely unknown. In this study we demonstrate different ways of silver deposition on DNA template and report the influence of silver nanostructures formation on DNA conformational state. Metallization of DNA chain proceeds by two different scenarios depending on DNA conformation. If DNA chain is unfolded (elongated) chain, silver reduction leads to the nucleation of silver nanoparticles and their growth on DNA scaffold. Silver nanoparticles assemble on negatively charged DNA template due to electrostatic interactions. During formation of silver nanoparticles, DNA chain, similarly to other polyelectrolytes, plays a role of stabilizing agent, and silver nanoparticles formed in DNA solutions are smaller and have narrower size distributions as compared to the particles formed in DNA-free solutions. Since positive change of thus formed silver nanoparticles is rather low, DNA chain remains in unfolded conformation no matter how high is a concentration of silver nanoparticles.

On the other hand, when DNA molecule has been compacted into tight condensate, naturally of a toroid shape, deposition of silver on compacted DNA chain proceeds in a different manner without discretion into nanoparticles. As a result of such silver metal deposition, DNA-templated silver nanorings are formed.

By comparison of UV-Vis spectra changes, the detection of transition point between unfolded and compact DNA conformations becomes possible. Metallization of unfolded DNA chain brings nanoparticles of about 30-50 nm size, while deposition of silver metal on a compact DNA condensate gives 100-150 nm metal rings that are distinguished by optical properties. The approach of different scenario of metallization can be used for detection of conformational changes in biopolymers.

1. INTRODUCTION

Nanostructures of noble metals with well-defined shape and size increasingly attract the attention of scientists in fields of catalysis, electronics, photonics, information storage, optoelectronics, biological labeling, etc. Further development and practical applications of nanostructures is expected to increase rapidly because of their interesting optical, electronic, and magnetic properties. In this context, an important knowledge in the direct preparation of metallic nanostructures of control size and shape has been developed for the past few years and various morphologies can now be prepared in a controlled way, such as nanoparticles (spheroids), nanocubes [1], nanoprisms [2], nanoplates [3] or nanobelts [3]. However, since these techniques are based on directed growth of particles in the reaction medium, they can only lead to shapes of simple topology, such as spheroids, ellipsoids, or polyhedrons. In contrast, nanoparticles with a toroid shape (nanoring) can not be produced by direct growth technique. Thus, the only way to produce such a morphology is to use a toroidal template of nanoscale dimensions. A beautiful method to prepare micro-scale silver and golden rings has been demonstrated independently by Xia and co-workers [4] and Yan and Goedel [5] which is based on the use of an array of nanospheres as a template for reduction of noble metal on surface of nanoparticles and further etching of nanoparticles. However, such rings have a size ranging from 0.5 to several microns and can not be dispersed directly in a water solution. On the other hand, specific interaction DNA and silver makes DNA an ideal template to build silver nanostructures. This has been used successfully to produce nanoparticles arrays on a DNA scaffold [6] or DNA-templated silver nanowires [7]. However, the ability of long DNA chains to form toroidal condensates [8] after folding transition of DNA chains (DNA condensation) has not been hitherto noticed by materials scientists. This ability of DNA to condense into well-defined toroids is a unique opportunity to use them as templates to create silver toroidal nanostructures (nanorings) of controlled shape and dimensions. In this paper, we describe a one-pot, two-step, simple preparation of well-defined silver nanorings dispersed in water, base on the use of DNA condensates as a nanostructure template.

2. EXPERIMENTAL SECTION

2.1 Materials.

Bacteriophage T4 DNA (166,000 base pairs, Nippon Gene Co., LTD, Japan); $AgNO_3$ (99.9999% purity, Aldrich, Japan), spermine (N,N'-bis(3-aminopropyl)-1,4-diaminobutane, Naclai Tesque, Japan), fluorescent dye YOYO-1 (1,1'-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis-4-[3-methyl-2,3-dihydro-(benzo-1,3-oxazole)-2-methylidene]-quinolinium tetraiodide) (Molecular Probes, USA), and

sodium borohydride, (Naclai Tesque, Kyoto, Japan) were as received.

2.2 Methods.

TEM observations were performed using a JEM-1200EX microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 100 kV. We used carbon-coated grids with a mesh size 300 at room temperature. Each grid was placed for 3 minutes on top of a 15 μL droplet of DNA solution on a Parafilm sheet and solution was blotted with filter paper before microscopic observation. Staining of sample by placing grid for 15 s on a 15 μL droplet of uranyl acetate (1% in water) was used for observations of compact toroidal DNA without silver shell or nanostructures having unfolded DNA chain.

Fluorescent microscopic observations were performed using an Axiovert 135 TV (Carl Zeiss, Germany) microscope equipped with a 100× oil-immersed lens and. Fluorescent images were recorded using EB-CCD camera and image processor Argus 10 (Hamamatsu Photonics, Hamamatsu, Japan).

UV-Vis spectra were recorded on a Jasco U-550 UV/VIS spectrophotometer in 1.0×0.2×0.5 cm quartz cells

3. RESULTS AND DISCUSSION

3.1. Preparation of silver nanoring.

A remarkable property of DNA molecules to be condensed by positively charged compounds into toroidal condensates has been discovered 30 years ago. [8] In vitro, multivalent cations induce DNA negative charge neutralization and successive DNA chain self-assembling into tightly packed toroidal condensates. The appearance of such toroidal morphology is assigned to the native rigidity of DNA double strand.

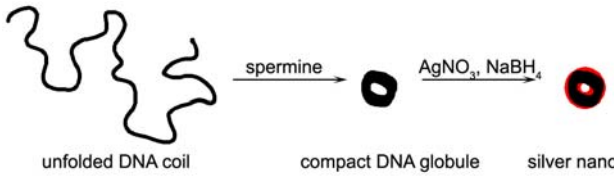

Figure 1. Schematic representation of the silver nanorings synthesis: Unfolded DNA chain, DNA toroidal condensate formed after the addition of a condensing agent (in this study, spermine, noted SPM^{4+}, a tetravalent polycation) and DNA-templated silver nanorings (silver shell is shown as red) formed after Ag^+ reduction by $NaBH_4$.

Figure 1 shows the process of preparing DNA-templated silver nanorings. First, unfolded DNA molecules (T4 DNA, 166,000 base pairs, contour length 57 μm, 1 μM in nucleotides in distilled water) were compacted by addition of tetravalent cation spermine SPM^{4+} (N,N'-Bis(3-aminopropyl)-1,4-diaminobutane, 25-50 μM) to get uniform toroids with average inner diameter of 50 nm and outer one of 90 nm. Transmission electron microscope (TEM) image of T4 DNA toroid is shown on Figure 2A. Prepared in such a way DNA toroids were used as templates for further preparation of silver nanorings. Silver nitrate solution was added to the solution of DNA toroids and then quickly reduced by adding the common reducing agent sodium borohydride ($NaBH_4$). It is well-known that an unfolded DNA chain acts as a source of nucleation centers for Ag^0 nanostructure formation due to binding of Ag^+ to DNA nucleotides. Once the DNA chain has been folded, the resulting toroidal condensate is usually described as having a neutral, very dense, crystal-like core, however, the surface of DNA toroid has a residual negative charge [9]. Therefore, we assume that the surface of the DNA toroid acts as a source of nucleation centers, as depicted in Figure 1. After reduction of Ag^+ to Ag^0 by $NaBH_4$, DNA toroids were covered by silver metal shell to give final silver nanorings (DNA@silver nanorings, Figure 1). Transmission electron micrograph of silver DNA@silver rings templated by a T4 DNA toroid is shown in Figure 2B.

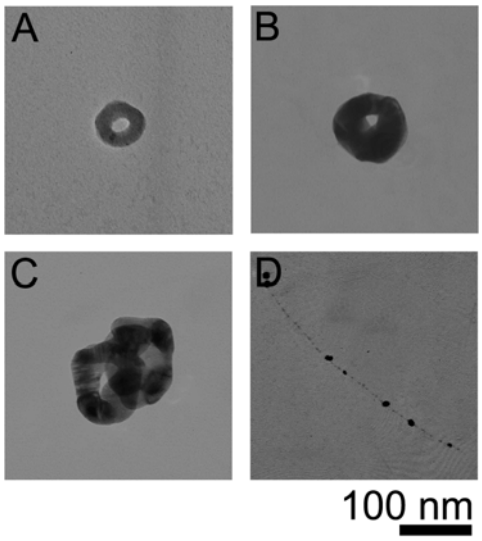

Figure 2. Transmission electron microscopy (TEM) images of various T4 DNA/silver nanostructures. A. Single-chain DNA toroidal condensate obtained by compaction of T4 DNA (1 μM nucleotides) with spermine (20 μM) in pure water. B. DNA-templated silver nanorings obtained after the successive addition into the DNA/spermine solution (image A) of $AgNO_3$ (100 μM) and $NaBH_4$ (100 μM). C. Typical multi-ring nanostructure obtained under the conditions of B at high DNA concentration (10 μM). D. Fragment of unfolded DNA chain complexed with silver nanoparticles observed under the conditions of B except a spermine concentration of 100 μM.

3.2 Optimal conditions for silver deposition on DNA condensate.

As a general rule, synthesis of uniform nanostructures requires well-determined conditions (concentration of reagents, order and time of mixing, etc.) In our four-component system, precisely adjusted conditions are

also important to achieve satisfactory shape of silver nanorings. These conditions can be summarized as follows. First, the concentration of DNA should be not higher than 1 μM unless one is aimed to synthesize multi-ring nanostructures, because DNA toroids tend to aggregate on the stage of compaction for larger DNA concentration. Figure 2C shows such multi-ring structure containing two toroids. Second, the deficit or excess of condensing agent (here, spermine) should be avoided because DNA condensates exist only in a precise range of condensing agent concentrations. In our experimental conditions, spermine concentration [SPM^{4+}] should be between 5 and 50 μM. For [SPM^{4+}] < 5 μM, DNA neutralization by SPM^{4+} binding is not enough (< 90%) and all DNA chains are in the elongated coil state. For [SPM^{4+}] > 50 μM, DNA chains condensed for lower [SPM^{4+}] unfold into the elongated coil state due to the so-called reentrant transition. In these conditions (deficit or excess of spermine), the addition of silver nitrate and further reduction by $NaBH_4$ leads to the formation of silver nanoparticles on the unfolded DNA chain as shown in Figure 2D. Third, the molar ratio between DNA, $AgNO_3$ and $NaBH_4$ mainly controls the shape and the characteristic sizes of nanorings.

In our experiments, a 100-fold molar excess of $AgNO_3$ to DNA nucleotides (100 μM and from equivalent to 5-fold excess of $NaBH_4$ to $AgNO_3$ were the optimal conditions to insure a satisfactory toroidal shape of silver nanorings. For higher $AgNO_3$ concentration, the resulted nanorings are very thick or even contain no hole. On the other hand, if $NaBH_4$ to $AgNO_3$ ratio is significantly below equivalent, the growth of individual silver nanoparticles on DNA toroid is induced rather than homogeneous growth of the silver shell. This leads to the formation of irregular rings (flower-like shape). When the experimental conditions have been optimized as described above ([T4 DNA] 1 μM [spermine] 20 μM, [$AgNO_3$] and [$NaBH_4$] both 100 μM), the silver nanorings have a well-defined toroidal shape (Figures 2B), with an average outer diameter of 93±7 nm and an inner diameter of 22±4 nm. The difference between average parameters of initial DNA toroids and final nanorings suggests the formation of a silver shell with a thickness of about 5 nm.

3.3 Protection of DNA by silver ring.

Silver shell of DNA@silver nanorings can be used for DNA encapsulation to protect it from modification of the DNA environment (pH, chemical composition, biochemical environment, enzymes) or to block the DNA activity (chemical or biological).

For instance, the protection from a chemical modification of the DNA environment was confirmed by the fluorescent microscopy (FM) observations of single-chain DNA (1 μM) labeled by the fluorescent dye DAPI (1 μM) using a classical procedure described elsewhere. In the absence of condensing agents, all DNA chains were unfolded and were observed as an elongated coil with large intra-chain fluctuations and a very slow diffusion motion (Figure 3A).

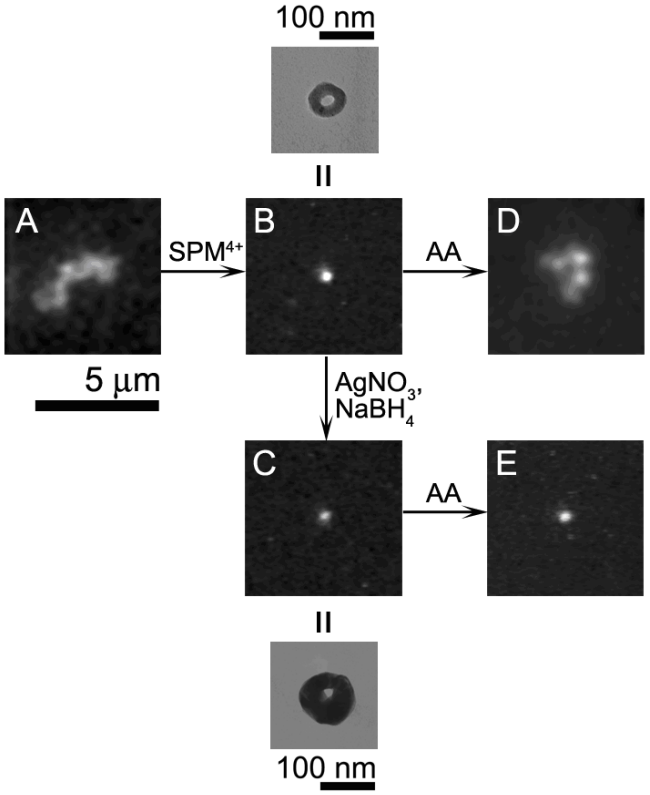

Figure 3. Fluorescence microscopy (FM) images of single molecules of T4 DNA (1 μM) labeled with fluorescent dye YOYO (0.2 μM) for various aqueous environments. A. Elongated coil conformation in pure water. B. Compact DNA (globule) in the presence of 20 μM of spermine. C. Compact DNA covered by silver (silver nanoring) observed after addition of $AgNO_3$ (100 μM) and $NaBH_4$ (100 μM) into solution of image B. D. After addition of acetic anhydride (AA, 100 μM) into solution of image B, all DNA molecules unfold into a coil state. E. After addition of AA (100 μM) into solution of image C, all DNA molecules are still in a compact state. Top and bottom transmission microscope images are given for illustration of nanostructures corresponding to globules of DNA observed by FM.

When spermine was added (concentration), FM shows that all DNA chains fold abruptly into very fast-diffusing globules that correspond to the DNA toroidal condensates (Figure 3B). After further addition of $AgNO_3$ and reduction by $NaBH_4$, the observation of DNA condensates (i.e., DNA @silver nanorings) was still possible. (Figure 3C) It is important to note that the silver nanorings diffuse freely in the solution almost as fast as the original DNA toroidal condensates which indicate that (i) they have an average size of 100 nm and (ii) they are well dispersed in the water solution. Then, as a model of chemical modification of DNA environment, we added acetyl anhydride (AA, 100 μM) to the DNA medium before and after the silver treatment. Addition of AA induces a partial acetylation of free spermine in the solution and, thereby, displaces the

equilibrium towards the unfolded form of DNA. When AA was added before silver treatment, FM observations showed that all DNA condensates unfold into the initial elongated coil state (Figure 3D). However, the addition of AA after silver modification has essentially no effect on the behavior of DNA condensates that still diffuse fast and freely in the solution. TEM observations on the same solution confirmed the integrity (in shape and size) of the silver-coated DNA toroids. These observations show that, in principle, the encapsulation of DNA into silver capsule protects compacted DNA form chemical modifications of the environment.

The potential scope of nanostructures that can be obtained by DNA condensate-templated method is rather broad. If DNA molecules are longer than 400 base pairs, DNA condensed by a majority of multications adopts toroidal conformation which can be used as templates for silver (or other suitable noble metal) nanorings. Basically, the diameter of nanorings can be varied from 30 nm up to about 200 nm [10, 11]. Furthermore, under specific conditions DNA can adopt several different morphologies: rods, rackets, multi-toroidal condensates, and toroids-on-a string structures which can also serve as seeds to get corresponding nanostructures.

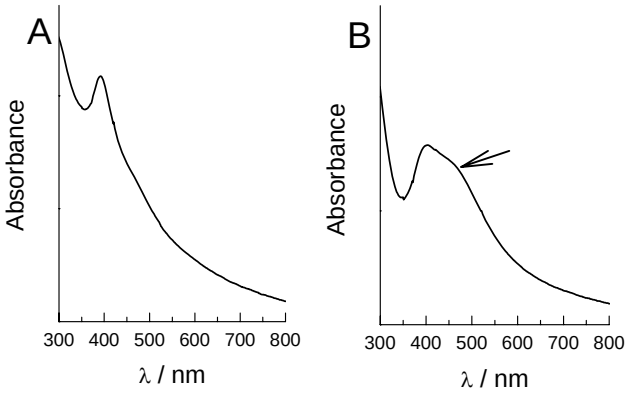

Figure 4. UV-Vis spectra of the control solution without DNA (A) and the silver nanorings solution (B). The arrow indicates the characteristic long wavelength absorption shoulder (470 nm) inherent to the silver ring morphology.

Optical properties of thus prepared silver nanorings (Figure 4B) in solution differ from those of corresponding solutions of silver nanostructures prepared without DNA toroidal seeds (Figure 4A). UV-Vis absorption spectra of nanorings are characterized by a broad absorption with a maximum at 400-500 nm. Two closely located overlapping bands can be distinguished and assigned to the typical absorption for silver nanoparticles (400 nm) and absorption resulting from the toroidal shape of silver rings (about 470 nm), respectively (Figure 4B). The second long wavelength band, specific for the silver rings, was not observed in the absence of DNA toroidal seeds. Spectra of similar shapes were reported for nanorods made of noble metals with low aspect ratios.

In conclusion, the unique ability of DNA molecule to be organized into toroidal condensates of well-defined shape and size upon interaction with multications provides one with the opportunity to create monodisperse silver rings of nanometer size. Adaptation of presented DNA-Ag nanorings concept together with already known principles in synthesis of nanostructures (directed growth, specific adsorption, templated assembling) is awaited to gain new, yet impossible to prepare, sophisticated nanostructures of noble metals. This method might be also used to protect DNA from chemical or biological modifications of DNA environment or to block DNA activity by encapsulation into DNA@silver nanorings.

4. ACKNOWLEDGEMENTS

Authors thank Prof. T. Kanbe ((Nagoya University, Japan)) for help with electron microscopy observations and discussions. This work was supported in part by the fellowship No. P04154 from Japan International Science and Technology Exchange Center (JISTEC), the fellowship No. P03200 from Japanese Society for Promotion of Science (JSPS), and the Grant-in-Aid for the 21st Century COE "Center for Diversity and Universality in Physics" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

5. REFERENCES

[1] Y. Sun, Y. Xia, Science 2002, 298, 2176.
[2] C. Xue, Z. Li, C. A. Mirkin, Small 2005, 1, 513.
[3] B. Wiley, Y. Sun, B. Mayers, Y. Xia, Chem. Eur. J. 2005, 11, 454.
[4] J. M. McLellan, M. Geissler, Y. Xia, J. Am. Chem. Soc. 2004, 126, 10830.
[5] F. Yan, W. A. Goedel, Nano Lett. 2004, 4, 1193.
[6] H. Nakao, H. Shiigi, Y. Yamamoto, S. Tokonami, T. Nagaoka, S. Sugiyama, T. Ohtani, Nano Lett. 2003, 3, 1391.
[7] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 1998, 391, 775.
[8] L. C. Gosule, J. A. Schellman, Nature 1976, 259, 333.
[9] Y. Yamasaki, Y. Teramoto, K. Yoshikawa, Biophys. J. 2001, 80, 2823.
[10] C. C. Conwell, I. D. Vilfan, N. V. Hud, Proc. Natl. Acad. Sci. USA 2003, 100, 9296.
[11] Y. Yoshikawa, K. Yoshikawa, T. Kanbe, Langmuir 1999, 15, 4085.