Template Synthesis

Sol–Gel Reaction Using DNA as a Template: An Attempt Toward Transcription of DNA into Inorganic Materials

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The creation of nanosized inorganic superstructures is a challenging research target not only from a purely scientific viewpoint, in an attempt to make artificial fossils, but also from its great potential for application, such as for catalysts, nanowires, circuits, molecular containers, and complicated patterning, which are regarded to be indispensable tools for nanotechnology.[1] However, an essential difference in the research approaches exists between "organic" and "inorganic" materials: that is, a diversity of supramolecular structures can be created by the self-assembly of designed "organic" building blocks, whereas it seems very difficult or nearly impossible to create supramolecular "inorganic" materials in a diverse and regulated fashion because of their inability to self-assemble. Accordingly, the sole solution to overcome this difficulty in designing supramolecular "inorganic" materials is to use an appropriate "organic" template with diverse and well-regulated shapes onto which "inorganic" materials can be deposited by utilizing some specific interaction.

Recently, we and other research groups have demonstrated that organogel fibers, which are formed from the self-assembly of low-molecular-weight compounds, act as excellent templates for the creation of silica fibers by sol–gel polycondensation of tetraethoxysilane (TEOS).[2] Very interestingly, it was shown that sol–gel polycondensation of gelated TEOS solutions affords novel silica materials with lamellar,[2a,b] linear fiber,[2c,d] or helical[2e–h] structures as a result of their supramolecular structures acting as a template containing a hollow center during the TEOS polycondensation process. One may now propose, therefore, that the unique superstructures constructed in the organogels are readily transcribed into the silica structures: this means that superstructures of organic assemblies that are temporarily formed by noncovalent interactions could be permanently fixed as inorganic materials.

Biomolecules exhibit various unique higher-order structures, so they should act as fascinating templates to create such inorganic superstructures. In fact, it has been demonstrated by several research groups that protein cages,[3] biolipid cylinders,[4] multicellular superstructures,[5] and collagen fibers[6] can be utilized as templates for the direct deposition of silica or other metal clusters. Among these biomolecules, DNA is one of the most attractive templates because its double-stranded structure has a well-regulated micrometer length and uniform 2-nm diameter which cannot be prepared by artificial polymers or low-molecular-weight gels. Furthermore, one can find several topologically different higher-order DNA structures in the native DNA system. For example, plasmid DNA, produced by *Escherichica coli*, adopts a coiled-coil structure with a well-regulated length and width under appropriate biological conditions, and the morphology can be readily changed to the closed circular structure by chemical or enzymatic treatment. It thus occurred to us that if plasmid DNA could be used as a template for sol–gel polycondensation, it would give unique silica structures reflecting the higher-order structures of DNA. Herein we report several novel findings related to this research aim: 1) plasmid DNA can be solublilized into organic solvents by appropriate modification with cationic surfactants, 2) when the overall charge of the DNA–surfactant complex is positive, it can act as an active template for the sol–gel reaction, and 3) the topologically different structures in plasmid DNA can be successfully transcribed into resultant silica structures. These findings imply that the different higher-order silica structures could be created from the same DNA template through its higher-order conformational change.

To use DNA as a template we had to overcome two problems which arise from the mismatching properties between DNA and TEOS. Firstly, it is known that when sol–gel polycondensation is carried out in neutral or alkaline media, the propagating silica species are considered to be anionic. Hence, only a molecular assembly having a cationic surface can act as the template to adsorb the anionic silica particles. Since DNA itself is an anionic polymer, one must transform the "anionic" to a "cationic" species. Secondly, TEOS is soluble in organic solvents, whereas DNA itself is soluble virtually only in water, hence some appropriate solvent system which can dissolve both components had to be found. One potential solution to overcome these problems is to dissolve DNA in an organic solvent by replacing the sodium counterions with cationic amphiphiles.[7] One might expect that the resultant DNA–amphiphile complex would gain not only moderate solubility in organic solvents but also the ability to adsorb the anionic silica particles. Previously, we mixed DNA with an excess amount of *n*-octyl-1,8-diammonium dibromide (**1**) with the expectation that one ammonium cation would form an ion pair with one phosphate anion and the resultant DNA–**1** complex would become overall positively charged because of the presence of another ammonium cation. The analysis of the obtained precipitate revealed, however, that the two ammonium groups form ion pairs with the two phosphate groups in an intramolecular manner and the resultant precipitate is apparently neutral (Figure 1a). This failure brought forth an alternative idea, that is, provided that intermolecular ion-pair formation proceeds in preference to an intramolecular one, it follows that the second cationic group should remain without forming an ion pair with the

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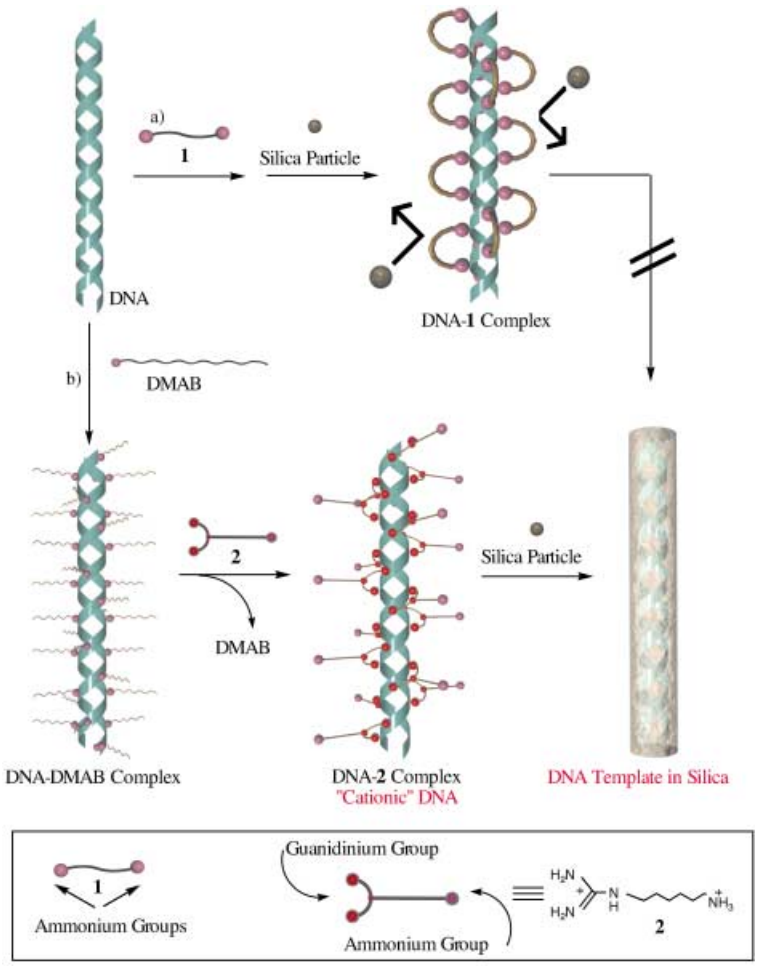

Figure 1. Schematic representation of the transformation of the DNA surface. See text for details.

phosphate group. We thus designed **2** bearing one ammonium group and one guanidinium group with the expectation that intermolecular formation of an ion pair with the guanidinium group would proceed in preference to an intramolecular one with the ammonium group (Figure 1b). By utilizing this strategy we have found that DNA can act as a new "cationic" template for sol–gel transcription and the resultant silica materials transcribe the higher-order structures of DNA very precisely.

Firstly, we prepared the plasmid DNA–amphiphile complex by mixing DNA and cationic didodecyldimethylammonium bromide (DMAB) according to the reported method.[7] The plasmid DNA aqueous solution (45.0 μL, 0.67 μg μL^{-1}) containing 10 mM Tris-HCl (Tris = tris(hydroxymethyl)aminomethane) and 1.0 mM ethylenediamine tetraacetate (EDTA) was added to the DMAB solution (100 mM, 15.0 μL) and the mixture was stirred at room temperature. The resultant white precipitate was collected by centrifugation (9000 rpm, 50 min) and washed with distilled water several times. Freeze-dry treatment of this sample afforded a white powder. The product (DNA–DMAB complex) was easily solubilized into a mixture of chloroform and methanol (3:1, v/v). The CD and UV/Vis spectra of the thus obtained DNA–DMAB complex are shown in Figure 2a, b, respectively (the final concentration was adjusted to 1.0 mM/base). The CD spectrum shows a positive Cotton effect at 280 nm and a negative Cotton effect at 260 nm. A peak at around 260–300 nm is evident in the UV/Vis spectrum which is attributed to DNA bases. These spectral features observed in organic solution are similar to those observed in aqueous solution.[8] The spectral similarities indicate that the prepared plasmid DNA–DMAB complex maintains the morphology similar to that in aqueous solution. For the subsequent experiments we employed a 1.0 mM chloroform:methanol (3:1, v/v) solution as a stock solution.

Secondly, we carried out sol–gel polycondensation using the DNA–DMAB complex as a template. Water (6.3 μL), benzylamine (5.0 μL), and TEOS (10.0 μL) were added sequentially to the DNA–DMAB solution (500 μL). After mixing, the resultant solution was left at room temperature for three days. Although this is the procedure frequently employed to create superstructures of silica materials using an organogel as a template, we could not observe any specific silica structure by SEM or TEM. This result shows that the DNA–DMAB complex, expected to be neutral overall, cannot act as a template. Taking this observation into consideration, we added **2**, which has the ammonium group at one end and the guanidinium group at the other end. It is well-known that the guanidinium group is complementary to the phosphate group and the interaction is much stronger than that with the ammonium group. One can

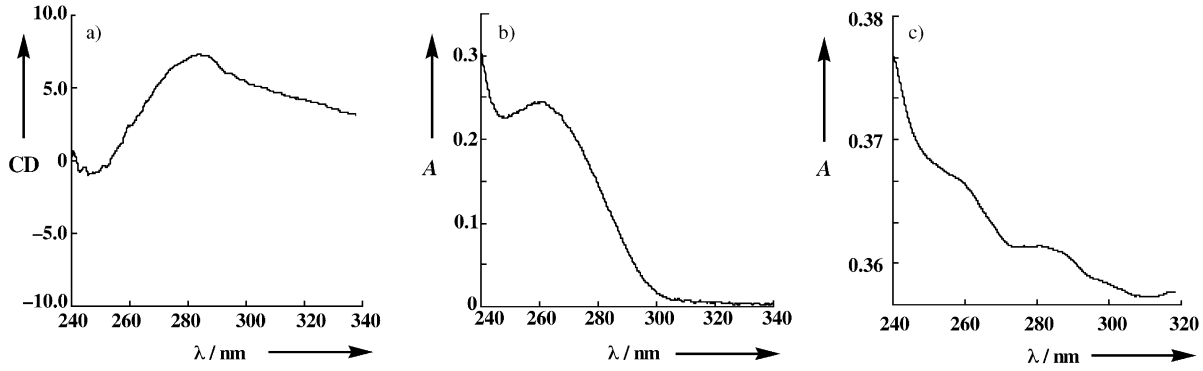

Figure 2. a) CD spectrum and b) UV/Vis spectrum of the DNA–DMAB complex ($CHCl_3$:MeOH (3:1, v/v)), 1.0 mM/base, 5.0 mM, 25 °C; c) UV/Vis spectrum of the obtained silica.

thus expect that: 1) when **2** is added to the DNA–DMAB complex solution, the DMAB is exchanged with **2** because of the stronger guanidinium–phosphate interaction, 2) the resultant DNA–**2** complex becomes "cationic" overall (Figure 1b), and 3) the oligomeric silica particles should be adsorbed to the DNA–**2** complex, because of the silica–ammonium electrostatic interaction. To test this intriguing hypothesis, we carried out in situ cation exchange by mixing the DNA–DMAB complex (500 μL, chloroform:methanol (3:1 v/v)) with **2** (5 μL, 100 mM, one equivalent/base) in methanol. This cation exchange proceeded without the formation of any precipitate, unless a very high DNA concentration was used with an excess amount of **2**. Then, we carried out sol–gel polycondensation using the resultant DNA–**2** complex as a template according to the general procedure. The obtained silica was analyzed after washing it with distilled water and methanol. Figure 2c shows the UV/Vis spectrum of the resultant silica composite. The spectrum is somewhat broadened, but the basic spectral features of the DNA–**2** solution (Figure 2b) are evident, thus indicating that DNA is incorporated into the silica.

To obtain further evidence that the DNA really acted as a template to create this silica structure, we analyzed the composite by SEM and TEM. Figure 3 shows the SEM images

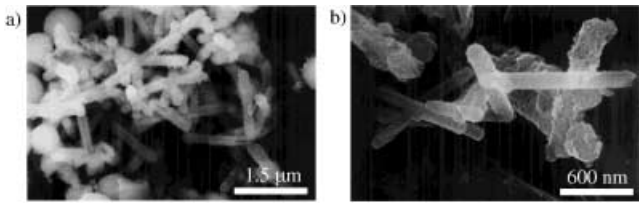

Figure 3. SEM pictures of the rodlike silica obtained.

of the obtained silica composite: the unique, rodlike silica structures of uniform length (ca. 1 μm) can be observed. It is well-known that plasmid DNA adopts a coiled-coil (super-coil) structure under the appropriate pH conditions or salt concentrations. In a separate study with TEM analysis, we confirmed that the DNA–**2** complex mostly exists in the coiled-coil structure in solution and the length is estimated to be about 800 nm (not shown). This structural similarity between the DNA–**2** complex and the observed silica composite supports the view that the DNA–**2** complex acted as a template during the polycondensation reaction of TEOS. In addition, many small spherical silica particles around the rodlike silica can also be recognized (Figure 3a). Formation of these spherical silica particles is explained as follows: DMAB, which is dissociated from the first DNA–DMAB complex by exchange with **2**, forms the spherical aggregates (micelles), which act as templates for formation of these spherical silica particles. As a reference experiment, we carried out sol–gel polycondensation in the presence of only DMAB as well as with a mixture of DMAB and **1** under the same reaction conditions. As expected, formation of similar spherical silica particles was recognized by both SEM and TEM.

To observe the inner structure of the silica composite we used TEM, which usually gives higher resolution and more useful information than SEM. Figure 4 shows the TEM images of the obtained silica composite, in which uniform rodlike silica structures (Figure 4a) can be recognized. The

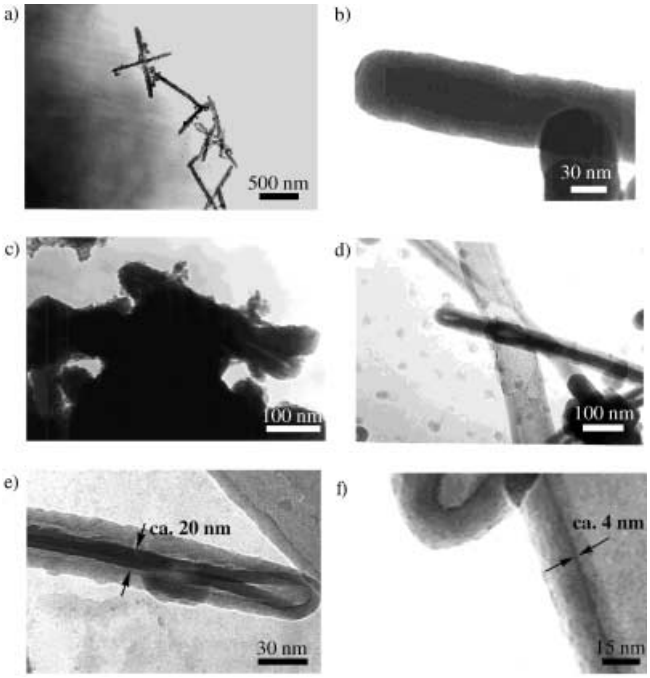

Figure 4. TEM pictures of a) the rodlike silica, b) close-up image of the inner hollow of the rodlike silica, c) rodlike silica after calcination, d) silica structures obtained from the partial coiled-coil template, e) close-up image of the core silica obtained from the partial coiled-coil template, and f) close-up image of the silica obtained from the DNA dulplex template.

presence of a hollow with a diameter of approximately 10 nm inside the silica rods is confirmed (Figure 4b). The diameter is very close to that of the plasmid DNA–**2** complex in solution (ca. 7 nm). The hollow structure of the silica rods can be recognized even after calcination (Figure 4c). These findings unambiguously support the view that the plasmid DNA–**2** (super-coil structure) complex works as an efficient template for the formation of these rodlike silica composites (Figure 5a).

Conformational relaxation of the coiled-coil structure in plasmid DNA may be induced by extraction into organic solution with a surfactant, thus giving rise to a morphological change into a partial coiled-coil DNA. One may expect, therefore, that if these transformed DNA morphologies were also useful as templates, several new silica structures could be created. Furthermore, it is expected that the silica particles are predominantly adsorbed onto the surface of the partial coiled-coil DNA–**2** complex, because such relaxation exposes the anionic charges of the DNA on the surface and exchange with **2** can occur more preferably. If this is the case, one can obtain the TEM pictures in which the template–silica interface is more deeply stained by silica particles (Figure 5b). Actually, we observed several silica rods in which the

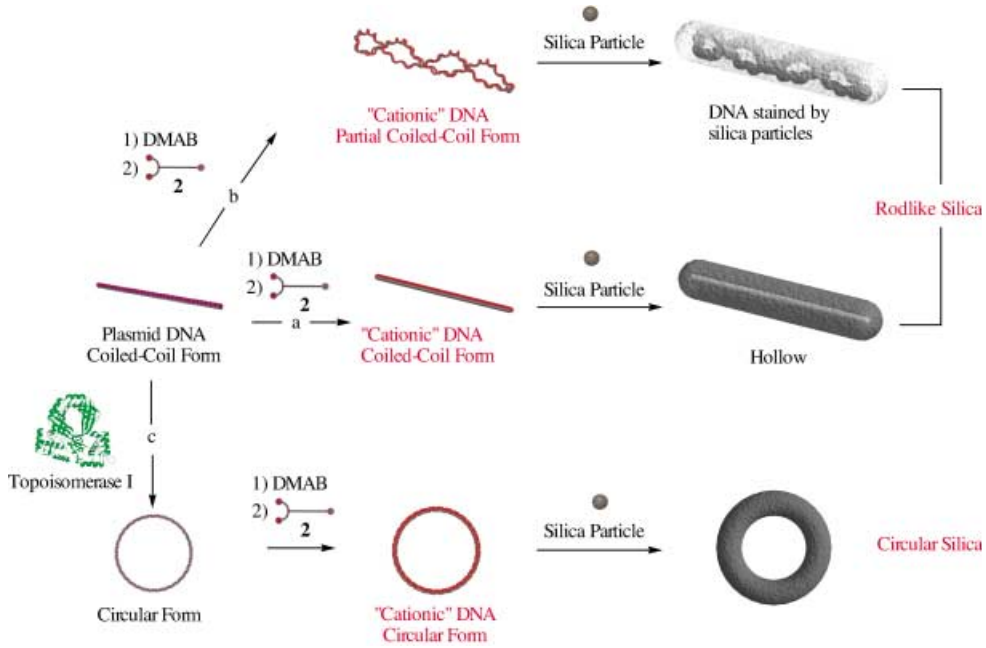

Figure 5. Formation of the different ordered silica structures created from the same template through conformational changes.

morphological pattern of partial coiled-coil DNA is shadowed more darkly than the outer surface (Figures 4d,e and 5b). It should be noticed that these silica rods commonly include hairpin or internal loop DNA templates. Very interestingly, even the structure created from the template effect of one double-stranded DNA can be found (Figure 4f): the diameter of the smallest silica-stained fiber is approximately 4 nm, which can be regarded as the template effect arising from one DNA double strand (2 nm diameter).

It is known that relaxation of the coiled-coil structure finally results in the circular form. This fact further stimulated us to utilize the circular DNA structure as a template for sol–gel polycondensation, with the expectation that the circular-shaped silica would be obtained (Figure 5c). Topoisomerase I is an enzyme that is able to induce such relaxation of the coiled-coil structure.[9] The relaxation process by topoisomerase I is known to proceed almost quantitatively under mild conditions. Accordingly, we first treated plasmid DNA with topoisomerase I for 1 h at 37 °C under the conventional reaction conditions.[9] The topological change from the coiled-coil structure to the circular one was confirmed by the gel mobility shift assay (see the Supporting Information). Then, the sol–gel polycondensation reaction was carried out using the resultant circular plasmid DNA after extraction into the organic phase with DMAB followed by cation exchange with **2** according to the same procedure as above. Figure 6 shows SEM and TEM images of the obtained silica composite. Many toroidal-shaped silica structures with diameters of 500–800 nm (center to center) can be observed (Figure 6a): these values are very reasonable, provided that plasmid DNA adopts the circular form. The central cavity can be also recognized in the close-up picture (Figure 6b). Figure 6c and d show TEM images of the same silica composite before and after calcination, respectively. The similar toroidal silica structure can also be recognized in these TEM images. The

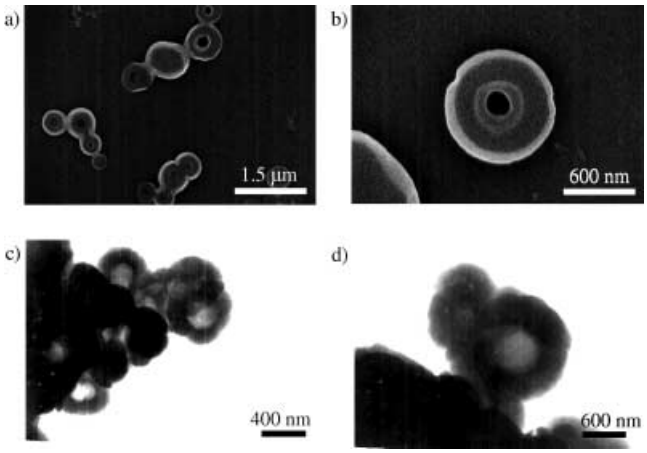

Figure 6. a) SEM picture of the circular silica, b) close-up SEM image of the circular silica, c) TEM picture of the circular silica, and d) TEM picture of the circular silica after calcination.

results clearly support the view that circular plasmid DNA acted as the template for creating the toroidal silica structure.

In conclusion, we have demonstrated that plasmid DNA can act as an attractive, new template for sol–gel polycondensation of TEOS after treatment by two cation-exchange steps. The obtained silica structures are drastically changed and reflect the conformationally transformed template structures. To the best of our knowledge, this is the first study showing that DNA can work as a template for sol–gel polycondensation. We believe that this is the first step to immobilize the memory of DNA on an inorganic material, as in the formation of fossils. We are now planning to apply the present concept to more complicated but sophisticated templating systems using the rationally designed natural DNA and artificial DNA structures.

Experimental Section

2: *N*-(6-Aminohexyl)carbonic acid *tert*-butyl ester (0.30 g, 1.72 mmol) and triethylamine (240 μL, 1.72 mmol) were dissolved in MeOH (30 mL). *O*-methylisourea hydrogensulfate (0.36 g) was added to this mixture and the resultant solution was stirred at room temperature. The MeOH was removed under reduced pressure after 12 h. The reaction mixture was subjected to column chromatography using $CHCl_3$:MeOH (3:1, v/v) as eluant. After removing the solvent, a colorless oil was obtained (170 mg). The product obtained (100 mg, 0.3 mmol) was then dissolved in dried CH_2Cl_2. Trifluoroacetic acid (TFA, 50 μL, 2.5 equiv) was added to this solution and the mixture was stirred at room temperature. After 6 h, the solvent and exces TFA were removed under reduced pressure to give **2** as a colorless oil. TEM and SEM observations were carried out on a JEOL TEM-2010 (accelerate voltage 120 kV) and Hitachi S-5000, respectively. CD and UV/Vis spectroscopic studies were performed on a JASCO J-720WI and SHIMADZU UV-2500PC, respectively.

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