Higher-order Conformations of DNA Are Useful as Templates to Create Various Superstructural Poly(pyrrole) Morphologies

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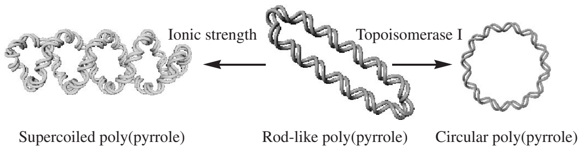
It was found that plasmid DNA can act as an attractive template for oxidative polymerization of pyrrole and result in the novel higher-order superstructures composed of the conductive polymers. The TEM and SEM observation estabilished that poly(pyrrole) grows up to nanosized rod-like, circular, and supercoiled structures reflecting the higher-order conformation of plasmid DNA. The obtained polymer structures are drastically changed reflecting the controlled DNA morphologies used as their templates.

Recently, we and others have explored a new method to transcribe a variety of organic superstructures into inorganic materials by a sol-gel reaction of metal alkoxides ("sol-gel transcription"), by which one can control the morphology of inorganic compounds and create various new superstructural inorganic materials.[1–4] The primary driving force operating in this sol-gel transcription is considered to be an electrostatic interaction between "anionic" silica nanoparticles and "cationic" organic assemblies acting as templates. This implies that "anionic" superstructures would be also useful as templates and could be transcribed into some "cationic" polymer-forming material. Thus, it occurred to us that the morphology of poly(pyrrole) would be controllable, applying this template method to its chemical and electrochemical polymerization process:[5] as oxidative polymerization of pyrrole produces "cationic" intermediates, the "anionic" assemblies should act as an appropriate template due to the mutual electrostatic attractive force. Here, we noticed that a polymeric template which is not yet applied to this system but would be the most suitable and attractive candidate for the template is a "polynucleotide".[6] Among these biological molecules, DNAs exhibit various unique higher-order structures, so that they should act as fascinating templates to create novel poly(pyrrole)-based superstructures.[7] We thus employed plasmid DNA as a template and carried out oxidative polymerization of pyrrole by chemical and electrochemical methods. It is shown in Scheme 1 that we can find the complicated, higher-order DNA structures in the native DNA system.[8] This is the first example that these higher-order conformational varieties are successfully transcribed into conductive polymers by a templating method.

The chemical oxidation of pyrrole (0.06 mol·dm^{-3}) in the absence and the presence of ColE1 plasmid DNA (11 μg·mL^{-1}) was carried out with ammonium peroxodisulfate (APS, 0.01 mol·dm^{-3}) for 20 min at 25 °C. It is well-known that plasmid DNA adopts the supercoiled structure in the appropriate salt concentration. To observe the inner and outer structures of poly(pyrrole), TEM and SEM were used. Figure 1 shows the electron micrograph images of the obtained poly(pyrrole). As can be seen from Figures 1a and 1b, one can recognize the rod-like structures in these TEM images. Furthermore, it is clearly recognized that the hollow with ca. 10 nm diameter exists inside the rod-like polymers. The diameter and the length (ca. 50–300 nm) of the hollow are well consistent with those of plasmid DNA in solution. It is undoubted that dark parts reflect poly(pyrrole) and bright parts reflect plasmid DNA because these TEM pictures were taken without any staining treatment. These findings unambiguously confirm the view that the plasmid DNA works as a template for formation of the rod-like polymer structures. As a result of measuring XPS, poly(pyrrole)·plasmid DNA composite is formed from 3 pyrrole units per one phosphate group of plasmid DNA.



Scheme 1. Higher-order conformations of DNA.

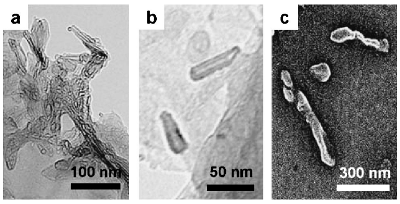


Figure 1. TEM images (a and b) and SEM image (c) of poly(pyrrole)·plasmid DNA composite yielded by chemical oxidation with APS.

It is known that relaxation of the supercoiled structure finally gives the circular form. This fact further stimulated us to utilize the circular DNA structure as a template for oxidative polymerization, which is expected to give the circular-shaped poly(pyrrole) polymer. Topoisomerase I is an enzyme that can relax the supercoiled structure. The relaxation process by Topoisomerase I is known to proceed almost quantitatively under the physiological mild conditions. Accordingly, we first incubated plasmid DNA with Topoisomerase I for 1 h at 37 °C under appropriate reaction conditions. Then, the chemical oxidation was carried out with APS. Figure 2 shows TEM and SEM images of poly(pyrrole)·plasmid DNA composite thus obtained. One can observe many circular structures with ca. 30–100 nm diameters in these TEM images (Figures 2a and 2b). As evidenced from Figure 2, this poly(pyrrole) has a circular structure, indicating that the circular plasmid DNA acts as a template. Furthermore, these images indicate that a single plasmid molecule of 6700 base pairs (ca. 700 nm) condenses into a single toroid of ca. 100 nm diameter, when measured from center-to-center. It

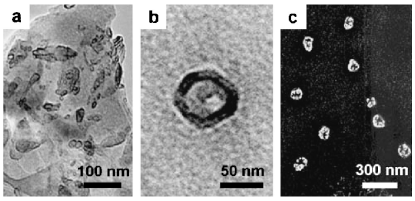


Figure 2. TEM images (a and b) and SEM image (c) of poly(pyrrole)·plasmid DNA composite yielded by chemical oxidation after relaxation with Topoisomerase I.

is known that the higher-order DNA condensation requires the presence of cations,[9] and APS and poly(pyrrole) play the role in the present system.

An SEM image of the poly(pyrrole)·plasmid DNA composite prepared by chemical oxidation with APS under the ionic conditions (LiCl addition) resulted in a supercoiled structure, indicating that the supercoiled plasmid DNA produced by the influence of 0.05 mol·dm^{-3} LiCl acted as a template (Figure 3a). One may expect that the similar fabrication would be possible by electrochemical polymerization of pyrrole. Thus, electrochemical polymerization of pyrrole was carried out in a CV cell in a voltage range of 0–0.8 V (vs. Ag/AgCl) with a scan rate of 50 mV·s^{-1} at 25 °C (scan cycle: 30 cycles) under the same conditions (LiCl, 0.05 mol·dm^{-3}). The typical SEM images of the plasmid DNA-deposited ITO electrode surface are also shown to be a supercoiled structure in Figure 3b. In Figures 3a and 3b, one can recognize that the size obtained from chemical oxidation is somewhat larger than that obtained from electrochemical oxidation. This is because chemical oxidation proceeds continuously, whereas electrochemical oxidation proceeds only under the oxidative treatment. It is undoubted, therefore, that both chemical and electrochemical methods afford basically the same composite morphology. The difference between Figure 1 (prepared without LiCl) and Figure 3 (with LiCl) further supports the view that the higher-order superstructures of plasmid DNA act as templates.

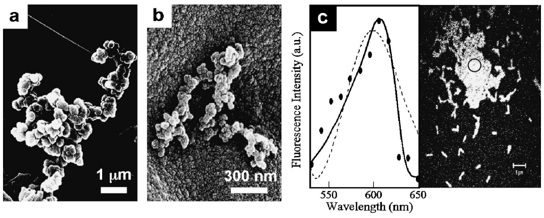


Figure 3. SEM images of poly(pyrrole)·plasmid DNA composite in the presence of LiCl yielded by chemical (a) and electrochemical oxidation (b); CLSM image of the poly(pyrrole)·plasmid DNA composite film binding EB and its fluorescence spectrum obtained by exciting the circle at 470 nm (—); only EB solution (- - -) (c).

To obtain further evidence that these supercoiled structures consist of a composite of poly(pyrrole) and plasmid DNA, we carried out two additional experiments. Firstly, the modified ITO electrode was immersed in an aqueous solution containing ethidium bromide (EB, 0.5 mg·mL^{-1}), a typical DNA intercalator for 64 h at 25 °C. Then, the electrode was rinsed for 30 min with water. A new UV–vis absorption maximum appeared at 502 nm, which is ascribable to EB. Secondly, this EB-stained electrode was submitted to confocal laser scanning microscope (CLSM: Figure 3c). When the sample was photoirradiated by UV light in the dark, we could observe red color emission, a typical fluorescence color of EB. Furthermore, the large aggregates consisting of fabricated supercoils observed by SEM were again confirmed by the images of CLSM. Such spectral changes were not observed at all for the poly(pyrrole)-modified ITO electrode prepared under the same conditions in the absence of plasmid DNA and even though it was immersed in a DNA-containing aqueous solution. The results clearly establish that the poly(pyrrole) surface has no specific affinity with DNA and cannot bind DNA or preserve it from the water rinse. One can propose, therefore, that the DNA is deposited as a poly(pyrrole) composite through the oxidative polymerization process and responsible for the binding of EB to this modified film.

In conclusion, the present study has demonstrated that as the plasmid DNA is useful as a template, one can not only immobilize the higher-order DNA structures in the poly(pyrrole) matrix but also create novel superstructures from poly(pyrrole). One may regard, therefore, that this is a novel and general transcription process of anionic templates to oxidizable monomers through oxidative polymerization. So far, it has been believed that in oxidative polymerization of pyrrole, the easiness in the preparation method is a merit whereas the difficulty in the morphological control is a serious demerit. The convenience of the fabrication method and the easiness of the morphology control as attained in the present system seem to promise the broad applications of these conductive polymers as functional materials. We now believe that this is the first step to immobilize the memory of DNA on the conductive polymer.

References and Notes

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