Single Molecular Morphology of Porphyrin/DNA Complex

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A Porphyrin/DNA complex stabilized by both ionic bonds and stacking of π conjugates is reported herein. Morphology observation on the single molecular level revealed that the complex exhibits rigid behavior and inhomogeneity with phase separation. These characteristics are the indication of the presence of stacking interaction between porphyrins along the DNA scaffold.

One-dimensional molecular complexes are attracting much interest for the design of nanostructured materials with electronic and magnetic functions.[1] Low-dimensional structures are usually available as the structural motifs in crystals, and accordingly have been a subject of solid-state physics and chemistry. Recently, the physics and chemistry of single molecules have emerged as a new field of material science. A method to isolate molecular complexes on the single molecular level and maintain the molecular structure on a solid surface would provide a new family of functional molecular wires. The porphyrin nanostructure has been investigated extensively because of the attractive photoelectric properties originating from its stacked π-electron system, which extends to the molecular plane.[2] In order to create a one-dimensional porphyrin architecture, ionic assembly of porphyrins with a DNA scaffold is advantageous to high-density integration. This is because one phosphate group per base pair can bond with one functional cation.[3] Herein, we describe the single molecular morphology of porphyrin/DNA complex nanowires consisting of a π-stacked porphyrin assembly deposited on a solid surface. Atomic force microscopy (AFM) observations of the porphyrin/DNA complex reveal the characteristic structure of π–π stacking interactions between porphyrin molecules.

The porphyrin/DNA complex was constructed using, 5,10,15,20-tetrakis[4-trimethylammoniophenyl]porphyrin-tetratoluene-4-sulfonate (TMAP, purchased from STERM) (Figure 1a),[4,5] and poly(dA)poly(dT) (50-mer, purchased from Amersharm Pharmacia Biotech). TMAP and DNA were dissolved in doubly distilled water at concentrations of 2×10^{-4} mol/L (M) and 20 units, respectively, and maintained for one day after stirring. A concentration of one unit is equal to 50 μg/mL of solution of DNA, which can be converted to 1.6×10^{-4} mol/L (M) for the number of bases (phosphate groups). Various ratios of the solution were mixed by stirring in order to examine the different compositions of the TMAP/DNA complex. The mixed solutions were then diluted 100 times with water, and 10 drops of the solution were placed onto freshly cleaved mica in order to deposit the complex. After 2 min of incubation, the mica surface was dried under nitrogen flow. The complex formation in the solution was confirmed by spectroscopic data obtained using a UV–vis absorption spectrometer (U-3300, Hitachi) and a CD spectrometer (J-720, Jasco). The morphology of the TMAP/DNA complexes was observed by AFM (Model Nanoscope IIIa Dimension 3000, Digital Instruments) operated in tapping mode with a silicon single crystal probe under ambient pressure.

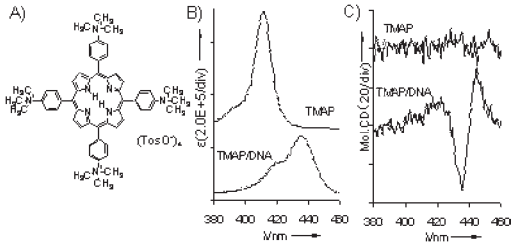

Figure 1. A) Molecular structure of the free-base porphyrin TMAP, B) UV–vis absorption, and C) CD spectra for the Soret band of TMAP alone (without DNA) and the TMAP/DNA complex in aqueous solution.

Figure 1B shows the absorption spectra of TMAP and the TMAP/DNA complex in the Soret band region. Aqueous TMAP solution without DNA shows only one peak at 411 nm. Upon addition of DNA to the TMAP solution, a red-shifted peak appeared at 435 nm, indicating the formation of the TMAP/DNA complex. Furthermore, the peak attributed to TMAP at 411 nm disappeared upon addition of DNA. The peak at 435 nm shows maximum intensity at concentrations of 2×10^{-6} M of TMAP and 0.2 units of DNA. These concentrations are corresponding to the number ratio of 1/8 for TMAP molecules to phosphate groups in DNA. Considering the theory developed by Kasha,[6] the red shift of TMAP in the Soret band indicates the presence of a large dipole coupling interaction between π-stacked[7] TMAP bound to the DNA scaffold.

Figure 1C shows the circular dichroism (CD) spectra corresponding to the wavelength region of the visible absorption spectra in Figure 1B. The CD spectrum indicates the presence of a chiral arrangement of TMAP in the TMAP/DNA complex. This peak for TMAP is dextrorotatory, which is in accord with the chirality of the DNA double helix. These findings suggest that the porphyrin molecules aggregate along the DNA scaffold in a chiral arrangement. The titrimetric study of the complexing TMAP and DNA and possible structure models of TMAP/DNA complexes were reported elsewhere.[8]

In an effort to examine whether the TMAP/DNA complexes can be isolated on a solid surface at the single molecular level without decomposition of the complex, the morphology of the complex on a mica surface was compared with that of each TMAP and DNA deposited by itself on the surface. Figure 2A shows that TMAP deposited on the surface gave 0.5–1.5 nm aggregates. Figure 2B indicates that a single molecule (not bundled) of DNA adsorbed to the surface is 0.25 nm in height. This value is similar to that reported for poly(dA)poly(dT) adsorbed to mica substrates.[9] However, the morphology and height of the TMAP/DNA complexes indicate quite different features from those of the poly(dA)poly(dT), as shown in Figure 2C.

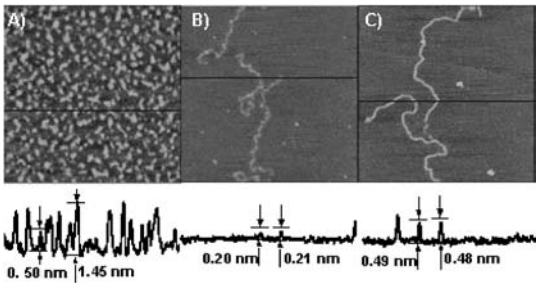

Figure 2. AFM images of A) TMAP coagulate deposited from the solution of TMAP alone (2×10^{-6} M), B) DNA molecules deposited from the solution of DNA alone (0.2 units), and C) TMAP/DNA complex molecules deposited from the mixed solution TMAP and DNA (2×10^{-6} M and 0.2 units, respectively). Images were taken in tapping mode in air, and the scan area was $500 \times 500\,nm^2$.

Although the concentration of TMAP in the TMAP/DNA-mixed solution was the same as that of the sample in Figure 2A (TMAP alone), TMAP aggregates were not observed on the surfaces where TMAP/DNA complexes were deposited, as shown in Figure 2C. This result suggests that almost all TMAP molecules were completely incorporated into DNA molecules. This consideration is further supported by the fact that the height of the TMAP/DNA complex (0.4–0.6 nm) was about two times higher than that of DNA (0.2–0.3 nm). Furthermore, the image of a DNA molecule shows random-coil behavior, as shown in Figure 2B, whereas that of the TMAP/DNA complex indicates a rigid-rod feature. This rigidity is attributed to the stacking interaction between TMAPs, because TMAPs are bond to the phosphates scaffold of DNA and surround the DNA strand.

The morphology of the TMAP/DNA complexes is significantly different from that of DNA, not only on the single molecular level, but also from the viewpoint of its cohesion behavior. In order to investigate the cohesion behavior, we examined the effect of DNA concentration on the morphology of DNA alone and of the TMAP/DNA complex. Figure 3A shows the AFM images of DNA alone deposited on a mica substrate from a solution with a concentration of 1.6 units, a concentration eight times higher than that of the solution used for the sample shown in Figure 2B. Under these conditions, DNA molecules do not exist as single molecules, but only as aggregates consisting of inhomogeneous knots and bundles with various heights. This finding indicates that the affinity between DNA molecules is stronger than tendency of DNA to stick the surface.

However, the presence of small amount of TMAP changes the morphology drastically, as shown in Figure 3B. The well-dispersed random-coil and rigid-rod molecules were determined to have heights of 0.2–0.4 and 0.4–0.8 nm, respectively. In comparison with the height analysis in Figure 2, these two classes of heights correspond to DNA alone and the TMAP/DNA complex, respectively.

The coexistence of unreacted DNA with the TMAP/DNA complex cannot be understood simply by the low concentration of TMAP. The TMAP concentration is only 2×10^{-6} M (the same as that of the sample in Figure 2C), which is equivalent to 1/64 of the number of phosphate groups on the DNA. However, the TMAP/DNA complex formed by homogeneous assembly is indistinguishable from unreacted DNA by AFM observation. On the contrary, TMAP-bound and -unbound DNA can be easily distinguished as shown in Figure 3B. Furthermore, TMAP-bound region can be clearly seen even in a single molecules of DNA (Figure 3c). The black arrows indicate the boundary between the unreacted DNA and the DNA that has formed a complex. Such phase-separation behavior in a single DNA molecule suggests that TMAP/DNA complex formation is driven by the attractive interactions between TMAPs, namely π stack. The direct observation of a morphology at the single molecular level, as well as this phase-separation behavior, are a new stage of the investigation of low-dimensional complex chemistry.

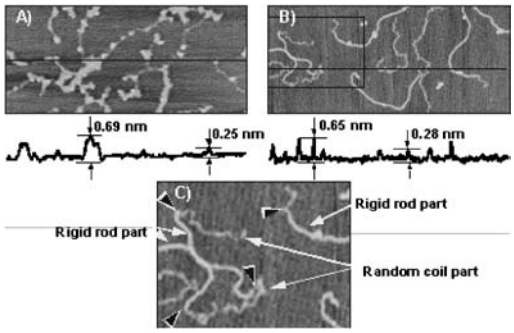

Figure 3. AFM images of A) DNA aggregates deposited from the DNA (concentration, 1.6 units) and B) TMAP/DNA complex molecules deposited from the mixed solution of TMAP and DNA (concentration of 2×10^{-6} M and 1.6 units, respectively). The scan areas of both A) and B) were $1\,\mu m \times 440$ nm. C) Magnified image of B) represents the rigid and random-coil regions. The images were taken in tapping mode in air.

We thank Dr. Tetsuo Osa of Tohoku University for helpful discussions and advices.

References

1 a) O. Kahn, C. J. Martinez, *Science* **1998**, *279*, 44. b) O. Kahn, *Acc. Chem. Res.* **2000**, *33*, 647. c) H. Okamoto, M. Yamada, *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2023.
2 A. P. H. J. Schenning, F. B. G. Benneker, H. P. M. Geurts, X. Y. Liu, R. J. M. Nolte, *J. Am. Chem. Soc.* **1996**, *118*, 8549.
3 H. Nakayama, H. Ohno, Y. Okahata, *Chem. Commun.* **2001**, 2300.
4 a) J. E. Mcclure, L. Baudouin, *Biopolymers* **1997**, *42*, 203. b) M. C. Carvlin, R. J. Fiel, *Nucleic Acids Res.* **1983**, *11*, 6121.
5 a) L. Young, K. Jin, C. Tae, S. Rita, K. K. Seog, *J. Am. Chem. Soc.* **2003**, *125*, 8106. b) L. M. Scolaro, A. Romeo, R. F. Pasternack, *J. Am. Chem. Soc.* **2004**, *126*, 7178.
6 M. Kasha, H. R. Rawls, M. A. El-Bayoumi, *Pure Appl. Chem.* **1965**, *11*, 371.
7 a) E. E. Jelly, *Nature* **1936**, *138*, 1009. b) G. Scheibe, *Angew. Chem.* **1936**, *49*, 563.
8 To appear in *Tetrahedron Lett.* December issue, **2005**.
9 H. G. Hansma, I. Revenco, K. Kim, D. E. Laney, *Nucleic Acids Res.* **1996**, *24*, 713.

Published on the web (Advance View) December 10, 2005; DOI 10.1246/cl.2006.88