J|A|C|S
ARTICLES
Published on Web 03/13/2007

Instantaneous Inclusion of a Polynucleotide and Hydrophobic Guest Molecules into a Helical Core of Cationic β-1,3-Glucan Polysaccharide

Masato Ikeda,[†] Teruaki Hasegawa,[†,||] Munenori Numata,[†] Kouta Sugikawa,[†] Kazuo Sakurai,[‡] Michiya Fujiki,[§] and Seiji Shinkai*,[†]

Contribution from the Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Motooka 744, Nishi-ku, Fukuoka 819-0395, Japan, Department of Chemical Processes and Environments, Faculty of Environmental Engineering, The University of Kitakyushu, Hibikino 1-1, Wakamatsu-ku, Kitakyushu, Fukuoka 808-0315, Japan, and Graduate School of Materials Science, Nara Institute of Science and Technology, Takayama 8916-5, Ikoma, Nara 630-0101, Japan

Received November 24, 2006; Revised Manuscript Received January 18, 2007; E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp

Abstract: We succeeded in the quantitative and selective introduction of an ammonium cationic group into the C6 position of Curdlan (CUR) by "Click Chemistry", and the obtained cationic Curdlan (CUR-N^+) showed good solubility in water. ORD studies suggested that CUR-N^+ adopts a single-stranded structure, different from a right-handed, triple-stranded helical structure of β-1,3-glucan polysaccharides in water. It has been revealed that the polymeric complexes of CUR-N^+ with polymeric guest molecules, such as polycytidylic acid (poly(C)), permethyldecasilane (PMDS), and single-walled carbon nanotubes (SWNTs), can be easily obtained by just mixing them in water with sonication. The characterization of the resultant CUR-N^+−poly(C) complexes by UV−vis, CD spectroscopic measurements, and AFM and TEM observations revealed that they have stoichiometric, nanosized fibrous structures. From these experimental results as well as our precedent studies (e.g., refs 6 and 23), we propose that the complexation would be driven by the cooperative action of (1) the hydrogen-bonding interaction between the OH group at the C2 position and hydrogen-bonding sites of the cytosine ring (ref 6d), (2) the electrostatic interaction between the ammonium cation and the phosphate anion (ref 23), as well as (3) the background hydrophobic interaction. In addition, the complexed polynucleotide chain showed a strong resistance against enzymatic hydrolysis. Likewise, the dispersion of PMDS and SWNTs in water by CUR-N^+ and the fibrous structures of the complexes were confirmed by spectroscopic measurements as well as microscopic observations. These binding properties of CUR-N^+, which can proceed spontaneously in water, clearly differ from those of schizophyllan (SPG), which inevitably require a denature−renature process corresponding to a conversion of a triple strand to single strands induced by DMSO or base for inclusion of polymeric guest molecules.

Introduction

Helical polymers have attracted significant interest in recent years because of their potential applications to chiral sensing and separation.[1] Native Curdlan (CUR), consisting of a β-1,3-glucan main chain as shown in Figure 1, forms a right-handed, triple-stranded helical structure, but is insoluble in water.[2] Hence, several attempts to improve the solubility have been reported to date.[2,3] One possible attempt was to lower the molecular weight by hydrolytic cleavage of the glycoside linkage.[4] The second attempt is regioselective modification of CUR, but the successful examples are still rare. The OH group at the C2

[†] Kyushu University.
[‡] The University of Kitakyushu.
[§] Nara Institute of Science and Technology.
[||] Present address: Department of Life Sciences, Faculty of Life Sciences, Toyo University, Izumino 1-1-1, Itakuramachi, Oora-gun, Gunma 374-0193, Japan.

(1) (a) Green, M. M.; Peterson, N. C.; Sato, T.; Teramoto, A.; Cook, R.; Lifson, S. *Science* **1995**, *268*, 1860−1866. (b) Okamoto, Y.; Yashima, E. *Angew. Chem., Int. Ed.* **1998**, *37*, 1020−1043. (c) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574. (d) Nakano, T.; Okamoto, Y. *Chem. Rev.* **2001**, *101*, 4013−4038. (e) Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M.; Sommerdijk, N. A. J. M. *Chem. Rev.* **2001**, *101*, 4039−4070. (f) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011. (g) Yashima, E.; Maeda, K.; Nishimura, T. *Chem.-Eur. J.* **2004**, *10*, 42−51. For recent examples, see: (h) Onouchi, H.; Miyagawa, T.; Morino, K.; Yashima, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 2381−2384. (i) Kim, O.-K.; Je, J.; Jernigan, G.; Buckley, L.; Whitten, D. *J. Am. Chem. Soc.* **2006**, *128*, 510−516.

(2) Lee, I.-Y. In *Biotechnology of Biopolymers −From Synthesis to Patents*; Steinbüchel, A., Doi, Y., Eds.; Wiley−VCH: Weinheim, 2005; Chapter 16, pp 457−480.

(3) (a) Yoshida, T.; Yasuda, Y.; Mimura, T.; Kaneko, Y.; Nakashima, H.; Yamamoto, N.; Uryu, T. *Carbohydr. Res.* **1995**, *276*, 425−436. (b) Gao, Y.; Fukuda, A.; Katsuraya, K.; Kaneko, Y.; Miura, T.; Nakashima, H.; Uryu, T. *Macromolecules* **1997**, *30*, 3224−3228. (c) Borjihan, G.; Zhong, G.; Baigude, H.; Nakashima, H.; Uryu, T. *Polym. Adv. Technol.* **2003**, *14*, 326−329. (d) Koumoto, K.; Umeda, M.; Numata, M.; Matsumoto, T.; Sakurai, K.; Kunitake, T.; Shinkai, S. *Carbohydr. Res.* **2004**, *339*, 161−167. (e) Jin, Y.; Zhang, H.; Yin, Y.; Nishinari, K. *Carbohydr. Res.* **2006**, *341*, 90−99.

(4) (a) Koumoto, K.; Kimura, T.; Kobayashi, H.; Sakurai, K.; Shinkai S. *Chem. Lett.* **2001**, 908−909. (b) Koumoto, K.; Sakurai, K.; Shinkai, S.; Kunitake, T. *Polym. Prep. (Am. Chem. Soc., Div. Polym. Chem.)* **2002**, *43*, 721−722.

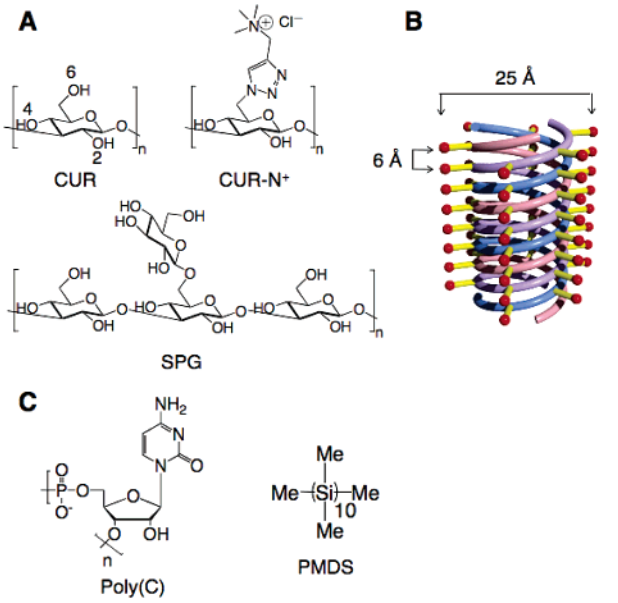
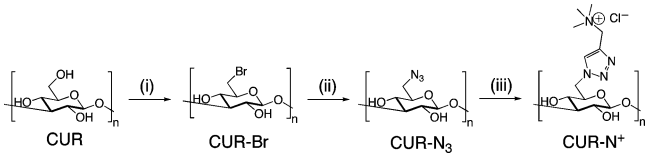


Figure 1. (A) Repeating unit of Curdlan (CUR), cationic Curdlan (CUR-N^+), and schizophyllan (SPG); (B) schematic illustration of a triple-stranded helical structure of CUR derivative synthesized by "Click Chemistry" (red balls denote side-chains introduced into C6 position); and (C) chemical structure of poly(C) and PMDS.

position of β-1,3-glucan, such as CUR and SPG, plays an important role to stabilize the intriguing triple-stranded helical structure, whereas the OH group at the C6 position oriented toward outside the helix.[5] Furthermore, it has been proposed that the complex formation between β-1,3-glucan polysaccharides and polynucleotide is driven by the hydrogen-bonding interaction between the OH group at the C2 position and the nucleic acid base.[6] Therefore, it is crucial to introduce a desired functional group into Curdlan selectively to the C6 position without inferring the OH group at the C2 position to maintain the unique binding properties of β-1,3-glucan toward not only polynucleotides[6,7] but hydrophobic guest molecules, such as single-walled carbon nanotubes (SWNTs),[8] polysilanes,[9] polymers,[10] functional molecules,[11] and Au nanoparticles.[12] Recently, we explored a new methodology to introduce a variety of

Scheme 1. Synthesis of CUR-N^{+a}



[a] (i) PPh_3, LiCl, DMF, room temperature, 3 h, then CBr_4, 60 °C, 24 h; (ii) NaN_3, DMSO, 80 °C, 36 h; (iii) 3-trimethylammonium-1-propyne chloride, $CuBr_2$, ascorbic acid, propylamine, 90% aqueous DMSO, room temperature, 24 h.

functional groups into the C6 position of Curdlan quantitatively and selectively by "Click Chemistry"[13,14] (Scheme 1). According to this method, we herein introduce ammonium groups into the C6 position of Curdlan to make Curdlan soluble in water and investigate molecular recognition properties of the obtained water-soluble CUR-N^+ toward various guest molecules.

Schizophyllan (SPG), a natural polysaccharide produced by fungus *Schizophyllum commune*, consists of a β-1,3-glucan main chain bearing a β-1,6-glucoside side-chain at every third glucose unit as shown in Figure 1A. A so-called denature−renature process, which is the structural transition from a random-coil state to a triple-stranded helix state triggered by the condition such as solvents, pH, or temperature,[15] is a key process for complex formation between SPG and guest molecules (Figure 2C). Because the triple-stranded helical structure of SPG in water shows sufficient stability, SPG in the form of triple-stranded helix cannot include guest molecules.

The electrostatic repulsion between the cationic groups of CUR-N^+ should provide a strong influence on its conformation in water, because the cationic groups of CUR-N^+ are located at the distance of approximately 6 Å from each other in the triple-stranded helical structure (Figure 1B). One can presume, therefore, that the triple-stranded helical structure of CUR-N^+ is significantly destabilized to be transformed to a loosely tied triple-stranded structure or a single-stranded structure in water. If this is the case, CUR-N^+ will show binding properties toward guest molecules different from those of SPG. In fact, optical

(5) (a) Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: Amsterdam, 1998. (b) Sathyanarayana, B. K.; Rao, V. S. R. *Biopolymers* **1971**, *10*, 1605−1615. (c) Deslandes, Y.; Marchessault, R. H.; Sarko, A. *Macromolecules* **1980**, *13*, 1466−1471.
(6) (a) Sakurai, K.; Shinkai, S. *J. Am. Chem. Soc.* **2000**, *122*, 4520−4521. (b) Sakurai, K.; Mizu, M.; Shinkai, S. *Biomacromolecules* **2001**, *2*, 641−650. (c) Bae, A.-H.; Lee, S.-W.; Ikeda, M.; Sano, M.; Shinkai, S.; Sakurai, K. *Carbohydr. Res.* **2004**, *339*, 251−258. We have recently shown the result of MOPAC calculation on CUR−poly(C) complex in 2:1 stoichiometry, which is based on the right-handed, triple-stranded helical structure of β-1,3-polysaccharide,[5b] evidenced by TEM and AFM observations,[6c] and analysis of X-ray fiber diffraction pattern as well as spectroscopic measurement[6a,b] of the complex. We therein propose that two types of hydrogen bond are formed between the OH group at the C2 position and hydrogen-bonding sites of the cytosine ring. (d) Miyoshi, K.; Uezu, K.; Sakurai, K.; Shinkai, S. *Biomacromolecules* **2005**, *6*, 1540−1546.
(7) For a review, see: Sakurai, K.; Uezu, K.; Numata, M.; Hasegawa, T.; Li, C.; Kaneko, K.; Shinkai, S. *Chem. Commun.* **2005**, 4383−4398.
(8) (a) Numata, M.; Asai, M.; Kaneko, K.; Hasegawa, T.; Fujita, N.; Kitada, Y.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2004**, *33*, 232−233. (b) Hasegawa, T.; Fujisawa, T.; Numata, M.; Umeda, M.; Matsumoto, T.; Kimura, T.; Okumura, S.; Sakurai, K.; Shinkai, S. *Chem. Commun.* **2004**, 2150−2151. (c) Numata, M.; Asai, M.; Kaneko, K.; Bae, A.-H.; Hasegawa, T.; Sakurai, K.; Shinkai, S. *J. Am. Chem. Soc.* **2005**, *127*, 5875−5884.
(9) Haraguchi, S.; Hasegawa, T.; Numata, M.; Fujiki, M.; Uezu, K.; Sakurai, K.; Shinkai, S. *Org. Lett.* **2005**, *7*, 5605−5608.
(10) (a) Numata, M.; Hasegawa, T.; Fujisawa, T.; Sakurai, K.; Shinkai, S. *Org. Lett.* **2004**, *6*, 4447−4450. (b) Li, C.; Numata, M.; Bae, A.-H.; Sakurai, K.; Shinkai, S. *J. Am. Chem. Soc.* **2005**, *127*, 4548−4549.
(11) (a) Hasegawa, T.; Haraguchi, S.; Numata, M.; Fujisawa, T.; Li, C.; Kaneko, K.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2005**, *34*, 40−41. (b) Hasegawa, T.; Fujisawa, T.; Numata, M.; Li, C.; Bae, A.-H.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2005**, *34*, 1118−1119. (c) Hasegawa, T.; Haraguchi, S.; Numata, M.; Li, C.; Bae, A.-H.; Fujisawa, T.; Kaneko, K.; Sakurai, K.; Shinkai, S. *Org. Bioorg. Chem.* **2005**, *3*, 4321−4328. (d) Numata, M.; Li, C.; Bae, A.-H.; Kaneko, K.; Sakurai, K.; Shinkai, S. *Chem. Commun.* **2005**, 4655−4657. (e) Numata, M.; Tamasue, S.; Fujisawa, T.; Haraguchi, S.; Hasegawa, T.; Bae, A.-H.; Li, C.; Sakurai, K.; Shinkai, S. *Org. Lett.* **2006**, *8*, 5533−5536.
(12) Bae, A.-H.; Numata, M.; Hasegawa, T.; Li, C.; Kaneko, K.; Sakurai, K.; Shinkai, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 2030−2033.
(13) (a) Hasegawa, T.; Umeda, M.; Numata, M.; Li, C.; Bae, A.-H.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2006**, *35*, 82−83. (b) Hasegawa, T.; Umeda, M.; Numata, M.; Li, C.; Bae, A.-H.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Carbohydr. Res.* **2006**, *341*, 35−40.
(14) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599. Selected examples on the application of "Click Chemistry" to polymers, see: (b) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 15020−15021. (c) Malkoch, M.; Thibault, R. J.; Drockenmuller, E.; Messerschmidt, M.; Voit, B.; Russell, T. P.; Hawker, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 14942−14949. (d) Parrish, B.; Breitenkamp, R. B.; Emrick, T. *J. Am. Chem. Soc.* **2005**, *127*, 7404−7410. (e) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823−4830.
(15) (a) Norisuye, T.; Yanaki, T.; Fujita, H. *J. Polym. Sci., Polym. Phys. Ed.* **1980**, *18*, 547−558. (b) Yanaki, T.; Norisuye, T.; Fujita, H. *Macromolecules* **1980**, *13*, 1462−1466. (c) Bluhm, T. L.; Deslandes, Y.; Marchessault, R. H.; Pérez, S.; Pinaudo, M. *Carbohydr. Res.* **1982**, *100*, 117−130. (d) Kitamura, S.; Hirano, T.; Takeo, K.; Fukuda, H.; Takahashi, K.; Falch, B. H.; Stokke, B. T. *Biopolymers* **1996**, *39*, 407−416. (e) McIntire, T. M.; Brant, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 6909−6919.

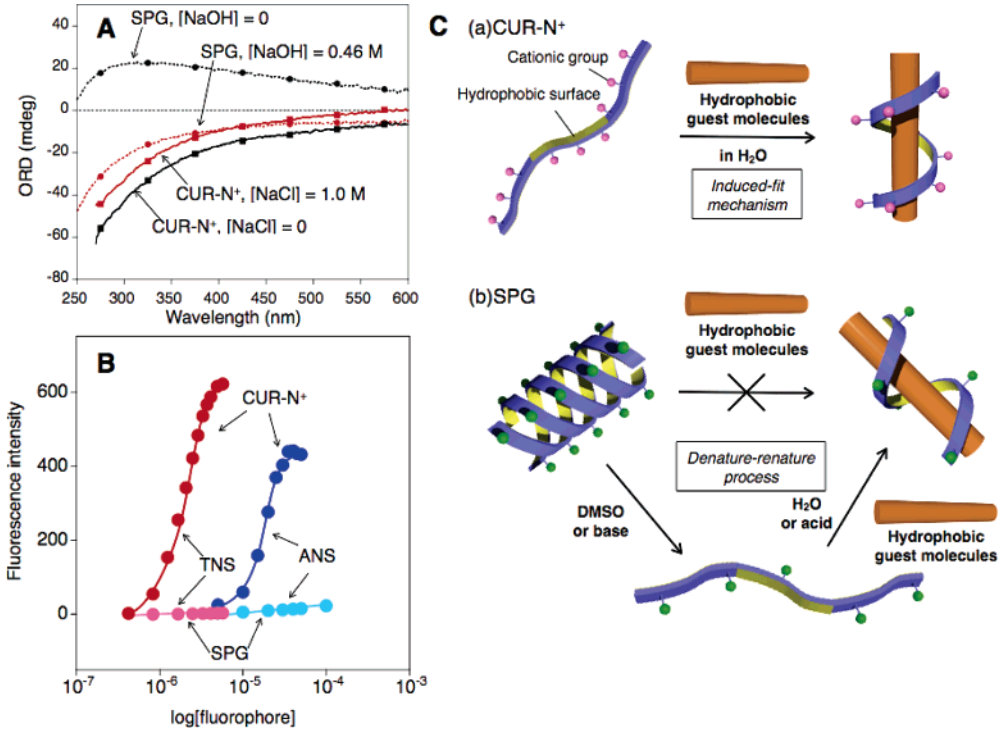


Figure 2. (A) ORD curves of CUR-N^+ and SPG in aqueous solution (5 mg/mL) at various conditions at 25 °C with a 1-cm cell; (B) plots of fluorescence intensities at 480 nm for ANS (λ_{ex} = 370 nm) and at 450 nm for TNS (λ_{ex} = 370 nm) versus log fluorophore concentration in the presence of CUR-N^+ (10 μM (monomer unit)) or SPG (10 μM (monomer unit)) in aqueous solution at ambient temperature (entire spectra are shown in Figures S5 and S6, Supporting Information); and (C) schematic illustration of the composites formation between β-1,3-glucans ((a) CUR-N^+ and (b) SPG) and a hydrophobic guest molecule.

rotatory dispersion (ORD) spectra indicate that the structure of CUR-N^+ is definitely different from that of SPG in water. Furthermore, the fluorescence probe experiments indicate that CUR-N^+ has an accessible hydrophobic region. We have then evaluated the binding properties of CUR-N^+ toward guest molecules, such as poly(C), polysilane, and SWNTs, in water. Interestingly, we found that complex formation of CUR-N^+ and the guest molecules proceeds spontaneously without the denature−renature process that is indispensable for SPG to bind the guest molecules.

Results and Discussion

Synthesis and Characterization of CUR-N^+. The reaction cascade is shown in Scheme 1. The synthesis of CUR-N_3 was reported previously.[3a,13] The selective coupling between CUR-N_3 and alkyne-terminated ammonium chloride[16] was carried out in a 9/1 (v/v) DMSO/water mixed solvent with propylamine as a base and $CuBr_2$-ascorbic acid as a catalyst ("Click Chemistry").[13,14] The product was purified by dialysis (cutoff MW 8000) against aqueous NaCl and water, and the quantitative and regiospecific formation of 1,2,3-triazoles was confirmed by quantitative consumption of N_3 group as evidenced from IR (Figure S3 in the Supporting Information) as well as ^{1}H NMR (δ = 8.45 ppm for H^5; Figure S1 in the Supporting Information) and ^{13}C NMR spectra (δ = 138.06 and 128.64 ppm for C^4 and C^5; Figure S2 in the Supporting Information). Molecular weight of the obtained CUR-N^+ was estimated by SEC (Shodex OHpak SB-806M HQ, 0.1 M aqueous $NaNO_3$, 40 °C, pullulan standards) to be 3.2 × 10^4 (PDI = 1.6) (Figure S4 in the Supporting Information). The CUR-N^+ showed a very good solubility in water (50 mg/mL) but was not soluble in pure DMSO.

As the absorption band of the triazole ring of CUR-N^+ appeared in shorter wavelength region (λ_{max} = 219 nm), the CD spectroscopic study on CUR-N^+ at various conditions was difficult. We thus measured the optical rotatory dispersion (ORD) spectra of CUR-N^+ at various conditions (Figure 2A). In the form of triple-stranded helix, the ORD spectra of β-1,3-glucan polysaccharides such as SPG and CUR have positive values at the wavelength region from 600 to 200 nm.[17,18] However, CUR-N^+ in water shows a negative sign at this wavelength region. According to the literature,[19] the negative sign of β-1,3-glucan polysaccharides can be ascribed to the single-stranded form. Next, we evaluated the effect of added NaCl on the ORD sign of CUR-N^+, expecting that it may reduce electrostatic repulsion. The intensity increased gradually with increasing NaCl concentration but did not reach the value of SPG in water. This difference implies that formation of the triple-stranded helical structure from CUR-N^+ is strongly suppressed by electrostatic repulsion among the cationic charges of the side-chains.

Clearer evidence for the difference in the structural character between CUR-N^+ and SPG was obtained from the investigation

(16) Nguyen, H.-K.; Fournier, O.; Asseline, U.; Dupret, D.; Thuong, N. T. *Nucleic Acids Res.* **1999**, *27*, 1492−1498.

(17) (a) Ito, T.; Teramoto, A.; Matsuo, T.; Suga, H. *Macromolecules* **1986**, *19*, 1234−1240. (b) Sakurai, S.; Shinkai, S. *Carbohydr. Res.* **2000**, *324*, 136−140.

(18) Ogawa, K.; Watanabe, K.; Tsurugi, J.; Ono, S. *Carbohydr. Res.* **1972**, *23*, 399−405.

(19) (a) Duda, C. A.; Stevens, E. S. *Biopolymers* **1991**, *31*, 1379−1385. Meijer et al. reported that the structural properties of chiral polyethylene oxides, double-stranded or random-coiled structure, in water can be clarified by means of ORD. (b) Janssen, H. M.; Peeters, E.; van Zundert, M. F.; van Genderen, M. H. P.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 122−125.

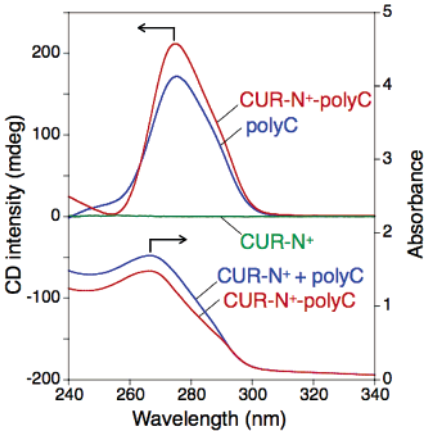


Figure 3. UV−vis and CD spectra of CUR-N^+ (1.3 mM (monomer unit)), poly(C) (0.24 mM (monomer unit)), and CUR-N^+−poly(C) (1.3 mM (monomer unit), 0.24 mM (monomer unit)) in 1.0 mM Tris-HCl buffer (pH 8.0) at 5 °C with a 1-cm cell (CUR-N^+ + poly(C) in absorption spectra is corresponding to the sum of the individual spectra).

on the binding properties of CUR-N^+ and SPG toward fluorescence probes. 1-Anilinonaphthalene-8-sulfonate (ANS) and 2-*p*-toluidylnaphthalene-6-sulfonate (TNS) are well-known as powerful fluorescent probes to detect the microenvironment in biological macromolecules and membranes: the fluorescence intensity of these compounds is enhanced when they bind to the hydrophobic region of proteins and membranes.[20] Figure 2B shows the concentration dependence of the fluorescence intensity in the presence of CUR-N^+ or SPG. The fluorescence intensity was scarcely increased in the presence of SPG, indicating that the fluorescent probes cannot bind SPG in water without the denature−renature process (Figure 2C).[21] In sharp contrast, the fluorescence intensity of both fluorescent probes was remarkably increased in the presence of CUR-N^+ accompanied with the blue shift of the fluorescence maxima (Figures S5 and S6 in the Supporting Information). These results indicate that the conformation of CUR-N^+ has the dynamic nature to accommodate hydrophobic molecules in the hydrophobic region of CUR-N^+ (Figure 2C). Moreover, the apparent binding affinity of TNS is higher than that of ANS, and only CUR-N^+−TNS complex shows induced CD signals (Figure S7 in the Supporting Information). This feature suggests that the hydrophobic region of CUR-N^+ retains the ability to discriminate the structure of hydrophobic guest molecules and to induce chirality into the hydrophobic guest molecules.[21a] In this paper, we focus on the study of the binding properties of CUR-N^+ toward polynucleotide and hydrophobic guest molecules in water.

Complexation Behaviors of CUR-N^+ toward Poly(C) Analyzed by UV−Vis and CD Spectra. Among polynucleotides that can form complexes with SPG, polycytidylic acid (poly(C)) showed the simplest behavior and therefore has most extensively been studied.[6,7,22] Figure 3 shows the UV−vis and CD spectra of poly(C) in the absence or presence of CUR-N^+ in Tris-HCl buffer (pH 8.0) at 5 °C. The solution of the CUR-N^+−poly(C) complex was stable for more than 1 month at 5 °C without precipitation. Obviously, the CD intensity of CUR-N^+ itself is negligible in this region, so that the CD bands observed in Figure 3 are ascribable to poly(C). Upon the addition of CUR-N^+, the 275 nm band increased by 23% and a new band appeared at around 240 nm. The CD spectrum of the CUR-N^+−poly(C) complex is slightly different from that of the SPG−poly(C) complex at 5 °C. In particular, the CD band at around 240 nm shifted toward shorter wavelength as compared to that of the SPG−poly(C) complex at 5 °C. The origin of the CD band at around 240 nm is not clear; however, our previous works suggest that the ion pair formation between the ammonium cations of CUR-N^+ and the phosphate anions of poly(C) contribute to the shape of CD spectra.[23] The absorbance of the CUR-N^+−poly(C) complex at 270 nm decreased by 15% as compared to that of the sum of the individual spectra, indicating that $\pi-\pi$ stacking of the cytosine rings of poly(C) is strengthened in the presence of CUR-N^+. These results indicate that CUR-N^+ forms a macromolecular complex with poly(C) and the complex would have a structure similar to that of the SPG−poly(C) complex.[6] In contrast, our previous works revealed that the CD intensity of poly(C) decreases significantly in the presence of PDDA (poly(diallydimethylammonium chloride)), which is a typical cationic polyelectrolyte.[6a,b] Likewise, poly(C) in the presence of chitosan, which is a cationic polysaccharide obtained from chitin, showed a decrease in the CD intensity (Figure S9 in the Supporting Information). In addition, AFM observation of the complex of chitosan and poly(C) on mica displays globular objects, which are typically observed for complexes of chitosan and polyanions (Figure S9 in the Supporting Information).[24] We can propose, therefore, that the enhancement of CD intensity of poly(C) is unique, retaining a characteristic behavior for β-1,3-glucan.

It is notable that the complexation between CUR-N^+ and poly(C) proceeds spontaneously in the aqueous solution by mixing each aqueous solution, whereas the denature−renature process induced with the aid of DMSO or NaOH is indispensable for SPG to bind poly(C) in the hydrophobic region of SPG. This unique binding property of CUR-N^+ toward poly(C) supports the view that CUR-N^+ possesses the dynamic structural property to accommodate guest molecules, as proposed by the ORD and the fluorescence studies of CUR-N^+.

To obtain further insight into the complexation behavior of CUR-N^+ and poly(C), we examined the temperature dependence of CD spectra of the CUR-N^+−poly(C) complex. In the presence of 5 equiv of CUR-N^+ against poly(C), the CD intensity at 275 nm decreased with an increase in temperature accompanied with a distinct transition (Figure 4). The process was reversible, and isodichroic points were clearly observed. Thus, the observed transition can be deduced to a reversible binding between CUR-N^+ and poly(C). However, the CD intensity of poly(C) in the presence of CUR-N^+ at 70 °C was significantly larger than that of poly(C) in the absence of CUR-N^+. This feature indicates that CUR-N^+ still interacts with poly(C) at such elevated temperature.

From our recent investigations,[6] it has been proposed that SPG forms a well-defined helical macromolecular complex with

(20) Johnson, J. D.; El-Bayoumi, M. A.; Weber, L. D.; Tulinsky, A. *Biochemistry* **1979**, *18*, 1292−1296.
(21) (a) Shimada, N.; Okobira, T.; Takeda, Y.; Shinkai, S.; Sakurai, K. *J. Polym. Sci., Part A: Polym. Chem.*, submitted. Jacobs et al. reported that a single-stranded rich form of SPG binds to aniline blue and its fluorescence is enhanced. (b) Young, S.-H.; Jacobs, R. R. *Carbohydr. Res.* **1998**, *310*, 91−99.
(22) Sletmoen, M.; Stokke, B. T. *Biopolymers* **2005**, *79*, 115−127.
(23) (a) Koumoto, K.; Kimura, T.; Mizu, M.; Sakurai, K.; Shinkai, S. *Chem. Commun.* **2001**, 1962−1963. (b) Nagasaki, T.; Hojo, M.; Uno, A.; Satoh, T.; Koumoto, K.; Mizu, M.; Sakurai, K.; Shinkai, S. *Bioconjugate Chem.* **2004**, *15*, 249−259.
(24) Philippova, O. E.; Akitaya, T.; Mullagaliev, I. R.; Khokhlov, A. R.; Yoshikawa, K. *Macromolecules* **2005**, *38*, 9359−9365.

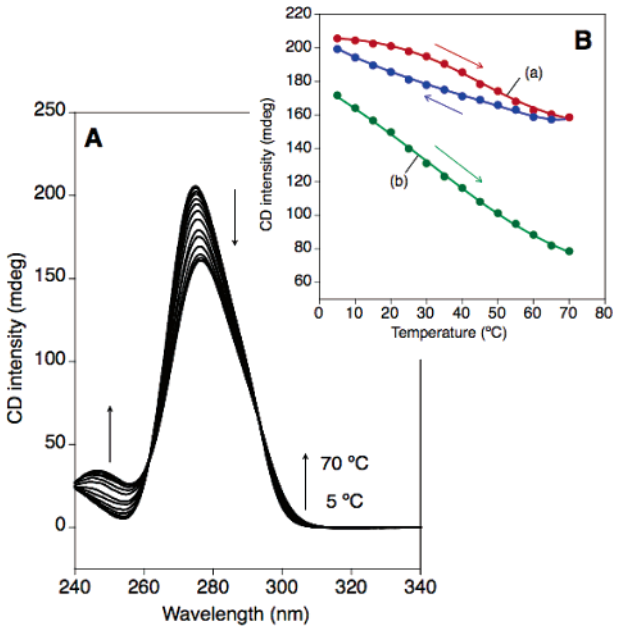


Figure 4. (A) Temperature dependence of the CD spectra of CUR-N^+−poly(C) (1.3 mM, 0.24 mM); and (B) the plots of CD intensity at 275 nm of (a) CUR-N^+−poly(C) complex and (b) poly(C) against temperature.

poly(C) consisting of two strands of SPG and one strand of poly(C), and the complex is dissociated completely into each component by raising the solution temperature. Taken this binding mode into account, one can guess that the transition observed for the CUR-N^+−poly(C) complex will correspond to the dissociation of one strand of CUR-N^+ from CUR-N^+−poly(C) complex consisting of two strands of CUR-N^+ and one strand of poly(C). In the dissociation of the SPG−poly(C) complex, the 2:1 complex decomposed into the individual polymers in one step, which made the transition very clear.[6a] In the present system, on the other hand, the relatively weak transition can be explained by the small number of hydrogen bonds being dissociated in the transition from the 2:1 complex to the 1:1 complex. To confirm this hypothesis, we examined the temperature dependences of CD spectra of poly(C) in the presence of various amounts of CUR-N^+.

Figure 5 shows plots of the CD intensity against the temperature (Figure 5A) and the molar ratio plots of CUR-N^+−poly(C) complex at 5 and 70 °C (Figure 5B). The molar ratio plot at 5 °C gives the break point at [CUR-N^+]/[poly(C)] = 2, indicating that CUR-N^+ can form the complex with poly(C) in 2:1 stoichiometry. In contrast, the molar ratio plot at 70 °C gives the break point at [CUR-N^+]/[poly(C)] = 1, indicating that CUR-N^+ can form the complex with poly(C) in 1:1 stoichiometry. These results support the view that CUR-N^+ can form a complex in 2:1 or 1:1 stoichiometry depending on the feed and the solution temperature. Figure 5C illustrates these complexation behaviors between CUR-N^+ and poly(C). We consider that the action of three different interactions (electrostatic, hydrogen-bonding, and hydrophobic) between CUR-N^+ and poly(C) is the origin of such unique complexation behaviors.

Microscopic Observation of the Fibrous Architecture of the Complex Using AFM and TEM. To obtain further structural information on the CUR-N^+−poly(C) complex, we carried out microscopic observations by means of AFM and TEM. Figure 6A and B displays the typical AFM images of CUR-N^+ and poly(C), respectively. From the AFM image of CUR-N^+ (Figure 6A), the average height of the observed fibrous objects was approximately 0.5 nm, which is smaller than that expected from the diameter of the triple-stranded helical structure (approximately 2.5 nm for a triple-stranded helical structure of CUR-N^+ obtained by a molecular mechanics calculation).[6c,14e,25] In addition, the apparent persistence length of CUR-N^+ observed in its AFM image was not as long as that expected for a stiff triple-stranded helical structure (150 nm for t-SPG).[14e] These findings suggest that CUR-N^+ exists as a single-stranded structure under this condition or on this substrate surface, which is in accord with the ORD studies on CUR-N^+. The AFM image of poly(C) showed only circular dots (Figure 6B), which were frequently observed for a flexible-chain polymer and consistent with the previous studies on single-stranded polynucleotides.[26]

In sharp contrast, fibrous objects were observed in the AFM images of CUR-N^+−poly(C) complexes (Figure 6C−E). The TEM images of the CUR-N^+−poly(C) complexes also present the fibrous objects (Figure 7C−E). In [CUR-N^+]:[poly(C)] = 1:1 feed ratio (Figure 6C), the fibrous objects possessed almost uniform height, the average height being approximately 1.0 nm. In [CUR-N^+]:[poly(C)] = 2:1 and 5:1 feed ratios (Figure 6D and E), the fibrous objects also possessed almost uniform height, the average height being approximately 1.5 nm. This difference in the height of the fibrous objects observed for [CUR-N^+]/[poly(C)] = 1 and more than 2 suggests that CUR-N^+ can form two different types of macromolecular complexes with poly(C), depending on the conditions as suggested by the spectroscopic analyses. Figure 6F represents an energy-minimized structure of a 2:1 CUR-N^+−poly(C) complex, which is generated on the basis of the assumption that CUR-N^+ forms a structure similar to that of the SPG−poly(C) complex in 2:1 stoichiometry.[6c,d] The diameter (2.5 nm) of the model is compatible with the height estimated by the cross-section analyses of the AFM images in Figure 6D and E. Additionally, the length of the fibrous objects in Figure 6E ranged from 50 to 200 nm, which could be assigned to the length of one strand of poly(C). Because poly(C) used in this study has 570 bases, the length of the extended single chain would be about 200 nm. These results suggest that the CUR-N^+−poly(C) complex in 2:1 stoichiometry has a well-defined structure, which is compatible with that of the SPG−poly(C) complex. Under the condition of [CUR-N^+] ≈ [poly(C)], the electrostatic interaction might contribute strongly to the complex formation between CUR-N^+ and poly(C), and subsequent charge neutralization could facilitate the entanglement of the individual fibrous structures, as observed in Figure 6C and D. The CUR-N^+−poly(C) complexes, indeed, showed an increase in their hydrodynamic diameter when the value of [CUR-N^+]/[poly(C)] was close to unity, which was evaluated by dynamic light scattering (DLS) measurements (Figure S10 in the Supporting Information).

Normally, a polyion complex between a polycation and a polyanion emerges as a globular structure depending on the rigidity of each polymer.[27,28] Especially, most of such studies have focused on the compaction of double-stranded DNA

(25) Müller, D. J.; Engel, A. *Biophys. J.* **1997**, *73*, 1633−1644.
(26) Fritz, J.; Anselmetti, D.; Jarchow, J.; Fernàndez-Busquets, X. *J. Struct. Biol.* **1997**, *119*, 165−171.
(27) (a) Thünemann, A. F.; Müller, M.; Dautzenberg, H.; Joanny, J.-F.; Löwen, H. *Adv. Polym. Sci.* **2004**, *166*, 113−171. (b) Maurstad, G.; Danielsen, S.; Stokke, B. T. *J. Phys. Chem. B* **2003**, *107*, 8172−8180.

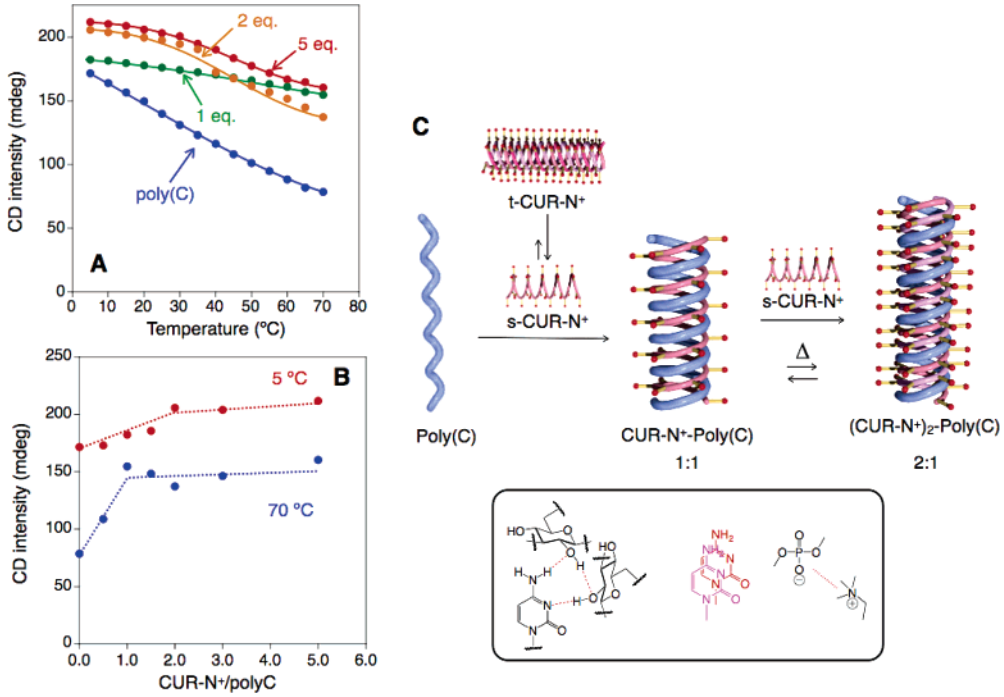


Figure 5. (A) Temperature dependence of CD intensity at 275 nm for the various molar ratios of CUR-N^+ against poly(C); (B) plots of CD intensity at 5 and 70 °C against the molar ratio [CUR-N^+]/[poly(C)] (the lines are for the guidance of eyes); and (C) schematic illustration of a proposed mechanism for the formation of CUR-N^+−poly(C) complexes shown with their ideal structures and possible interactions.

(dsDNA). The nonspecific binding of polycations, such as poly(L-lysine) and poly(ethyleneimine), with the dsDNA results in compactions, which mainly give rise to toroidal or linear morphologies.[28c,d] In contrast, the CUR-N^+−poly(C) complexes likely have two different types of stoichiometry as well as the nanosized fibrous structure, which could be a consequence of the cooperative action of hydrogen bonding[6d] and electrostatic interactions[23,29] in addition to the background hydrophobic effect. We believe that the stereoregularity of CUR-N^+ would be one of the most important factors to retain the ability of β-1,3-glucan to form macromolecular inclusion complexes with hydrophobic guest molecules, in which the hydrophobic region such as cytosine rings would be located inside the hydrophobic core of the complex and shielded from hydrophilic outside. This complexation mode clearly differs from the conventional polyion complexation, which is predominantly driven by the electrostatic interaction.

Resistance of the Complexed Polynucleotide Chain against the Enzymatic Hydrolysis. For the future application of CUR-N^+ as gene carriers, we evaluated whether the complexed polynucleotide chain can resist enzymatic hydrolysis. The hydrolysis of poly(C) by RNase A can be traced by a change in the absorbance at 260 nm, which is correlated with the production of cytidine-3′-monophosphate (CMP).[30] Figure 8 shows the time courses of the absorbance at 260 nm. Under the condition recorded in the caption of Figure 8, more than 95% of naked poly(C) was hydrolyzed by RNase A after 5 min, whereas almost no change was observed for the CUR-N^+−poly(C) complex. This remarkable difference indicates that the complexed polynucleotide aquires a strong resistance against the hydrolysis by RNase A. This feature can be interpreted by the presence of excess cationic charges surrounding the CUR-N^+−poly(C) complex, which can significantly reduce the binding affinity between the RNase A and CUR-N^+−poly(C) complex.

Dissolution of Permethyldecasilane (PMDS) into Aqueous Solution by CUR-N^+. Polysilanes and oligosilanes are unique synthetic polymers, which show interesting photo- and electrochemical properties arising from the σ-conjugation of Si−Si bonds along the main chain.[31] Furthermore, the conformation is sensitive to the chiral environment.[32] Recently, we[9] and others[33] have demonstrated that SPG can behave as an effective host for the encapsulation of oligosilanes. Because PMDS was insoluble in water, we applied a biphasic system with hexane/water, in which PMDS exists in a hexane layer and SPG exists in an aqueous NaOH layer with a single-stranded structure. The

(28) (a) Kabanov, A. V.; Kabanov, V. A. *Bioconjugate Chem.* **1995**, *6*, 7−20. (b) Kabanov, V. A.; Sergeyev, V. G.; Pyshkina, O. A.; Zinchenko, A. A.; Zezin, A. B.; Joosten, J. G. H.; Brackman, J.; Yoshikawa, K. *Macromolecules* **2000**, *33*, 9587−9593. (c) Kwoh, D. Y.; Coffin, C. C.; Lollo, C. P.; Jovenal, J.; Banaszczyk, M. G.; Mullen, P.; Phillips, A.; Amini, A.; Fabrycki, J.; Bartholomew, R. M.; Brostoff, S. W.; Carlo, D. J. *Biochim. Biophys. Acta* **1999**, *1444*, 171−190. (d) Dunlap, D. D.; Maggi, A. R.; Soria, M.; Monaco, L. *Nucleic Acids Res.* **1997**, *25*, 3095−3101.

(29) Salt concentration will reduce the strength of interaction arising from the electrostatic interaction between CUR-N^+ and poly(C). In fact, the CD intensity at 275 nm of CUR-N^+−poly(C) complex decreased abruptly from 15 °C in the presence of 150 mM NaCl (Figure S8 in the Supporting Information).

(30) Mizu, M.; Koumoto, K.; Kimura, T.; Sakurai, K.; Shinkai, S. *Biomaterials* **2004**, *25*, 3109−3116.

(31) (a) Peng, W.; Motonaga, M.; Koe, J. *J. Am. Chem. Soc.* **2004**, *126*, 13822−13826. (b) Nakashima, H.; Fujiki, M.; Koe, J. R.; Motonaga, M. *J. Am. Chem. Soc.* **2001**, *123*, 1963−1969.

(32) (a) Fujiki, M. *J. Am. Chem. Soc.* **1994**, *116*, 6017−6018. (b) Fujiki, M.; Koe, J. R.; Terao, K.; Sato, T.; Teramoto, A.; Watanabe, J. *Polym. J.* **2003**, *35*, 297−344 and references cited therein.

(33) Tanaka et al. reported that complex between permethyldecasilane bering a naphthyl group at the one end and SPG can be obtained by direct mixing in water without the denature−renature process. (a) Sanji, T.; Kato, N.; Tanaka, M. *Chem. Lett.* **2005**, *8*, 1144−1145. (b) Sanji, T.; Kato, N.; Kato, M.; Tanaka, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 7301−7304. However, no detectable CD is observed for PDMS by mixing and sonication with t-SPG in water from our study.[9] Thus, it can be concluded that the denature−renature process is crucial for effective guest inclusion inside the hydrophobic and helical interior of SPG.

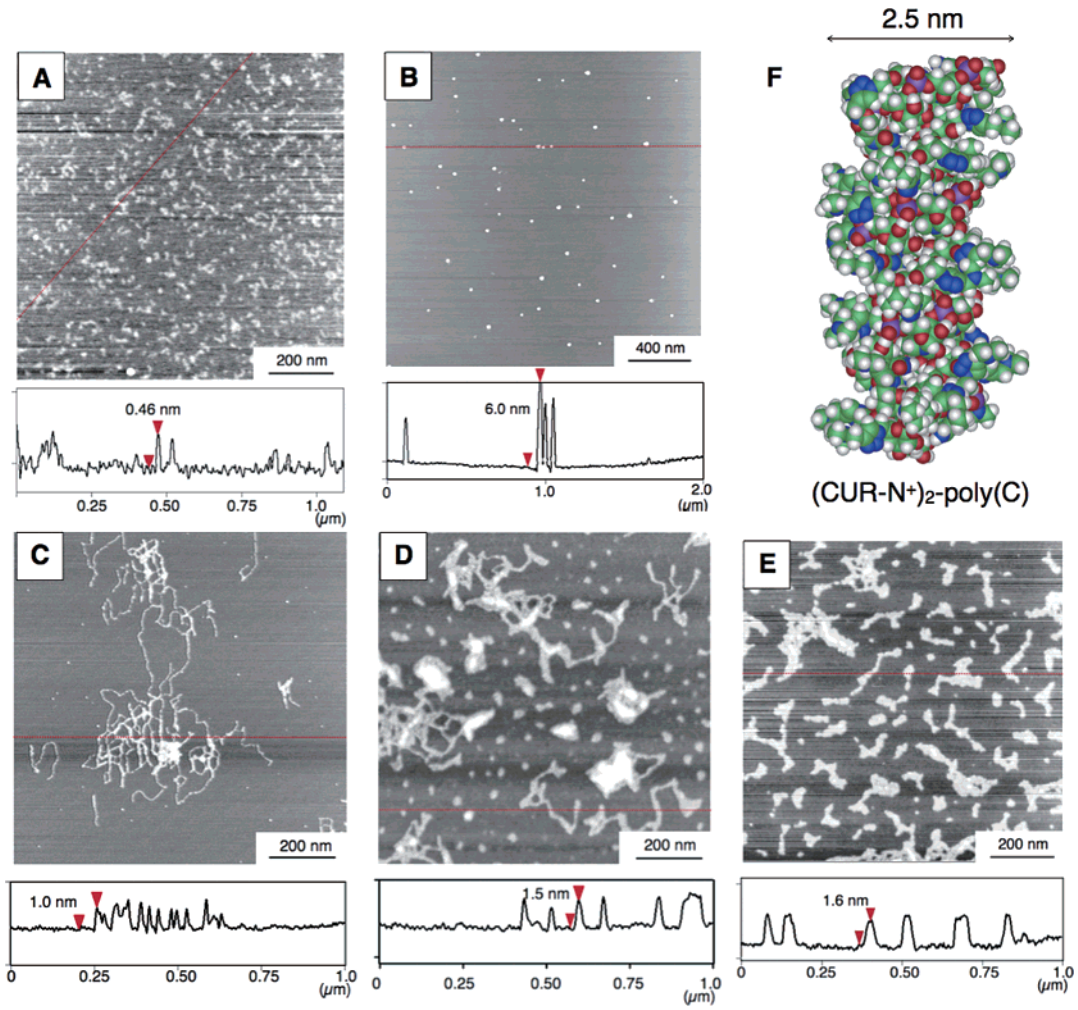


Figure 6. The AFM height images on mica of CUR-N^+ (A), poly(C) (B), and CUR-N^+−poly(C) ((C), (D), and (E) for [CUR-N^+]:[poly(C)] of 1:1, 2:1, and 5:1 feed ratio, respectively) and the corresponding cross-section profiles along the red line in the images (see experimental section for details of the sample preparation) and (F) a computer-generated CPK model for an ideal 2:1 complex of CUR-N^+−poly(C) (see experimental section).

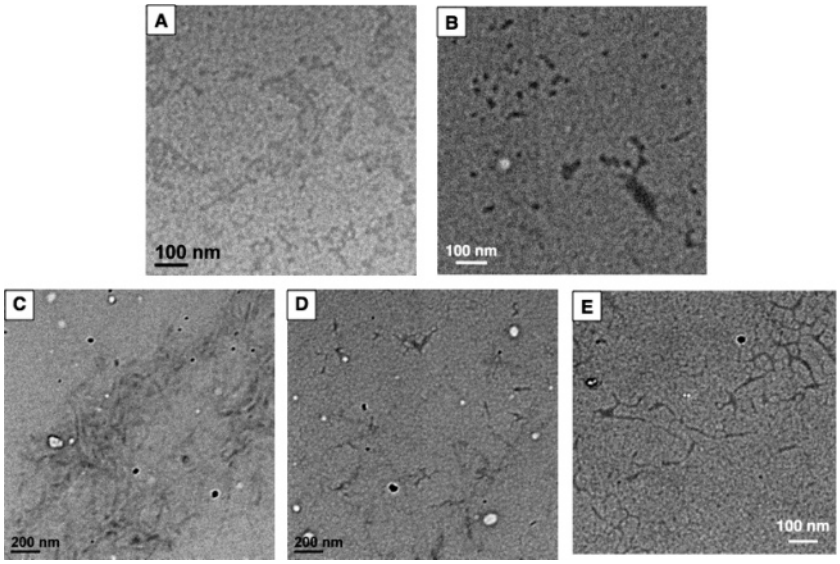


Figure 7. The TEM images of CUR-N^+ (A), poly(C) (B), and CUR-N^+−poly(C) (C, D, and E for CUR-N^+:poly(C) of 1:1, 2:1, and 5:1 feed ratio, respectively) (see experimental section for details of the sample preparation).

CD active and water-soluble SPG−PMDS composite was obtained by neutralization of the biphasic solution by acetic acid.[9]

Here, we tried to dissolve PMDS into water using CUR-N^+ by simple mixing with sonication. A powder of PMDS was dispersed in water containing CUR-N^+ by sonication with a

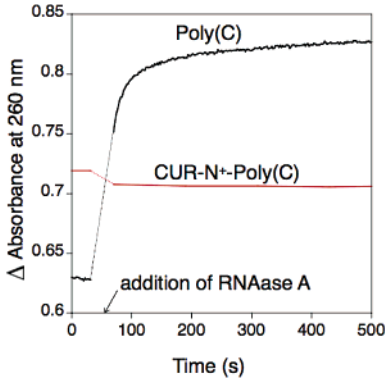


Figure 8. Time courses of the UV absorbance at 260 nm by the addition of RNase A solution (2.0×10^{-4} gL^{-1}) to the poly(C) (0.12 mM) and CUR-N^+−poly(C) solution (0.60 mM, 0.12 mM) in 1.0 mM Tris-HCl buffer (pH 8.0) at 25 °C.

sonication probe in a water bath. The solution was centrifuged to removed uncomplexed PMDS, and the 1/4 diluted solution was subjected to spectroscopic and microscopic experiments (the details are described in the experimental section). By comparing the extinction coefficient of uncomplexed PMDS dissolved in hexane, we estimated that approximately 5% of PMDS was dissolved into water by CUR-N^+ by these treatments. This value indicates that approximately 0.8 equiv of PMDS against CUR-N^+ in monomer unit ($SiMe_2$ unit for PMDS and glucose unit for CUR-N^+, respectively) is included in CUR-N^+−PDMS composites. The experiment was repeated two times to confirm the reproductivity. As seen in Figure 9A, a broad and red-shifted absorption band centered at 287 nm assignable to the $\sigma-\sigma^*$ transition of PMDS (280 nm in hexane) and the corresponding fluorescence emission centered at 316 nm (310 nm in hexane) were observed. Additionally, a broad bisignate CD band (288 nm ($g(\Delta\epsilon/\epsilon) = -0.5 \times 10^{-4}$), 316 nm ($g(\Delta\epsilon/\epsilon) = +0.8 \times 10^{-4}$)) was observed. The dissymmetry ratios (g) of CUR-N^+−PDMS composites are smaller by a factor of ca. 4 than those of SPG−PMDS composites, indicating that the PMDS included in CUR-N^+ consists of a mixture of a few conformations. The broad absorption band of CUR-N^+−PMDS composites as compared to that of SPG−PMDS composites also suggests that the PMDS inside CUR-N^+ is not comprised of a single conformer. It is still not clear why CUR-N^+−PMDS composite has a different pattern of CD spectrum as compared to that of SPG−PMDS composites. Most likely, the electrostatic repulsion among cationic side-chains of CUR-N^+ prevents CUR-N^+−PDMS composites from the formation of a rigid supramolecular helical structure as SPG−PMDS composites.

To obtain a visual image on the morphology of CUR-N^+−PMDS composites, TEM microscopic observation was performed. Figure 9B shows the typical TEM image of CUR-N^+−PMDS composites, which presents fibrous architectures of several hundred nanometers in length (Figure S11 in the Supporting Information). These results demonstrate that CUR-N^+ can act as an effective one-dimensional host molecule toward PMDS, which avoids the complicated procedure of SPG described above.

Dissolution of ag-SWNTs into Aqueous Solution by CUR-N^+. Since the discovery of SWNTs,[34] much attention has been paid to the characterization of the individual SWNTs.[35] How-

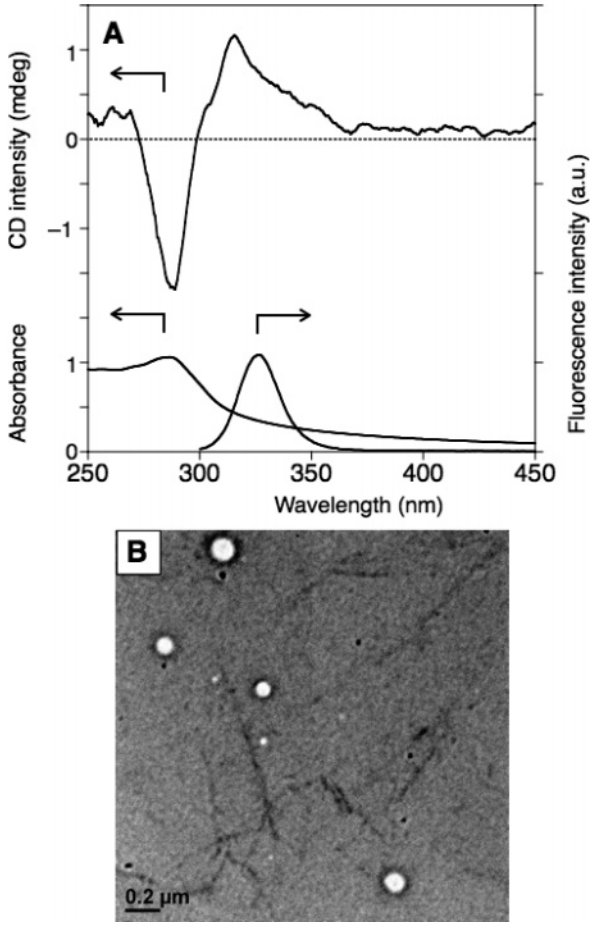


Figure 9. (A) UV−vis, fluorescence ($\lambda_{ex} = 290$ nm), and CD spectra of CUR-N^+−PMDS composite ([CUR-N^+] = 3.0×10^{-4} M (monomer unit), [PMDS] = 2.5×10^{-4} M (monomer unit); the details are described in the experimental section) in an aqueous solution at ambient temperature; and (B) the TEM image of CUR-N^+−PMDS composite after treatment with phosphotungstic acid.

ever, the macroscopic figure of SWNTs is isotropic and randomly bundled. Thus, processing SWNTs for practical uses is a major challenging theme.[36] Recently, several groups have succeeded in dispersing SWNTs individually into water by wrapping with helical polymers, such as amylose[37a,b] double-stranded or single-stranded DNA.[37c,d] We also showed that SPG and CUR can dissolve as-grown and cut SWNTs (ag-SWNTs and c-SWNTs (1−2 μm), respectively) effectively in water and investigated their composite structures thoroughly by micro-

(34) Iijima, S. *Nature* **1991**, *354*, 56−58.

(35) (a) Treacy, M. M. J.; Ebbesen, T. W.; Gibson, J. M. *Nature* **1996**, *381*, 678−680. (b) Tans, S. J.; Devoret, M. H.; Dai, H.; Thess, A.; Smalley, R. E.; Geerlings, L. J.; Dekker, C. *Nature* **1997**, *386*, 474−477.

(36) SWNTs dissolution by polymeric materials. (a) O'Connell, M. J.; Boul, P.; Ericson, L. M.; Huffman, C.; Wan, Y.; Haroz, E.; Kuper, C.; Tour, J.; Ausman, K. D.; Smalley, R. E. *Chem. Phys. Lett.* **2001**, *342*, 265−271. (b) Yudasaka, M.; Zhang, M.; Jabs, C.; Iijima, S. *Appl. Phys. A* **2000**, *71*, 449−451. (c) Star, A.; Stoddart, J. F.; Steuerman, D.; Diehl, M.; Boukai, A.; Wong, E. W.; Yang, X.; Chung, S.-W.; Choi, H.; Heath, J. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 1721−1725. (d) Chen, J.; Liu, H.; Weimer, W. A.; Halls, M. D.; Waldeck, D. H.; Walker, G. C. *J. Am. Chem. Soc.* **2002**, *124*, 9034−9035. (e) Takahashi, T.; Tsunoda, K.; Yajima, H.; Ishii, T. *Chem. Lett.* **2002**, *31*, 690−691. (f) Dieckmann, G. R.; Dalton, A. B.; Johnson, P. A.; Razal, J.; Chen, J.; Giordano, G. M.; Munoz, E.; Musselman, I. H.; Baughaman, R. H.; Draper, R. K. *J. Am. Chem. Soc.* **2003**, *125*, 1770−1777. (g) Zorbas, V.; Ortiz-Acevedo, A.; Dalton, A. B.; Yoshida, M. M.; Dieckmann, G. R.; Draper, R. K.; Baughman, R. H.; J.-Yacaman, M.; Musselman, I. H. *J. Am. Chem. Soc.* **2004**, *126*, 7222−7227. Recently, Noy et al. reported that the conformation of polyeletrolytes is important for the wrapping of SWNTs. (h) Huang, S.-C. J.; Artyukhin, A. B.; Wang, Y.; Ju, J.-W.; Stroeve, P.; Noy, A. *J. Am. Chem. Soc.* **2005**, *127*, 14176−14177.

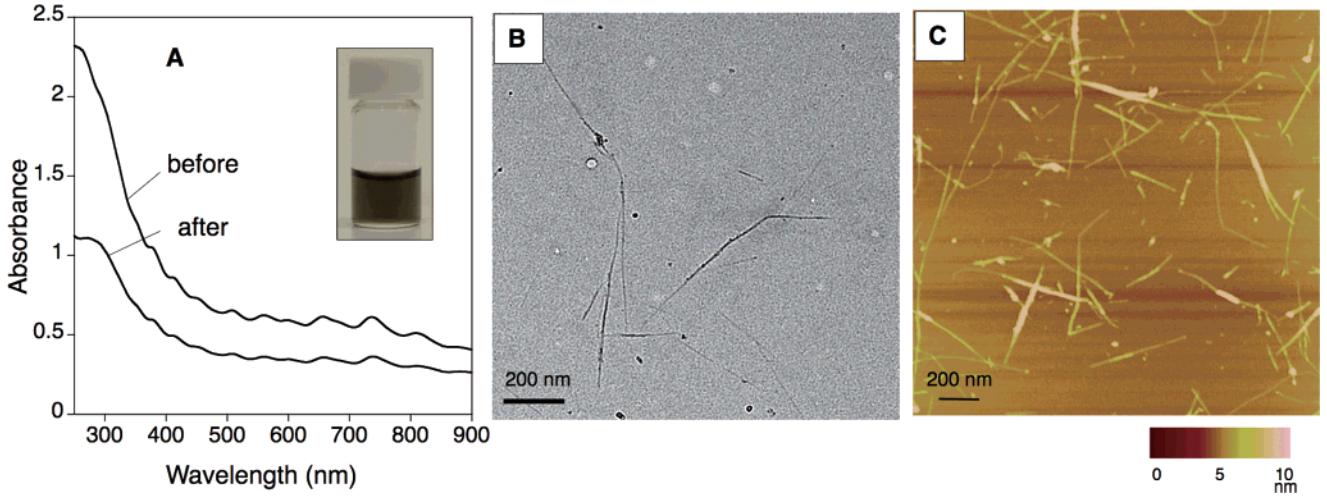


Figure 10. (A) Vis−NIR absorption spectra of CUR-N^+−SWNTs composite in an aqueous solution (1-mm cell) at ambient temperature before and after chromatographic purification (the details are described in the experimental section; inset is a photograph of CUR-N^+−SWNTs composite in an aqueous solution before chromatographic purification) and (B) TEM and (C) AFM height images of CUR-N^+−SWNTs composite after the chromatographic purification.

scopic techniques.[8] Yet again, the denature−renature process is indispensable for the dissolution of both types of SWNTs.

Here, we tried to dissolve SWNTs in water by CUR-N^+ without the denature−renature process. A powder of ag-SWNTs was dispersed in water containing CUR-N^+ by sonication with a sonication probe in a water bath. The solution gradually became dark as the sonication time progressed. The solution was then diluted with water and subjected to a centrifugation process (the details are described in the experimental section). The obtained aqueous solution of CUR-N^+−ag-SWNTs composite was stable for at least 1 month without precipitation. Figure 10 shows the vis−NIR spectrum of the solution of CUR-N^+−ag-SWNTs composite, which has a typical band pattern characteristic of ag-SWNTs.[8c] AFM images of the sample prepared by casting the CUR-N^+−ag-SWNTs composite solution on mica show bundles of several pieces of SWNTs as well as almost individual pieces of SWNTs wrapped by CUR-N^+ (Figure S12 in the Supporting Information).

The obtained ag-SWNTs−CURN$^+$ composite showed good solubility in water. Chromatographic purification based on the size-exclusion mode was thus applied to isolate the individual pieces of SWNTs wrapped by CUR-N^+. The vis−NIR spectrum of a fractionated solution was basically the same as that before the purification. Figure 10B and C represents typical TEM and AFM height images of the purified ag-SWNTs−CURN$^+$ composite. These images clearly indicate that the individual pieces of SWNTs wrapped by CUR-N^+ are eluted almost exclusively. Thereby, one can confirm that water-soluble CUR-N^+−ag-SWNTs composites are successfully prepared without the denature−renature process. This mild and simple procedure is a promising method to arrange functional groups around one piece of SWNTs without destructing the unique π-conjugate properties of the SWNTs.

(37) (a) Star, A.; Steuerman, D. W.; Heath, J. R.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 2508−2512. (b) Kim, O.-K.; Je, J.; Baldwin, J. W.; Kooi, S.; Pehrsson, P. E.; Buckley, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 4426−4427. (c) Nakashima, N.; Okuzono, S.; Murakami, H.; Nakai, T.; Yoshikawa, K. *Chem. Lett.* **2003**, *32*, 456−457. (d) Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; Mclean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. *Nat. Mater.* **2003**, *2*, 338−342.

Summary and Conclusions

In conclusion, we have succeeded in the quantitative and selective introduction of an ammonium cationic group into the C6 position of CUR by "Click Chemistry". The obtained CUR-N^+ showed good solubility in water. It was revealed that the CUR-N^+ forms two types of macromolecular complex with poly(C) depending on the feed ratio, and the resultant complexes have nanosized fibrous structures, which could be a consequence of the cooperative action of (1) hydrogen bonding, (2) electrostatic interactions, as well as (3) the hydrophobic effect. Moreover, the complexed polynucleotide chain showed strong resistance against enzymatic hydrolysis. Therefore, we believe that the cationic Curdlan (CUR-N^+) and its analogues would become potential candidates for gene carriers. Likewise, the dissolution of functional polymers, such as polysilane and SWNTs, into water has been successfully achieved. We propose that the stereoregularity and site-specific modification of CUR-N^+ would play an important role to form the macromolecular inclusion complexes with the hydrophobic guest molecules. Most noteworthy is the finding that we can obtain the complexes between CUR-N^+ and the hydrophobic guest molecules easily by just mixing each solution or a powder in water without DMSO or NaOH as denaturants. This advantage is very important for future applications as a one-dimensional host molecule to develop functional nanofibers and subsequently manipulate the nanofibers into nanomaterials. These features indicate that CUR-N^+ can be regarded as a single molecular cylindrical micelle in a sense. We thus believe that the present inclusion phenomena have broad potential for chemical and biological purposes. The research in these directions is now underway in our laboratory.

Acknowledgment. We thank Prof. T. Nagasaki for valuable discussions and comments. We gratefully acknowledge financial support from the Japan Science and Technology Agency, SORST program, and the 21st Century COE program, "Functional Innovation of Molecular Informatics", of the Ministry of Education, Culture, Science, Sports, and Technology (Japan).

Supporting Information Available: Experimental details and figures showing ^{1}H, ^{13}C NMR, and FTIR spectra, and SEC chromatogram of CUR-N^{+}, UV−vis and fluorescence spectra of ANS and TNS in the presence of CUR-N^{+} and SPG, CD spectrum of TNS in the presence of CUR-N^{+}, CD spectrum and AFM height image of chitosan−poly(C) composite, CD spectrum of CUR-N^{+}−poly(C) composite in the presence of NaCl, DLS analyses of the CUR-N^{+}−poly(C) system, unstained TEM image of CUR-N^{+}−PMDS complex, and AFM height image of CUR-N^{+}−ag-SWNTs composites. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0684343