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PH-Controlled Carbon Nanotube Aggregation/Dispersion Based on Intermolecular I-Motif DNA Formation

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In this work, we report a new strategy to manipulate the aggregation and dispersion of carbon nanotube in solution via formation of intermolecular i-motif (four-stranded C-quadruplex) structures in a pH dependent manner. Firstly, single-stranded (ss) DNAs containing two stretches of cytosine (C)-rich domains are covalently linked to carbon nanotubes. At pH 8.0, DNAs are at random coil state, which enhance the dispersion of multi-wall carbon nanotubes (MWNTs) in water; after changing pH to 5.0, the intermolecular i-motif structures formed by the C-rich ssDNAs on neighboring carbon nanotube could drive the MWNTs aggregate. This process is reversible and the transition process has been verified by circular dichroism (CD) spectroscopy, gel electrophoresis and transmission electron microscopy (TEM). Considering the mechanical properties of carbon nanotube, this finding will benefit many application research fields, such as artificial muscle, functional nano-devices and so on.

Keywords: Intermolecular I-Motif, Carbon Nanotube, pH Control.

1. INTRODUCTION

Carbon nanotubes (CNTs) have drawn significant research interests since they were firstly reported by Iijima in 1991. Owing to the outstanding chemical and physical properties, carbon nanotubes have been used to realize many applications, including electronics, optics, mechanics and biosenseing.[1–4] To achieve these goals, it is crucial to make it clear that how to control the carbon nanotubes assembling into desired large architectures. Encouragingly, recent work has shown us the ability of controlling the carbon nanotubes self-assembly process via small molecules,[5] polymers[6] and biomolecules, such as proteins[7] and DNA molecules.[8]

Among those candidate biomolecules, DNA molecules have been proved the most promising tool in nanotechnology field, because of their unique properties, such as sequence programming, base pair recognition and tunable conformation. Recently, several groups used DNA molecules to successfully disperse and assemble carbon nanotubes. For example: Zheng et al. reported a method to disperse single-walled carbon nanotubes (SWNTs) in water by DNA wrapping.[9] Mao and his co-workers explored a new way to manipulate the aggregation and dispersion process of SWNTs by DNA hybridization[8a] and demonstrated reversing the process by changing temperature: when the temperature was higher than the sequence melting temperature(Tm), SWNTs showed individual state, in contrast, SWNTs were in aggregation state.

Recently, Deng and his co-workers developed an elegant method to realize controllable self-assembly of single-walled carbon nanotubes:[8b] by introducing a "toehold", they could regulate the hybridization kinetics to control the rate of SWNTs assembly process and they also tested the ability of this elaborate system to work as a sensing unit. Mao's and Deng's work are both based on the DNA strands-exchanging mechanism. Herein, we put forward a different design to control the MWNTs aggregation/dispersion process based on intermolecular i-motif DNA formation instead of adding other complementary DNA. The i-motif structure is formed by four stretches of C-rich DNA domains which can only be stabilized at lower pH.[10] The intramolecular i-motif DNA has been employed to facilitate a nanomachine driven by pH, which is strong, reliable and has been widely used in constructing different nanodevices.[11] Meanwhile, the intermolecular i-motif structure has also been demonstrated the ability to pull gold nanopaticles together in the solution[12] and formed a pH-trigged, fast-responding DNA hydrogel.[13] In this work, single-stranded (ss) DNAs

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containing two stretches of cytosine (C)-rich domains are covalently linked to carbon nanotubes via chemical reaction. Circular dichroism (CD) spectroscopy confirms that the intermolecular i-motif structures formed between the C-rich domains of the ssDNAs on neighboring carbon nanotube is driven by environmental pH change. Transmission electron microscopy (TEM) and gel electrophoresis techniques are applied to characterize the reversible aggregation/dispersion process.

2. EXPERIMENTAL DETAILS

The mutil-walled carbon nanotubes (MWNTs) in this work were purchased in Timesnano Inc (Chengdu, China). The diameter of MWNTs is about 10 nm and the purity is larger than 90 percent. 20 mg MWNT was mixed with a solution of sulfuric (H_2SO_4) acids 60 ml and nitric (HNO_3) acids 20 ml in a 3:1 ratio, and was heated at 100 °C for 1 h. This step could introduce carboxyl group to MWNTs and shorten MWNTs. The functionalized MWNTs were filtered and washed with deionized water until neutral. Modified singe strand DNA (i-motif DNA: 5′-NH2-TTTTTTTTTCCCCTAACCCC-3′; control DNA: 5′-NH2-TTTTTTTTTCACTCACCACT-3′) was synthesized by TaKaRa Biotech (Dalian, China). Excess amino-modified single strand DNA and carboxy-functionalized MWNTs were mixed together for four hours to form conjugation with the aid of ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) in the Tris buffer (pH = 7.5). Then the reaction mixture was loaded in a Micron YM-100 (Millipore Company) and centrifuged for 20 min at 4,400 rpm to remove unreacted DNA. The DNA-MWNTs pad was re-dispersed in 50 mM MES buffer (pH = 7.0 NaCl 10 mM). The final concentration of the DNA-MWNTs conjugates was estimated to be 0.1 mg/ml.

Transmission electron microscopy experiments were carried out on FEI T20 electron microscope operated at 200 kV with a CCD camera. Samples were prepared by placing a drop of the DNA-MWNT conjugates onto transmission electron microscopy copper grids (400 meshes, carbon coated). The grids were air-dried for 12 h. For gel electrophoresis experiment, 5 μl samples were loaded into 0.5% agarose gel and run in 50 mM TBE buffer at 8 V/cm for 5 minutes. All CD spectra were recorded on a Jasco–810 spectropolarimeter equipped with a programmable temperature control unit. All the pH values mentioned were calibrated by a micro pH meter (FE 20 from METTLER TOLEDO company). The photos in this paper were captured by a Cannon IXUS 70 digital camera.

3. RESULTS AND DISCUSSION

Figure 1 illustrates the working scheme of our system, which contains only a single type of MWNT chemically linked with amino-modified ssDNAs containing two stretches of CCCC domains (namely, the i-motif DNA). The two C-rich domains act like a nanoscale "hook" to assemble the MWNTs. The two C-rich domains are spaced out by the 9 thymine bases spacer to increase their accessibility by reducing steric hindrance. At pH 8, the i-motif DNAs are in random coils, so the MWNTs are seperated because of the electrostatic repulsion between negatively charged carboxyl groups on their surface. The solution shows black as the MWNTs are well dispersed. When the pH is lowered, the cytosines in the C-rich domains become partially protonated, leading to the formation of a C≡CH+ hydrogen bond between protonated (CH+) and nonprotonated cytosines (C), and hence intermolecular i-motifs are formed between the C-rich domains of neighboring MWNTs. This could pull the MWNTs together and assemble them into aggregates, which can be seen by naked eyes. Also, the process can be reversible. Adding alkali to the

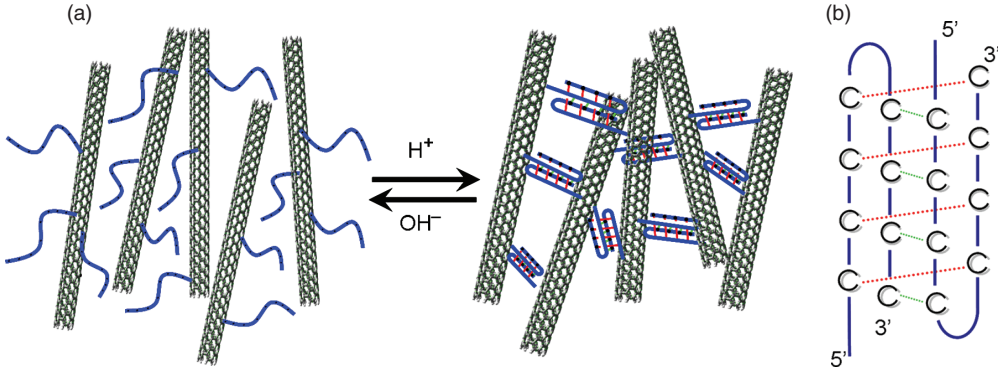

Fig. 1. (a) Schematic illustration of the transition process between aggregation and dispersion states. At high pH, all DNAs on the MWNTs are random coils, so the MWNTs are separated individually. At low pH, the formation of intermolecular i-motifs leads to the assembly of MWNTs into aggregates. The two states can be switched by varying the solution pH by adding acid or alkali to the system. (B) Schematic illustration of the intermolecular i-motif structure formed by two half i-motif DNAs from neighboring MWNTs, where the two C-rich domains of the i-motif DNAs act like nanoscale hooks to assemble the MWNTs.

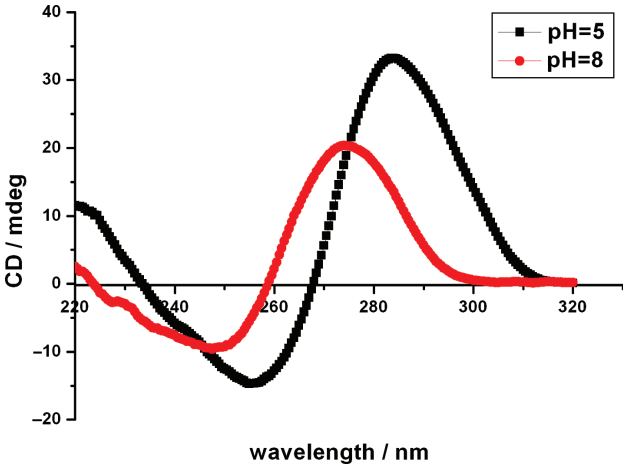

Fig. 2. CD spectra of i-motif DNA at different environmental pHs.

system deprotonates the protonated cytosines and breaks up the intermolecular i-motifs and further disassembles the MWNT aggregate.

To test the feasibility of our design, circular dichroism (CD) spectroscopy was used to verify the formation of i-motif structure. At pH 5.0, the system has a CD spectrum which shows the distinct characteristics of the i-motif structure, with a strong positive band near 285 nm, a smaller negative band around 260 nm, and a crossover around 270 nm. This really indicates the C-rich ssDNAs linking to MWNT could form the i-motif structure and have the potential ability to pull MWNT together. At pH 8.0, CD spectroscopy shows distinct characteristics of a B-form duplex DNA structure with a positive band near 275 nm, a negative band near 245 nm, and a crossover at 258 nm. In particular, the crossover at 258 nm rules out a random-coil structure which could help to break down the aggregates and the system shows homogeneous black solution.

Gel electrophoresis is a powerful technique to analyze the behaviors of the charged object in the electric field. In this technique, charged particles migrate in a porous matrix (gel) under an electric field; particle mobility depends on their charge and size. Recently, gel electrophoresis was found use in colloidal science and it was applied to isolate gold nanocrystal/DNA conjugates since the binding of DNA to nanoparticles should produce a significant shift in their electrophoretic mobility.[14] Inspired by that, we try to characterize the reversible assembly process by gel electrophoresis method. Firstly, the MWNTs functionalized with carboxyl group can not run in the gel. This is mainly because the carboxyl groups introduced by oxidation reaction can not provide enough negative charge to give a proper charge-mass ratio and hence there shows no obvious shift in the gel. Then, we check it out how the DNA-MWNT conjugates perform in the gel electrophoresis process. Herein, we design a control DNA with the sequence 5′-NH2-TTTTTTTTTCACTCACCACT-3′ which can't form any secondary structure at slight acidic environment. Observed from the agarose gel in Figure 3, lane 1 and lane 2 are loaded with control DNA-MWNT conjugates at pH 8 and pH 5 respectively, which both show continuous smear. This is mainly because the length of MWNTs is not uniform. Thus, linking to the same control DNA, MWNTs with different length will certainly have different electrophoretic mobility and appear smear in the agarose gel. The i-motif DNA-MWNT conjugates perform totally different from the control DNA-MWNT conjugates in the gel electrophoresis process at pH 5 and pH 8. Lower pH will induce the formation of the i-motif structure and this i-moif DNA is functionalized as a linker to join the adjacent MWNT together and finally form aggregate. In the gel electrophoresis process, this aggregate can not penetrate into the gel. When the pH value raise to 8, the i-motif structure is broken down and the i-motif DNA shows random single strand state. Therefore, the aggregate is collapsed into individual MWNT and show continuous smear in the gel as the control did. This pH-dependent behavior in the gel electrophoresis really confirms that the MWNT aggregation/dispersion process is driven by the conformation change of i-motif DNA. This gel electrophoresis method is also used in the previous work to demonstrate DNA-CNT assembly process.[8] By alternating addition of HCl and NaOH, three cycles are demonstrated in the gel electrophoresis. From Figure 3 (lane 3–8), we can suggest that the cycle efficiency is fairly good. It is worthy to note that the gel electrophoresis was carried out in the TBE buffer (pH = 8.0) and in this situation, the MWNT

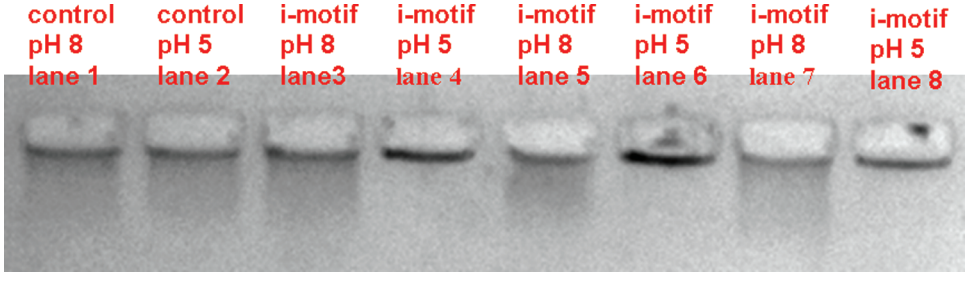

Fig. 3. 0.5% agarose gel electrophoresis analysis of reversible MWNT aggregation-dispersion process by environmental pH change. Lanes 1 and 2 correspond to control DNA-MWNTs conjugates at pH 8 and 5 respectively. Lanes 3–6 correspond to i-motif DNA-MWNTs conjugates at pH 8 and 5 for three cycles.

aggregate will slowly collapse, so it is crucial to load sample and run the agrose gel more quickly and carefully.

After the aggregation happens, DNA-MWNTs conjugates should tend to precipitate from the solution. In addition to the gel electrophoresis experiment, we have also used a centrifugation-assisted precipitation technique[8b] to visually observe the precipitate formed as a result of the aggregation of the DNA-MWNT conjugates as well as the re-dispersion of the as-formed precipitate. Figure 4 (inset pictures) illustrates how the i-motif DNA-MWNT conjugates perform after centrifuging at 2000 g for five minutes at pH 5 and pH 8 respectively. This result reveals the sharp contrast between the dispersed and aggregated states of the DNA-MWNT conjugates.

To further investigate the DNA-MWNT aggregates formed by i-motif formation, transmission electron microscopy (TEM) technique is used to observe the DNA-MWNT at micro scale. Two samples at pH 8 and 5 were put on transmission electron microscopy grids and imaged respectively. Representative TEM graphs are shown in Figure 4: it is clear to see that the MWNTs are well dispersed at pH 8. And they entangle with each other and assemble into aggregates at pH 5. This finding thus agrees very well with the results from gel electrophoresis and confirms that the observed gel electrophoretic mobility shift indeed results from the MWNTs assembly.

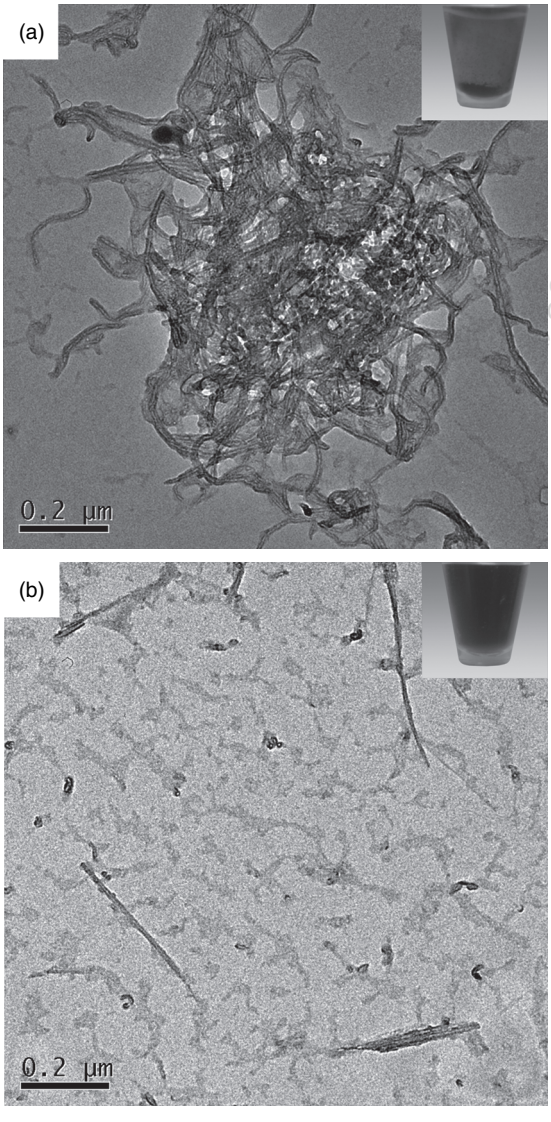

Fig. 4. Representative TEM images of the DNA-MWNTs conjugates at (a) pH 8 and (b) pH 5. Inset pictures show the DNA-MWNTs conjugates centrifuging at 2000 g for five minutes at (a) pH 8 and (b) pH 5.

4. CONCLUSIONS

In conclusion, we have demonstrated that MWNTs modified with ssDNA containing two stretches of C-rich domains can be controllably aggregated by changing environmental pH to 5. The aggregation is driven by the formation of intermolecular i-motif DNA and thus happens only at pHs below the pKa of the i-motif. The aggregation is specific to the two C-rich domains, and if other control DNA takes place of i-motif DNA, there is no formation of intermolecular i-motif structure and no aggregation of MWNTs. The aggregation is reversible and can be switched by changing the environmental pH, and the reverible process can be visualized by centrifugation-assisted precipitation technique. Considering the outstanding physical and chemical properties of carbon nanotubes, if this system is integrated with the new i-motif DNA structure modulating methods,[15] some new electronic and optics devices will be achieved. Due to the reversible process between assembly and disassembly state, our design provides a kinetic two-state transition system which could benefit many application research fields, such as artificial muscle and so on.

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References and Notes

1. H. Dai, *Acc. Chem. Res.* 35, 1035 **(2002)**.
2. M. Ouyang, J. L. Huang, and C. M. Lieber, *Acc. Chem. Res.* 35, 1018 **(2002)**.
3. P. W. Barone and M. S. Strano, *Angew. Chem., Int. Ed.* 45, 8138 **(2006)**.
4. D. A. Heller, E. S. Jeng, T. K. Yeung, B. M. Martinez, A. E. Moll, J. B. Gastala, and M. S. Strano, *Science* 311, 508 **(2006)**.
5. K. Nobusawa, A. Ikeda, J. I. Kikuchi, S. I. Kawano, N. Fujita, and S. Shinkai, *Angew. Chem., Int. Ed.* 47, 4577 **(2006)**.
6. K. C. Etika, F. D. Jochum, P. Theato, and J. C. Grunlan, *J. Am. Chem. Soc.* 131, 13598 **(2009)**.
7. M. J. Pender, L. A. Soward, J. D. Hartgerink, M. O. Stone, and R. R. Naik, *Nano Lett.* 6, 40 **(2006)**.
8. (a) Y. Chen, H. Liu, T. Ye, J. Kim, and C. Mao, *J. Am. Chem. Soc.* 129, 8696 **(2007)**; (b) Y. Li, X. Han, and Z. Deng, *Angew. Chem., Int. Ed.* 46, 1 **(2007)**.

9. M. Zheng, A. Jaagota, E. D. Semke, B. A. Diner, R. S. Mclean, S. R. Lustig, R. E. Richardson, and N. G. Tassi, *Nat Mat.* 2, 338 **(2003)**.
10. D. Liu and S. Balasubramanian, *Angew. Chem., Int. Ed.* 42, 5734 **(2003)**.
11. (a) H. Liu and D. Liu, *Chem. Comm.* 2625 **(2009)**; (b) S. Wang, H. Liu, D. Liu, X. Ma, X. Fang, and L. Jiang, *Angew. Chem., Int. Ed.* 46, 3915 **(2007)**; (c) Y. Mao, D. Liu, S. Wang, S. Luo, W. Wang, Y. Yang, Q. Ouyang, and L. Jiang, *Nucleic Acids Res.* 35, e33 **(2007)**; (d) W. Shu, D. Liu, M. Watari, C. K. Riener, T. Strunz, M. E. Welland, S. Balasubramanian, and R. A. McKendry, *J. Am. Chem. Soc.* 127, 17054 **(2005)**.
12. W. Wang, H. Liu, D. Liu, Y. Xu, Y. Yang, and D. Zhou, *Langmuir* 23, 11956 **(2007)**.
13. E. Cheng, Y. Xing, P. Chen, Y. Yang, Y. Sun, D. Zhou, L. Xu, Q. Fan, and D. Liu, *Angew. Chem., Int. Ed.* 48, 7660 **(2009)**.
14. D. Zanchet, C. M. Micheel, W. J. Parak, D. Gerion, and A. P. Alivisatos, *Nano Lett.* 1, 32 **(2001)**.
15. H. Liu, Y. Xu, F. Li, Y. Yang, W. Wang, Y. Song, and D. Liu, *Angew. Chem., Int. Ed.* 46, 2515 **(2007)**.

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