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Water-Solubilization of Nucleotides-Coated Single-Walled Carbon Nanotubes Using a High-Speed Vibration Milling Technique

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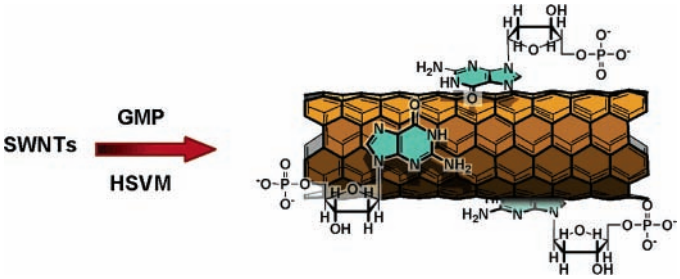
ABSTRACT



Pristine single-walled carbon nanotubes (SWNTs) and nucleotides mixed using a mechanochemical high-speed vibration milling technique (HSVM) are soluble in an aqueous solution, and the solubilities of SWNTs depend significantly on the number of phosphate groups and the kinds of bases employed.

The easy availability of single-wall carbon nanotubes (SWNTs) has spurred and sustained exploration of their outstanding unique physical and chemical properties.[1,2] However, despite their vast potential, applications have remained extremely limited because of the difficulty in dissolving them in an aqueous solution. Several lines of effort have been devoted to compensation for this drawback through covalent functionalization using hydrophilic substituents,[3] mixing with water-soluble polymers,[4] and chemical or mechanical cutting.[5,6] We have reported higher solubilization and debundling of purified SWNTs[5] from the formation of SWNT·cyclodextrin (CD) complexes using a mechanochemical high-speed vibration milling technique (HSVM).[7,8] The purified SWNTs, which were cut by

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ultrasonication in strong acid, introduce oxygen-containing groups such as phenolic hydroxides and carboxylic acids.[5] Formation of a hydrogen bond between the SWNTs and CDs was important for SWNTs solubilization. Thereby, the pristine SWNTs were only slightly dissolved in an aqueous solution by CDs using a HSVM because of few oxygen-containing groups in the SWNTs surface and the length of unshortened SWNTs. Herein, we employed various nucleotides as solubilizing agents not only because of high water-solubility and lack of toxicity but also because of their potential for hydrophobic and $\pi-\pi$ interactions[9] between their bases and SWNT surfaces.

Mixtures of pristine SWNTs (1.00 mg)[10] and nucleotides (0.67 mmol) were placed in an agate capsule together with two agate mixing balls. This study used each of the following nucleotides, adenosine 5′-monophosphate (AMP), adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP), guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP), and uridine 5′-monophosphate (UMP), and ribose 5-phosphate (Ribose 5-P) as solubilizing agents. The mixtures were tested for their solublity of nanotubes and for the spectral properties of those suspensions. They were mixed vigorously by mixing at 1800 rpm for 20 min using a high-speed vibration mill (MM200, Retsch Co. Ltd.). The solid mixtures were dissolved in 1.0 mL of an aqueous solution to produce a black emulsion. After centrifugation (8000 rpm, 10 min, 20 °C), all nondispersed SWNTs were removed from the solutions (Figure 1a−h). Measurements using UV−vis

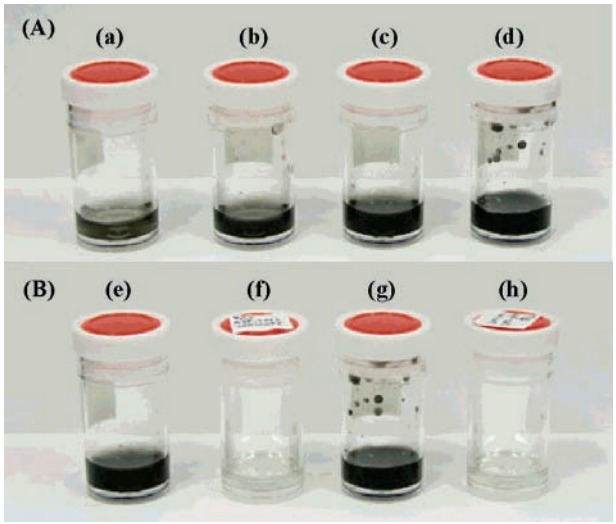

Figure 1. Photographs of SWNTs in an aqueous solution (A) after HSVM in the presence of (a) AMP, (b) ADP, (c) ATP, (d) GMP, and (B) (e) and (g) after HSVM and (f) and (h) after sonication in the presence of (e) and (f) ATP, and (g) and (h) GMP.

absorption, near-IR, and Raman spectroscopy evidenced the presence of SWNTs in the aqueous solutions. Figure 2 shows the UV−vis absorption spectra of these solutions. Solubilities of the SWNTs were determined by measuring the absorbance of their solutions at 500 nm and by using a specific extinction

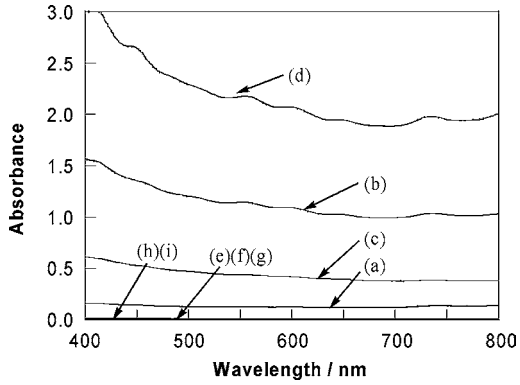

Figure 2. UV−vis absorption spectra of SWNTs in an aqueous solution after HSVM in the presence of (a) AMP, (b) ADP, (c) ATP, (d) GMP, (e) CMP, (f) UMP, and (g) ribose 5-P and after sonication in the presence of (h) ATP and (i) GMP at 25 °C (1 mm cell).

coefficient for SWNTs of $\epsilon_{500} = 2.86 \times 10^4$ cm^2 g^{-1}.[11] Table 1 summarizes these results. Figure S1 shows near-IR spectra of the ADP-SWNT, ATP-SWNT, and GMP-SWNT solutions. The characteristic absorption band corresponding to the van Hove singularities are apparent in both the 800 and 1400 nm regions, which are fundamentally similar to those of the reported spectra.[4i−k] Moreover, in the Raman spectra, these samples have nearly identical sharp peaks with a shoulder near the high frequency of 1588 cm^{-1}. They are assigned to the tangential modes of the graphite (Figure S2).[4i−k] The low-frequency range of 160−200 cm^{-1} is the radial breathing modes[4i−k] whose frequencies are dependent on the tube diameter. No recognizable difference exists between them. These spectral data indicate solubilization of SWNTs by several nucleotides.

First, AMP, ADP, and ATP were chosen as solubilizing agents to estimate the effects of various phosphates of these adenylic acids on SWNT solubility. The order of SWNTs solubility is ADP > ATP > AMP (SWNT solubilities 0.38, 0.16, and 0.04 g dm^{-3}). This observation leads to the consideration that the resultant mixtures have good solubili-

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(10) We purchased the SWNTs used in this study from Carbon Nanotechnologies Inc., TX.

(11) Concentrations of SWNTs were determined using a specific extinction coefficient for pristine SWNTs of $\epsilon_{500} = 2.86 \times 10^4$ cm^2 g^{-1}. Bahr, J. L.; Mickelson, E T.; Bronikowski, M. J.; Smalley, R. E.; Tour, F. M. *Chem. Commun.* **2001**, 193.

Table 1. Absorbance and Solubility (g dm^{-3}) of SWNTs in an Aqueous Solution by HSVM and Sonication with Solubilizing Agents

solubilizing agent[a]	methods	A_{500}[b,c]	SWNTs solubilities[c,d] (g dm^{-3}) (extractability)
AMP	HSVM	0.12	0.04 (4%)
ADP	HSVM	1.10	0.38 (38%)
ATP	HSVM	0.45	0.16 (16%)
ATP	sonication	0.01	0.00 (0%)
GMP	HSVM	2.23	0.78 (78%)
GMP	sonication	0.02	0.01 (1%)
CMP	HSVM	0.00	0.00 (0%)
UMP	HSVM	0.00	0.00 (0%)
ribose 5-P	HSVM	0.00	0.00 (0%)

[a] [solubilizing agent] = 0.67 mol dm^{-3}. [b] 1 mm cell; saturated solution; 25 °C. [c] Each experimant was carried out three times. [d] $\epsilon_{500} = 2.86 \times 10^4$ cm^2 g^{-1}.

ties due to the hydrophilicity of the solubilizing reagents. However, the reason for the highest solubilities in ADP is not clear at this time. Results confirmed by ^{31}P NMR spectroscopy show that ADP and ATP did not decompose to AMP after a HSVM (Figure S3). In contrast, an experiment using adenosine without a phosphate group was impossible under the same conditions because of the poor solubility of adenosine itself in an aqueous solution. These results show definitively that phosphate groups in solubilizing agents are required to solubilize SWNTs in an aqueous solution.

Second, AMP, GMP, CMP, and UMP were chosen as solubilizing agents to estimate the effect of different kinds of bases on SWNT solubility. Among these nucleoside monophosphates (NMP), AMP and GMP with purine components showed moderately high solubility of SWNTs (solubilities 0.04 and 0.78 g dm^{-3}). On the other hand, CMP and UMP with pyrimidine components hardly extracted any SWNTs (solubilities each 0.00 g dm^{-3}). Although this trend was apparent in the formations of tetrakis(4-*N*-methylpyridyl)porphyrine-nucleotide complexes and ethidium-deoxynucleotide complexes,[12,13] the association constants between purines and pyrimidines are much more similar compared with that of our system. The greatly different solubilities of SWNTs between pyrimidine and purine are attributable to the relative face-to-face $\pi-\pi$ stacking ability of the natural bases. For example, the relative stacking ability is qualitatively in the order purine-purine > pyrimidine-purine > pyrimidine-pyrimidine.[14] Moreover, ribose 5-P, without a base moiety, did not extract SWNTs (solubility 0.00 g dm^{-3}). These results indicate that bases in nucleotides play important roles in the interaction between SWNTs and nucleotides. Detail of the interaction between nucleotides and SWNTs are described below.

Finally, the solubilities of SWNTs by HSVM for 20 min were compared with those by sonication for 2 h.[15] The aqueous solutions of SWNTs by HSVM were obtained in the higher solubilities and in shorter time periods than those by sonication {ATP 0.16 (by HSVM) and 0.00 g dm^{-3} (by sonication); GMP 0.78 (by HSVM) and 0.01 g dm^{-3} (by sonication)}. These results indicate that the water-solubilization of SWNTs by HSVM is superior to that by sonication.

We measured the ^1H NMR spectra of the SWNT·nucleotide complexes in D_2O to further elucidate the interaction between the SWNTs and the nucleotides. In the absence of the SWNTs, the adenine H_2 and H_8 protons of ATP appeared as singlet peaks at 8.40 and 8.60 ppm in D_2O (Figures 3 and S4a). The peaks were shifted to higher

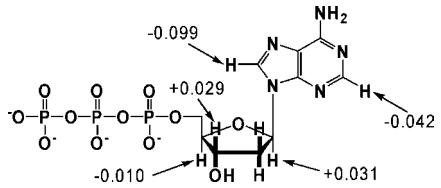

Figure 3. ^1H NMR chemical shift changes of ATP according to the addition of SWNTs in an aqueous solution (D_2O) at 25 °C: [ATP] = 5.5×10^{-3} mol dm^{-3}, [SWNT] = 0.04 g dm^{-3}, 600 MHz). The + sign denotes a downfield shift, and the − sign denotes an upfield shift (in ppm).

magnetic fields (8.30 and 8.56 ppm; $\Delta\delta = -0.099$ and -0.042 ppm, respectively) in the presence of the SWNTs (Figures 3 and S4b). On the other hand, no significant changes or lower magnetic field shifts are observed for the D-ribose H_1', H_2', H_3', H_4', and H_5' protons ($\Delta\delta = -0.10$ to $+0.31$ ppm). These results strongly support the consideration that the formation of hydrophobic and $\pi-\pi$ interactions occurs between adenine moiety and SWNT surfaces.

Despite further addition of ATP, signals for free ATP and those for the complex did not appear separately; for example, the peaks for the adenine H_2 and H_8 appeared between those of free and complex species (8.34 and 8.59 ppm, respectively). These results strongly indicate that the complexation-decomplexation exchange occurs more rapidly than the ^1H NMR time-scale at room temperature and that the SWNT·ATP complex does not form covalent bonds between the NH_2 moiety of adenine and the SWNTs surface by HSVM (Figure S2c).

Morphology of the SWNTs was observed by TEM (Figure 4a−d) after washing on the grid with ultrapure water.[16] Figure 4a and 4c show TEM micrographs in the region in

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(15) Dispersions were typically formed by adding the pristine SWNTs (1.00 mg) and nucleotides (0.67 mmol) and then 1.0 mL of an aqueous solution to a 10 mL glass vial. The solution was sonicated using an ultrasonic bath (180 W, 42 kHz, 5510 Branson Ultrasonic Corp.) for 2 h. Samples were then centrifuged in an microcentrifuge (Eppendorf AG) for 10 min at 8000 rpm.

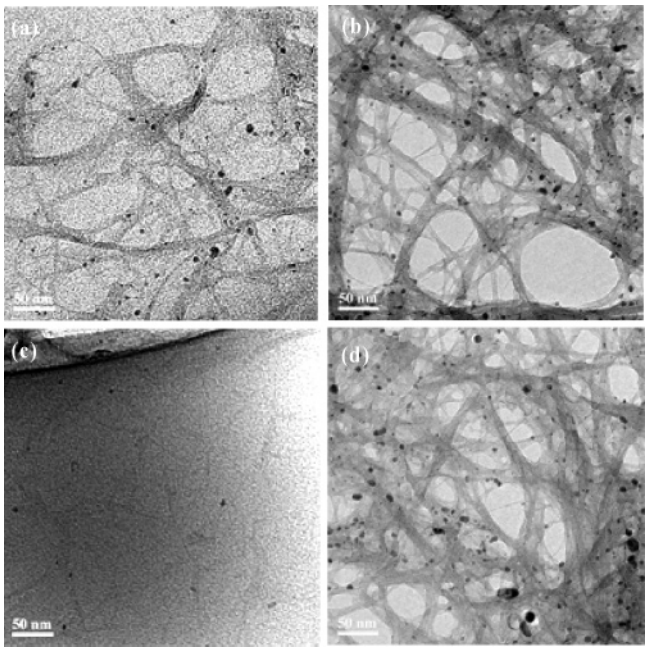

Figure 4. TEM images of SWNTs: (a) and (b) the SWNT·ATP complex; (c) and (d) the SWNT·GMP complex; (a) and (c) the region in which ATP and GMP remained; (b) and (d) the region in which ATP and GMP only slightly remained.

which the ATP and GMP remained; Figure 4b and 4d show those in the region where the ATP and GMP only slightly remained. Figure 4a shows that a TEM micrograph in which the SWNT·ATP complex with 10−30 nm mean diameter is visible. In addition, SWNT·GMP complexes with a mean diameter of around 3 nm are visible in the TEM micrographs (Figure 4c), indicating that some SWNTs were well dispersed in an aqueous solution and were debundled. These different morphologies of the SWNT·AMP and the SWNT·GMP complexes are attributable to the differences of interaction between the base and the surface of SWNTs and engender the different solubilities of SWNTs. The low contrast of the TEM micrographs is attributable to the SWNTs' existence in the membranes of the excess ATP or GMP. On the other hand, Figure 4b and 4d show that the SWNTs appear in bundles with diameters of 10−50 and 20−30 nm, respectively, indicating that the absence of ATP or GMP coating the surface of SWNTs leads to the formation of large bundles. In Figure 4c, shortened SWNTs in the SWNT·GMP complex were observed. Whether the observation of short pieces results from "soft cutting"[6] or from debundling remains unclear.

In conclusion, the present study has dissolved pristine SWNTs in an aqueous solution with nucleotides as solubilizing agents using HSVM. Dramatic influences on the solubilities of SWNTs were apparent when the number of phosphate groups and the kinds of bases were changed. In particular, the SWNT·GMP complex can be dissolved in an aqueous solution at a concentration of 0.78 g dm^{-3} and a part of SWNTs was debundled. We suggest that HSVM is extremely useful for simple preparations of the solubilization of SWNTs in an aqueous solution and that debundling of SWNTs is extremely important for material preparation. These studies, which are currently under investigation in this laboratory, should be extended further to other solubilizing agents using HSVM.

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Supporting Information Available: Near-IR spectra of the ADP-SWNT, ATP-SWNT, and GMP-SWNT solutions; Raman spectra of the ADP-SWNT, ATP-SWNT, and GM-SWNT solutions; ^{31}P NMR spectra of ATP before and after HSWV; and ^{1}H NMR spectra of ATP and the SWNT·ATP complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The SWNT·ATP and SWNT·GMP complexes contain metal catalyst because of the use of pristin SWNTs. According to ref 17, we attempted to treat the SWNT·GMP complex with dilute HCl to remove Fe but failed. The SWNT·GMP complex produced a viscous precipitation on addition of HCl because of a decomposition of GMP by an acidic condition.

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