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Dispersion of Carbon Nanotubes in Water by Noncovalent Wrapping with Peptides Screened by Phage Display

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12-mer peptides, which were originally identified by affinity screening for carbon nanotubes (CNTs) and poly(phenylenevinylene) (PPV) from phage-displayed peptide libraries, were used as dispersants for single-walled CNTs (SWNTs). The amounts dispersed were then compared under the same experimental conditions. Among the various peptides, Mps01 (HNAYWHWPPSMT) showed the highest dispersion capability. Gly scanning revealed that the Trp and Pro residues were crucial for dispersion. Atomic force microscopy (AFM) confirmed that the majority of dispersed SWNTs were debundled and isolated via Mps01 wrapping. AFM height analysis also suggested that the majority of SWNTs were covered by a monolayer of Mps01.

Carbon nanotubes (CNTs)[1] are fascinating materials with remarkable mechanical[2] and electrical[3] properties. These properties make them promising candidates for a large number of applications including health, energy, and the environment.[4] As-synthesized CNTs are difficult to dissolve in any solvent due to bundling caused by strong van der Waals interactions between the CNT sidewalls, which often hinder these applications. Therefore, the solubilization of CNTs has been achieved either by covalent functionalization of the surface or by the noncovalent attachment of appropriate dispersants.[5] Both approaches have their merits and limitations.[6] Recent noncovalent approaches have involved the adsorption of functional molecules including surfactants,[3a] polycyclic aromatic compounds,[7] polymers,[8] carbohydrates,[9] DNA,[10] proteins,[11] and peptides,[12] which led to debundling and suspension of the CNTs.

Regarding the noncovalent binding of peptides, de novo design[12a–12e] and biological screening[12f–12i] have been used, and the possible interactions between the peptides and CNTs have been discussed. For the identification of the latter peptides, phage-displayed peptide libraries have been used for the affinity screening of the desired peptides for CNTs.[12f–12h,13] However, it is still unclear which peptide is the most efficient for the aqueous dispersion of CNTs due to the lack of comparative studies covering these screened peptides. Since the biological screening process selects only high-affinity binders, the dispersing capabilities of the selected peptides should be assessed separately without confounding the binding abilities. In addition, a comparison of the dispersing capabilities under identical experimental conditions would be informative for assigning the potentials of screened peptides.

Interestingly, our previous study revealed that the amino acid sequences of poly(phenylenevinylene) (PPV)-binding peptides screened from phage-displayed peptide libraries partly shared the sequences already identified for CNTs.[14] This might be reasonable, because PPV and CNTs have similar molecular structures enriched with π-conjugated carbons. This observation strongly prompted us to evaluate the CNT-dispersing capabilities of PPV-binding peptides as alternatives to CNT-binding peptides.

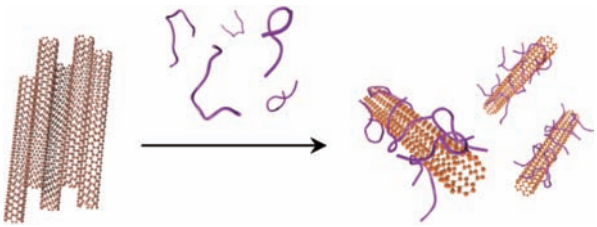

Figure 1. Schematic illustration of SWNT dispersion by noncovalent peptide wrapping.

In this study, we quantitatively analyzed dispersions of single-walled CNTs (SWNTs) with CNT- and PPV-binding peptides under identical experimental conditions (Figure 1), and the best dispersing system was further characterized in detail. Seven possible 12-mer peptides were examined, and were originally identified by affinity selection from phage-displayed peptide-libraries (for the sequences see Figure 2d). Mps01[15] and Hyp04[12g,14] have been identified for both CNTs and PPV. B3[12f] and P1[12h] are known to be CNT-binding peptides. Hyp01,[14] Lin01,[14] and Mps02[15b] are all known to be PPV-binding peptides. The common properties of these peptides are that they are rich in aromatic amino acids, which are anticipated to bind to the CNTs through π–π stacking interactions.

A typical experimental procedure is briefly described below (also see Supporting Information[16]). The HiPco SWNTs were weighed in a glass vial (1.0 ± 0.1 mg), and 1 mL of an aqueous solution of Mps01 (1 mM) was added. The resulting solution was gently sonicated for 1 h in a normal bath-type ultrasonic cleaner at 25 ± 5 °C. During the sonication, the aqueous solution changed from colorless to black (Figure 2a), indicating the solubilization of the SWNTs. After sonication, the solution was centrifuged until no precipitate could be found (20000 *g*, four times 15 min, total 60 min). The supernatant was homogenous and its color was still gray (Figure 2b), suggesting that the dispersion of the SWNTs was successfully achieved.

To further investigate this Mps01/SWNT dispersion, the visible–near infrared (vis–NIR) absorption spectra of the super-

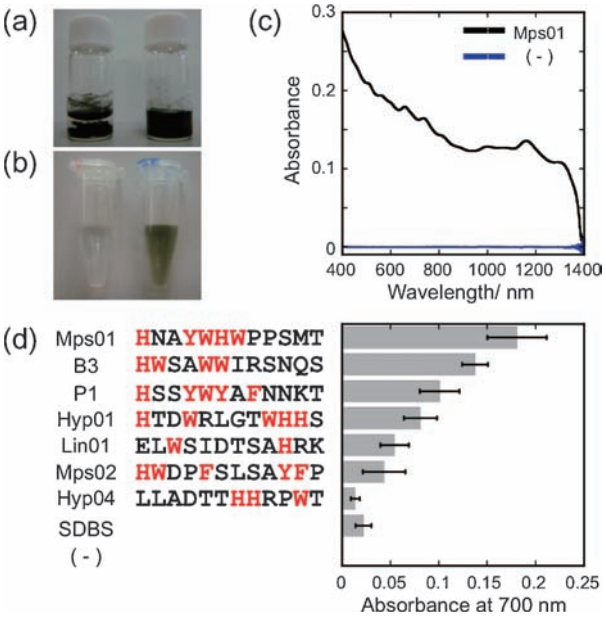

Figure 2. Dispersion of SWNTs in water via peptide wrapping. (a, b) Optical micrographs of the SWNT dispersions in the absence (left) and presence (right) of Mps01 (1 mM); (a) before and (b) after centrifugation. (c) Representative absorption spectra of the supernatants of SWNT dispersions prepared in the absence or presence of Mps01 (100 μM). (d) The name and sequence of the peptides and the dispersed amounts, which were determined from the absorbance at 700 nm. The concentrations of all peptides and SDBS were 100 and 438 μM, respectively. The aromatic amino acids are colored in red. The results represent average values with standard deviations from three independent measurements (mean ± SD).

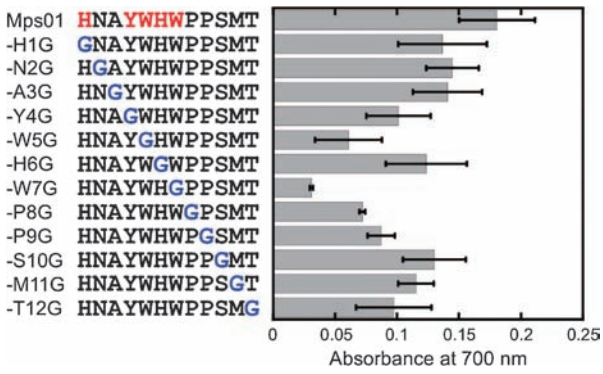

Figure 3. Results of the Gly-scanning experiments. The amount dispersed was determined from the absorbance at 700 nm when each Gly mutant of the Mps01 peptide was used as a dispersant. The results represent average values with standard deviations from three independent measurements (mean ± SD).

natant solutions were measured, as shown in Figure 2c. Typical absorption spectra for the SWNTs were then obtained. The multiple peaks in the absorption spectra can be attributed to existence of multitudinous SWNT species with different chiralities.[3b] In contrast, in the absence of the Mps01, no absorption derived from the SWNTs was observed (Figure 2c). Similarly, we prepared SWNTs solutions with various peptidyl dispersants (100 μM) and then compared the amounts dispersed (Figure 2d). Since the absorption intensity is proportional to the amount of SWNTs dispersed,[3] the absorbance at 700 nm was used as a measure of the SWNT concentration.[17] The order of dispersing capability was Mps01 > B3 > P1 > Hyp01 > Lin01 > Mps02 > SDBS > Hyp04. In fact, Mps01 showed a 13-fold greater dispersing capability than Hyp04. The amount dispersed when Mps01 was used as a dispersant was 10-fold greater than that of sodium dodecyl benzenesulfonate (SDBS) as a positive control. In this experiment, the concentration of SDBS was 438 μM (which corresponds to the same wt % as 100 μM Mps01). Notably, the critical micelle concentration (CMC) of SDBS is known to be ca. 2 mM,[18] and the dispersing capability of SDBS tends to decrease below the CMC. However, at such a dilute concentration range, many SWNTs were successfully dispersed when Mps01 was used as a dispersant.

It is widely recognized that aromatic amino acids are important for the interactions between the SWNTs and the peptide through π–π stacking interactions.[12c] Dieckmann and co-workers clearly showed that Trp, which has the largest aromatic surface among the natural amino acids, was particularly important for efficient dispersion.[12e] All of the peptides used in this study contained more than one Trp residue. However, the dispersing capabilities are not strictly correlated with the number of Trp or aromatic residues. This is probably because the order and spatial location of the aromatic amino acids are another important factor. Surprisingly, Hyp01 and Lin01, which were only selected in the biopanning targeted for PPV, showed practical dispersing capabilities greater than that of Hyp04, which is a known CNT-binding peptide. This result clearly shows that PPV-binding peptides can be alternatives to CNT-binding peptides in terms of SWNT dispersion in water.

Next, we evaluated the dispersion stability by recording the absorbance intensities at 700 nm over the course of 60 days (Figure S1[16]). The peptides with high dispersing capabilities (Mps01, B3, P1, and Hyp01) showed long-term stability; ca. 80% of the initially dispersed SWNTs remained even after 1 month standing at ambient temperature. On the other hand, peptides with relatively low dispersing capabilities (Lin01, Mps02, and Hyp04) led to complete precipitation within 1 month. These results suggest that the wrapping of the SWNT surface with these peptides was unstable or that these peptides tended to self-aggregate in water, followed by the precipitation of the dispersed SWNTs.

To investigate the contribution of each amino acid in the best sequence to the dispersing capability, Gly scanning of Mps01 was carried out (Figure 3). H1G denotes that a His residue located at 1st position from the N-terminus (H1) was substituted with a Gly. In those cases where H1G, N2G, A3G, H6G, and S10G were used as the dispersants, the amounts dispersed were equivalent to that of the original Mps01 within experimental error. These results indicated that H1, N2, A3, H6, and S10 were not essential for the water-dispersing capability of Mps01. However, the other seven substitutions (Y4G, W5G, W7G, P8G, P9G, M11G, and T12G) clearly decreased the amount dispersed, suggesting that these amino acids are important for the water-dispersing capability. In particular, when W5 and W7 were substituted with Gly, the SWNTs dispersed in water were clearly decreased. Therefore, the two Trp residues in the Mps01 sequence played a critical role in the

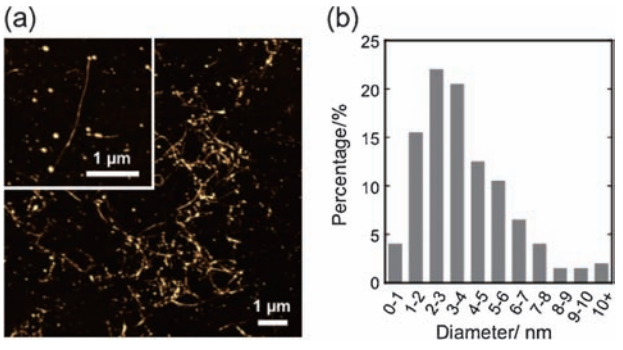

Figure 4. AFM measurements of Mps01/SWNTs. (a) AFM images of Mps01/SWNTs deposited on a mica surface. (b) Diameter distributions determined from the AFM height measurements ($N = 200$).

water-dispersing capability as expected from previous reports.[12e] Interestingly, in addition to the Trp residues, P8G and P9G also apparently reduced the amount dispersed. Pro often regulates the conformation of peptides by introducing a kinked structure into the backbones, implying the importance of the peptide's conformation for efficient dispersing capabilities.

From circular dichroism (CD) spectrometry observations (Figure S2[16]), it was suggested that Mps01, B3, P1, Hyp01, and Mps02 partially adopted specific conformations including α-helix, β-turn, and β-strand, while Lin01 and Hyp04 had a random coil conformation.[19,20] The peptides with specific conformations were likely to exhibit a higher dispersing capability, except for Mps02, probably due to the entropic benefit of preorganization during the binding event. These observations suggest that the binding capability is closely related to the dispersing capability.

Mps01-wrapped SWNTs were deposited on a mica surface and imaged with atomic force microscopy (AFM). From the AFM image, the majority of the SWNTs were isolated and debundled (Figure 4a). The heights of 200 SWNTs were then measured and analyzed statistically (Figure 4b). Fitting the data to a Poisson distribution revealed a diameter of 3.5 ± 1.9 nm (mean $\pm$ SD). Assuming that the HiPco SWNTs had an average diameter of 1.1 nm,[21] the mean thickness of the peptide layer was estimated to be 1.2 nm, which is approximately equal to the expected thickness for a monolayer of extended Mps01. This highly homogeneous coating of Mps01 led to an efficient and stable dispersion of the SWNTs in water.

In conclusion, we quantitatively analyzed the aqueous dispersion of SWNTs with 12-mer peptides, originally screened via phage display, under the same experimental conditions. Although all of the peptides were enriched in aromatic amino acids, each peptide exhibited completely different dispersing capabilities. Thus, it was proposed that not only the aromatic amino acid content but also the conformation of the peptides in water are important factors for the dispersing capability. The best dispersant, Mps01, was investigated in detail by a Gly-scanning experiment, supporting the importance of the Trp and Pro residues. AFM observations revealed that the SWNTs were sufficiently isolated by Mps01 wrapping. Fusing bioactive molecules to better peptides via chemical reactions or a genetically encoding method will enable further desired modifications of SWNTs in the near future.

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