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Coating Single-Walled Carbon Nanotubes with Phospholipids

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Single-walled carbon nanotubes (SWNTs), being hydrophobic by nature, aggregate in water to form large bundles. However, isolated SWNTs possess unique physical and chemical properties that are desirable for sensing and biological applications. Conventionally isolated SWNTs can be obtained by wrapping the tubes with biopolymers or surfactants. The binding modes proposed for these solubilization schemes, however, are less than comprehensive. Here we characterize the efficacies of solubilizing SWNTs through various types of phospholipids and other amphiphilic surfactants. Specifically, we demonstrate that lysophospholipids, or single-chained phospholipids offer unprecedented solubility for SWNTs, while double-chained phospholipids are ineffective in rendering SWNTs soluble. Using transmission electron microscopy (TEM) we show that lysophospholipids wrap SWNTs as striations whose size and regularity are affected by the polarity of the lysophospholipids. We further show that wrapping is only observed when SWNTs are in the lipid phase and not the vacuum phase, suggesting that the environment has a pertinent role in the binding process. Our findings shed light on the debate over the binding mechanism of amphiphilic polymers and cylindrical nanostructures and have implications on the design of novel supramolecular complexes and nanodevices.

Introduction

SWNTs are broadly used in such applications as nanoelectronics, chemical and biosensing, and mostly recently in gene and drug delivery.[1−12] However, the bottleneck for many of these applications that require water phase is the hydrophobicity of SWNTs.[13−15] Schemes to overcome hydrophobicity include covalent binding of organic molecules to SWNT surfaces and noncovalent wrapping of SWNTs by surfactants, synthetic molecules, or biopolymers.[13−16] In particular regard to the noncovalent schemes, the hydrophobic and the hydrophilic moieties of the attached molecules interact with the tube surfaces and the solution, respectively. These arrangements minimize alterations to the SWNT properties and allow for the SWNT solubility to be controlled by the parameters (temperature, pH, etc.) of the environment. For example, SWNTs may be disposed in a particular cell compartment if the conditions therein weaken the affinity of the organic molecules for the tubes.

It has been proposed that, when solubilizing SWNTs with lipids or other amphiphilic surfactants, the tubes may either form the core of cylindrical micelles of the surfactants or become adsorbed by the surfactants in either a hemimicellar or a randomly distributed form.[17−20] However, these models may be oversimplified since they do not consider the polarity and the number of the surfactant tails. These two aspects, however, are directly related to the physical characteristics and geometrical properties of the surfactants and thus may affect their binding mode and affinity for SWNTs. In this paper we investigate these issues using a variety of phospholipids and surfactants.

Another external factor that may affect the binding of surfactants to SWNT is the environment. While the binding experiments are usually performed in solution, SWNT−surfactant complexes have often been visualized by electron microscopy under vacuum conditions.[19,21] Thus, it is unclear to what extent the binding mode revealed in vacuum corresponds to that in solution; a previous TEM study found that lipids wrapped around nanotubes or nanotube bundles in striations.[19] Herein we show that this observation is true in the lipid phase; however, nanotubes are bare in the vacuum phase (definitions see section "binding mode"). We further show that striation coating of SWNTs is not universal for all lipids but is dependent on their polarity and geometry.

Experimental Section

Solubility Assays. Pristine SWNTs were synthesized using the arc-deposition method.[22] The average diameter of the SWNTs was approximately 1.4 nm measured by Raman spectroscopy, and the average molecular weight (MW) of the SWNTs was 1×10^6 Dalton (Da) estimated from TEM. Glycerol phospholipids and lysoglycerophopholipids were purchased from Avanti. SWNTs of 1 mg were placed in eppendorf tubes containing lysophosphatidylcholine LPC 18:0, dimyristoyl phosphatidyl choline PC 24:0, 1,2-dioleoylphosphatidylglycerol PG 36:2, and 1,2-dipalmityolphosphatidylethanolamine PE 32:0 of

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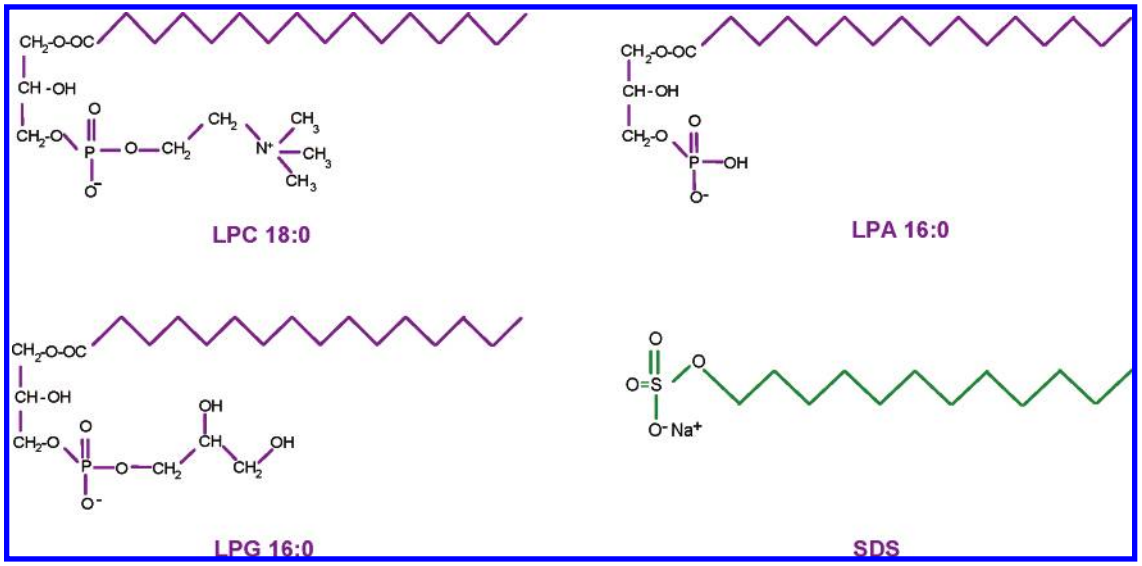

Figure 1. Structures of lysophospholipids LPC 18:0, LPA 16:0, and LPG 16:0 and surfactant SDS. The numbers "18" and "0" in LPC 18:0 denote the total number of carbon atoms and the total number of double bonds contained in the sum of the fatty acyl chains, respectively.

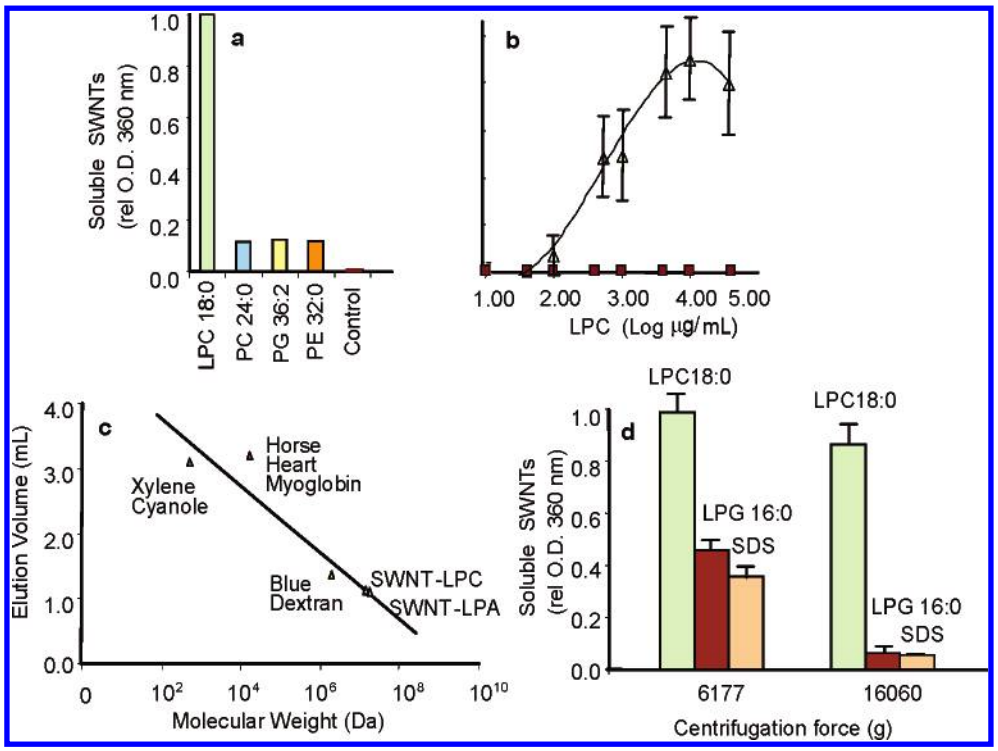

Figure 2. Characteristics of SWNTs solubilized with phospholipids. (a) Comparison of SWNT solubility with different aqueous phospholipid solutions. The label for the vertical axis "rel O.D." denotes relative optical density. (b) LPC solubilized SWNTs. Filled triangles: SWNTs with LPC; filled squares: LPC alone as a control for micelles. A control of LPC alone showed negligible absorbance at 360 nm. (c) Size exclusion chromatography measurements of SWNT−LPC 18:0 (centrifuged at 16 060 g for 3 min) and SWNT−LPA 16:0 complexes (centrifuged at 6177 g for 3 min). A linear curve between compound molecular weight in log scale and elution volume in mL was established using xylene cyanole FF with blue color (538.6 Da), horse heart myoglobin with reddish color (16951.5 Da), and blue dextran with blue color (2×10^6 Da). (d) Comparison of SWNT solubility in LPC, LPG, and SDS solutions.

10, 40, 100, 400 μg and 1, 4, 10, 40 mg in 1 mL phosphate buffered saline (PBS, pH = 7.4) solution. The eppendorf tubes were sonicated in a water bath for 1 h at room temperature. The solutions were centrifuged for 3 min at 6177 g RCF at the tip. Approximately 0.3 mL of the supernatant was transferred into a cuvette and read for optical density against a blank at 360 nm.

Size Exclusion Chromatography. Sepharose CL-4B beads (Sigma) having an exclusion limit MW of 20×10^6 Da were used for gel filtration chromatography in a 10×150 mm open column. The flow rate by gravity was approximately 0.5 mL/min. Samples in PBS of 40 μL were loaded into the column with 1 M acetic acid as the mobile phase. Molecular weight markers with visible colors were used for calibration. The lysophospholipid dispersed SWNTs were monitored visually by comparing their elution volume with those of standard compounds.

TEM Experiment. Buffered solutions of SWNT−LPC and SWNT−LPG were sonicated for 1 min. The solutions were placed on holey carbon grids for 1 min and the excess drawn off with filter paper. The grid was negatively stained with a 2% uranyl acetate solution for 1 min. The images were recorded

at magnification ranges from 400 000× to 600 000× with a Hitachi 7600 transmission electron microscope at 100 and 120 kV.

Results and Discussion

Solubility of SWNTs with Different Lipids. To measure the solubility provided by pure phospholipids, SWNTs were dispersed in PBS containing phospholipids of varying amounts. The concentrations of the lipids were kept above their CMCs (~1 mg/mL) for stable coating of the SWNTs. After sonication for 1 h at room temperature, SWNTs were found completely solubilized by LPC 18:0 (Figure 1). The weight ratio of solubilized SWNTs to LPC was approximately 1:10 corresponding to a molar ratio of 1:20 000 at saturation based on their molecular weights (Figure 2b), indicating the high binding capacity of SWNTs. Comparable solubility of SWNTs was also obtained with lysophosphatidic acid, LPA 16:0, and lysophosphatidylglycerol, LPG 18:0 (Figure 1), based on the same treatments.

From the elution volumes measured with chromatography, we estimated that the SWNT-LPC and the SWNT-LPA complexes had an average MW of 14×10^6 Da and 18×10^6 Da, respectively (Figure 2c). Since each SWNT carries approximately 20 000 LPC molecules and the molecular weight of each LPC is 523.7 Da, the "MW" of each SWNT−LPC complex is estimated at 11.5×10^6 Da, in agreement with our measured value of 14×10^6 Da. The discrepancy is mainly caused by the heterogeneous size distribution of the SWNTs and the occurrence of small SWNT bundles.

A comparison of SWNT solubility is given in Figure 2d for LPC, LPG, and surfactant sodium dodecyl sulfate or SDS (Figure 1), a routine solvent for SWNTs.[13] At 6177 g, on a per-molecule basis, LPC is approximately 2.5× more effective than SDS in dispersing SWNTs in PBS. At 16 060 g, LPC is approximately one order of magnitude more effective than SDS in dispersing SWNTs. This difference might be because LPC has a bulkier headgroup for interacting with water and a longer acyl chain for binding to SWNTs. The solubility of SWNTs with LPG is slightly better than SDS. In addition, solubilization of SWNTs with lysophospholipids was more effective than with nucleic acids,[11,16,22] and far more effective than with proteins.[3,14] The aqueous SWNT−lysophospholipid solutions were exceptionally stable for months at room temperature, a feature promising for their biological applications.

To investigate the effect of the hydrophobic lipid tail(s) on the ability of the lipids to solubilize SWNTs, we also tested how SWNT solubility changes upon addition of double-chained phospholipids. The phospholipids used are PC 24:0, which is zwitterionic at physiological pH, and PG 36:2 and PE 32:0, both of which are negatively charged at physiological pH. None of the above phospholipids yielded good solubility for SWNTs.

Binding Mode. To probe the mechanism of SWNT−lysophospholipid binding, zwitterionic LPC and net negatively charged LPG at physiological pH were bound to SWNTs and imaged with TEM (Figures 3a−c). One can see the formation of areas of tightly packed lysophospholipids in the dark/gray areas, our termed "lipid phase", in Figures 3a−c. The light/blank areas in Figures 3a−c correspond to lysophospholipid free regions or our termed "vacuum phase". In the lipid phase SWNTs are wrapped by striations of ~5 nm for LPC and 5−7 nm for LPG. Such organizations were previously reported[19] for the binding of SDS and synthetic lipids on carbon nanotubes. However, in the vacuum phase (Figures 3a,c) SWNTs are practically naked, indicating that the binding is controlled by the local lysophospholipid environment rather than by the specific interactions between lysophospholipids and SWNTs.

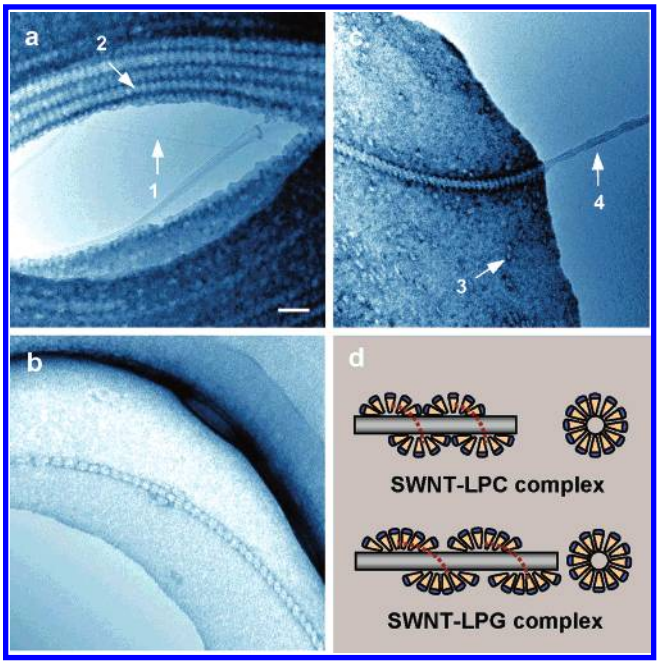

Figure 3. TEM images of SWNT−LPC (a and c) and SWNT−LPG (b) complexes. Numbers 1~4 in a and c correspond to (1) an isolated SWNT in the vacuum phase, (2) an LPC striation on an SWNT/SWNT bundle, (3) possibly an LPC micelle on the substrate in the lipid phase, and (4) an uncoated SWNT bundle in the vacuum phase. Note the less organized and wider striations of SWNT-LPG complexes in (b), as compared to those in (a) and (c) for SWNT−LPC. Scale bar: 20 nm (same for a−c). (d) Hypothesized microscopic binding modes of LPC and LPG with SWNTs. The lysophospholipids are shown as truncated triangles in brown, their headgroups are shown in blue, and the SWNTs in gray. The left section of (d) illustrates the proposed lipid spiral wrapping along the tube axis, while the right section shows their possible binding along the circumference of the tubes.

Neither LPC nor LPG binds to SWNTs in the vacuum phase, while both coat SWNTs in the lipid phase. LPC on a SWNT or a SWNT bundle displays such a consistent pattern along the tube(s) that striations remain approximately the same size (Figures 3a,c). By contrast, the binding of LPG to SWNTs in the lipid phase does not follow the same pattern (Figure 3b). The size and orientation of the striations change along the axis of SWNTs. These differences could be related to the different lysophospholipid organizations shown in their respective backgrounds. It can be seen (Figures 3a,c) that the lipid phase of LPC is composed of many large objects of ~5 nm which probably are micelles, while the lipid phase of LPG is homogeneous, probably composed of individual lysophospholipids. Another major difference in the binding of LPC vs LPG in the lipid phase is the shape of the striations. The crests of LPG striations are about 0.2 nm above the surface of SWNT(s), while the clefts almost touch the surface of SWNT(s) for LPC.

The observed periodic wrapping in the lipid phase strongly suggests that the microscopic binding mode is the "half-cylinders"[19] as illustrated in Figure 3d. Note that the macroscopic arcs formed by lysophospholipids along the SWNT axis are not semi-spherical but extended arcs, because the ends of lysophospholipid tails within an arc cannot occupy the same point and must offset along the tube axis. Furthermore, LPC on SWNTs (Figures 3a,c) exhibit macroscopic spirals possibly resulting from the wrapping of deformed long lipid half-cylinder(s). In some cases, the step of the spiral is equal to the

width of the half-cylinder, and thus a single half-cylinder could provide a complete coating of the tube. In the case of SWNT bundles, we observed binding with a larger step corresponding to double spiral wrapping.[19] We also noticed a ring binding mode and the rings in some cases were tilted with respect to tube axis. The wrapping mode is conserved along the tube as long as the tube diameter remains the same but may change when the bundle size varies (Figure 3a). By contrast, the TEM images with LPG (Figure 3b) exhibit alterations of wrapping mode along the tube axis. In addition, the average width of the LPG striations is noticeably larger than that of LPC (Figure 3b vs Figure 3a) resulting from the net negative charge of LPG headgroups. A macroscopic configuration such that LPG headgroups in consecutive half-cylinders are paired and are in contact with weakly polarizable SWNT surfaces is energetically unfavorable. To reduce the repulsion between the LPG headgroups, the SWNT−LPG system has to pay an energy penalty by distorting and/or broadening the striations (Figure 3d, lower section). Agreeably, our experimental data revealed that LPC bound to SWNTs better than to LPG (Figure 2d).

In addition, our study revealed that different lysophospholipids elucidated dissimilar SWNT solubility. The best result was obtained with zwitterionic LPC, which carries a large dipole moment. Thus, the electro neutrality of LPC permits tight packing or complete coating of a tube surface, while the large dipole moment of LPC favors interaction with water, which yields excellent solubility.

From a geometrical perspective, the three-dimensional (3D) structures of lysophospholipids can be approximated as cones (Figure 3d). Packing of conical objects normally results in spherically shaped objects, e.g., micelles, due to their curvophilicity.[24] By contrast, double-chained phospholipids are considered to be curvophobic; their 3D structures are approximated as cylinders and their packing assumes bilayers. Obviously, wrapping around a cylindrical object, i.e., a SWNT, is geometrically preferential for lysophospholipids but not for phospholipids.

The geometrical considerations provide further support for the microscopic binding mode shown in Figure 3d. "Half-cylinder" wrapping is the only microscopic mode that results in semi-spherical curvature along SWNT axis and along the circumference of SWNT. In addition to the packing consideration, we calculated the average number of LPC needed to coat an average SWNT assuming tight packing and the size of LPC headgroup of 0.6 nm. We found that "half-cylinder" binding results in 21 000:1 lipids/tube−a number that is in excellent agreement with our experimentally estimated ratio of 20 000:1.

Conclusions

Our solubility and TEM studies support the "half-cylinder" model for the binding of lipids to SWNTs, but suggest this model may only account for lipids with a large dipole moment and a conical geometry. We have shown that lysophospholipids provide exceptional solubility for SWNTs with extended stability. The biocompatibility of lysophospholipids is unsurpassed since they occur naturally in the cell membrane. The signaling capacity of lysophopholipids and the electronic property of SWNTs[2] may be combined for biosensing. Since the headgroups of lysophospholipids can be functionalized with antioxidants and monoclonal antibodies, SWNT−lysophospholipids may be utilized for nanomedicine.

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