9 Vesicular and Tubular Nanoassemblies of an Helical Amphiphilic Polyacetylene

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1 INTRODUCTION

Molecular self-assembly of small molecules to ordered macromolecular structures by noncovalent interactions is well known and often found in nature (Whitesides *et al.* 1991), in which hollow tubular structures of molecular sizes provide various biological functions: for example, scaffolding and packaging roles played by cystoskeletal microtubules and viral coat proteins, respectively, as well as the chemical transport and screening activities of membrane channels. Organic tubular assemblies are of interest owing to their numerous possible applications, many of which are obvious from the viewpoints of mimicry of biological systems (Eisengerg, B., 1998, Voges *et al.*, 1998, Zwickl *et al.*, 1999, Sigler *et al.*, 1998, Borgnia *et al.*, 1999). Much research has been focussed on the construction of simpler synthetic tubes for applications such as specific ion sensors, tailored molecular reaction vessels, and molecular sieves.

There are five possible ways for molecules to self-assembly into tubular structures (Bong *et al.*, 2001, Spector *et al.*, 1999, Yu and Eisengerg, 1998, Liang *et al.*, 2000): (1) helical molecules coil to form hollow, folded structures, (2) rod-like molecules assemble in a barrel-stave fashion to form molecular bundles, (3) macrocycles stack to form continuous tubes, (4) sector or wedge-shaped

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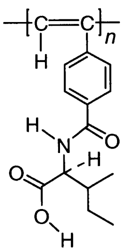
molecules assemble into discs that subsequently stack to form continuous cylinders, similar to macrocycles, and (5) amphiphilic molecules self-assemble into bilayers that organize into helical tubules via fusion of the bilayer or vesicular structures. Employing a biomimetic approach, in this paper, we demonstrate self-association of a nonbiological homopolymer, poly(4-ethynylbenzoyl-L-isoleucine) (**PEBIle**), whose molecular structure is illustrated in Chart 1, into a nanotubular structure. In addition to a vesicular structure, under transmission electron microscopic observation, both the morphologies are confirmed as hollow structures with similar diameters. The co-existence of these two structures reasonably explains their relation in the formation process: assembling of the nanotubes based on linear conjunction of the elementary vesicles. Such a process is comparatively close to the methodology 5 stated above. Further investigation on the nanotubular structure reveals the helical rotating pattern that has been rarely reported in scientific literature.

Chart 1



2 EXPERIMENTAL

PEBIle (M_w ~2.2×10^4) was synthesized by the similar procedures reported in our previous publications (Tang, 2001, Salhi *et al.*, 2000). Self-assembling of the polymer was carried out in an open atmosphere (humidity: 60–70%, temperature: ~20 °C). Each sample was prepared in the same location in the same platform. A given amount of the polymer was first completely dissolved in methanol. After filtration through a 0.1 mm filter to remove dust, the solution was placed onto a substrate, which was located on a flat and vibration-free platform, and the solvent was allowed to naturally evaporate at room temperature. For atomic force microscopy (AFM) analysis, 5 μL of the polymer solution was dropped on the surface of newly cleaved mica. AFM images were then obtained on a Digital Instrument Nano IIIa microscope of Digital Instrument Co. (Santa Babara, California) in a tapping mode using hard silicon tip. The images were collected with a maximum number of pixels (512 × 512) and were only processed by flattening. For transmission electron microscopy observations, 3 μL of the polymer solution was dropped on a carbon-coated copper grid. The transmission electron micrographs were taken on a JEOL 2010 transmission electron microscope (TEM) operating at 200 kV.

3 RESULTS AND DISCUSSION

In the structural hierarchy of proteins, α helix is a secondary structure, whose change causes further variations in the higher-level structures of the biopolymers. The change in the helicity of the polyacetylene chains (Tang, 2001) may also give rise to variations in their quaternary structures, which is indeed the case, as demonstrated by AFM images of the self-assembling morphologies of **PEBIle** formed by a methanol solution (Figure 1). In the AFM analysis, we allowed the polymer solutions deposited on mica to dry naturally. The formation of the morphologies thus must be very fast, because it needs a split second for a tiny amount (normally ~5 μL) of a volatile solvent to evaporate in open air at ambient temperature. In actuality, the high molecular weight polymers should start to "fold" and precipitate at an even earlier stage, well before all the solvent molecules evaporate.

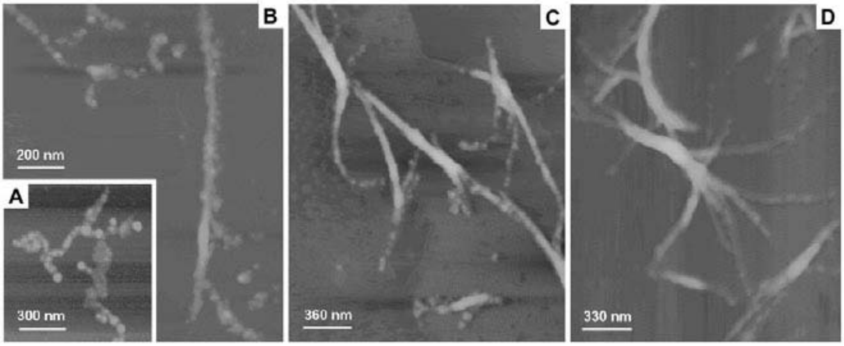


Figure 1. AFM height images of nanobeads and nanofibers formed upon natural evaporation of methanol solutions of **PEBIle** (7.8–19.3 μM) on the surfaces of newly cleaved mica under ambient conditions. The images were collected in tapping mode.

With L-isoleucine appendages, **PEBIle** in methanol displays an intense positive CD absorption at ~380 nm, meaning that its macromolecular chains adopt a predominant one-handed helical conformation in this solution (Tang, 2001). When 5 μL of a dilute methanol solution of **PEBIle** (7.8–19.3 μM) were dropped on freshly cleaved mica, fascinatingly, nanobead and nanofibrous structures were formed. Examples were given in Figure 1A: the polymer molecules self-assemble into small beads, which often link up together to form a necklace-like morphology. Without further collapsing into larger spherical structures, those beads are ~33–35 nm in length and 7–9 nm in height. Besides the spheres, interestingly, the nanofibers are simultaneously self-associated, whose widths are similar to those of the nanobeads (Figure 1B). Some of the nanofibers even branch with the necklace-like structure followed by disjointed individual beads, as revealed by panels B and C in Figure 1. Further bundling of the nanofibers readily produces helically twisted cables, as evidenced in Figures 1C and 1D. The loose polymer tails found at the ends of each cable clearly demonstrate that the formation of the multistranded cables is the result of the winding of the single fibers. In the whole folding process, the hydrogen bonds and shape complementarity are believed to serve as "sticky" adhesives to bundle and link up the polymer chains.

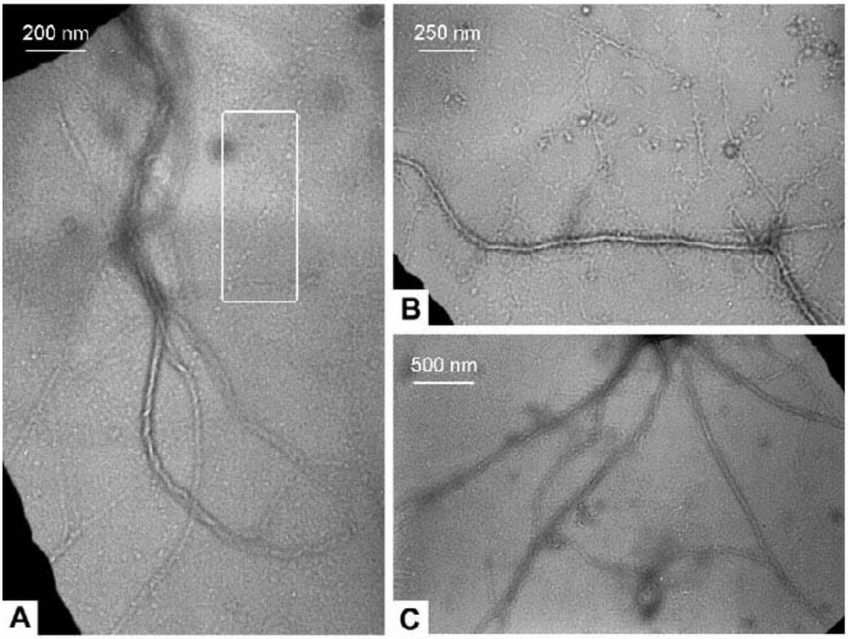


Figure 2. TEM images of vesicles and nanotubes formed upon natural evaporation of a methanol solution of **PEBIle** (19.3 μM) on a carbon-coated grid under ambient conditions.

Further investigation by TEM allows us to have a better understanding on the external as well as the internal structures of the nanoassemblies. As shown in Figure 2B, the self-associated morphologies resemble clearly the nanobeads and nanofibers observed under AFM. With a focus on their internal details, surprisingly, these morphologies are divulged as hollow in nature; they are thus in fact vesicles and nanotubes. The widths of the nanotubes with wall thickness of ~5.2 nm are found to be in the range of 12.5–50.0 nm, similar to the diameters of the vesicles formed. These values are on average smaller than those estimated by the AFM analysis; it is however not totally unexpected if taking into account that heir dimensions are determined by two dissimilar microscopic techniques. In fact, the AFM dimensions are normally larger than the real ones because of the contribution from the undesirable but inherent tip-broadening effect (Lashuel *et al.*, 2000, Cavalleri *et al.*, 2000, Leclere *et al.*, 2000).

The co-existence of these two structures reasonably accounts for their relationship in the formation process: assembling of the nanotubes based upon linear conjunction of the elementary vesicles, as imaged in the rectangle marked in Figure 2A. Panels A and C of Figure 2 illustrate some of helically twisted nanotubes stem from the larger helical cables, which further suggests that the winding process discussed above is stimulated by the small helical tubes.

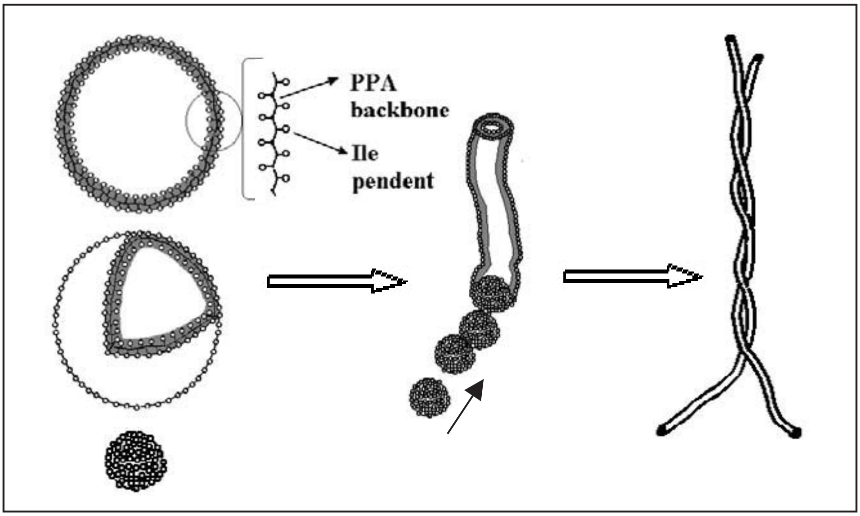


Figure 3. Schematic diagram of proposed process for the formation of vesicles, nanotubes, and helical nanofiber of **PEBIle**.

The formations of vesicular and tubular structures have been reported in studies of small molecule amphiphiles and copolymers (Bong *et al.*, 2001, Spector *et al.*, 1999, Yu and Eisengerg, 1998, Liang *et al.*, 2000, Meier, 2000, Zhang and Eisenberg, 1996, Stewart and Liu, 2000). It is generally agreed that the former is resulted from sealing of lamellar structures aligned by the amphiphilic molecules (Luisi and Straub, 1982, Moroi, 1992, Jones and Chapman, 1995). As described above, there are, however, various approaches to explain the case of the latter structure. For the copolymers, vesicles were found to be the precursors in the formation of tubules (Yu and Eisengerg, 1998, Liang *et al.*, 2000). Via a process of adhesive collisions, the vesicles can fuse together and stretch themselves to form the tubular structure. This collision and fusion mechanism has also been widely employed to explain micellar transition from spheres to rods (Luisi and Straub, 1982, Moroi, 1992, Jones and Chapman, 1995, Zhang and Eisenberg, 1999, Lee *et al.*, 2000, Crämer Pfanneüller *et al.*, 1996), which is thus not standing alone. In our present study, what we have observed under both AFM and TEM shows a great resembling, further affording the close relationship between these two assemblies. With respect to the amphiphilicity of **PEBIle**, association of the polar pendant groups forming compact shielding layers is an effective way to minimize the contact area of hydrophobic polymer backbone against a polar environment (Moroi, 1992). The shielding layers were therefore suggested to be the arrays forming outer and inner layers of bi- or multilayer sheets, allowing ready formation of the closed vesicles via the sealing of the sheets. Fusion of two vesicles is favored by the adhesive collision. When the degree of fusion reaches a critical point, the balance of interfacial energy to internal energy will no longer maintain the original structure, resulting in the transition of vesicular to hollow rod structure. The tubular morphology is therefore reasonably considered as elongation of the rod structure achieved by a linear fusion of more vesicles. The whole tube is expected to be fully covered by the amino acid appendages, and its affinity possibly behaves like a α-helix. Putting a few pieces of the helixes together may cause further variations in the higher-level structures of the biopolymers (Campbell, 1995). Bundling of the nanotubes indeed resembles this biological process, leading to the multistranded helical cables that are strongly adhered by interstrand hydrogen bonds between the pendant groups. A schematic diagram of the proposed process illustrating their formations is given in Figure 3.

It is worth noticing that the presence of necklace-like vesicles reasonably accounts for the incomplete transition of vesicle to tubular structure, offering a good support to our proposed mechanism. Such nanoassemblies are generally in accord with Eisenberg's hypothesis that tubular structure is arisen from coalescence of vesicles (Yu and Eisengerg, 1998). Their further mutated morphologies such as helical nanotube and multistranded tubular helix have, however, seldom been reported. We believe that the formation of such unique structures should thank for the predominant chain helicity of **PEBIle** induced by the optically active amino acid building blocks along its polyacetylene chains.

4 CONCLUSIONS

The molecular self-assembling study of the amphiphilic homopolymer, poly(4-ethynylbenzoyl-L-isoleucine) (**PEBIle**), demonstrates that its aggregation favors the formation of ordered nanovesicular and nanotubular structures upon evaporation of its methanol solutions. The AFM and TEM observations revealed the co-existence of the two organizational structures, strongly supporting that the tubules are resulted from the coalescence of the vesicles. Further variation of the tubular structure promoted the formation of the multistranded helical tubes by winding up the single-stranded nanotubules.

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