Self-assembly of Pure Nanotubes from a Single-Chain Diacetylene Amine Salt

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Introduction

The discovery that complex chiral diacetylene derivatives can self-assemble into nanostructures 1 has led to a search for simpler molecules that retain that activity. The search for novel materials is complicated because the factors governing self-assembly are complex and until the simple system is identified the underlying mechanisms are difficult to discover2-5. Unlike spherical liposomes, lipid tubules are thought to require chiral molecules in their formation and are expected to reflect the chiral nature of the lipids used. This chirality in molecular packing is reflected in helical markings often visible in electron micrographs of tubules and in large peaks observed in their circular dichroism spectra, both of which change handedness when the opposite enantiomer lipid is used 6. Lack of chirality does not in and of itself prevent self-organization since both chiral and achiral diacetylenic amphiphiles have been shown to form cylindrical microstructures from aqueous solution under quite specific conditions 7-9. The majority of work, however, has been concentrated on relatively complex chiral, multi-chain lipids as precursors for microstructured materials.

We attempted to synthesize the simple quaternary ammonium salt by direct quaternization of the diacetylene amine compound 2 (Figure 1). The reaction product was found to be a remarkably versatile nanostructure-forming moiety that when self-assembled could induce a visible color change. Surprisingly, a detailed analysis of the reaction product revealed a family of amine salt derivatives. Synthesis of seven individual amine, amine salt, and quaternary ammonium salt derivatives of diacetylene demonstrates that the only precursor that has the potential to form nanotubes (in a single 100% yield and uniform manner) is the secondary amine salt (compound 3). To our knowledge the remarkable self-assembly of this inexpensive and simple lipid is unprecedented and represents a real step toward the rational design of bioactive monodisperse nanostructures.

Synthesis of diacetylenic amine salts.
Method A. PDA modified with NHS in the presence of DEC was slowly added to 10-fold excess of ethylenediamine in dichloroethane. After the reaction, the mixture was washed with water. The organic phase was dried with sodium sulfate and rotary evaporated to yield a white powder. A quaternization reaction was performed by reacting the powdered compound 2 with ethyl bromide in chloroform/nitromethane (1:1) at room temperature for 24 hr. Reaction solvents were removed by rotary evaporation and the resulting...
white solid was dissolved in a small amount of chloroform followed by slow addition of hexane and drying in a vacuum oven at room temperature.  

Method B. PDA modified with NHS in the presence of DEC was slowly added to 10 times excess of N-ethylenediamidine or N,N-diethylhexylenediamidine in dichloroethane. After the reaction, the mixture was washed with water. The organic phase was dried with sodium sulfate and rotary evaporated to yield a white powder. Pure compound 3 was prepared from compound 6. Compound 6 was dissolved in methanol and an equal volume aqueous HBr was added. The methanol was removed by rotary evaporator. Hexane was added to the chloroform solution to precipitate compound 3 and the precipitate is dried in vacuuo at room temperature. For nanotube formation, 1 mg of the dried reaction products from Method A or B was suspended in 20 ml water and sonicated for 5 min at 25 °C in a sonic water bath. The solution was transferred onto plain glass slides and dried for 3 h at room temperature.

Results and Discussion

Our initial goal was to synthesize compound 5 in an attempt to develop a cell disrupting material that was also a chemically integrated biosensor. Initial experiments demonstrated that the reaction product from the Method A synthesis (Figure 1) had unexpectedly low solubility in water. The reaction product would not be expected to form nanostructures in solution since, until this work, achiral simple diacetylenes have not been shown to be “nanoactive”. Surprisingly scanning electron microscopy showed that, with the relatively simple processing, our synthetic product self-assembled into a remarkably monodisperse preparation of nanotubes in aqueous solution (Figure 2A). These monodisperse nanotubes were seen in the presence of amorphous material, but whenever a nanotube was formed it was of a uniform diameter. Mass spectroscopy of the quaternization reaction product showed that a mixture of amine salts was present (10% of 2, 60% of 3, 30% of 4, and less than 1% of 5) raising the fascinating question of whether one of the compounds was exclusively responsible for tube formation or whether the tubes consisted of mixed populations of derivatized PDA’s. We therefore synthesized each of the compounds as shown in Figure 1, Method B, and processed each one individually. Only two of the compounds formed any structured material. The amine HBr salt of compound 2 formed the cauliflower structures and compound 3 formed the nanotubes seen in Figure 2B. Compound 4 produced only amorphous material presumably because the head group is less hydrophilic. Interestingly, the quaternary ammonium salt (compound 5), was in fact a liquid at room temperature which
explains its absence from the original nanotube forming mixture. Carefully controlling the process conditions with compound 3 has allowed us to make preparations in which essentially 100% of the material self-assembles into nanotubes with identical diameters. Under the SEM, the nanotubes are monodisperse with respect to wall thickness (27 nm) and internal diameter (35 nm) (Figure 2B). The tubes formed via Method A and B are identical in size. The precise structure of these remarkable “nano-macaroni” structures is revealed in the TEM and SAXS data reported below.

TEM observations of both naked nanotubes and nanotubes after staining with phosphotungstic acid reveal a hollow inner core and a wall consisting of 5 lipid bilayers (Figure 3A). The structure in solution was further characterized by SAXS. The results (Figure 3B) suggest that the equilibrium spacing of the tubule bilayers in excess water is 57.8 Å. Although the diameter of the tubes is uniform throughout the sample, the length varies, with a mean of approximately 1 µm.

As mentioned above, exposure to UV light induces a polymerization through the triple bonds of the diacetylene groups on adjacent molecules. This polymerization has been shown to be accompanied by a color change. Nanotubes were dried onto glass surfaces and UV irradiated. This resulted in a color change from white to dark blue. Exposure of the polymerized nanotubes to detergents and strong acids induced a color change from blue to red or yellow (data not shown). Nanotubes recovered from the polymerization and examined by SEM were indistinguishable from non-cross linked tubes.

In summary, the self-assembly of certain diacetylene amine salts under carefully controlled conditions induces the formation of an array of nanostructures. We have produced nanotubes with uniform diameters from a single starting material. Interest in the versatility of diacetylene amine salts as platform compounds for nanomaterial design and synthesis is enhanced by their biocidal nature and the ability of polymerized diacetylene amine salts to signal their interaction with cells.

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References and Notes