Role of Water in Directing Diphenylalanine Assembly into Nanotubes and Nanowires

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Nanotubes (NTs) and nanowires (NWs) are typical one-dimensional nanomaterials, which have been used extensively as components in various nanodevices owing to their unique properties that arise from their anisotropic structure. NTs that bear hollow tubular space can serve in nanofluidic transport, as nanotemplates/nanoreactors, and as catalyst supports/adsorbents because of their large surface area. NWs with highly rigid mechanical properties may serve in composite reinforcement, colloidal liquid-crystal systems, and energy devices. Due to such morphology-dependent properties, much research effort has been devoted to trying to achieve NT and NW morphology control. Self-assembly processes of biological molecules, which allow for building-block arrangements, have facilitated control of the morphologies of NTs and NWs when assisted by the adjustment of preparative conditions. Such assembly is environmentally benign, which is extremely advantageous for biocompatible applications. Despite the enormous scientific interest in such nanostructural properties and the prospect of utility in current and emerging applications, mechanisms of formation for the observed morphologies based on molecular-scale building-block arrangements are still unclear in many cases due to the lack of atomic-scale structural information.

Given this paucity of mechanistic information, in this Communication we set out to try to better understand the self-assembly of an aromatic dipeptide consisting of two covalently linked phenylalanine units (diphenylalanine), which is a key structural motif in the Alzheimer’s β-amyloid polypeptide. This system serves as an excellent model because it has been reported to form well-defined NTs and NWs, nanoribbons and nanovesicles via transition from NTs and such structural and mechanistic considerations are related to investigations into the potential causes of neurodegenerative diseases in humans. Both NT and NW morphologies are formed by the self-assembly of diphenylalanine molecules, in which many interactions such as hydrogen bonding, aromatic stacking, and electrostatic interactions are involved. Among them, the existence of both intermolecular hydrogen bonds between peptid-peptide and peptide-water molecules and aromatic interactions (π...π) between aromatic side chains has been reported, and aromatic interactions were observed to be critical for the formation of amyloid fibrils in Alzheimer’s disease. In particular, based on the crystal structure, Görbitz provided the evidence that aromatic stacking of diphenylalanine molecules in a hierarchical array surrounding water clusters tightly holds the hydrogen-bonded peptide main chains in NTs; in this way, fibrils are formed along the water channel. Further, Gazit et al. investigated the dynamics of nanotube assembly from diphenylalanine using NMR spectroscopy; this study revealed that in NT formation, diphenylalanine molecules migrate out of their water solvation shells to form aggregates, reflected by a decrease in the integral of the diphenylalanine signal and a concomitant increase in the integral of the water signal. Thus, this is known as a nucleation-dependent process, common for highly ordered self-assembly processes. As this example shows, a structural investigation of nano/microscale morphologies on the molecular level can provide important clues in the understanding of the phenomenon of morphological evolution.

In this Communication, we demonstrate that NW morphologies can be obtained by using diphenylalanine in the aqueous phase at high ionic strength. The prepared NWs can then be readily disintegrated and used to form NTs by adjusting the aqueous conditions of preparation. The prepared morphologies of both NWs and NTs are analyzed on the molecular level by using Rietveld refinement of powder X-ray diffraction (PXRD) patterns and subsequently the maximum entropy method (MEM), which reveals an interesting fact that the spatial arrangements of diphenylalanine in both morphologies are closely related to central water clusters via intermolecular hydrogen bonds. Atomic-scale differences between NT and NW morphologies are observed appreciably only in the a and b directions as the expansion (5.5% from NT to NW) of the hydrophilic channel that encloses the central water column (between N2...N2), whereas the aromatic stacking distance along the c direction shows a considerably smaller change (0.1% contraction from NT to NW). Combining the information about
preparation conditions and structural details on the molecular level may prove helpful in tracking the formation mechanism of each morphology. On the basis of our findings, as well as those from Gazit et al.,[11b] the formation of NWs and NTs seems to be under kinetic and thermodynamic control, respectively, in that each morphology could be prepared depending on the free-water contents in the reaction medium. Indeed, increasing the concentration of diphenylalanine relative to the available water concentration led to the formation of NWs whereas decreasing the concentration led to the formation of NTs. The thermal stability of NTs over NWs from thermogravimetric analysis (TGA) data also clearly supports those features. We believe that the free-water content plays an important role in the self-assembly of diphenylalanine molecules into NTs and NWs.

The synthetic procedure for the aqueous formation of NTs and NWs is illustrated in Figure 1 and is described as follows: diphenylalanine is dissolved in trifluoroacetic acid (TFA) solution, which yields a transparent solution. Thereafter, a NH4OH solution is slowly added to the reaction mixture under vigorous stirring. When the pH of the reaction mixture approaches the isoelectric (pI) value of 5.5, the solution becomes highly turbid, indicating a rapid assembly of diphenylalanine into NWs. The presence of NWs was confirmed by scanning electron microscopy (SEM). The production of NWs at the pI value suggests that electrostatic interaction via deprotonation of the carboxylic acid functional group may play a crucial role in the self-assembly. Unlike NWs, NTs are prepared simply by sonication and subsequent annealing in deionized water at low concentrations of diphenylalanine. At high diphenylalanine concentrations (>2.5 mg mL\(^{-1}\)), NWs rather than NTs are formed as the major product. Notably, the morphological evolution between NTs and NWs is interconvertible in a sense: the dried NWs can be converted into NTs by sonication and annealing in deionized water. Conversely, the NWs can be readily prepared from dried NTs by dissolving NTs in TFA and titrating the sample with NH4OH. The morphological evolution was confirmed by SEM and is shown schematically in Figure S1 (Supporting Information).

To obtain PXRD data, samples of NTs and NWs were prepared by drying the dispersions and finely grinding the residual powder. The PXRD data were collected at room temperature (RT); detailed experimental conditions and structural parameters are provided in Table S1 (Supporting Information). Pawley refinements[10] were performed to optimize the lattice parameters and Rietveld refinements (Fig. 2) in considering all possible geometrical degrees of freedom by using the single-crystal structure of diphenylalanine.[11c] A superposition of the refined structures (Fig. 3a and 3b) clearly reveals that the NW diphenylalanine molecules are translationally shifted away from the central water molecules, compared with those for the NT. The most prominent differences are found in the region between the amine hydrogen atoms at the N-terminus of diphenylalanine and the oxygen atoms of water (Fig. 3c and 3d). Four distinct intermolecular hydrogen bonds (HB1–4) are detected in this region; internuclear distances are listed in Table S2 (Supporting Information). Since the strength of intermolecular hydrogen bonds is a function of distance, the increased interaction distances in NWs, relative to the those in the NTs, suggest a generalized weakening of hydrogen bonds in NWs, which may be compensated for by increased electrostatic interaction.

To account for the differences in hydrogen bonding interactions in detail, an electron density map was constructed through MEM analysis for the Rietveld refinement results (Fig. 4). Figure 4a and c represents electron-density distributions for the (001) plane for NTs and NWs, respectively. In these (001) slices, amine nitrogen atoms of the diphenylalanine in NTs and NWs are cleaved at values of...
$z = 0.794$ (NTs) and $z = 0.935$ (NWs), which are fractional coordinates of corresponding nitrogen atoms along the $z$ direction. The nitrogen atom of the amine group and the oxygen atoms of the water, involved in intermolecular hydrogen bonding, are indicated with black ‘N’ and ‘O’ labels. The electron density associated with hydrogen bonds in the NTs is about 3.5 times higher than that in the NWs, which is consistent with the notion that NTs have more closely packed 2D contours about the hydrogen bonds than NWs do. In addition, 2D contours around the water molecule (dotted grey circle) also show higher electron densities for NTs than for NWs.

Figure 4b and 4d represent electron-density distributions on the (110) plane for NTs and NWs, respectively. Two unit cells elongated ($C_2$) in the $c$ direction are shown for better visualization. In these (110) slices, hydrogen bonds are cleaved by the (110) plane, which is 19.5 Å away from the lattice origin. Black arrows indicate the electron density of hydrogen bonds (dotted black rectangle). From the viewpoint of the (110) slices, the electron densities in the NTs are also confirmed to be about 3 times higher than those in the NWs. This difference is true even when the distance of (110) slices is scanned from 19.0 to 20.0 Å. Interestingly, the reduced electron densities of hydrogen bonds in NWs are likely to be closely related to the significantly reduced occupancy in one water oxygen atom (O1G). The occupancy value of O1G is close to 0 for the NWs after Rietveld refinement; therefore, only half the hydrogen bonds seem to be effective when considering occupancy and distance factors. The total occupancies of water oxygen atoms in one asymmetric unit of NWs are ~20% less than those of NTs: the total water oxygen atom occupancy in one asymmetric unit of NWs and NTs is 2.22 and 2.76, respectively.

Besides hydrogen bonds between a diphenylalanine and a water molecule, NTs show stronger hydrogen bonds than NWs in another hydrogen bond motif, namely, the $\text{NH}_2 - \text{H} \cdots \text{OO} \text{C}$ head-to-tail chains formed between two diphenylalanine molecules. This type of hydrogen bond encloses central water molecules in forming a helical architecture with its hydrophilic parts (for both NTs and NWs). Figure S2a and S2b (Supporting Information) reveals this architecture of NTs in the $\overline{1}0$ direction. There are three hydrogen bonds (HB5–7, Table S2), in which the O1 atom of the carboxylate group serves as a hydrogen bond bridge that interconnects layers (HB6 and HB7), and the O2 atom holds two adjacent diphenylalanine molecules in the same layer (HB5). In the case of NWs, the weakening of one hydrogen bond (HB5) makes a slightly looser lateral packing, wherein the O1 atom is mainly in the hydrogen bonding network between two diphenylalanine molecules. As mentioned previously, aromatic stacking ($\pi \cdots \pi$) between aromatic side chains is also an important driving force in maintaining structural integrity. However, our results show that the translational shift along the $c$ direction from NTs to NWs is negligible (0.1% contraction), and thus both morphologies have nearly the same aromatic stacking distance. The diameter of the...
hydrophilic channel that encloses the central water column (between \(N_2\)–\(N_2\)) is 12.217 and 12.886 Å for NTs and NWs, respectively, indicating a notable expansion (~5.5% from NT to NW) in spacing between the diphenylalanine molecules in the NWs.

To assess the stability in both hierarchical structures, TGA was performed. TGA data reveal a weight loss in the NT sample at 586 °C from the lattice origin in NTs, \(z = 0.935\) from the lattice origin in NWs. Contours are plotted up to 7.0 e Å\(^{-3}\) and the isosurface level at the pI condition prepared by adding \(NH_4OH\) solution to a TFA stable than NTs in the absence of solvent. After NWs were formed (110) slices: 0.42, 0.47 for NTs and 0.12, 0.17 for NWs.

**Experimental**

**Materials:** The lyophilized form of the \(NH_2-Phe-Phe-COOH\) dipeptide was purchased from Bachem (Bubendorf, Switzerland). Water was deionized by using Milipore MilliQ. \(NH_3\) solution and trifluoroacetic acid were purchased from Junsei Chemical Co. (Japan) and Samchun Pure Chemical Co. (Korea), respectively.

**Preparation of Peptide NTs:** A deionized water solution of \(NH_2-Phe-Phe-COOH\) (diphenylalanine) was prepared (2 mg mL\(^{-1}\)) via sonication (20 min) with subsequent annealing (30 min). Peptide NTs were formed during cooling to ambient temperature. When the diphenylalanine molecules are dissolved in deionized water in the preparation of NTs, the pH of the solution approaches the isoelectric point and shows concentration dependence. A solution of diphenylalanine (2 mg mL\(^{-1}\)) in deionized water resulted in a pH of approximately 5.3. From this observation, the diphenylalanine molecules in the NTs were also assumed to be in their zwitterionic form.

**Preparation of Peptide NWs:** A portion of aromatic diphenylalanine was dissolved in trifluoroacetic acid and subsequently titrated by adding a portion of ammonia solution (approx. 30 wt%) to reach the isoelectric
point of pH 5.5. After this titration, the final diphenylalanine concentration in the reaction mixture was 5.26 mg mL⁻¹. NWs could then self-assemble under isoelectric conditions. Excess salt was removed by washing with deionized water.

Control of NT and NW Morphologies: The independently prepared and dried NTs and NWs could be converted: the NTs could be converted to NWs, and NWs to NTs, by applying respective preparation conditions. Sonication and annealing of the dispersed NWs in deionized water yielded the NT morphology (Fig. S1a, Supporting Information). Conversely, dissolving the dried NTs in TFA and titrating the solution with NH₄OH solution yielded the NW morphology (Fig. S1b, Supporting Information).

In the case of NWs, even this morphological evolution from NWs to NTs was observed under its original buffer condition as follows: NWs can be converted into NTs in situ without changing the reaction medium by sonication and annealing the reaction mixture.

Scanning Electron Microscopy: The morphology of peptide NTs or NWs was analyzed by a field-emission scanning electron microscope (Hitachi S-4800, Japan). A carbon coating was used to enhance scattering contrast and electric conductivity (CED O30 carbon evaporator, Bal-tec, Germany).

Collection and Refinement of PXRD Patterns: High-resolution PXRD data of NT and NW samples were collected at the 8C2 high-resolution powder diffraction beamline of the Pohang Accelerator Laboratory in Pohang, Korea. The incident X-ray was vertically collimated by a mirror and monochromatized to the wavelength of 1.5489 Å by using a double-crystal Si(111) monochromator. The diffraction patterns were scanned over 2θ values ranging from 2.00° up to 70.00° in increments of 0.02° (room temperature). The samples for PXRD were prepared by evaporating water and fully grinding the remaining powder. The sample holder on the instrument was rotated about a vector normal to the surface during measurement to increase sampling statistics and to reduce preferred orientation effects. The obtained experimental powder diffraction patterns are in good agreement with a simulated powder diffraction pattern of the previously reported single-crystal structural data (CCDC 163340) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif [11e]. With this unit cell structure, we performed Pawley refinement to optimize the lattice parameters iteratively until no change was obtained in the Rwp value. The orthogonal polynomials with 20 coefficients were used as the basis set for fitting the experimental background. In Rietveld refinements, the positions and torsional angles of diphenylalanine molecules were explicitly considered as structural degrees of freedom. The March–Dollase function was selected for the preferred orientation correction and an anisotropic temperature factor was applied for all heavy atoms. For water molecules, isotropic temperature factors and occupancies were refined only. Rietveld refinement gave good convergence even when all parameters were refined simultaneously. The final refined parameters are summarized in Table S1 (Supporting Information) and selected intermolecular hydrogen bond distances are listed in Table S2 (Supporting Information). Most of the molecular modeling, Pawley refinements, Rietveld refinements, and electron-density calculations via the MEM were carried out using Rietan-FP [18] and Reflex, a software package for crystal structure determination from powder diffraction patterns, implemented in MS modeling v4.2 (Accelrys Inc.) [19]. The visualization and analysis of the electron-density map from MEM were performed by using VESTA program [20].

Thermogravimetric Analysis of NTs and NWs: Small amounts of dried samples of each were heated from 303 to 773 K with a constant heating rate of 5 K min⁻¹.

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