

Thermal and Chemical Stability of Diphenylalanine Peptide Nanotubes: Implications for Nanotechnological Applications

Lihl Adler-Abramovich,[†] Meital Reches,[†] Victoria L. Sedman,[‡] Stephanie Allen,[‡] Saul J. B. Tendler,[‡] and Ehud Gazit^{*†}

Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel, and Laboratory of Biophysics and Surface Analysis, School of Pharmacy, The University of Nottingham, Nottingham NG7 2RD, U.K.

Received September 4, 2005. In Final Form: November 18, 2005

The diphenylalanine peptide, the core recognition motif of the β -amyloid polypeptide, efficiently self-assembles into discrete, well-ordered nanotubes. Here, we describe the notable thermal and chemical stability of these tubular structures both in aqueous solution and under dry conditions. Scanning and transmission electron microscopy (SEM and TEM) as well as atomic force microscopy (AFM) revealed the stability of the nanotubes in aqueous solution at temperatures above the boiling point of water upon autoclave treatment. The nanotubes preserved their secondary structure at temperatures up to 90 °C, as shown by circular dichroism (CD) spectra. Cold field emission gun (CFEG) high-resolution scanning electron microscope (HRSEM) and thermogravimetric analysis (TGA) of the peptide nanotubes after dry heat revealed durability at higher temperature. It was shown that the thermal stability of diphenylalanine peptide nanotubes is significantly higher than that of a nonassembling dipeptide, dialanine. In addition to thermal stability, the peptide nanotubes were chemically stable in organic solvents such as ethanol, methanol, 2-propanol, acetone, and acetonitrile, as shown by SEM analysis. Moreover, the acetone environment enabled AFM imaging of the nanotubes in solution. The significant thermal and chemical stability of the peptide nanotubes demonstrated here points toward their possible use in conventional microelectronic and microelectromechanics processes and fabrication into functional nanotechnological devices.

Introduction

Self-assembled protein and peptide nanostructures are envisioned to serve as important building blocks in future nanotechnological devices and assemblies. Such assemblies offer the advantage of biocompatibility and chemical diversity while using efficient molecular recognition modules. Short peptides self-assemble into various forms in the nanoscale, including tapes, fibrils, tubes, and spheres.^{1–9} Yet, the inherent thermal and chemical instability of many protein structures raises a question regarding the compatibility of peptide structure with common lithographic techniques, as well as the long-term durability of such devices.

Self-assembly of proteins are common processes in many natural systems. Such a process, which plays a key pathogenic role, is the formation of amyloid fibrils, which are associated with a large number of major human diseases. A partial list includes Alzheimer's disease, type II diabetes, and prion diseases. Significant research efforts are being directed toward the

understanding of the mechanism of amyloid assembly.^{10–12} Amyloid fibrils made by different proteins have been characterized by atomic force microscopy (AFM) to gain a better insight into the amyloid formation mechanism and surface morphology.^{13–16} Short peptide fragments have been used in the study of amyloid formation in order to identify recognition motifs within amyloidogenic proteins.^{17–19}

Our studies on the mechanism of amyloid fibril formation led us to the unexpected discovery that diphenylalanine, NH₂–Phe–Phe–COOH, the core motif of the β -amyloid polypeptide, self-assembles into well-ordered nanotubular structures.²⁰ Diluting a concentrated fluorinated alcoholic solution of the dipeptide into aqueous solution resulted in its efficient assembly into long and hollow tubular nanostructures. A follow-up study by another group demonstrated that similar structures could be formed by solubilizing the peptide in water at 65 °C, followed by cooling the solution to room temperature.²¹ The formation of peptide

* To whom correspondence should be addressed. E-mail: ehudg@post.tau.ac.il. Tel: +972-3-640-9030. Fax: +972-3-640-5448.

[†] Tel Aviv University.

[‡] The University of Nottingham.

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nanotubes under mild conditions combined with their water solubility, exceptional persistence, and biocompatibility suggest that peptide nanotubes can serve as excellent building blocks for future nanotechnological applications.^{20,21} Peptide nanotubes not only serve as a degradable mold for the fabrication of silver nanowires²⁰ but also enable the formation of platinum nanoparticle composites.²¹ The results of recent studies demonstrated that peptide nanotubes can serve as part of an electrochemical biosensor platform.^{22,23} The tubular structures are remarkably rigid assemblies with a Young modulus of about 20 GPa.²⁴ Such unique properties suggest that peptide nanotubes can be used as a key element in such applications as environmental and medical biosensing, the fabrication of remarkably rigid biocompatible materials, and as a template for the nanofabrication of metallic and other inorganic materials.

The formation of well-ordered and discrete peptide nanotubes by self-assembly of the aromatic dipeptide phenylalanine is consistent with our earlier suggestion that the stacking of aromatic moieties contributes energy, as well as order and directionality, to the self-assembly of amyloidogenic structures.^{25–27} This notion has recently gained much experimental and theoretical support.^{28–33}

The contribution of specific interactions between aromatic moieties to protein stabilization at elevated temperature was well demonstrated in a study of the thermal stability of thermophilic proteins.³⁴ In that study, protein families, for which the crystal structures of both the thermophilic and their mesophilic homologues are known, were analyzed. In 17 different thermophilic protein families, the results showed the presence of aromatic clusters or enlarged aromatic networks that were absent in the corresponding mesophilic homologues.

In the current study, we probed the thermal and chemical stability of aromatic dipeptide nanotubes. The stability of the structures was studied using electron microscopy (EM) and AFM, as well as circular dichroism (CD) and thermogravimetric analysis (TGA) analysis. Taken together, the structures showed a remarkable stability when compared with other biomolecular assemblies. The properties of the peptide nanotubes together with their unique physical and chemical properties make them attractive building blocks for future nanotechnological applications.

Experimental Section

Preparation of Initial Solutions of Peptides. Peptides were purchased from Bachem (Bubendorf, Switzerland). Fresh stock solutions were prepared by dissolving the lyophilized form of the peptides in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma Aldrich) at a concentration of 100 mg/mL. To avoid any preaggregation, fresh stock solutions were prepared for each experiment.

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Instruments. AFM images were generated using a Nanoscope IIIa AFM (Digital Instruments, Veeco Metrology Group, Santa Barbara, CA).

Transmission electron microscopy (TEM) samples were viewed using a JEOL 1200EX electron microscope (JEOL, Tokyo, Japan) operating at 80 kV. Scanning electron microscopy (SEM) images were prepared using a JSM JEOL 6300 SEM (JEOL, Tokyo, Japan) operating at 5 kV. A Cold field emission gun (CFEG) high-resolution scanning electron microscope (HRSEM) (JEOL JSM-6700 field emission scanning electron microscope equipped with a cold field emission gun operating at 1 kV (JEOL, Tokyo, Japan) was used to view samples prepared under dry heating conditions. CD spectra at the far-UV region (200–250 nm) were recorded with an AVIV 202 spectropolarimeter (Aviv Instruments, Lakewood, NJ) equipped with a temperature-controlled cell using a cell of path length 0.5 cm; the bandwidth was 1 nm, and the averaging time was 4s for each measurement. TGA measurements were recorded on a TGA 2950 at a heating rate of 10 °C/min.

Thermal Stability of Autoclaved Nanotubes. Peptide stock solution was diluted in double distilled (dd) H₂O to a final concentration of 2 mg/mL, autoclaved at 121 °C, 1.2 atm, and aliquots (10 μL) were prepared for further analysis. For TEM, an aliquot (10 μL) of the autoclaved peptide nanotubes solution was placed onto a 400 mesh copper grid (SPI Supplies, West Chester, PA) covered by carbon stabilized Formvar film. Excess fluid was removed after 1 min, and the grid was negatively stained with 2% uranyl acetate in water. After 2 min, excess fluid was again removed from the grid and the samples were viewed. For SEM, an aliquot (10 μL) of the autoclaved peptide nanotubes solution was dried at room temperature on a microscope glass cover slip and viewed after coating with gold.

For AFM, an aliquot (10 μL) of the autoclaved peptide nanotubes solution was diluted to a final concentration of 0.5 mg/mL and then dropped onto a freshly cleaved mica and dried at room temperature. All imaging was performed in air in tapping mode using a “J” type scanner with heating stage using silicon nitride tapping probes (supplied by Veeco Metrology Group) mounted on cantilevers with spring constants of 34.4–37.2 N/m and a resonant frequency of 280–360 kHz. For imaging in solvent, a “J” type scanner was used in tapping mode using NP-sharpened silicon nitride tapping probes (supplied by Veeco Metrology Group) mounted on cantilevers with spring constants of 0.032 N/m.

For CD spectra, peptide stock solution was diluted in double distilled (dd) H₂O to a final concentration of 2 mg/mL and then immediately diluted to a final concentration of 0.04 mg/mL. The CD spectra of peptide nanotubes that self-assembled from the dipeptides NH₂–(L)Phe–(L)Phe–COOH or NH₂–(D)Phe–(D)Phe–COOH were recorded at 25, 37, 60, 70, 80, and 90 °C. A control dipeptide that does not self-assemble, NH₂–(L)Ala–(L)Ala–COOH, was incubated under the same conditions, and its CD spectra at 25 °C was recorded.

Thermal Stability of the Nanotubes in Heated Aqueous Solution. Peptide stock solution was diluted in double distilled (dd) H₂O to a final concentration of 2 mg/mL, incubated in a water bath at 80 °C for 1 h, and aliquots (10 μL) were prepared for further analysis. For TEM, an aliquot (10 μL) of the incubated peptide nanotube solution was placed onto a 400 mesh copper grid (SPI Supplies, West Chester, PA) covered by carbon-stabilized Formvar film. Excess fluid was removed after 1 min, and the grid was negatively stained with 2% uranyl acetate in water. After 2 min, excess fluid was again removed from the grid and the samples were viewed. For SEM, an aliquot (10 μL) of the incubated peptide nanotubes solution was dried at room temperature on a microscope glass cover slip and viewed after coating with gold.

Thermal Stability under Dry Conditions. The peptide stock solution was first diluted to 2 mg/mL and then immediately diluted to a final concentration of 0.5 mg/mL. An aliquot (10 μL) was dried on freshly cleaved mica, under vacuum in a desiccator at room temperature and then heated for 10 min at the required temperature. The samples were viewed using CFEG HRSEM. For TGA, the diluted peptide nanotubes solution (2 mg/mL) was incubated for 5 h at room temperature and then lyophilized.

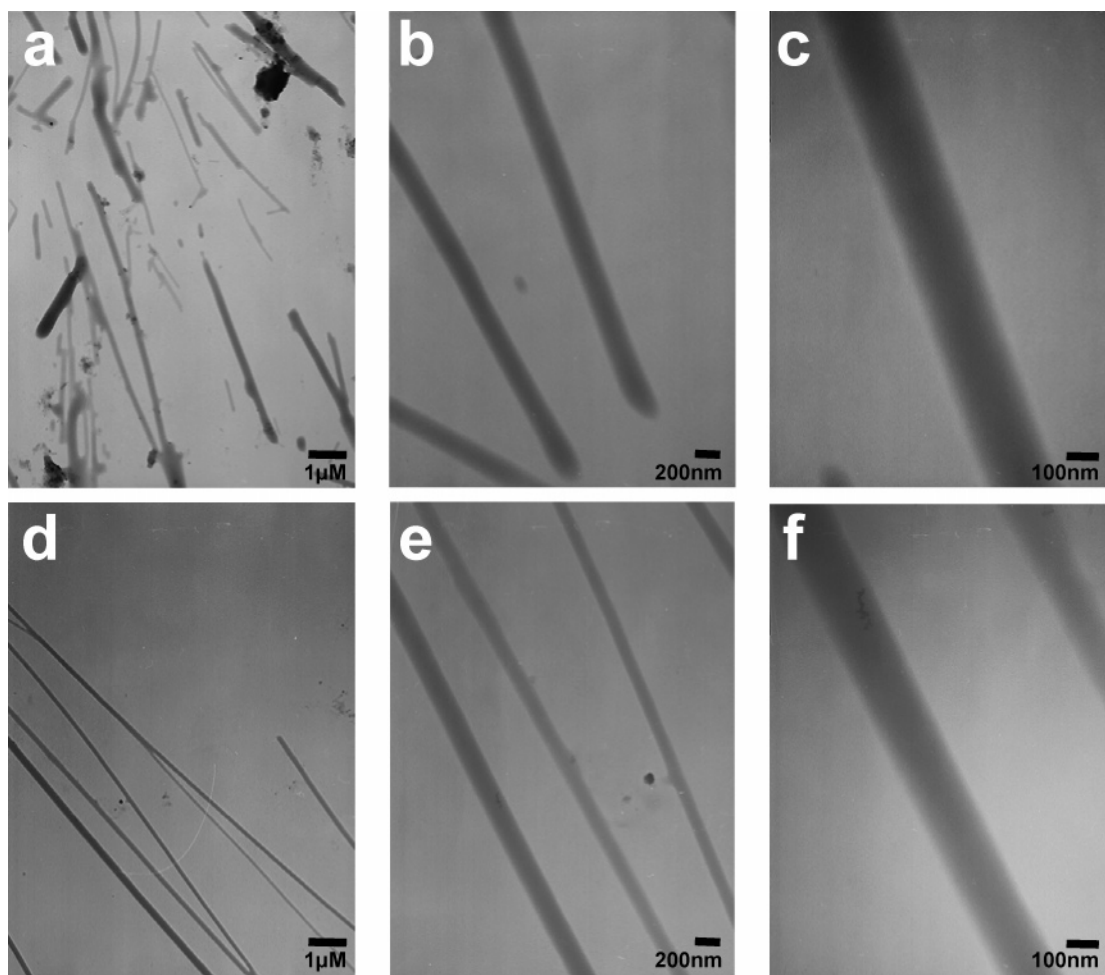


Figure 1. TEM micrographs of autoclaved nanotubes (121 °C, 1.2 atm). Untreated control (a–c) and autoclaved (d–f) samples of peptide nanotubes viewed at magnifications of $\times 1000$ (a and d), $\times 10\,000$ (b and e), or $\times 50\,000$ (c and f).

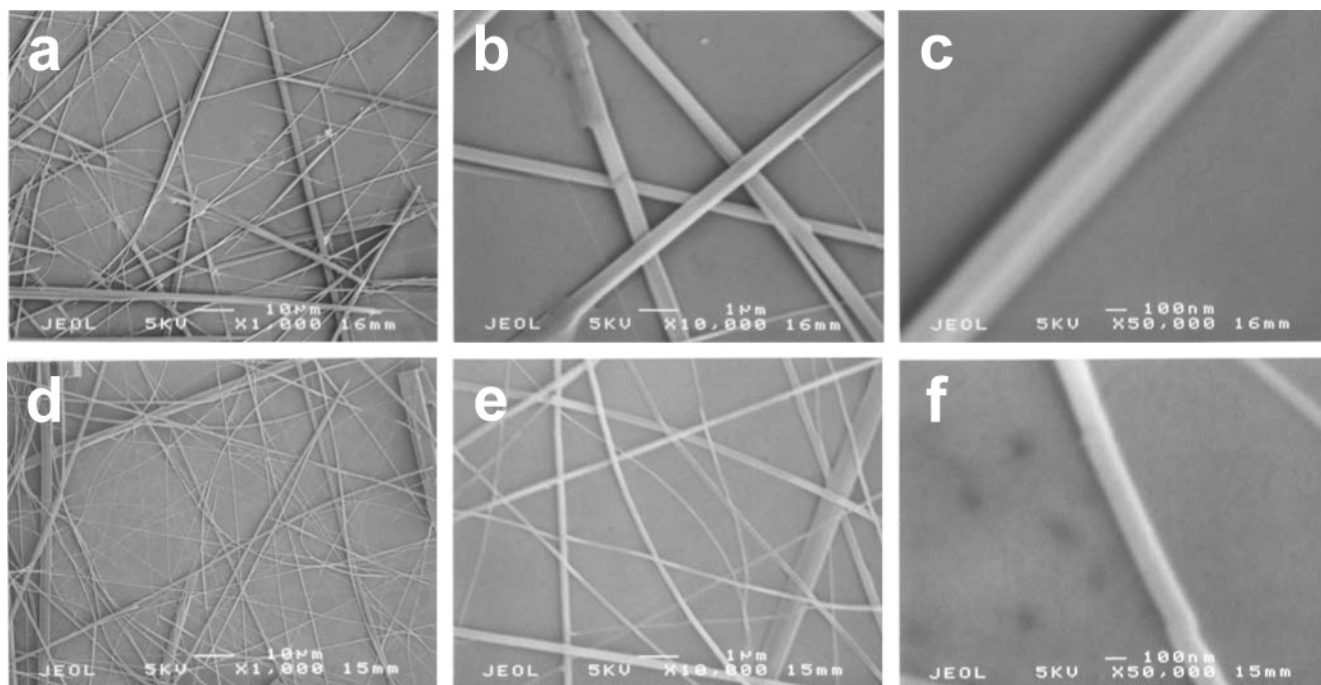


Figure 2. SEM micrographs of autoclaved peptide nanotubes. Untreated control (a–c) and autoclaved (d–f) samples of peptide nanotubes viewed at magnifications of $\times 1000$ (a and d), $\times 10\,000$ (b and e), or $\times 50\,000$ (c and f).

Conformational Stability in Organic Solvent. An aliquot (10 μL) of the diluted nanotubes solution (2 mg/mL) was air-dried on freshly cleaved mica, placed for 10 min in acetone or acetonitrile,

and then analyzed by SEM. In addition, 50 μL of the peptide nanotubes solutions were mixed with 50 μL of each of the following solvents: ethanol, methanol, 2-propanol, acetone, and acetonitrile and incubated

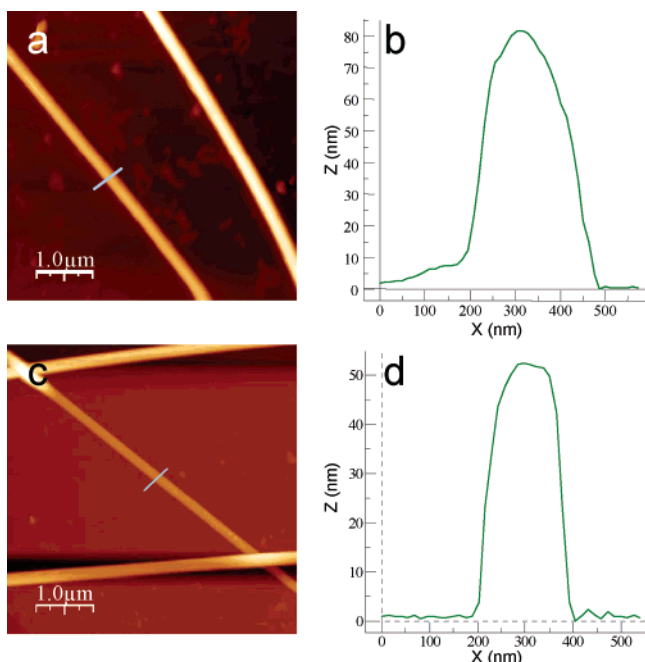


Figure 3. AFM images of autoclaved peptide nanotubes. (a) Topography image ($5 \mu\text{m} \times 5 \mu\text{m}$) of untreated peptide nanotubes. (b) Line plot of height across the axis of the nanotubes seen in Figure 3a. (c) Topography image ($5 \mu\text{m} \times 5 \mu\text{m}$) of autoclaved peptide nanotubes. (d) Line plot of height across the axis of the nanotubes seen in Figure 3c.

at room temperature for half an hour. For SEM analysis, an aliquot ($10 \mu\text{L}$) of the incubated peptide nanotube solution was air-dried on a microscope glass cover slip and viewed after coating with gold.

Imaging the Peptide Nanotubes in Aqueous Environment.

For AFM analysis, the diluted peptide nanotubes solution (2 mg/mL) was diluted in acetone to a final acetone concentration of 50%. The samples were dropped onto freshly cleaved mica and imaged.

Results

Thermal Stability of the Nanotubes under Wet Heating Conditions. We have previously reported that diphenylalanine nanotubular structures retain their intact structure in boiling silver nitrate solution, while examining the ability of the nanotubes to serve as molds for casting metal nanowires.²⁰ In this set of experiments, we have studied their thermal stability in aqueous solution at temperatures above the boiling point of water at atmospheric pressure or in a high-pressure autoclave. Ultrastructural analysis by TEM revealed that the structure of the peptide nanotubes is preserved after standard sterilization at 121°C and 1.2 atm (Figure 1). Scanning electron micrographs (Figure 2) confirmed the similarity of the three-dimensional (3D) structures of untreated (parts a–c of Figure 2) and autoclaved (parts d–f of Figure 2) peptide nanotubes.

Figure 3 shows AFM images of peptide nanotubes self-assembled at room temperature (parts a and b of Figure 3) and after autoclaving (parts c and d of Figure 3). The results confirm the durability of the structure during autoclave treatment. Moreover, the line across the axis of the treated and untreated peptide nanotubes shows their similarity in height, 50 and 80 nm, respectively, along the Z-axis, indicating that the macroscale structure remains intact after autoclave treatment (parts b and d of Figure 3). However, given that the nanotubes are not identical, no detailed comparison can be made.

The CD spectra, which was used to compare the secondary structure of the nanotubes at increasing temperatures, were similar at all temperatures, showing peaks at 200 nm and at 217 nm

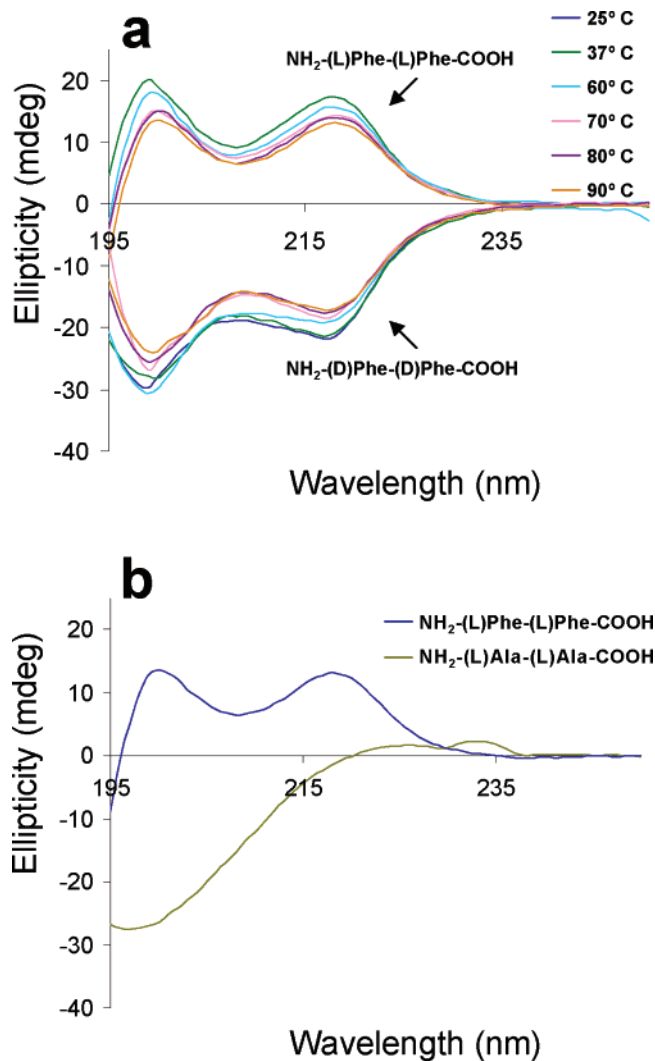


Figure 4. CD spectra of peptide nanotubes in solution, self-assembled from the dipeptides $\text{NH}_2\text{-(L)Phe-(L)Phe-COOH}$ or $\text{NH}_2\text{-(D)Phe-(D)Phe-COOH}$. (a) CD spectra of $\text{NH}_2\text{-(L)Phe-(L)Phe-COOH}$ (upper) and $\text{NH}_2\text{-(D)Phe-(D)Phe-COOH}$ (lower) dipeptide nanotubes at 25, 37, 60, 70, 80, and 90°C . (b) CD spectra of the $\text{NH}_2\text{-(L)Phe-(L)Phe-COOH}$ dipeptide nanotubes and the non-self-assembling dipeptide $\text{NH}_2\text{-(L)Ala-(L)Ala-COOH}$ at 25°C .

(Figure 4a). The difference between the spectra of the $\text{NH}_2\text{-(L)Phe-(L)Phe-COOH}$ and $\text{NH}_2\text{-(D)Phe-(D)Phe-COOH}$ dipeptide nanotubes was seen in their ellipticity, which showed positive values for the former and negative for the latter. The CD spectra for the control dipeptide, $\text{NH}_2\text{-(L)Ala-(L)Ala-COOH}$, showed a peak at 200 nm (Figure 4b). To further monitor the stability of the peptide nanotubes in heated aqueous solution, we placed the peptide nanotubes in 80°C water bath for 1 h. SEM analysis confirmed the stability of the peptide nanotubes (Figure 5) as compared to peptide nanotubes that were not thermally treated (parts a–c of Figure 2).

Thermal Stability of the Tubes upon Dry Heating Conditions. To examine the effect of dry heat CFEG HRSEM, was utilized to examine the nanotubes. We observed that, up to a temperature of 150°C , the peptide nanotubes were clearly stable and showed the same morphology as that observed for untreated tubes (Figure 6). A significant destruction of the structures did occur, however, when the nanotubes were heated to $200\text{--}300^\circ\text{C}$ (Figure 6). At higher temperatures, almost no peptide material was observed. When nanotubes were heated to 200°C and above, the nanotubes showed sensitivity to the electron beam.

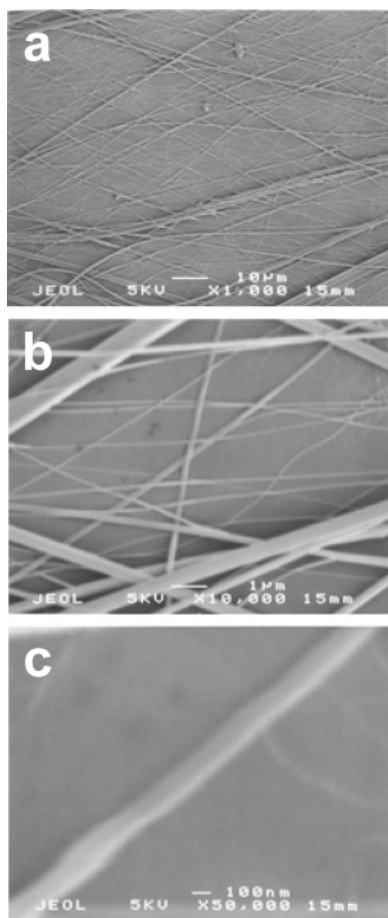


Figure 5. SEM micrographs of peptide nanotubes treated at 80 °C water bath for 1 h, viewed at magnifications of $\times 1000$ (a), $\times 10\,000$ (b), or 50 000 (c).

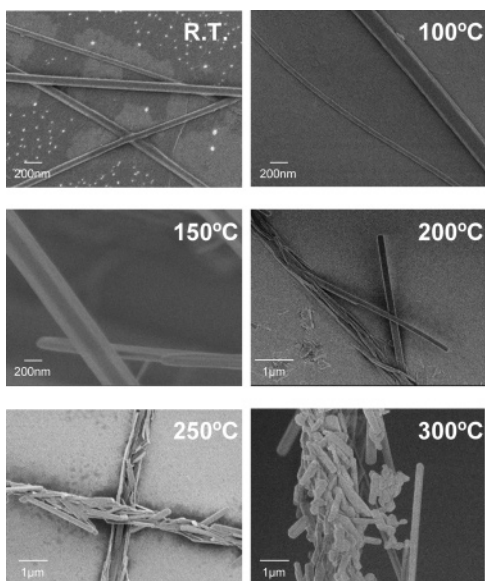


Figure 6. CFEG micrographs of peptide nanotubes at room temperature (upper left-hand corner) and CFEG HRSEM micrographs of peptide nanotubes heated under dry conditions to 100, 150, 200, 250, and 300 °C, respectively.

To determine the exact parameters of the physical stability of the self-assembled peptide nanotubes, we used TGA to monitor the weight as a function of increasing temperature. The TGA thermogram of the $\text{NH}_2\text{-Phe-Phe-COOH}$ nanotubes initially showed a certain amount of weight decrease ($\sim 15\%$) above 50

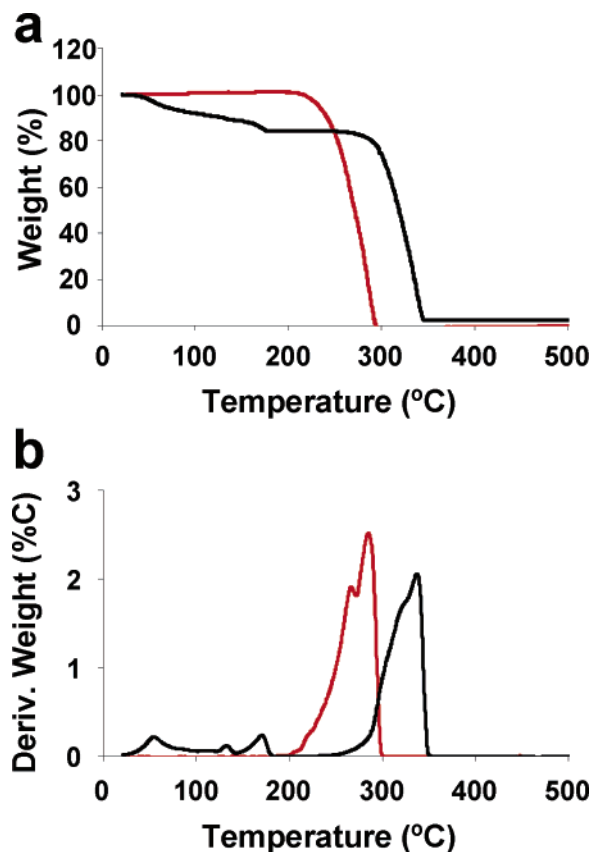


Figure 7. TGA of (a) Diphenylalanine nanotubes (black line) showing stability up to 300 °C, and the dialanine peptide (red line) showing an initial weight loss at ~ 220 °C; (b) Derivative weight showing the stability of the diphenylalanine (black line) to a higher temperature than dialanine (red line).

°C (Figure 7a), presumably from the evaporation of water molecules that interact with the lumen of the nanotubes. A major decrease in weight was observed only above 300 °C. No significant initial weight decrease occurred in the control peptide $\text{NH}_2\text{-Ala-Ala-COOH}$. The $\text{NH}_2\text{-Ala-Ala-COOH}$ peptide was used to present a generic dipeptide that could not form any structures at the nanoscale under the experimental conditions but that is yet quite similar to the studied peptides in its overall physicochemical profile. This finding is consistent with the inability of $\text{NH}_2\text{-Ala-Ala-COOH}$ to form ordered structures that would allow the cooperative adhesion of water molecules. Nevertheless, the weight of the $\text{NH}_2\text{-Ala-Ala-COOH}$ dipeptide did decrease at temperatures ranging from 250 to 290 °C and reached a low plateau at ~ 290 °C, a temperature significantly lower than that found for the $\text{NH}_2\text{-Phe-Phe-COOH}$ nanotubes. The thermogram of the $\text{NH}_2\text{-Phe-Phe-COOH}$ nanotubes demonstrated a similar degradation, but at temperatures ranging from 300 to 340 °C, reaching a low plateau around 340 °C. The plot of the weight-change derivative (Figure 7b) showed major peaks of 286 and 338 °C for $\text{NH}_2\text{-Ala-Ala-COOH}$ and $\text{NH}_2\text{-Phe-Phe-COOH}$, respectively. When compared with the monomer, a virtually linear transformation of the self-assembled structures was seen in the TGA thermogram at temperatures above 50 °C (Figure 7).

Conformational Stability in Organic Solvents. To study the compatibility of the nanotubes to conditions that are used for conventional lithography processes, we studied the stability of the nanotubes in organic solvents. Peptide nanotube solution was mixed with different solvents and incubated at room temperature for half an hour. SEM analysis revealed chemical

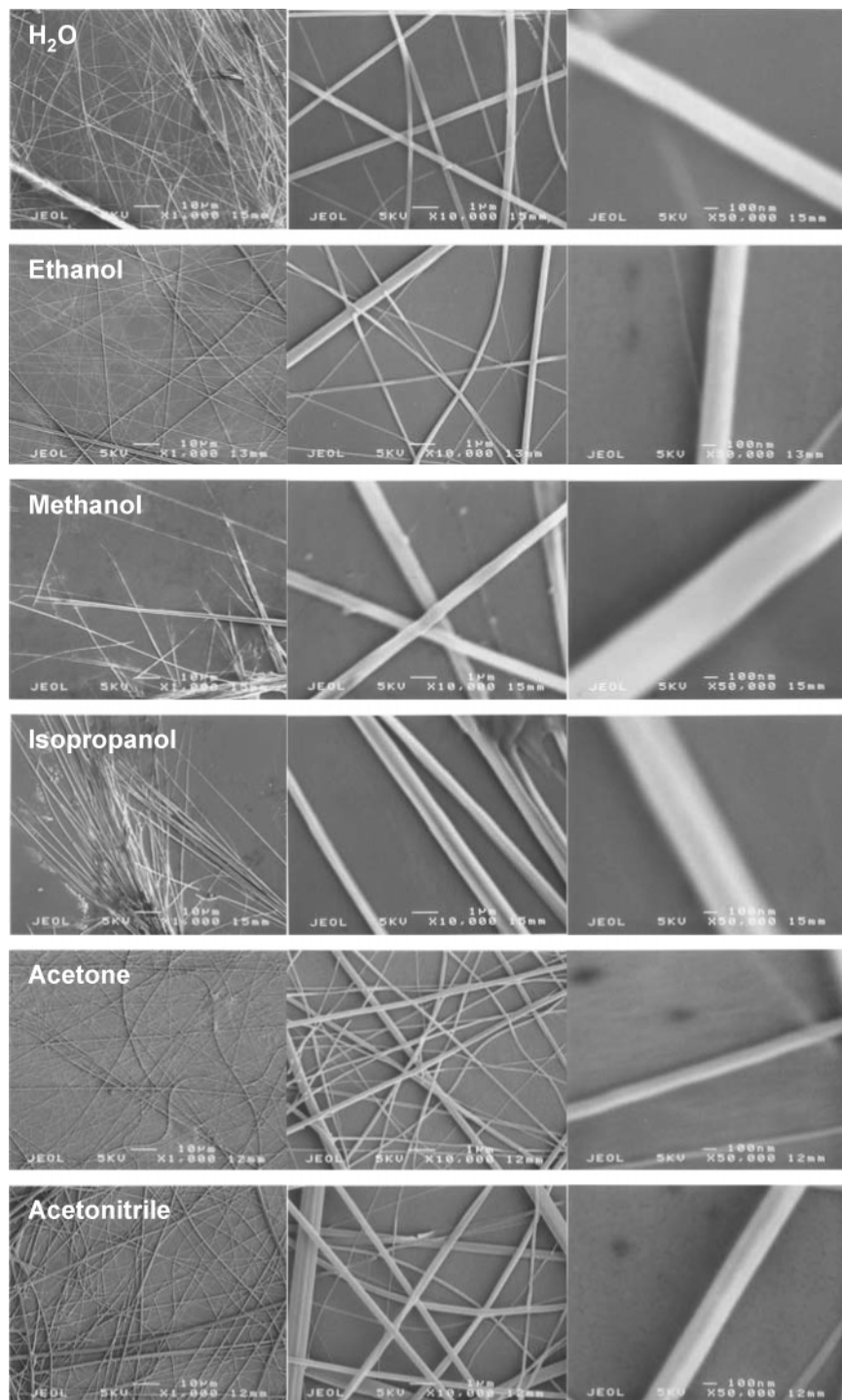


Figure 8. SEM images showing ultrastructure stability of the diphenylalanine nanotubes. Peptide nanotubes in H₂O and peptide nanotubes after half an hour incubation with ethanol, methanol, 2-propanol, acetone, and acetonitrile.

stability of the nanotubes to each of the following solvents: acetone, acetonitrile, ethanol, methanol, and 2-propanol (Figure 8).

Furthermore, peptide nanotubes were deposited on a mica surface and submerged for 30 min in acetone or acetonitrile prior to imaging using SEM. The nanotubes completely retained their structure in acetone as well as acetonitrile (data not shown).

Imaging of the Peptide Nanotubes in Solution. Imaging in liquid is important but difficult as the conventional use of EM requires drying of the sample followed by coating with conducting material. In this study, we have used AFM analysis in order to image the peptide nanotubes in a hydrated environment. However,

imaging in solution is often problematic, as the sample must be immobilized to a surface in order to gain stable images. Various substrates such as gold, silicon, and mica were investigated but did not result in the physisorption of nanotubes to substrate, only dilution of the nanotubes by acetone lead to a clear AFM image on mica. The nanotubes were diluted to a final acetone concentration of 50% acetone and revealed a clear tubular structure in liquid environment (Figure 9).

Discussion

The diphenylalanine peptide, NH₂-Phe-Phe-COOH, the core motif of the β -amyloid polypeptide, efficiently self-assembles

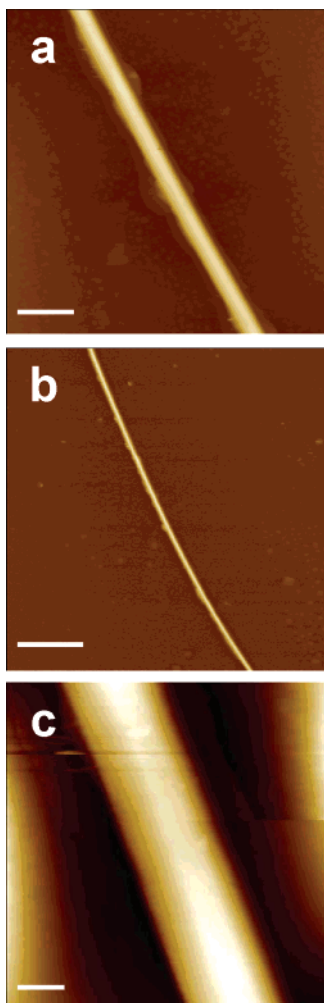


Figure 9. AFM images of diphenylalanine nanotubes in solution. Diphenylalanine nanotubes solution diluted with acetone to a final acetone concentration of 50% then imaged in aqueous environments on a mica substrate. (a) Topography image ($20\ \mu\text{m} \times 20\ \mu\text{m}$), bar = $4\ \mu\text{m}$. (b) Topography image ($30\ \mu\text{m} \times 30\ \mu\text{m}$), bar = $1\ \mu\text{m}$. (c) Topography image ($1\ \mu\text{m} \times 1\ \mu\text{m}$), bar = $200\ \text{nm}$.

into highly persistent, discrete nanotubes. We undertook this study to investigate their possible use in conventional microelectronic and microelectromechanics processes for fabrication into functional nanotechnological devices. An earlier report from our laboratory described the assembly of enzymatically stable nanotubes from proteolytically stable building blocks based on the D-amino acid analogue of the dipeptide, $\text{NH}_2\text{-(D)-Phe-(D)-Phe-COOH}$. The nanotubes formed by this dipeptide have the same structural features as the corresponding L-amino acid peptide.²⁰ In the current study, we investigated the thermal and chemical conformational stability of diphenylalanine peptide nanotubes to gain further insight into their physical properties for their potential integration into nanotechnological devices and assemblies. Several studies have shown the possible use of protein- and peptide- as well as nucleic acids-based bionanometric materials for applications ranging from molecular electronics to drug delivery.

The results of the present study indicate a marked structural stability of the diphenylalanine nanostructures in conditions of wet heat ($121\ ^\circ\text{C}$, $1.2\ \text{atm}$) (Figures 1–5) or dry heat (Figures 6–7).

SEM and TEM (Figures 1 and 2, respectively) as well as AFM (Figure 3) revealed the stability of the nanotubes in aqueous solution at temperatures above the boiling point of water or upon

autoclave treatment. The resemblance in CD spectra peak values of the heated $\text{NH}_2\text{-(L)-Phe-(L)-Phe-COOH}$ and the $\text{NH}_2\text{-(D)-Phe-(D)-Phe-COOH}$ dipeptides, which self-assemble into nanotubes structures, indicates that the secondary structure for each dipeptide remains intact over temperatures ranging from $25\ ^\circ\text{C}$ up to $90\ ^\circ\text{C}$. The peak at $200\ \text{nm}$ seen in the CD spectrum of a control dipeptide, $\text{NH}_2\text{-Ala-Ala-COOH}$, reflects a random coil secondary conformation (Figure 4b), which is consistent with the results of TEM and SEM analyses showing the inability of this peptide to form ordered nanoscopic structures.

The advantage of CD spectrometry is that it allows the analysis of the structures in solution. The CD spectra of the heated diphenylalanine nanotubes solution confirmed the images produced by EM and AFM that showed an intact structure after autoclave treatment, clearly supporting the unique stability of the supramolecular peptide structures in contrast to many naturally occurring protein structures. The compatibility of the nanotubes with sterilization procedures can be highly useful for the integration of the nanotubes into biosensors or for other applications requiring aseptic conditions.

In dry heat conditions, CFEG HRSEM indicated the thermal stability of the nanotubes, in that the durability of the tubes persisted until $150\ ^\circ\text{C}$, with degradation occurring only above $200\ ^\circ\text{C}$ (Figure 6).

The results of TGA indicate that the thermal stability of diphenylalanine peptide nanotubes is significantly higher than that of a nonassembling $\text{NH}_2\text{-Ala-Ala-COOH}$ dipeptide. The TGA results for the dry $\text{NH}_2\text{-Ala-Ala-COOH}$ peptide are consistent with its lack of ordered structure as shown by electron microscopy. Hence, the overlapping thermograms probably reflect the inherent instability of a monomeric dipeptide. As compared with the TGA thermogram of the monomer, the almost linear transformation of the diphenylalanine nanotubes above $50\ ^\circ\text{C}$ (Figure 7) indicates that their thermal stability is significantly higher than that of a similar hydrophobic dipeptide that does not form nanoscale ordered structures. We assume that this increase can be attributed to a well-organized aromatic stacking network that mediates the formation of the structures.^{25,26}

We previously demonstrated the stability of nanoparticles under extreme chemical conditions—from acidic (10% TFA) to alkaline (1 M NaOH).²⁶ Here, we assessed the stability of the nanotubes upon exposure to organic solvents that are used in the lithography process. The results of SEM analysis indicate that after treatment with ethanol, methanol, 2-propanol, acetone, and acetonitrile, the tubes completely retain their ultrastructure (Figure 8).

Imaging of nanostructures in solution is highly important in order to view the specimens in their natural state and gain a better insight of the ultrastructure in the liquid environment. The conventional imaging of biological nanometric assemblies in solution is undertaken using EM, which requires drying and coating with a conductive material. One way to deal with this problem is to image the biological samples using AFM in a liquid environment. The AFM, which is a scanning probe microscope, has several advantages over the electron microscope. Unlike the electron microscope, which provides a 2D image of a sample, the AFM provides a true 3D surface profile. More important in our case is that samples viewed by an AFM do not require any special treatment which might change the specimen properties. While EM needs a vacuum environment for proper operation, the AFM can work perfectly well in an ambient or even liquid environment and can be used to image nonconductive materials. In this study, we were interested in examining the peptide nanotubes in their natural aqueous environment. For that aim, we used AFM analysis in solution. To obtain a stable AFM

image of the nanotubes in an aqueous or semihydrated environment, various substrates, including gold, silicon, and mica, were assayed. However, these various substrates alone did not result in sufficient immobilization of the specimen to enable stable imaging. Only while diluting the peptide nanotubes solution with acetone to a final acetone concentration of 50% acetone and placing on mica substrate lead to a stable and clear AFM image. The image revealed a clear tubular structure of the nanotubes (Figure 9).

Diphenylalanine peptide nanotubes represent the core recognition motif of Alzheimer's β -amyloid polypeptide. The unique physical properties of the nanotubes reported here mirror the structural stability of amyloid fibrils.³⁵ Scheibel et al. recently demonstrated a formation of conductive nanowires by the coating of amyloid fibrils. The metallic nanowires were formed by gold deposition on a yeast prion-amyloid fiber, Sup35p. The amyloid fibrils are reportedly stable under a wide variety of harsh physical conditions.³⁵ Other studies have demonstrated that the infectivity of prion protein assemblies is retained at high temperature and pressure upon autoclave treatment.³⁶

Taken together, our results clearly demonstrate the remarkable thermal and chemical stability of peptide nanotubes in wet or dry conditions, pointing to their potential use in common lithographic procedures. The well-known advantage of biological materials and biological scaffolds is their molecular recognition properties and chemical diversity. The main disadvantage, however, is their relative instability. The diphenylalanine peptide, $\text{NH}_2\text{-Phe-Phe-COOH}$, retains the advantages of its biological characteristics on one hand and its high conformational stability on

the other. This combination makes it an excellent candidate for industrial nanotechnological applications.

Summary

Well-ordered and discrete peptide nanotubes are formed by the self-assembly of the diphenylalanine core recognition motif of Alzheimer's β -amyloid polypeptide. In this study, we characterized self-assembled peptide nanostructures, focusing on the thermal and chemical conformational stability of diphenylalanine nanotubes. The results presented here using several microscopic techniques, as well as spectroscopy, demonstrate the conformational stability of the peptide nanotubes to sterilization procedures in aqueous solution, as well as their thermal stability at high temperatures under dry conditions and chemical stability in organic solvents. We believe that information gained from this study will lead to a better understanding of the potential use of peptide nanotubes in future applications, such as micro- and nanoelectromechanics (MEMS and NEMS, respectively) and for medical devices.

Acknowledgment. The authors thank Yaacov Delarea for his help with the TEM experiments, Dr. Zehava Barkay for HRSEM analysis, Dr. Diana Golodnitsky for performing the TGA experiments, and members of the Gazit laboratory for helpful discussions. This research was supported by a start-up grant from the German-Israel Research Foundation (G.I.F.) to E.G. The work of L.A.A. was supported by the Marian Gertner Institute for Medical Nanosystems. V.S. thanks the U.K. Biotechnology and Biological Sciences Research Council for funding her studentship.

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