Nanostructured Peptide Fibrils Formed at the Organic–Aqueous Interface and Their Use as Templates To Prepare Inorganic Nanostructures

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ABSTRACT Formation of fibril-type nanostructures of the Alzheimer's β-amyloid diphenylalanine (L-Phe-L-Phe, FF) at the organic–aqueous interface and the factors affecting their structures have been investigated. Such nanostructures are also formed by bovine serum albumin and bovine pancreas insulin. The concentration of the precursor taken in the aqueous layer plays an important role in determining the morphology of the nanostructures. The addition of curcumin to the organic layer changes the structure of the self-assembled one-dimensional aggregates of diphenylalanine. By coating the diphenylalanine dipeptide fibrils with appropriate precursors followed by calcination in air, it has been possible to obtain one-dimensional nanostructures of inorganic materials.

KEYWORDS: organic–aqueous interface • diphenylalanine • albumin • insulin • nanostructures

INTRODUCTION

Self-assembled nanostructures can be formed by a variety of inorganic and organic building blocks, of which peptides are among the most useful ones. Peptides can spontaneously associate to form nanotubes, nanospheres, nanofibrils, nanotapes, and other ordered structures at the nanoscale level (1). Gadhiri and co-workers (2–4) used the concept of alternating D- and L-amino acids in the context of a cyclic peptide to form a planar ring that self-assembled, one on top of the other, to form nanotubular structures of the desired diameter. Gazit and co-workers (5) observed the self-assembly of a short peptide, the Alzheimer's β-amyloid diphenylalanine (L-Phe-L-Phe, FF) structural motif, into nanotubes and related one-dimensional (1D) structures in an aqueous solution. A vertically aligned nanoforest of nanotubes (6) and nanotube-based films (7) has also formed by this peptide. Controlled patterning of the dipeptide nanotubes has been accomplished by inkjet printing technology (8). Gorbitz (9, 10) has described the crystal structures of the different hydrophobic dipeptides and shown how hydrophilic channels embedded in a hydrophobic matrix by the peptide side chain are created along with three-dimensional aromatic stacking. The dipeptide tubes are stable under extreme physical and chemical conditions (11). Besides the simple dipeptides, insulin is known to aggregate to form mechanically strong amyloid fibrils (12–14). Nanostructures with different dimensionalities found by other peptides such as bolaamphiphile peptides (15), peptide-conjugated amphiphiles (16), and conjugated peptides (17) have also been explored.

In view of the great interest created by peptide-based nanostructures and fibrils, we have explored the formation of such structures at the oil–water (organic–aqueous) interface. The organic–aqueous interface has been exploited recently for generating ultrathin nanocrystalline films of a variety of inorganic materials (18, 19), but the interface has not been examined as a medium for the self-organization of biomaterials. We have investigated the formation of thin films comprising 1D nanostructures of the L-Phe-L-Phe structural motif at the organic–aqueous interface and characterized them by field-emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and UV–visible spectroscopy. We have examined the effects of the precursor concentration and the addition of curcumin on the formation of the peptide 1D nanostructures resembling amyloid-type fibrils. Nanostructures of bovine albumin and bovine insulin have also been prepared at the organic–aqueous interface.

It has been suggested that nanoscale fibers and tubes can be used as templates to form 1D inorganic nanostructures (20). Thus, the reduction of silver ions in association with dipeptide nanotubes, followed by enzymatic degradation of the peptide backbone, results in discrete silver nanowires (5). Silver-filled peptide nanotubes have been coated with gold to achieve trilayer coaxial nanocables (21). Composites of dipeptide nanotubes and platinum nanoparticles are obtained by reducing platinum salt at the nanotube surface (22). Collagen-related peptide fibers as well as self-assembled yeast amyloid fibers have also been used as templates to

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form metal nanowires (23, 24). We have used the dipeptide fibrils found at the organic–aqueous interface as templates to form 1D nanostructures of silica as well as gold and platinum. This was done by coating the fibrils with the precursor compounds and calcining in air at 400 °C.

EXPERIMENTAL SECTION

Diphenylalanine 1D fibrils were prepared at the organic–aqueous interface as follows. A total of 10 mg of diphenylalanine (phenylalanine–phenylalanine, NH_2-L-Phe-L-Phe-COOH; Bachem, Switzerland) was dissolved in 2 mL of water at 50 °C in a 5 mL beaker under sonication, and 2 mL of toluene was added to form an organic layer above the water layer. After 10 min, a thin white film formed at the interface. The film was lifted onto a Si(111) or quartz substrate for characterization. To obtain longer and more dense 1D nanostructures of diphenylalanine, aggregation processes were continued for longer durations (30, 50, 80, and 150 min), keeping the other conditions the same. Films were also prepared without sonication of the water layer before the addition of toluene. The effect of the concentration of the precursor dipeptide in the water layer was examined by decreasing its concentration to 0.5 mg/mL, keeping the other reaction parameters the same. The effect of curcumin on the formation of 1D fibrils was examined by adding 2 mL of a 10^{-6} M solution of curcumin in toluene, keeping the other parameters the same. Considering the volume of the organic layer (2 mL), the concentration works out to be 10^{-6} M, while the dipeptide concentration is 0.016 M in the water layer (2 mL).

A total of 10 mg of bovine serum albumin (Sigma-Aldrich, India) was dissolved in 2 mL of water in a 5 mL beaker, and 2 mL of toluene was added on the top of the water layer. After 20 h, a thin film formed at the interface. The film was lifted onto Si(111) for characterization. The concentration of bovine albumin in the water layer was decreased to 1 mg/mL, keeping the other parameters the same to examine the effect of dilution on the structures at the interface. A total of 10 mg of bovine pancreas insulin (Sigma-Aldrich, India) was dissolved in 2 mL of an aqueous acidic solution (pH = 2), and 2 mL of toluene was added on top of the water layer in a sealed beaker, which was kept at 40 °C. After 3 days, a thin film formed at the interface.

In order to examine the formation of 1D nanostructures of gold by using diphenylalanine fibrils as templates, 2 mg of a diphenylalanine 1D nanostructure as a dried powder was treated with $HAuCl_4$ (20 μL, 20 mM aqueous solution) and ascorbic acid (20 μL, 15 mM), followed by heating at 400 °C in air for 2 h. To prepare a 1D nanostructure of platinum, H_2PtCl_6 was used in place of $HAuCl_4$, keeping the other conditions the same. To prepare a 1D nanostructure of SiO_2, 2 mg of a diphenylalanine 1D nanostructure as a dried powder was treated with 10 μL of tetraethylorthosilicate (TEOS) followed by heating at 400 °C in air for 2 h.

Characterization of the thin films at the interface was carried out by transferring them onto Si(111). A field-emission scanning electron microscope (FEI Nova-Nano SEM-600, The Netherlands) was used to look at their morphology. In the case of peptide and amyloid nanostructures, films were coated with gold to render them conducting for FESEM studies. Energy-dispersive X-ray analysis (EDAX) was carried out by using the EDAX system attached to the FESEM. Transmission electron microscopy (TEM) studies of the nanostructures of gold, platinum, and SiO_2 were carried out with a JEOL JEM 3010 instrument operating at an accelerating voltage of 300 kV. Powder XRD patterns of nanostructures were recorded with a Philips X'Pert diffractometer employing the Bragg–Brentano configuration using Cu Kα radiation. TGA of peptide nanostructures was carried out with a Mettler Toledo thermal analyzer under a O_2 atmosphere with a scan rate of 2 °C/min. UV–visible spectra of the thin films of diphenylalanine 1D nanostructures were recorded after different times of aggregation using a Perkin-Elmer Lambda 900 UV–visible–near-IR spectrometer.

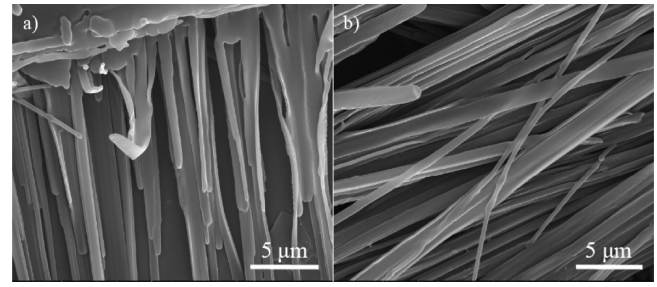

FIGURE 1. FESEM images of diphenylalanine 1D fibrils obtained at the toluene–water interface after (a) 10 min and (b) 150 min of the aggregation process.

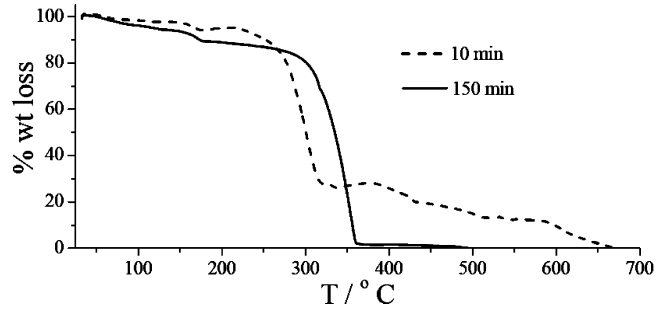

FIGURE 2. TGA curves of diphenylalanine 1D fibrils obtained at the interface after 10 and 150 min of the aggregation process.

RESULTS AND DISCUSSION

Peptide Nanostructures at the Interface. Gazit and co-workers (5–8, 11) have described various aspects of the formation of diphenylalanine peptide nanotubes in aqueous solution. We have investigated the formation of thin films comprising 1D nanostructures by the same dipeptide at the toluene–water interface under different conditions. Parts a and b of Figure 1 show the FESEM images of the dipeptide 1D nanostructures obtained at the interface after 10 and 150 min of the aggregation, respectively, starting with the initial dipeptide concentration of 5 mg/mL in water. After 10 min of aggregation, 1D dipeptide fibrils at the interface were found to have diameters in the 500–600 nm range and lengths in the 20–25 μm range. Upon an increase in the aggregation time to 150 min, the diameter and length increased to 1–1.2 μm and 80–100 μm, respectively. Powder XRD patterns (see Figure S1 in theSupporting Information) of dipeptide 1D fibrils obtained at the organic–aqueous interface could be indexed on the hexagonal structure (space group $P6_1$) as reported in the literature (6, 7). In Figure 2, we show the TGA curves of the dipeptide 1D fibrils obtained after 10 and 150 min of agrregation. We see that the 1D nanostructures are stable up to 300 °C. With an increase in the aggregation time, the thermal stability of these nanostructures increases sufficiently, accompanying their increased dimensions. Figure 3 shows the time evolution of the UV–visible spectra of the thin films of diphenylalanine peptide 1D fibrils. The intensity of the benzenoid band around 250 nm increases with the aggregation time. This is consistent with the increased dimensions of the

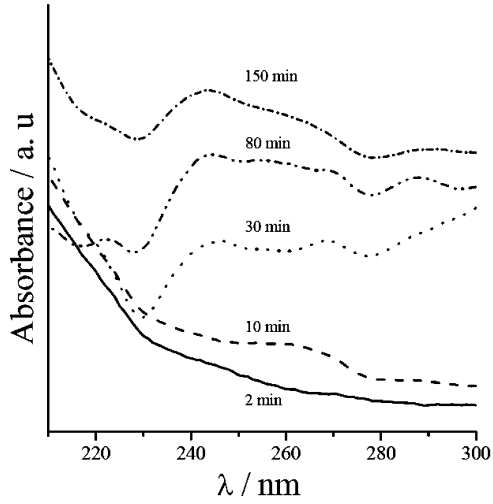

FIGURE 3. Time evolution of the UV−visble spectra of the thin films comprising 1D fibrils of diphenylalanine.

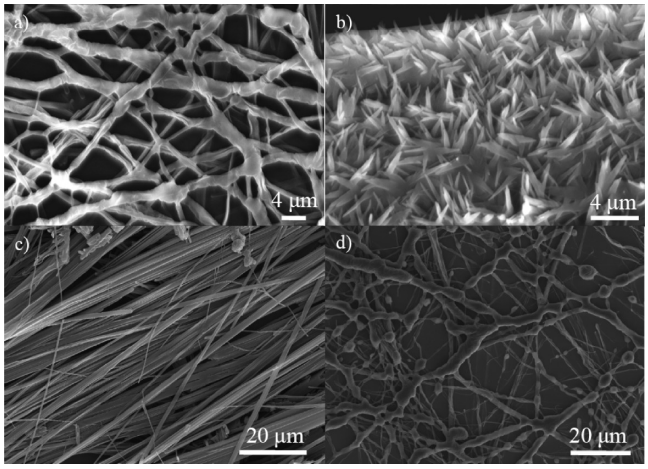

FIGURE 4. (a) FESEM image of a network-like nanostructure of diphenylalanine obtained at the interface after 150 min of the aggregation when initial sonication of the water layer was avoided. (b) FESEM image of a 1D nanostructure of diphenylalanine obtained at the interface after 150 min of aggregation with low precursor concentration (0.5 mg/mL in water layer). FESEM images of diphenylalanine 1D fibrils obtained at the interface in the (c) absence and (d) presence of curcumin in the toluene layer.

structures and the suggestion that aromatic $\pi-\pi$ stacking plays a crucial role in the formation of the 1D nanostructure (10). As the reaction proceeds for longer fibrils, there is greater $\pi-\pi$ stacking and associated thermodynamic stability of the nanostructures.

The morphology of the dipeptide nanostructures at the toluene−water interface also depends on the conditions employed. Figure 4a shows the FESEM image of the product formed after 150 min of the aggregation in the absence of initial sonication of the aqueous layer. We see that there is no network-type structure found when sonication was performed. When the precursor dipeptide concentration in the aqueous layer was decreased, keeping the other parameters the same, shorter and thinner 1D nanostructures occur at the interface after 150 min of aggregation. It can be seen from the FESEM image in Figure 4b that the diameter and length of the 1D nanostructures are 400−500 nm and 2−3 μm, respectively.

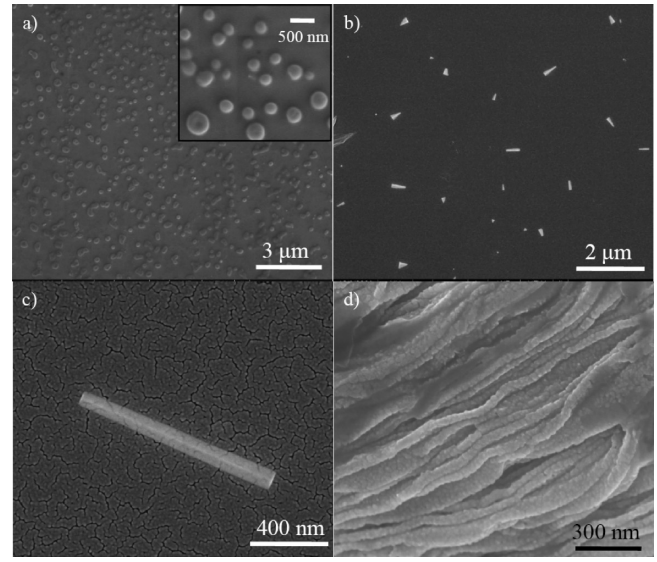

FIGURE 5. FESEM images of bovine albumin (a) nanovesicles and (b) 1D nanostructures obtained at the toluene−water interface. The inset in part a shows a high-magnification FESEM image of bovine albumin nanovesicles. (c) High-magnification FESEM image of a bovine albumin 1D structure. (d) FESEM image of bovine insulin fibrils obtained at the toluene−water interface.

It has been previously shown that diphenylalanine can self-assemble into short peptide nanotubes or a forest of peptide nanotubes when aqueous or fluorinated alcohol solutions of diphenylalanine are deposited on solid surfaces and the solvent is evaporated (5, 6). In the case of the organic−aqueous interface, thin films or networks of longer dipeptide fibrils are generally formed depending upon the conditions employed. We have always found peptide fibrils of different dimensions rather than nanotubes. TGA and UV−visible data suggest that aromatic $\pi-\pi$ stacking plays an important role in the formation of the 1D nanostructures at the interface.

Curcumin from turmeric is considered to be of high therapeutic value, and its use in modern medicine has been extensively studied. It is considered to be useful in the treatment of Alzheimer's disease (25). Curcumin appears to bind to small β-amyloid species to block aggregation and fibril formation (26). We considered it interesting to study the effect of curcumin on the formation of 1D fibrils of the Alzheimer's β-amyloid structural motif at the organic−aqueous interface. Parts c and d of Figure 4 show the FESEM images of dipeptide nanostructures obtained at the toluene−water interface after 150 min of aggregation in the absence and presence of curcumin in the toluene layer. In the presence of curcumin, short fibrils along with a few vesicles rather than robust arrays of dipeptide fibrils formed at the interface. It seems that curcumin does affect the aggregation and longer fibril formation of diphenylalanine at the interface.

We have extended our investigation to two other amyloid polypeptides, bovine albumin and bovine insulin. Figure 5a shows the FESEM image of the film formed by bovine albumin at the toluene−water interface. When the concentration of the albumin in the water layer was 5 mg/mL, we observed nanovesicles at the interface with diameters in the

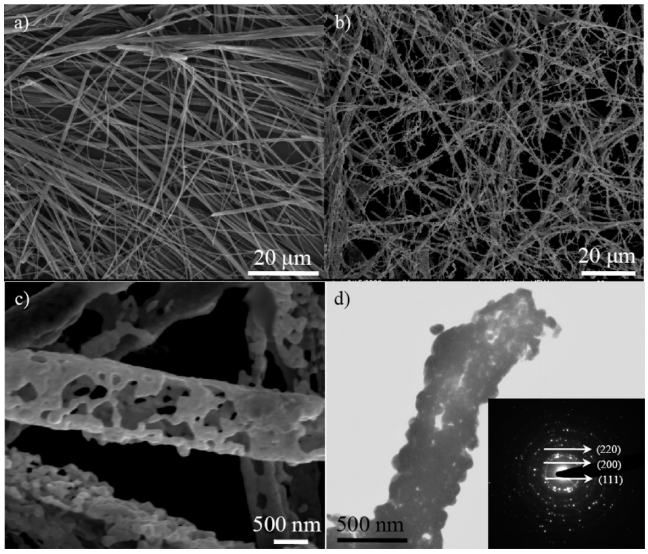

FIGURE 6. (a) FESEM image of diphenylalanine 1D fibrils before $HAuCl_4$ and ascorbic acid treatment. (b) FESEM image of a 1D nanostructure of gold after $HAuCl_4$ and ascorbic acid treatment followed by calcining in air. (c) High-magnification FESEM image of a 1D nanostructure of gold. (d) TEM image of a 1D nanostructure of gold. The inset in part d shows the indexed SAED pattern of a 1D nanostructure of gold.

350−500 nm range (see the inset of Figure 5a). When we decreased the concentration of the bovine albumin in the water layer to 1 mg/mL, 1D nanostructures of bovine album formed, as can be seen from Figure 5b. A typical high-magnification FESEM image of 1D structure (length 1μm; diameter 80 nm) of bovine albumin is shown in Figure 5c.

Bovine pancreas insulin also forms 1D structures at the toluene−water interface. In Figure 5d, we show a high-magnification FESEM image of bovine insulin nanofibrils formed at the interface after 3 days.

Use of Peptide Fibrils as Templates. We have obtained 1D nanostructures of gold by coating the diphenylalanine fibrils with $HAuCl_4$ followed by reduction with ascorbic acid. Upon calcination at 400 °C in air to decompose the peptide backbone to gaseous oxides, we obtain the gold nanostructures. Figure 6a shows the FESEM image of starting diphenylalanine 1D fibrils, and Figure 6b shows the FESEM image of 1D nanostructures of gold obtained by the procedure described above. The high-magnification FESEM image of these nanostructures in Figure 6c confirms its porous and tubular nature. The XRD pattern of the 1D structure showed that it was in agreement with the well-known literature cubic structure (space group $Fm\bar{3}m$, a = 4.074 Å). The TEM image of the 1D structure of gold in Figure 6d reveals that the walls of the nanostructure comprise small gold nanocrystals. The selected-area electron diffraction (SAED) pattern of the gold structure, shown as an inset in Figure 6d, reveals the polycrystalline nature of these structures. The pattern could be indexed on the basis of the cubic $Fm\bar{3}m$ structure.

We have also obtained 1D nanostructures of platinum by using diphenylalanine fibrils as templates. Figure 7a shows the FESEM image of the platinum structures. The high-magnification FESEM image of 1D structures of platinum in

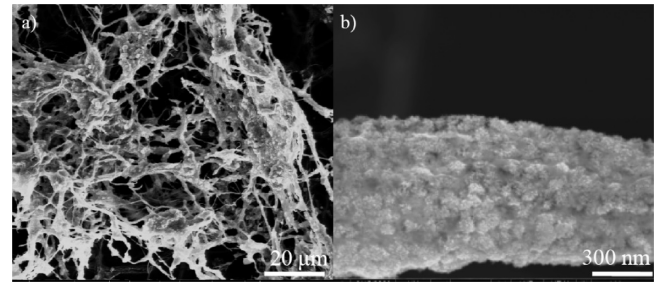

FIGURE 7. (a) FESEM image of a 1D nanostructure of platinum. (b) High-magnification FESEM image of a 1D nanostructure of platinum.

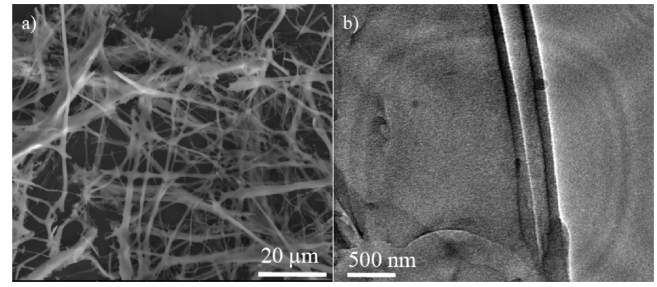

FIGURE 8. (a) FESEM image of a 1D nanostructure of SiO_2. (b) TEM image of nanotubular SiO_2.

Figure 7b confirms the presence of small platinum nanocrystals along the walls of the structure. The XRD pattern of 1D structures was in agreement with the cubic structure (space group $Fm\bar{3}m$, a = 3.924 Å).

We have obtained 1D nanostructures of SiO_2 by coating the diphenylalanine fibrils with TEOS followed by heating in air at 400 °C. The XRD pattern of the structures reveals the amorphous nature of SiO_2. In Figure 8a, we show the FESEM image of the SiO_2 1D structure. The TEM image of the structure shown in Figure 8b reveals the tubular nature.

CONCLUSIONS

The present study shows how L-Phe-L-Phe, bovine albumin, and bovine insulin form thin films comprising 1D nanostructures at the toluene−water interface. The formation of these nanostructures depends on the concentration of the precursors and other factors. Curcumin changes the structure of the self-assembled aggregates of the diphenylalanine fibrils. By using the diphenylalanine fibrils as templates, it has been possible to make 1D nanostructures of gold, platinum, and SiO_2.

Supporting Information Available: Indexed XRD pattern of a diphenylalanine 1D nanostructure obtained at the interface after 150 min of the aggregation process. This material is available free of charge via the Internet at http://pubs.acs.org.

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