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Organic Solvents Mediate Self-assembly of GAV-9 Peptide on Mica Surface

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Abstract Self-assembly of peptides into fibrils and other morphologies has attracted much attention in many fields, especially in nanofabrication, pathology and biochemistry. In this paper, self-assembly of GAV-9 peptide in organic solvents, ethanol and acetone, was investigated using atomic force microscopy (AFM) and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The results indicated that GAV-9 self-assembled into various nanostructures in both solvents after deposited and evaporated on mica. Fibrils with β-sheet conformation were observed in both solvents when the peptide concentration was higher than 280 μM. However, ordered fibrils with β-sheet conformation were formed in ethanol, but not in acetone, with a peptide concentration ranging from 7 μM to 28 μM. We attribute the formation of various nanostructures to the different physicochemical properties of the polar organic solvents on the self-assembly of GAV-9 peptide.

Key words self-assembly; GAV-9 peptide; organic solvents; ATR-FTIR; AFM

In recent years, peptide self-assembly has attracted great attention from scientists in various fields, such as nanotechnology, pathology and biochemistry [1–6]. In particular, self-assembly of peptides has been considered as a potential approach to the fabrication of nanostructures [7–13]. In this regard, methodologies that can direct the formation of the peptides into different nanostructures are particularly interesting. For example, our previous study has shown how the interfacial hydrophobicity/hydrophilicity influences the nanostructures formed on solid substrates [10].

It has been found that solvents also play an important role in the diversities of the self-assembled peptide structures [9–12,14]. It has been reported that the insulin protein shows different morphologies of both hierarchical aggregates and fibrils with the presence of various organic media as co-solvents, in contrast to rather less aggregate types in its aqueous solution [15]. In addition, Grudzielanek *et al*. reported that the fibrils grown in ethanol and water show significantly different ability to "seed" amyloid formation [15]. The influence of nonaqueous solvents on protein self-assembly has also been widely investigated [16].

In the present study, we investigated the self-assembly of GAV-9 peptide [10,17] in ethanol and acetone using atomic force microscopy (AFM) and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The results indicated that different morphologies of GAV-9 peptide were formed with the presence of these solvents on mica.

Materials and Methods

Materials

GAV-9 peptide (NH_2-VGGAVVAGV-$CONH_2$) was synthesized using the Boc solid-phase method on an ABI 433 A peptide synthesizer (Applied Biosystems, Foster City, USA) and cleaved from the 4-methylbenzhydrylamine (MBHA) resin (100–200 mesh; Fluka, Buchs, Switzerland) with hydrogen fluoride. The peptide was purified through

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a TSK-40 (S) column (2.0 C, 98 cm; Tosoh, Tokyo, Japan). Ethanol (HPLC grade) was purchased from Sigma (St. Louis, USA). Acetone was purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

Methods

To prepare samples, a drop of 1 μl GAV-9 peptide in ethanol or acetone solution with different concentrations was deposited on a freshly cleaved mica substrate and dried in air by evaporation. The samples were imaged by AFM (Nanoscope IIIa Multi-mode; Veeco, Santa Barbara, USA) equipped with a J scanner under the tapping mode. Silicon cantilevers (NSG 11; NT-MDT, Zelenograd, Russia) with a nominal constant of 2.5–10 N/m and a typical resonant frequency of 150 kHz were used. The temperature and relative humidity for the operations were 20±2 ºC and 30%±5%, respectively.

The solid-state ATR-FTIR spectra of the samples were taken using a Nicolet Avatar 370 FTIR with a deturiated triglycine sulphate detector and a ZnSe-window, single-bounce, ATR accessory (Nicolet Omni Sampler, Madison, USA) installed into the sample compartment. All spectra were collected by 32 scans and a resolution of 8 cm^{-1} from 4000 to 400 cm^{-1}. The final spectra were smoothed to reduce noise and were inverted to absorbance.

Results and Discussion

Self-assembly of GAV-9 peptide with ethanol or acetone as solvent

GAV-9 peptide is a conserved consensus of three neurodegenerative disease-related proteins: α-synuclein, amyloid β protein, and prion protein, which are capable of forming amyloid fibrils *in vivo* and *in vitro*. Our previous study [10] revealed the formation of well-defined nanofilaments when GAV-9 peptide assembles on solid surfaces in aqueous buffer. The height of the nanofilament is approximately 3 nm on mica or 1 nm on highly ordered pyrolytic graphite (HOPG), which indicates that peptides take an "upright" orientation on mica but "lying down" on HOPG. However, when a drop of the peptide in organic solvent was evaporated on mica substrate, more complicated morphologies were formed. As shown in **Fig. 1(A)**, when the concentration of GAV-9 was 1.4 μM, the peptide self-assembled into rod-like structures upon evaporation of the solvent. The top side of the rod was smooth, with a mean roughness of approximately 0.1 nm. The rod showed an average height of approximately 4 nm, and sometimes overlapped in a layer-by-layer fashion with a height more than 30 nm. When the peptide concentration increased, there were fibrils with various dimensions formed on the mica substrate as well as the rod-like structures. As shown in **Fig. 1(B)**, some were short fibrils with a height less than 1 nm, others were longer fibrils with a height more than 3 nm. Like the nanofilaments formed in water on HOPG, these thin fibrils were not stable and could be easily damaged when they were imaged with a hard AFM tip. In another case, the mica surface was covered with a layer of fibrils when the peptide concentration increased to 28 μM [**Fig. 1(C)**]. The fibrils showed a three-fold symmetry orientation that reflected epitaxy of the underlying substrate. This kind of substrate-assisted self-assembly of the peptide was very similar to our previous result [10]. However, if the peptide concentration was too high, the fibrils formed on the substrate would show no preference in orientation, indicating the decrease of the influence of the substrate [**Fig. 1(D)**].

Unlike that in ethanol, GAV-9 peptide in acetone had different self-assembly behavior in the concentration range 1.4–28 μM. On the samples prepared with low concentration peptide, there were more homogeneous rod-like structures but fewer aggregates than in ethanol [**Fig. 2 (A)**]. The dimensions of the rod were similar to those in ethanol. There were also layer-by-layer structures when the concentration of the GAV-9 peptide increased to 7 μM, as shown in **Fig. 2(B)**. As the peptide concentration increased [**Fig. 2(C)**], so did the layer-by-layer structures and aggregates observed on mica. There were many randomly deposited fibrils and a few aggregates on mica when the peptide concentration reached 280 μM [**Fig. 2 (D)**], which was a similar result to that shown in **Fig. 1 (D)**.

Interestingly, only randomly oriented fibrils were found on the samples prepared from GAV-9 peptide in acetone. This fact indicated that the self-assembly process was not influenced by the mica substrate. It could be partly attributed to the fast evaporation of the acetone during the self-assembly process. In our experiments, it only took approximately 3 s to evaporate the acetone drops, compared to approximately 30 s for the ethanol drops. In such a fast evaporation process, the peptide concentration increased immediately so that it tended to form randomly oriented fibrils. However, the different physicochemical properties of ethanol and acetone might also influence the self-assembly of GAV-9 peptide. Ethanol is both a donor and acceptor of hydrogen bonds, whereas acetone is only an acceptor. There are more hydrogen bonds in ethanol,

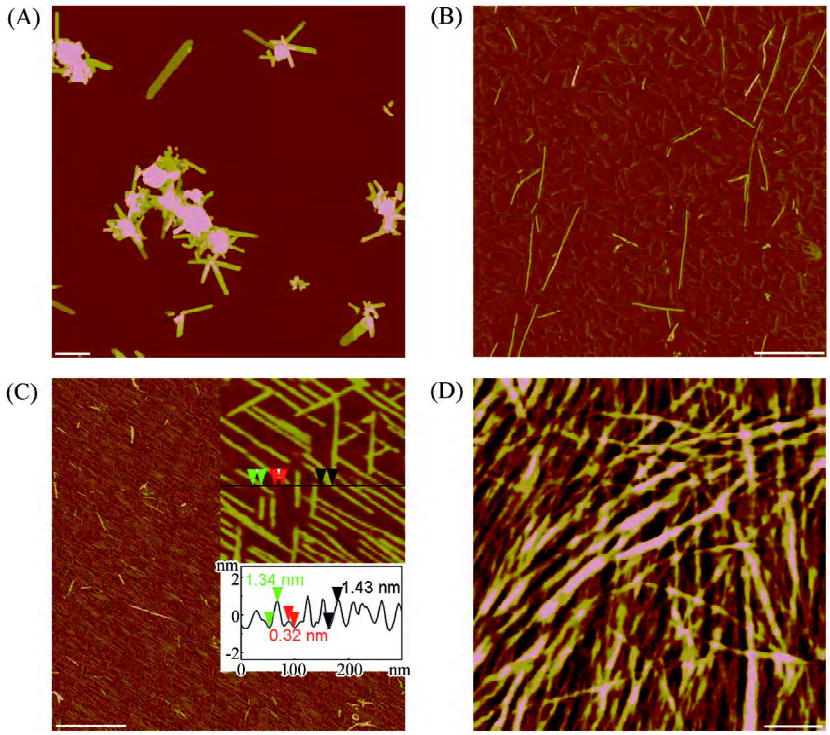

Fig. 1 AFM topography images of the samples prepared from GAV-9 peptide at different concentrations in ethanol

(A) 1.4 μM, (B) 7 μM, (C) 28 μM, and (D) 280 μM. The inset in (C) is a zoom picture and section analysis of the black line marked on it. The scale bars are 1 μm in (A), (B) and (C), and 500 nm in (D), respectively.

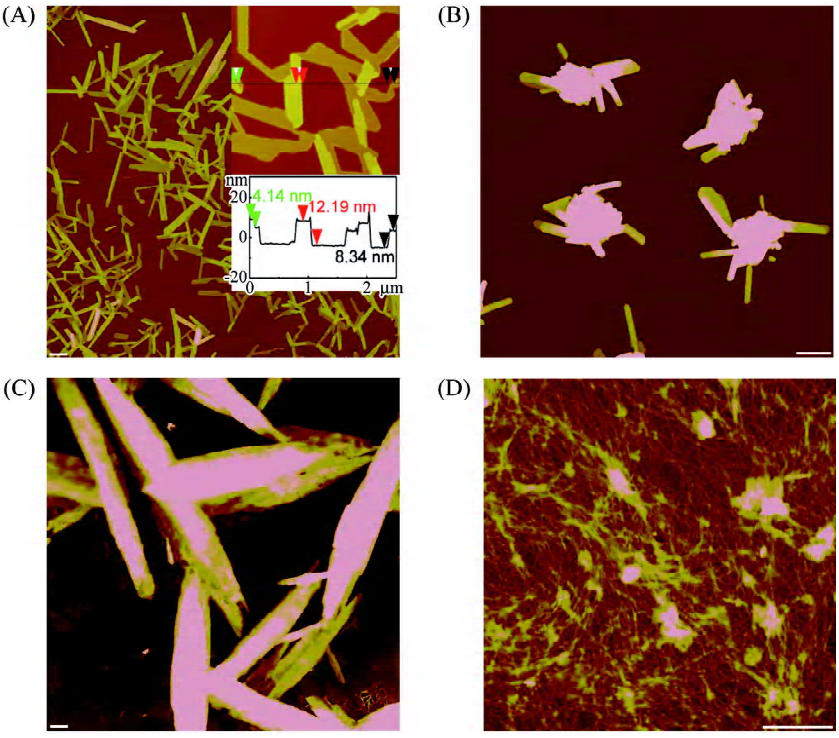

Fig. 2 AFM topography images of the samples prepared from GAV-9 peptide at different concentrations in acetone

(A) 1.4 μM, (B) 7 μM, (C) 28 μM, and (D) 280 μM. The inset in (A) is a zoom picture and section analysis of the black line marked on it. The scale bars are 1 μm in (A), (B), and (C), and 500 nm in (D), respectively.

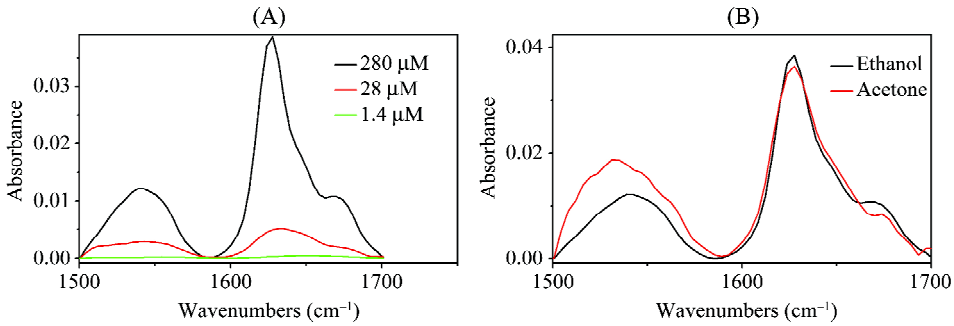

Fig. 3 ATR-FTIR spectra of GAV-9 peptide in ethanol and acetone

(A) IR spectrum of GAV-9 peptide in ethanol. (B) IR spectrum of GAV-9 peptide with a concentration of 280 μM in ethanol and acetone, respectively.

which might facilitate the formation of ordered fibrils on the polar substrate.

Conformational studies of GAV-9 peptide in ethanol and acetone by FTIR spectra

In this study, ATR-FTIR spectroscopy was used to probe the conformation of the peptide adsorbed on mica after the organic solvents evaporated. When the concentration of the GAV-9 peptide increased from 1.4 μM to 280 μM in ethanol, as shown in **Fig. 3(A)**, the amide I' band shifted to 1626 cm^{-1} and its peak width became narrow. At the same time, a more obvious shoulder peak appeared near 1670 cm^{-1}, which indicated the formation of the β-sheet conformation [18]. The increasing intensity of the peak near 1630 and 1670 cm^{-1}, along with the increasing peptide concentration, was consistent with the increasing amount of GAV-9 peptide fibrils on mica. This result indicated that these fibrils had β-sheet conformation. The GAV-9 peptide in acetone had similar results to those in ethanol.

Conclusion

In conclusion, we have shown that organic solvents can influence the self-assembly of GAV-9 peptide on a mica surface. In ethanol, which is both a donor and acceptor of hydrogen bonds, GAV-9 peptide self-assembled into rod-like structures, ordered β-sheet fibrils, and random fibrils according to different peptide concentrations. In acetone, which is just an acceptor of hydrogen bonds, GAV-9 peptide self-assembled into rod-like structures and randomly oriented fibrils. ATR-FTIR experiments indicated the formation of β-sheet conformation in samples prepared from both of the solvents. Our results could be used to fabricate controlled, shape-specific nano-architectures by self-assembly of peptides in different solvents.

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