Vancomycin-induced morphological transformation of self-assembled amphiphilic diacetylene supramolecules

Kun Ha Park, Ji-Seok Lee, Hyunwook Park, Eun-Hye Oh and Jong-Man Kim*

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Reversible ribbon-sphere microstructural transformation of dipeptide-containing diacetylene supramolecules was observed by specific ligand–receptor interactions.

Amphiphilic diacetylene lipids have been intensely investigated as supramolecular scaffolds due to the unique properties associated with the photopolymerizable diacetylene moieties as well as their ability to form well-defined nano/microstructures.[1–3] Although numerous diacetylene-based supramolecular structures have been uncovered, molecular recognition induced transitions of their microscopic morphology have not been observed. Only one example of a pH-induced nanostructural transformation from helical ribbons to nanofibers has been described.[4] Below, we describe the results of a recent investigation in which we have demonstrated that a reversible ribbon–spherical morphological transition of a specifically designed dipeptide containing diacetylene is promoted by its interaction with vancomycin and a vacomycin-specific ligand. It should be noted that an elegant example of Vancomycin-induced sol–gel transformation of a fluorenyl-containing dipeptide was recently described.[5]

The glycopeptide antibiotic vancomycin (Van) inhibits bacterial cell wall synthesis by specific binding to D-Ala-D-Ala dipeptide termini of peptidoglycan precursors.[6] Although model studies with Van and bacterial membrane mimics derived from D-Ala-D-Ala-containing lipids have been carried out,[7] the effects of this antibiotic on morphological changes of lipid assemblies has not been reported. Owing to the importance of Van in the treatment of severe bacterial infections, knowledge about Van-induced microscopic changes in lipid assemblies is significant. At the outset of these efforts, we felt that D-Ala-D-Ala dipeptide terminated, diacetylene lipids would form well-defined supramolecular structures as a result of intermolecular H-bonding interactions as has been observed in other amino acid terminated diacetylene lipids.[8] Consequently, if Van binding is able to disrupt well-ordered lipid assemblies, changes in the microscopic morphology in lipid assemblies are expected. The effects of Van binding on structural transitions of photopolymerizable diacetylene moieties and the reversibility of the process was addressed in the current investigation.

In order to investigate the above-mentioned issues, the peptide headgroup containing the 10,12-pentacosadiynoic acid (PCDA)-derived diacetylene amphiphile, PCDA-D-Ala-D-Ala **2**, was prepared along with its enantiomer PCDA-L-Ala-L-Ala **1** as a control (Fig. 1).†‡ A clear solution is obtained when dispersed mixtures (4 mg in 10 mL of 30% H_2O–EtOH) of each of the dipeptide-derived diacetylene amphiphiles **1** or **2** is heated to 60 °C. Upon cooling of each solution to room temperature, a solid precipitate of the recovered lipid is formed (>90% yield, Fig. 2(A) and (B)). As expected, no differences between the precipitation behaviors of the enantiomers **1** and **2** are observed. In contrast, strikingly different results are obtained when mixtures of the two diacetylene lipids are mixed with Van (1 molar equiv.).§ Heating and cooling of a solution containing diacetylene lipid PCDA-L-Ala-L-Ala **1** and Van yields a white solid precipitate (Fig. 2(C)) while a suspension is formed when a solution of PCDA-D-Ala-D-Ala **2** and Van are subjected to the same conditions (Fig. 2(D)).

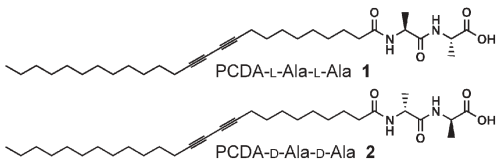

Fig. 1 Structures of dipeptide-containing diacetylene lipids.

Scanning electron microscope (SEM) analysis was carried out to gain structural information about the precipitates (Fig. 3(A), (B) and (C)) and dispersed solids (Fig. 3(D)) formed in the heating–cooling cycles described above. The SEM images show that ribbon-shaped structures of micrometer-range widths and 100–150 nm thicknesses are generated upon cooling solutions of **1** and **2** in the absence of Van (Fig. 3(A) and (B)). As anticipated, no significant morphological difference exists between the enantiomers **1** and **2**. The SEM image of the solid precipitate formed on cooling a solution of PCDA-L-Ala-L-Ala **1** in the presence of Van reveals that it also has a ribbon-like structure (Fig. 3(C)). However, the morphology of the supramolecules that originated by cooling a

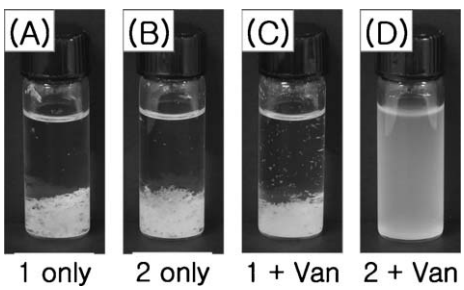

Fig. 2 Photographs of solutions containing dipeptide-derived diacetylene lipids **1** and **2** after standing at room temperature for 6 h in the absence (A and B) and presence (C and D) of Van. All solutions were clear and transparent before cooling.

Department of Chemical Engineering, Hanyang University, Seoul, 133-791, Korea. E-mail: jmk@hanyang.ac.kr; Fax: +82-2-2298-4101; Tel: +82-2-2220-0522

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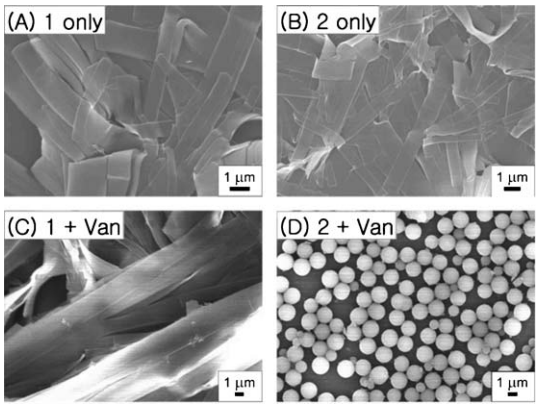

Fig. 3 SEM images of solid precipitates (A, B and C) and dispersed particles (D) obtained as described in Fig. 2.

mixture of the enantiomeric diacetylene amphiphile, PCDA-D-Ala-D-Ala **2**, and Van is strikingly different. In this case, the SEM image (Fig. 3(D)) shows that spheres with micrometer diameters are generated exclusively.

The unique Van-induced morphological transformation of PCDA-D-Ala-D-Ala **2** was further demonstrated by using fluorescence microscopy. It is known that self-assembled diacetylenes can be polymerized by UV light and the resulting polydiacetylenes (PDAs) in their 'red-phase' fluoresce.[9,3b] Samples of **1** and **2** in the presence and absence of Van, prepared by using the heating–cooling cycle described above, were irradiated with 254 nm UV light (1 mW cm^{-2}) for 300 s and then subjected to fluorescence microscope analysis. The images obtained (Fig. 4) show a clear difference between the two materials generated. The bundles of fibrous PDAs were detected from **1** and **2** which were prepared in the absence of Van (Fig. 4(A) and (B)). The fibrous bundles (Fig. 4(B)) detected when **2** is precipitated in the absence of Van, are not present in the PDAs obtained from **2** in the presence of Van (Fig. 4(D)). Instead weak fluorescence signals from microdots are observed.

In order to determine if the Van-induced morphological change occurs on preformed diacetylene supramolecules, ribbon-shaped supramolecular aggregates of PCDA-D-Ala-D-Ala **2** were

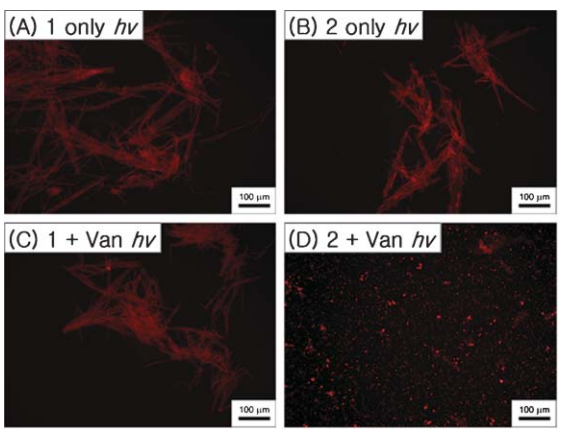

Fig. 4 Fluorescence microscopic images obtained with polymerized PCDA-L-Ala-L-Ala **1** and PCDA-D-Ala-D-Ala **2** in the absence (A and B) and presence (C and D) of Van.

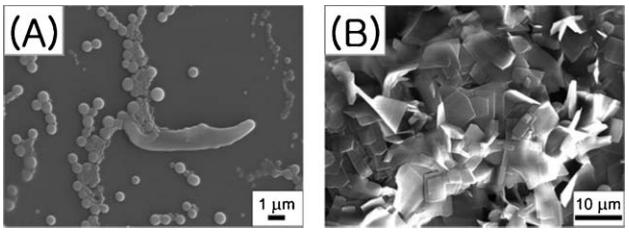

Fig. 5 SEM images obtained after addition of Van (A) and Ac-Lys(Ac)-D-Ala-D-Ala (B) to the solutions obtained as described in Fig. 2(B) and (D), respectively.

generated in the absence of Van. The solid was collected by centrifugation, washed with 30% H_2O–EtOH, and fully dispersed in 30% H_2O–EtOH containing 1 molar equiv. of Van at room temperature for 1 h. The SEM image of the material produced in this manner shows that spherical vesicles are formed (Fig. 5(A)). The effect of photopolymerizable diacetylene moieties on the morphological transition was tested by adding Van to the solution containing UV-irradiated ribbon aggregates. As expected, no ribbon-to-sphere transition was observed with the polymerized diacetylene assembly (data not shown).

The final phase of current investigation focused on the possibility of reversible sphere-to-ribbon transition. Thus, Ac-Lys(Ac)-D-Ala-D-Ala,[10] a substance known to tightly bind to Van was added to a dispersion of the spherical **2**–Van complex prepared as described in Fig. 3(D). SEM analysis shows that 1 h following addition of this blocked tripeptide (5 mol. equiv.) to a dispersion of the spherical **2**–Van complex, spherical structures have disappeared and ribbon-like structures associated with **2** are regenerated (Fig. 5(B)). Thus, a reverse sphere-to-ribbon transformation of the **2**–Van complex is promoted by addition of Van-specific ligand Ac-Lys(Ac)-D-Ala-D-Ala.

The results presented above show that reversible morphological transitions of diacetylene supramolecules can be promoted by designed ligand–receptor interactions. In the case of dipeptide lipid PCDA-D-Ala-D-Ala **2**, the Van-induced transition is the result of disruption of intermolecular hydrogen bonds which cause formation of layered ribbon-like structures (Fig. 6). Binding of Van to the D-Ala-D-Ala motif of **2** changes the effective size and H-bonding properties of dipeptide headgroup in a way that blocks formation of ribbon-shaped morphologies and enhances the creation of spherical microstructures. The fact that addition of the Van-specific ligand Ac-Lys(Ac)-D-Ala-D-Ala to the **2**–Van

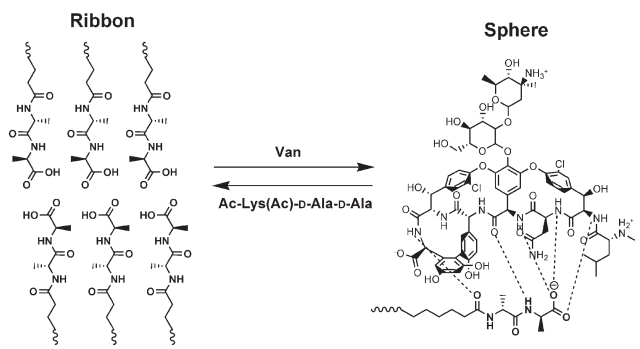

Fig. 6 A schematic representation of interactions occur in ribbon-like (left) and spherical (right) supramolecular structures.

complex causes regeneration of ribbon-shaped microstructures demonstrates that the morphological changes are reversible.

In summary, results arising from this investigation conclusively demonstrate that it is possible to design amphiphilic diacetylene lipids that undergo morphological transformations in response to specific ligand–receptor binding interactions. In addition, the study has shown that this process can be reversible. Lastly, since Van is a potent and important antibacterial agent, the observation that Van-binding induces a microstructural transformation of the D-Ala-D-Ala dipeptide-containing lipid assembly should be of general relevance to Van-related research areas.

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Notes and references

† *Materials*: 10,12-pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals. H-Ala-Ala-OH (L-Ala-L-Ala), H-D-Ala-D-Ala-OH (D-Ala-D-Ala) and Ac-Lys(Ac)-D-Ala-D-Ala-OH (Ac-Lys(Ac)-D-Ala-D-Ala) were purchased from Bachem. Vancomycin hydrochloride (Van) was purchased from Sigma. *N*-Hydroxysuccinimide ester of 10,12-pentacosadiynoic acid (PCDA-NHS) was prepared as described in the literature.[3d]

‡ *Synthesis of PCDA-L-Ala-L-Ala **1***: H-Ala-Ala-OH (204 mg, 1.27 mmol) was dissolved in 7 mL of THF–water solution and TEA (0.44 mL, 3.18 mmol), PCDA-NHS (500 mg, 1.06 mmol) were added. After stirring at room temperature for 24 h, the solvent was removed *in vacuo*. To the solid residue was added 200 mL of water and the precipitate was collected, washed several times with cold chloroform. After drying *in vacuo*, 357 mg (65%) of PCDA-L-Ala-L-Ala was obtained. A similar procedure was applied to synthesize PCDA-D-Ala-D-Ala **2**.

PCDA-L-Ala-L-Ala **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H), 1.12–1.64 (m, 38H), 2.19–2.31 (m, 6H), 4.47–4.64 (m, 2H), 6.23 (d, 1H), 7.01 (d, 1H), 7.26 (br s, 1H). FT-MS: calc. M = 516.3927, obs. MH^+ = 517.3999.

§ *Supramolecular ribbons and spheres*: A dipeptide-terminated diacetylene monomer (4 mg), PCDA-L-Ala-L-Ala **1** or PCDA-D-Ala-D-Ala **2** was dispersed in 10 mL of 30% H_2O–EtOH. The mixture was heated to 60 °C with vigorous stirring till it became clear. The solution was filtered to remove aggregates with a cotton filter and cooled down to room temperature. Similar procedures were repeated in the presence of Van (1 equivalent to the diacetylene lipid used). SEM images of diacetylene supramolecules were obtained on a JEOL (JSM-6330F) FE-SEM. Samples were freshly made and deposited dropwise on silicon wafers with a 20 μL micropipette. The wafers deposited with diacetylene supramolecules were then kept *in vacuo* for at least 12 h, followed by coating with Pt for 5 min. SEM images were examined at an accelerating voltage of 10 or 15 kV. An optical and fluorescent microscope (Olympus BX51 W/DP70) was used to observe the fluorescence images.

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