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Non-equilibrium structures of α-helix rod type hydrophobic polypeptide molecules on mica surfaces

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A hydrophobic polypeptide, poly(γ-benzyl-L-glutamate) (PBLG), has a solid and rod-like α-helix structure. According to this rigid molecular conformation, this type of peptide molecules forms good Langmuir-Brodgett (LB) thin films. In this study, non-equilibrium self-assembled structures, called dissipated structures, of PBLG were observed by putting and drying a 1,2-dichloroethane drop of PBLG on a hydrophilic mica surface. AFM images of the dried sample on mica clearly show the multi-layered structure of PBLG molecules with honeycomb structures under high humidity conditions. On the contrary, under low humidity conditions, densely packed cholesteric liquid crystal-like structures were observed without holes.

Key words: polypeptide, polymer rod, hydrophobic, Marangoni effect, hierarchical

1. INTRODUCTION

One of the typical non-equilibrium structures, called as dissipated structures, can often be observed in our actual world. For example, the structures of beautiful tears of wine observed on the inside surface of wine glasses and coffee stains on a mug, are generated according to Marangoni effect by convection. Recently, this self-assemble structure has been studied intensively in order to generate ordered patterns just by casting solutions which are immiscible with water onto a substrate. During the vaporization of the solvent, the aqueous vapor in the atmosphere coheres and forms small water drops on the solution. After complete evaporation of the solution, self-assembled structures of the solutes remain on the substrate. Under an optimized condition, highly ordered polymer patterns can be generated by these steps. The ordered dissipated structures are now considered as a useful self-assemble technology to create a new field of polymer materials. [1,2] However, in many cases, soft type polymers, such as vinyl polymers or polyesters are used to form such structures. In such cases, amphiphilic molecules, such as cationic surfactants, must be added to the solution to keep the water droplets small.

In this study, a polypeptide with a rigid and straight structure, poly(γ-benzyl-L-glutamate) (PBLG) is selected as a material polymer. Such polypeptides can be used as functional materials or templates. [3-5] PBLG can be easily synthesized and is highly hydrophobic. This polypeptide molecule has a stable α-helix conformation and a rod-like structure. PBLG molecules with a uniform molecular weight can form an extremely ordered ladder-like monolayer packing structure by Langmuir-Brodgett technique. Such monolayers can be accumulated readily onto solid substrates and show clear structural colors. [3] This accumulated PBLG multilayer films can be used as chemical sensors with extremely high sensitivity to detect dioxins for example.

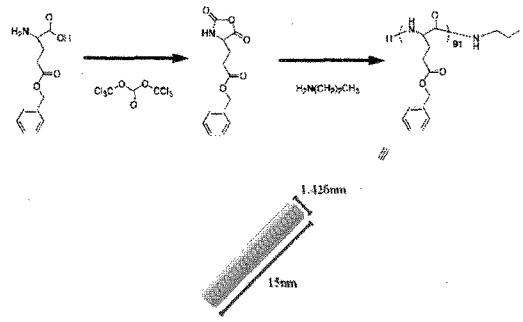
Casting a PBLG solution in 1,2-dichloroethane onto hydrophilic surface under various humidity conditions were investigated. The obtained layer structures were observed by AFM.

2. EXPERIMENTAL

2.1. Preparation of poly(γ-benzyl-L-glutamate) (PBLG)

PBLG was prepared by an NCA (N-carboxylic acid anhydrate) polymerization technique. Into 200 cm^3 of carefully dried tetrahydrofuran (THF), 5.0 g of benzyl-L-glutamate (BLG) was injected and the turbid dispersion was stirred at 60 °C under N_2 for 90 min. Then, trihosgen (one third (mol/mol) of BLG) was injected into this dispersion and again stirred for 2 h. Then the atmosphere was changed into nitrogen for safe. The dispersion was then evaporated and dried *n*-hexane was added. After 24 h, the product, γ-benzyl-L-glutamate-N-carboxylic acid anhydrate (BLG-NCA) was collected. Into a 1 cm^3 of DMF solution of propylamine (5.67 mg in 10 cm^3), a DMF solution (20 cm^3) of BLG-NCA (2.52g) was injected dropwise. After 72 h, the precipitates (PBLG) were collected and washed by ethanol. The average degree of polymerzation of the PBLG used in this study was 91, which was evaluated from ^{1}H NMR (200 MHz, in DMSO). Its secondary structure of α-helix was confirmed from Circular Dichroism spectroscopy.

Scheme 1 Preparation scheme of $PBLG_{91}$



2.2. Preparation of PBLG thin films by a casting method and observation of their structures

Thin films of PBLG were obtained simply by casting a 5.0×10^{-3} cm^3 of 1,2-dichloroethane (CH_2ClCH_2Cl) solution of PBLG (0.2 g dm^{-3}) onto a freshly craved mica substrate under lower (40 – 50 %) and higher (80 – 90 %) humidity conditions at room temperature. The surface structure of the prepared films was observed by vibration mode AFM (Nanoprobe IIIa, Digital Instrument Co.) in an ambient atmosphere. The AFM probes used in this study were super-sharp tips for vibration mode made of a Si single crystal (Nippon Veeco). The mica substrates were supplied by Nisshin EM.

3. RESULTS AND DISCUSSION

3.1. PBLG layer structures on mica surfaces under lower humidity condition (40 – 50 % at room temperature)

1,2-dichloroethane solution of PBLG was casted onto hydrophilic mica surfaces under a lower humidity conditions. The relative humidity of the atmosphere was controlled between 40 and 50 % at room temperature.

PBLG forms an α-helix polypeptide conformation and has a rigid and rod-like structure as shown in Scheme 1. The diameter and the length of PBLG molecule used in this study ($PBLG_{91}$) are ca. 1.4 nm and ca. 15 nm, respectively. The surface was scanned by AFM with a super-sharp type tip. In Figure 1, the AFM image of a PBLG layer thus obtained is shown. In this image, densely packed ribbon type structures are clearly observed. Although some small black circles can be found in the image, no obvious large pore structure is observed in this image. At a high concentration, PBLG molecules, with a rod like structure and a constant molecular length, should behave as cholesteric liquid crystals. In the AFM image of Figure 1, the width of one bright line is *ca.* 36 nm, which corresponds almost twice of the molecular length (15 nm) of $PBLG_{91}$. This image can be considered as the dried form of the cholesteric structure of $PBLG_{91}$. The height of these ribbons is *ca.* 15 nm. Almost ten layers of PBLG were accumulated onto mica surface.

3.2. PBLG layer structures on mica surfaces under higher humidity condition (80 – 90 % at room temperature)

We also casted 1,2-dichloroethane solution of PBLG onto a mica substrate at higher humidity condition at room temperature to prepare PBLG thin layers.

In this case, the obtained AFM image is clearly different from that observed under a lower humidity condition. In Figure 2, double separated layers can be observed. The upper layer forms quasi honeycomb structures with a pore size of μm range. And in a larger image (the upper image in Figure 2), not all honeycomb holes are located separately but some connecting holes can be observed. The under layer has also honeycomb structure, but the honeycomb holes are obviously smaller than those in the upper layer, and the atomic force topography scan indicates that their honeycomb pore size is 200 - 400 nm.

These honeycomb structures generated by Marangoni effect. Under such high humidity conditions, the condensation of water molecules into a cooled surface of a water-immiscible solvent, 1,2dichloroethane, during the evaporation of PBLG solution. Condensed water formed droplets on the layer surface and they were the template of the honeycomb structures observed in these AFM images.

Shimomura et al. reported such honeycomb structures prepared by casting water-immiscible solutions of various polymers and polymer complexes. [1,2,6] In many cases, such honeycomb patterns were obtained by polymer complexes. In such cases, chloroform is used. Chloroform is water-immiscible and

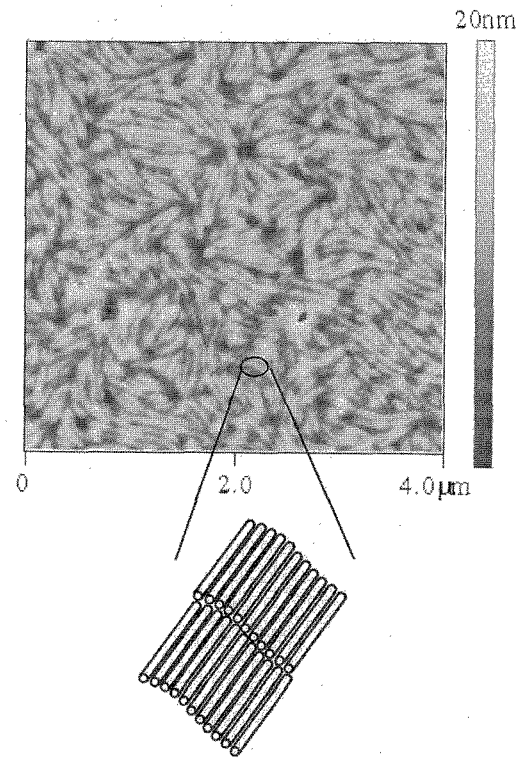


Figure 1. AFM images of PBLG thin layers prepared by casting 1,2-dichloroethane solution of $PBLG_{91}$ molecules (0.2 g dm^{-3}, 5.0×10^{-3} cm^3) casted onto mica under lower humidity condition (40- 50%RH) at room temperature.

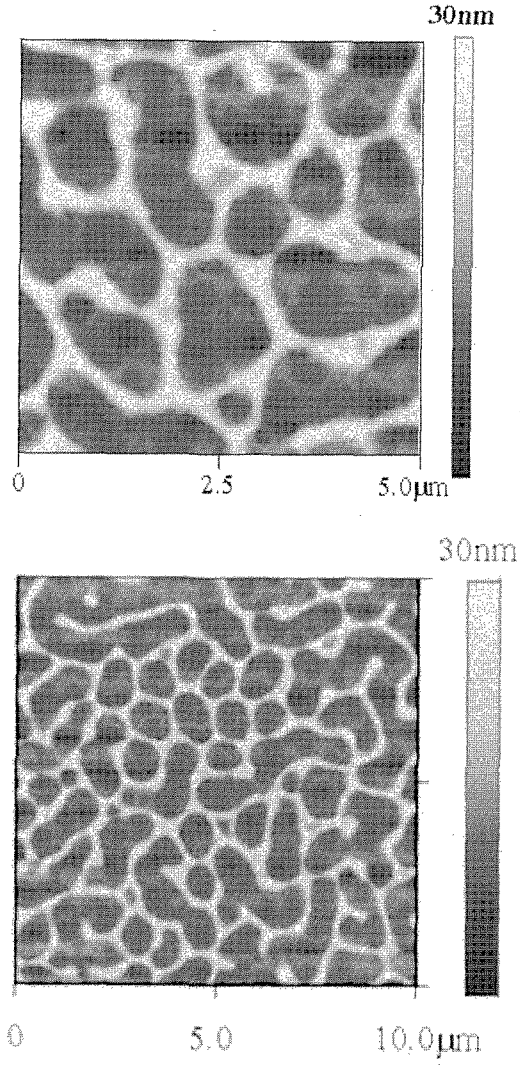


Figure 2. AFM images of PBLG thin layers prepared by casting 1,2-dichloroethane solution of $PBLG_{91}$ molecules (0.2 g dm^{-3}, 5.0×10^{-3} cm^3) casted onto mica under higher humidity condition at room temperature

its specific gravity is 1.484. Therefore, water droplets are deposited onto the surface of polymer complex solutions to form honeycomb structures.

On the other hand, without surfactant molecules, carbon disulfide is used to form a ordered honeycomb structure, probably according to its high interface tension against water. We have also proposed fluorocarbon solvents, possessing very high interface tension against water, to provide a perfectly ordered honeycomb structures of metal nanoparticles on a flat substrate.[7]

However, in this study, a rigid and rod like polymer PBLG was selected as a material polymer. Thanks to this rigid conformation, honeycomb structures could form even from 1,2-dichloroethane solution.

4. CONCLUSION

This is the first report of the formation of microporous films from hydrophobic polypeptide, PBLG, by using self-organization of water droplets during the evaporation of the solvent, 1,2-dichloroethane. The honeycomb structure was only found under a high humidity condition, that is, 80 – 90 %RH at room temperature. Thanks to the rigid rod-like structure of PBLG, even without surfactant molecules, honeycomb structures were observed. Under a lower humidity conditions, ordered and densely-packed ribbon structures of PBLG was observed in a thin layer. Possibly applications of such porous polypeptide films include membranes for biointerfaces and microreactors.

Acknowledgements:

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