Elastic Films

Self-Supporting Elastic Film without Covalent Linkages as a Hierarchically Integrated β-Sheet Assembly**

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In general, an amphiphilic molecule that can form an aggregate in a certain medium, especially an aqueous bilayer aggregate, forms a self-supporting film when the solution is cast onto an appropriate substrate then air dried.[1] The orderly molecular alignment within the cast film is useful for forming an ultrathin layer or coating with few defects. However, the supramolecular cast film has neither mechanical strength nor elasticity. Our aim in the present study is to improve the mechanical strength of the cast film, and to form an elastic supramolecular film that has the elastic properties of a macromolecular film. The prominent advantages of macromolecular films, namely, strength and elasticity, are ascribed to the covalent linkage that constitutes a sequence of the lowest unit molecule, the monomer. Therefore, an alternative interaction is needed to replace the covalent linkage to obtain an elastic supramolecular film. By using multiple H bonding as an alternative, Meijer and co-workers have demonstrated a supramolecular polymer.[2] The monomer of the supramolecular polymer has two sets of H-bonding blocks, for example, pyrimidon, at both edges of the molecule, which links the other molecules by multiple H bonding. The bora-type monomers yield a highly viscous, resinlike liquid. A similar substance has also been reported by Rebek, Jr. and co-workers.[3] Furthermore, the research group of Hutchison demonstrated that the self-assembled monolayer of a glycylglycine-containing amphiphile was stabilized by 3D networks of H bonding.[4] On the other hand, multiple H bonding has been observed as a β-sheet structure in the cast films of the tripeptide-containing amphiphiles.[5] These amphiphiles produce a self-supporting transparent film, but they are extremely brittle. This result means that another interaction that immobilizes the β-sheets will be needed for improving the mechanical strength of the supramolecular film. Thus, we attempted to use the side chains of the peptide part. Because the side chains of the adjacent amino-acid residues locate in opposite directions across the β-sheet plane,[6] the side chains could interlock with counterparts of the other β-sheet planes. This specific interdigitated structure should fasten the β-sheet planes when the peptide part contains at least three consecutive leucine residues as illustrated in Figure 1, which is similar to a zipper or a fastener. To reduce the confusion with the leucine zipper peptides, we prefer to call the aforementioned structure "leucine fastener". The leucine fastener will not be formed if a β-sheet structure is absent. Emphasis is placed on this hierarchy. However, until now, such hierarchic integration has not yet been realized. We demonstrate herein the hierarchic integration of supramolecular monomers, and aim to form a self-supporting elastic film without covalent linkage between the monomers.

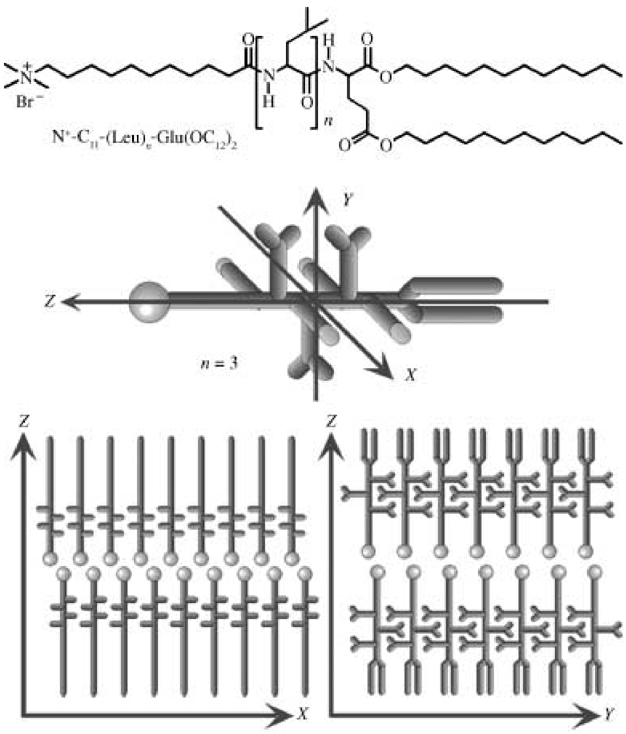

Figure 1. Schematic illustration of two-dimensional immobilization of trileucine-containing amphiphile. Parallel β-sheet form in the *X*–*Z* plane, and leucine fastener form in the *Y*–*Z* plane.

The supramolecular monomers used in the study are leucine-containing amphiphiles ($N^+C_{11}(Leu)_nGlu(OC_{12})_2$; $n = 2, 3, 4$). These amphiphiles dissolve in solvents with a low dielectric constant, namely, CCl_4, benzene, toluene, and cyclohexane, and form a gel at concentrations greater than 1 mM. In organic solvents with a higher dielectric constant, for example $CHCl_3$, the amphiphiles produce a clear solution. These results are consistent with our previous results with the other tripeptide or tetrapeptide-containing amphiphiles.[5,7]

We prepared the air-dried cast film from a CCl_4 gel and an isotropic $CHCl_3$ solution; the solutions were cast onto silicon-coated paper then air dried. The silicon-coated paper is available as cooking paper, which is helpful for the formation

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[**] This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (A), "Dynamic Control of Strongly Correlated Soft Materials" (No. 413/14045208), and a Grant-in-Aid for Scientific Research (No. 14550831) from the Ministry of Education, Science, Sports, Culture, and Technology, Japan.

DOI: 10.1002/ange.200352185

of a self-supporting film. The cast film was easily peeled off from the substrate by using tweezers. Although all of the leucine-containing amphiphiles formed a self-supporting transparent cast film, a flexible film was obtained from a $CHCl_3$ solution of the trileucine- or tetraleucine-containing amphiphile. The cast film of the tetraleucine-containing amphiphile formed from the $CHCl_3$ solution was extraordinary. This cast film, which was brittle immediately after peeling off from the silicon-coated paper, became flexible when the film was warmed to about 70 °C. The reason for this phenomenon is discussed below. The other films were brittle despite the thermal treatment. The characteristics of the $N^+C_{11}(Leu)_nGlu(OC_{12})_2$ cast films are summarized in Table 1.

Table 1: Characteristics of the Cast Films of N^+C_{11} $(Leu)_nGlu(OC_{12})_2$.

$N^+C_{11}(Leu)_nGlu(OC_{12})_2$	$CHCl_3$ solution	CCl_4 gel
$n=2$	brittle	brittle
$n=3$	nonbrittle	brittle
$n=4$	nonbrittle[a]	brittle

[a] This cast film was brittle immediately after peeling from the silicon-coated paper, but turned flexible when the film was warmed to ca. 70 °C.

Figure 2 depicts an example of the flexible cast film of $N^+C_{11}(Leu)_3Glu(OC_{12})_2$ formed from a $CHCl_3$ solution (10 mM). We can bend (Figure 2 A) and fold (Figure 2 B) the film without fragmentation. However, the film is not tensible and tears under strain. Although the tensile strength was inferior to a macromolecular film, importance is placed on the flexibility of the cast film.

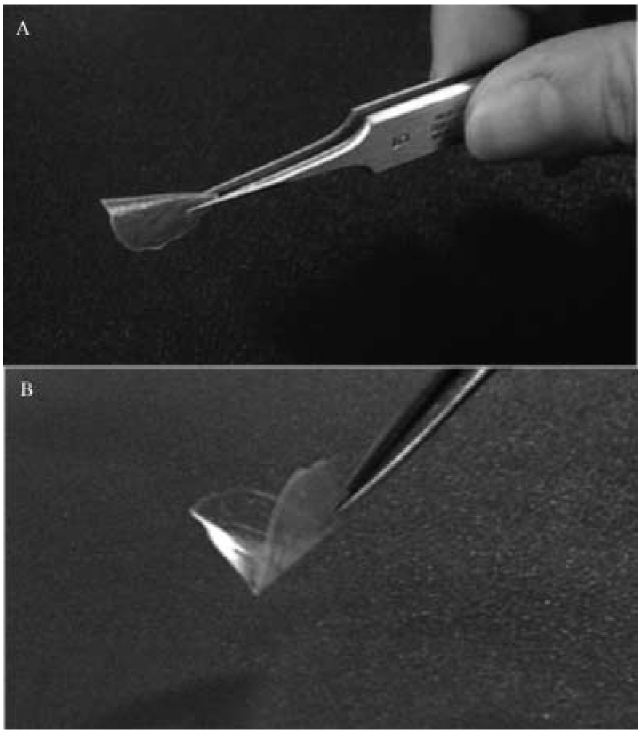
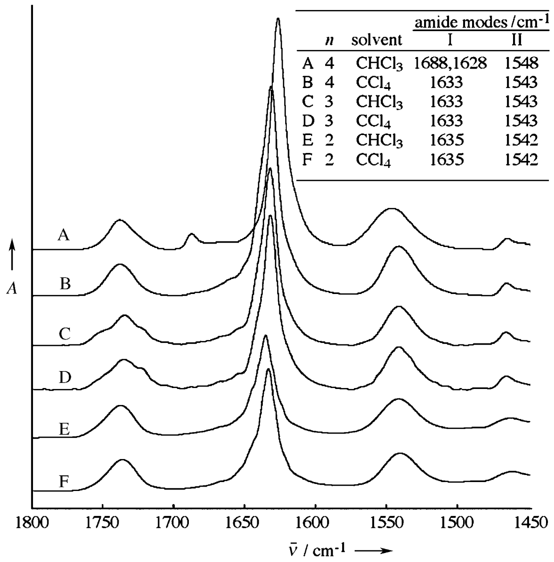


Figure 2. Flexible cast film formed from a $CHCl_3$ solution. We can bend (A) or fold (B) the cast film without fragmentation.

The interpeptide H bonding is not the cause for the elasticity as all of the cast films contain a β-sheet structure regardless of the elasticity. The secondary structure of the peptide-containing amphiphiles are easily diagnosed by Fourier transform infrared (FTIR) spectroscopy.[5] As shown in Figure 3, all of the cast films, except for the film formed on a CaF_2 window from the $CHCl_3$ solution of $N^+C_{11}(Leu)_4Glu(OC_{12})_2$, gave the same FTIR spectra, which was attributed to a parallel β-sheet structure. Strong and narrow amide I bands at 1630 cm^{-1} indicate the presence of a well-developed parallel β-sheet structure within every cast film, whereas the amide I bands accompanying a small peak at 1690 cm^{-1} meant that the cast film formed from the $CHCl_3$ solution of $N^+C_{11}(Leu)_4Glu(OC_{12})_2$ adopted an antiparallel β-sheet structure. Therefore, either the brittle or nonbrittle property should depend on another factor. The leucine fastener is thus most plausible factor, because the other peptide-containing amphiphiles without leucine residues formed only a fragile film. For example, pentapeptide-containing amphiphiles ($N^+C_{11}(Phe)_4Glu(OC_{12})_2$,[7] $N^+C_{11}(Ala)_4Glu(OC_{12})_2$[8]), tetrapeptide-containing amphiphiles ($N^+C_{11}(Phe)_3Glu(OC_{12})_2$,[7] $N^+C_{11}(Gly)_3Glu(OC_{12})_2$[8]), and the other tripeptide-containing amphiphiles previously reported,[5] which did not contain any leucinate residue, formed a self-supporting cast film, but never formed a flexible cast film. On the other hand, the leucine fastener needs at least three consecutive leucinate residues, because dileucine-containing amphiphiles could not form the specific interdigitated structure shown in Figure 1.



	n	solvent	amide modes /cm^{-1} I	amide modes /cm^{-1} II
A	4	$CHCl_3$	1688,1628	1548
B	4	CCl_4	1633	1543
C	3	$CHCl_3$	1633	1543
D	3	CCl_4	1633	1543
E	2	$CHCl_3$	1635	1542
F	2	CCl_4	1635	1542

Figure 3. FTIR spectra of $N^+C_{11}Leu_nGlu(OC_{12})_2$ cast films. A $CHCl_3$ or CCl_4 solution (2 mM) was spread onto a CaF_2 window then air dried. All of the spectra were measured at 25 ± 0.5 °C. Spectrum A ($n=4$, cast film from $CHCl_3$ solution) indicates formation of an antiparallel β-sheet structure, whereas the others (B–F) indicate formation of a parallel β-sheet structure. Details of the measurement and the diagnostics of the secondary structure in the peptide region have been reported elsewhere.[5]

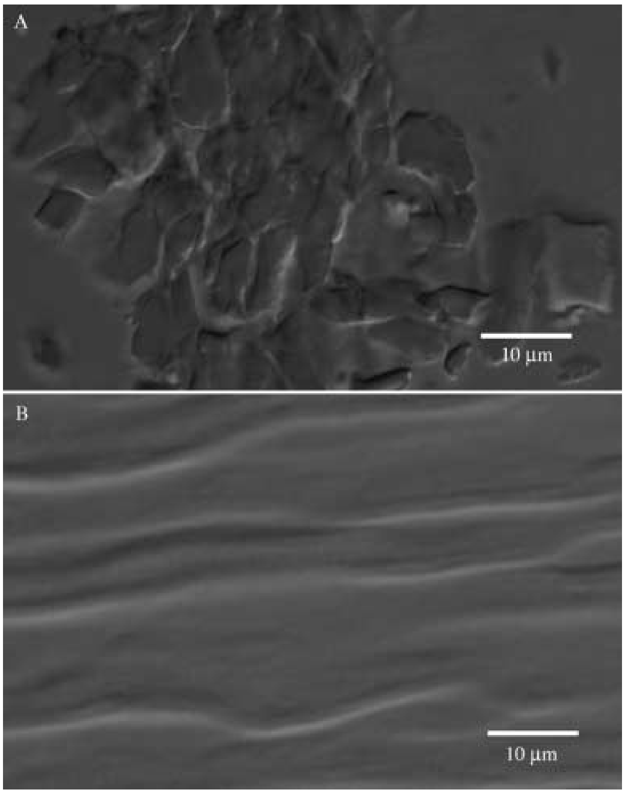


Figure 4. DIM pictures of the surface morphology. The cast films on slide-glass were prepared from a CCl_4 (A) or a $CHCl_3$ (B) solution of $N^+C_{11}Leu_3Glu(OC_{12})_2$.

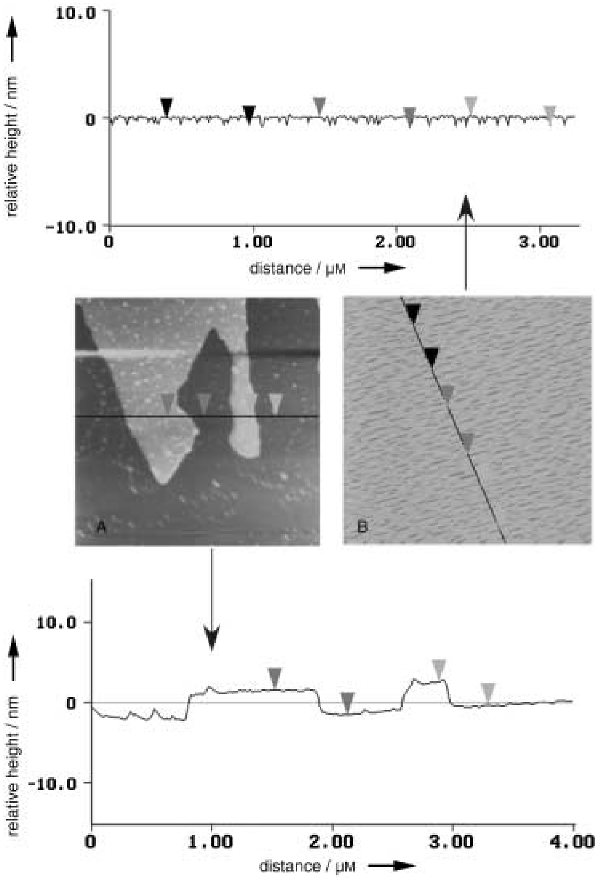


Figure 5. AFM images of the surface morphology and cross-section profiles. The cast films on freshly cleaved mica prepared from 10 mM of CCl_4 (A) or $CHCl_3$ (B) solution of $N^+C_{11}Leu_3Glu(OC_{12})_2$.

The next question is why did the CCl_4 gels of $N^+C_{11}(Leu)_n$-$Glu(OC_{12})_2$ ($n = 3$ and 4) not produce an elastic film? The answer was obtained from the observation of the surface morphology by using a differential interference contrast microscope (DIM) and an atomic force microscope (AFM). Figure 4 and Figure 5 show the DIM and AFM images, respectively, of the cast films of $N^+C_{11}(Leu)_3Glu(OC_{12})_2$. The surface morphology of the cast film prepared from the CCl_4 gel looked like a pile of platelets (Figure 4A). These platelets should be the aggregate formed in an original CCl_4 solution. On the other hand, the surface morphology of the cast film prepared from the $CHCl_3$ solution was a continuous sheet (Figure 4B). These images were consistent with the AFM images (Figure 5). A part of the platelet observed in Figure 4A is depicted in Figure 5A. This platelet should be an adsorbed monolayer or a tilted bilayer membrane, because the thickness of the platelet is about 3 nm, which corresponds to the expanded length of the $N^+C_{11}(Leu)_3Glu(OC_{12})_2$ molecule (about 4 nm). An important point is the discontinuity of the surface structure. The discontinuity should make the cast film fragile. On the other hand, the continuous sheet of the cast film from a $CHCl_3$ solution has a flat surface structure at the atomic level, although many clefts were observed. This flat surface morphology of the cast film formed from an isotropic $CHCl_3$ solution should reflect the flat surface of freshly cleaved mica. The clefts are very interesting, because their formation should be related to the continuous sheet formation process. Figure 6A shows the AFM image of the surface of the mica, on which a thinner $CHCl_3$ solution of $N^+C_{11}(Leu)_3Glu(OC_{12})_2$ (10^{-4} M) was spread then air dried. We consider that the rodlike objects are the single or doubled β-sheet as shown in Figure 6B. The fragmented β-sheet assemblages are closely packed when a solution with a higher concentration (10^{-3} M) was cast. In this process, clefts have been left behind, especially at both edges of the β-sheet (Figure 6C). Therefore, the H bonding could form along the direction of the clefts, and the leucine fastener is formed along the perpendicular direction to the β-sheet plane. The H bonding and the leucine fastener achieve a two-dimensional immobilization of $N^+C_{11}(Leu)_3Glu(OC_{12})_2$, which should be the reason for the elasticity of the cast film formed from the $CHCl_3$ solution.

At the present stage, there remains one question: why did the thermal treatment produce flexibility in the cast film of $N^+C_{11}(Leu)_4Glu(OC_{12})_2$ formed from the $CHCl_3$ solution? In general, an increase in H bonding tends to result in solidity rather than fluidity. As solidity results in the lose of elasticity, thermal treatment could transform the solid film into a fluid film. Therefore, the cast film of $N^+C_{11}(Leu)_4Glu(OC_{12})_2$ formed from $CHCl_3$ should be fluid at the alkyl chains by thermal treatment. Such a partial fluid state should be related

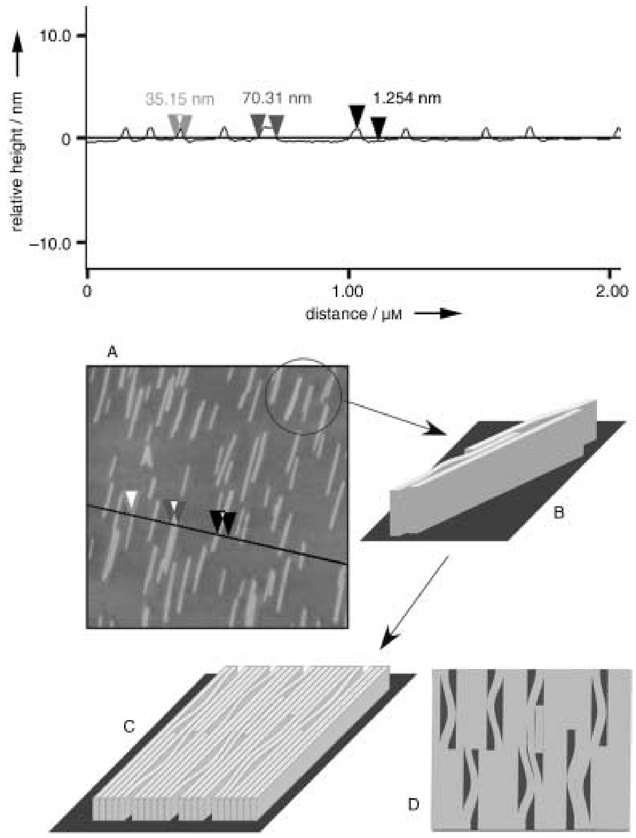


Figure 6. Plausible mechanism for the formation of a flat surface at the atomic level. The cast film formed from a thinner solution yields rodlike objects (A), which should be a single or stacked β-sheet (B). The cluster should accumulate when a thick solution was spread (C). Overhead scanning of (C) should give the image like (D), which bears a resemblance to Figure 5(B).

to a plastic crystal phase. We are now investigating such details of the thermal treatment.

In conclusion, we have demonstrated the first example of a self-supporting, elastic supramolecular film. The supramolecular monomer, tri- or tetraleucine-containing amphiphile self-assembles and forms a β-sheet. The β-sheets stack upon each other, which was immobilized by interlocking at the side chains of the leucinate residues. This hierarchic integration is characteristic of an elastic supramolecular film. Furthermore, the elastic film should not be a solid, but forms a metastable state, because crystallization definitely reduces film elasticity. To maintain the elasticity, some functional group that prevents the film from crystallizing will be needed.

Received: June 25, 2003 [Z52185]

Keywords: amphiphiles · hydrogen bonds · self-assembly · supramolecular chemistry · thin films

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