

## The monolayer oscillation and molecular recognition at oil/water interface.

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Amphiphilic  $\alpha$ -helical polypeptides, poly( $\gamma$ -methyl L-glutamate)s containing  $\beta$ -cyclodextrin (PMLG<sub>n</sub>-CyD (n=19, 34)), were prepared and the monolayer of polypeptides was formed at n-hexane/water interface. The interfacial pressure ( $\pi$ ) - area (A) isotherms of the monolayer showed that  $\alpha$ -helix rod of PMLG<sub>n</sub>-CyD could be vertically orientated at the oil/water interface by increasing the interfacial concentration of the polypeptide. When  $\alpha$ -helical rods were oriented almost normal to the interface, 1-adamantane carboxylic acid (ADC) was added as a guest molecule of CyD in the water phase. As a result, the oscillation of the interfacial pressure of the monolayer was induced. In the case of 2-*p*-toluidinylnaphthalene-6-sulfonate potassium salt (TNS) as a guest molecule of CyD, the period and the pattern of the oscillation was different from that of ADC guest system. This showed that the period and the pattern of the oscillation may be changed by the degree of affinity between the CyD and the guest molecule. It is also clear that degree of polymerization, n, of PMLG<sub>n</sub>-CyD may be one of the important factors that affect the stability of oscillation.

Key words: oscillation, molecular recognition, polypeptide, monolayer, oil/water interface

### 1. INTRODUCTION

It has been generally accepted that the oscillatory properties in nerve and muscle cells are essential for sensing of environmental stimuli, the transfer of generated signals, and the signal-cell response coupling. For example, the axonal membrane consisting of proteins and lipids can generate the electrical oscillatory properties of neurons [1]. It may say therefore that the biological oscillations for maintenance of life are based on the intelligent interfaces mainly consisting of polymeric and/or amphiphilic molecules.

These phenomena are more fundamentally recognized as dissipative structures far from equilibrium. Investigations on nonlinear or oscillatory phenomena related to polymeric systems at interfaces, which are important for an understanding of the mechanism of biological oscillation and the developments in a novel field of interfacial science and technology.

Up to now, many types of artificial oscillatory membranes have been reported and much attention has been paid for oscillatory phenomena in oil - water systems [2-7].

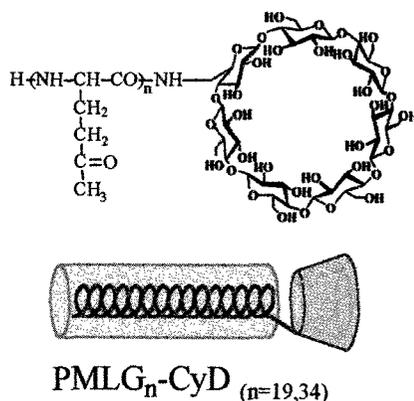
In recent years, we reported that the oscillation of interfacial pressure was induced by a host-guest interaction [8]. An amphiphilic polypeptide, which is hydrophobic poly( $\gamma$ -methyl L-glutamate)

containing  $\beta$ -cyclodextrin as a hydrophilic part at the terminal (PMLG<sub>n</sub>-CyD; n is the degree of polymerization), was prepared, and the monolayer of this polypeptide was formed at n-hexane/water interface. The  $\alpha$ -helix rod of PMLG<sub>n</sub>-CyD could be vertically orientated at the oil/water interface by increasing the interfacial concentration of the polypeptide [8,9]. When  $\alpha$ -helical rods were oriented almost normal to the interface, 2-*p*-toluidinylnaphthalene-6-sulfonate potassium salt (TNS) as a guest molecule of CyD was added into the water phase. As a result, the oscillation of the interfacial pressure of the monolayer was induced. A possible mechanism for this oscillation was proposed based on the polymer-polymer and host-guest interactions.

In this study, 1-adamantane carboxylic acid (ADC) was used as a guest molecule instead of TNS and the effect of degree of polymerization on this oscillation system was investigated. It is clear that the frequency and the pattern of this oscillation could be changed according to the binding constant between the CyD and the guest molecule. In addition, the degree of polymerization was one of the most important factors. That is, the longer  $\alpha$ -helix rod could stabilize the monolayer oscillation.

## 2. EXPERIMENTAL

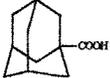
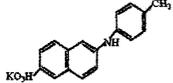
PMLG<sub>n</sub>-CyD was obtained by polymerization of N-carboxy L-glutamic acid anhydride  $\gamma$ -methyl ester with 6-amino-cyclodextrin as an initiator. The average degree of polymerization of PLMG moiety in the PMLG<sub>n</sub>-CyD was estimated to be 19 and 34, abbreviated as PLMG<sub>19</sub>-CyD and PLMG<sub>34</sub>-CyD, respectively, which were determined by high-resolution <sup>1</sup>H-NMR spectra (JEOL JNM-GX400) in trifluoroacetic acid (Scheme 1). The secondary structure of PMLG<sub>n</sub>-CyDs was confirmed to be  $\alpha$ -helix conformation by circular dichroism (CD) measurements (Jasco J-820K spectropolarimeter) in 2,2,2-trifluoroethanol (TFE).



Scheme 1 Chemical structure and schematic illustration of PMLG<sub>n</sub>-CyD

1-Adamantane carboxylic acid (ADC) (Nacalai Tesque, Inc., Japan) and 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) (Sigma Chemical Company) were used without further purification. Table 1 shows the chemical structure and binding constants with CyD [10].

Table 1 Guest molecules of CyD and their binding constant

Guest molecule	K
1-Adamantane carboxylic acid (ADC) 	$3.3 \times 10^4$
2- <i>p</i> -toluidinylnaphthalene-6-sulfonate potassium salt (TNS) 	$2.5 \times 10^3$

The interfacial pressure of PMLG<sub>n</sub>-CyD monolayer at n-hexane/water interface was measured by the Wilhelmy method using a Langmuir film balance (Nippon Laser & Electronics Lab., NL-LB240-MWA). A circular Teflon trough was filled with Milli-Q treated

water (25 mL), and then n-hexane (25 mL) was introduced above the water. A DMF solution of the polymer was put on the n-hexane/water interface from a micro syringe. This procedure was repeatedly performed to increase the interfacial concentration of PMLG<sub>n</sub>-CyD. Through the measurement, the area of the monolayer is fixed to be equal to that of cross-section of the circular trough. Therefore, the area occupied by one PMLG<sub>n</sub>-CyD molecule in the monolayer (A) can be determined by the variable interfacial concentration of PMLG<sub>n</sub>-CyD and the fixed monolayer area. Thus the interfacial pressure ( $\pi$ ) - area per molecule (A) isotherm was obtained.

When PMLG<sub>n</sub>-CyD monolayer was formed at n-hexane/water interface ( $\pi = 25$ ;  $\alpha$ -helical rod of PMLG<sub>n</sub>-CyD was almost perpendicular to the interface), an aqueous solution of guest molecules was directly injected into the water phase beneath the PMLG<sub>n</sub>-CyD monolayer. The induced oscillation of interfacial pressures of was monitored by the Langmuir film balance which was connected to a personal computer through a digital data recorder (MacLab ML740, ADInstruments Co. Ltd.). These measurements were carried out at room temperature.

## 3. RESULTS AND DISCUSSION

The molecular orientation of PMLG<sub>n</sub>-CyD at n-hexane/water interface was characterized by their  $\pi$ -A isotherms. These  $\pi$ -A isotherms for PLMG<sub>19</sub>-CyD and PLMG<sub>34</sub>-CyD monolayer (Figure 1) showed two step increasing parts, suggesting that the monolayer can take two possible molecular arrangements in the monolayer state at the oil/water interface. Extrapolations of each steep increasing part of the isotherm to  $\pi = 0$  gave two extrapolated values of area per PMLG<sub>n</sub>-CyD molecule,  $A_L$  and  $A_S$  denoted in Figure 1. The smaller area,  $A_S$ , observed for PMLG<sub>19</sub>-CyD and PMLG<sub>34</sub>-CyD was found to be consistent though lengths of their

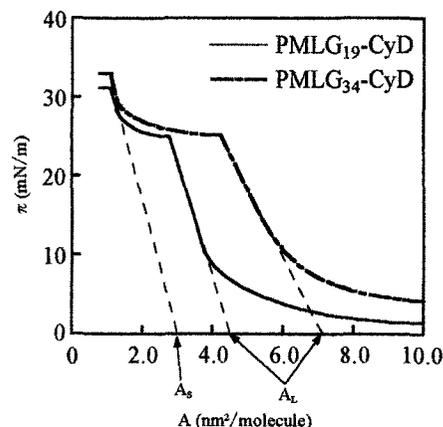


Figure 1 Interfacial pressure-area ( $\pi$ -A) isotherms of monolayer of PMLG<sub>n</sub>-CyD (n=19,34) at n-hexane/water interface.

helix rods are different each other ( $A_s = 3.05 \text{ nm}^2/\text{molecules}$ ). One of plausible molecular arrangements is that  $\alpha$ -helix rods of PMLG<sub>n</sub>-CyD oriented perpendicular to the interface by increasing the interfacial concentration of the polypeptide.

A guest molecule for the CyD, 1-adamantane carboxylic acid (ADC), was injected into the water phase beneath the PLMG<sub>34</sub>-CyD monolayer at  $\pi = 25 \text{ mN/m}$ , in which the  $\alpha$ -helix rod of PLMG<sub>34</sub>-CyD is almost perpendicular to the oil/water interface. The terminal CyD in the monolayer should be faced with the water phase because of its hydrophilic character. The concentration of ADC added in the water phase was  $2.0 \times 10^{-5} \text{ M}$ . Within a minute the monolayer began to oscillate resulting in rhythmic responses of the interfacial pressure. Figure 2 shows the oscillation of interfacial pressures observed for the PLMG<sub>34</sub>-CyD monolayer on addition of ADC. Interestingly, this oscillation is composed of three parts of positive peek, random peek and negative peek regions. At first, the positive peeks appeared, followed by the random region and finally the pattern was changed in a negative peek manner. The period of the oscillation was about 1 second.

A plausible molecular mechanism of the oscillation is proposed in Figure 3. At first, the hydrophilic terminal CyD bound to the hydrophobic ADC. As a result, the PMLG<sub>n</sub>-CyD may lose its amphiphilic character because of the binding of the hydrophobic guest. The monolayer moves to a n-hexane phase when the interfacial concentration of binding guest ADC reaches a critical value. Then the terminal CyD in the oil phase will release the guest due to low concentration of the guest in n-hexane phase. As a result, the monolayer may recover the original position. This process can produce the oscillation of the interfacial pressure. When the concentration of the guest in water phase is high, the monolayer may stay at n-hexane side of the interface (Figure 3(b)), because the CyD of the monolayer constantly interacts with the guest losing its hydrophilic character. When the monolayer temporary moves to the n-hexane/water interface ((b)→(a) in Figure3), the positive peek in the interface pressure will be appeared. Reversely, when the concentration gradient of the guest is decreased, the monolayer may predominantly stay at the interface, producing the negative peeks.

Furthermore, the oscillation in Fig. 2 is different from the oscillation induced by TNS (Figure 4). The period of the oscillation in Fig. 4 was found to be about 18 second. It is considered that the period and the pattern of this oscillation could be changed according to the binding constant between the CyD and the guest molecule. The binding constant of TNS for CyD is smaller than that of ADC. This implies that as the affinity

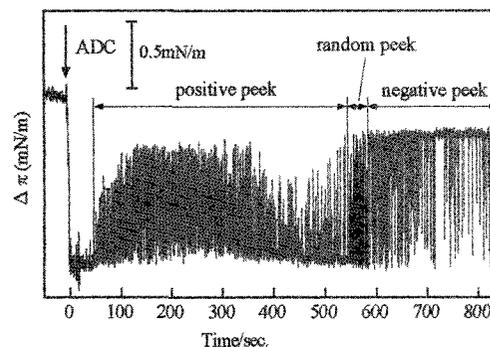


Figure 2 Oscillation of the interfacial pressure of PLMG<sub>34</sub>-CyD monolayer at n-hexane/water interface induced by the addition of ADC to the water phase ( $2.1 \times 10^{-5} \text{ M}$ ) at time = 0.

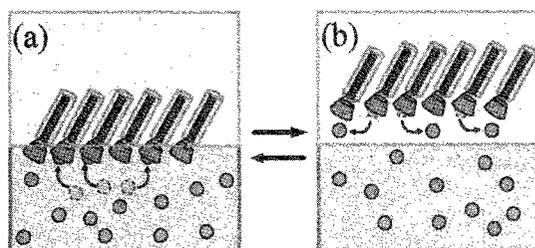


Figure 3 Schematic picture of the change in the location of the PMLG<sub>n</sub>-CyD monolayer resulting from the binding and releasing of the guest molecule across the oil / water interface.

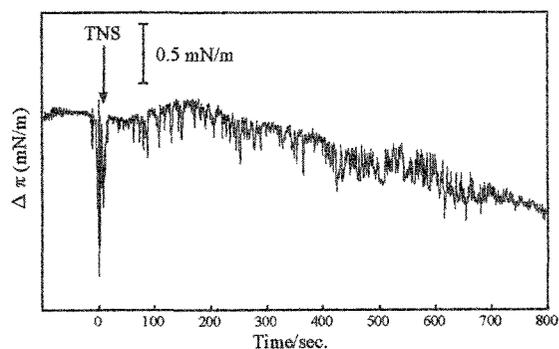


Figure 4 Oscillation of the interfacial pressure of PMLG<sub>19</sub>-CyD monolayer at n-hexane/water interface induced by the addition of TNS to the water phase ( $2.0 \times 10^{-6} \text{ M}$ ) at time=0.

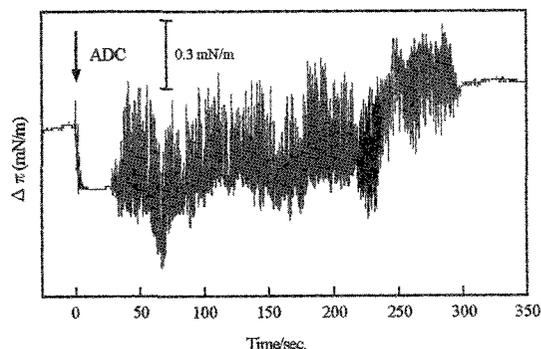


Figure 5 Oscillation of the interfacial pressure of PMLG<sub>19</sub>-CyD monolayer at n-hexane/water interface induced by the addition of ADC to the water phase ( $1.0 \times 10^{-5} \text{ M}$ ) at time = 0.

between CyD and guest is lower, the oscillation period becomes longer.

Figure 5 shows the ADC induced oscillation of the monolayer composed of PMLG<sub>n</sub>-CyD whose degree of polymerization was 19. The period was almost as same as that observed for PMLG<sub>34</sub>-CyD in Fig.2 (about 1 sec.). However, this oscillation seems to be unstable. This result implies that the stability of oscillating domain of the monolayer is closely related to the degree of polymerization of the polypeptide segment.

#### 4. CONCLUSIONS

The guest-induced oscillation of the interfacial pressure of the host containing monolayer could be observed at oil/water interface. When the guest molecules interact with the host CyD site in the monolayer and the chemical potential of the guest is enough across the interface to produce the oscillation, the movement of the monolayer to the oil phase occurred. On the contrary, the monolayer released the guest molecule based on the low binding ability in the oil phase; as a result, it recovered the original location at the oil/water interface. The cycle of changes in monolayer location could be monitored as the oscillation in the interfacial pressure. In addition, the oscillation pattern had a tendency to depend on the guest molecules. And it is also shown that the

long  $\alpha$ -helix rod formed a stable domain in the monolayer at oil/water interface producing a stable oscillation. This oscillation phenomenon is a novel type of chemical sensing systems where the chemical information is changeable to oscillation frequency.

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