

# The guest-induced oscillation of a monolayer composed of polypeptide containing $\beta$ -cyclodextrin at the terminal

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We prepared a rod-like amphiphile with a molecular recognition end group,  $\alpha$ -helical and hydrophobic poly( $\gamma$ -methyl L-glutamate) (PMG) containing hydrophilic  $\beta$ -cyclodextrin (CyD) as an active end group (PMG-CyD), and formed its monolayer at the *n*-hexane/water interface. The interfacial pressure ( $\pi$ )-area (*A*) isotherms of the monolayer showed that  $\alpha$ -helix rod of PMG-CyD could be vertically oriented at the oil/water interface, facing the hydrophilic terminal CyD group to the water phase, by increasing the interfacial concentration of the polypeptide. Under the condition 2-*p*-toluidinyl-naphthalene-6-sulfonate (TNS), an intimate guest molecule for the CyD in water was introduced into the water phase beneath the monolayer. Within a minute the monolayer began to oscillate which could be monitored by the rhythmic response of the interfacial pressure of the monolayer. The oscillation continued over ten minutes and then terminated. The mode of the oscillation was found to change with time, i.e., the initial stage showing a periodic sharp reduction in the interfacial pressure (period I), the second stage having sharp increase in the  $\pi$  value (period II), and the last stage of irregular oscillations (period III). The Fourier analysis of each period also supported the three stages during the oscillatory process. It was also found that when the  $\alpha$ -helix rod of PMG-CyD lay down in the monolayer, the guest TNS did not induce any changes in the interfacial tension. This nonlinear rhythmic interfacial phenomenon was explained in terms of the periodic movement of the PMG-CyD monolayer resulting from the binding and releasing of the guest TNS across the oil/water interface. © 1999 American Institute of Physics. [S1054-1500(99)00402-4]

**The orientation of macromolecules at interfaces, particularly in biological membranes, can play a key role in such important processes as molecular recognition and energy or information transfer. A simple model for such a system consists of rod-like, hydrophobic,  $\alpha$ -helical polymer attached to a hydrophilic end group that is capable of molecular recognition. We study the behavior of a monolayer of this polymer at an oil/water interface in the presence of a hydrophilic guest molecule that binds to the end group. When the polymer is appropriately oriented in the monolayer, the guest molecule induces oscillatory behavior analogous to that seen in biological systems.**

## I. INTRODUCTION

It has been generally accepted that the oscillatory properties in nerve and muscle cells are essential for the sensing of environmental stimuli, the transfer of a generated signal, and the coupling between the signals and the response system. For example, the axonal membrane consisting of a few nm thick layer of proteins complexed with lipids can generate the electrical oscillatory properties of neurons.<sup>1</sup> It may

say that the biological oscillations for maintenance of life are based on the interfaces having 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 as interfaces, therefore, are important for an understanding of the mechanism of biological oscillation and also for the developments in a novel field of interfacial science and technology.

Up to now, many types of artificial oscillatory membranes has been reported.<sup>2-11</sup> In 1955, Teorell<sup>2</sup> first reported an oscillatory response in electrical potential across porous membranes with fixed charges above a critical current value. A self-excitable system was also found by Kobatake<sup>5,6</sup> using amphiphilic dioleylphosphate doped Millipore membranes under the KCl concentration gradient. More recently, much attention has been paid for oscillatory phenomena in oil-water systems.<sup>12-17</sup> For example, Yoshikawa *et al.*<sup>13,14</sup> showed that oscillation in the electrical potential in a water-oil-water three-phases system could be observed in the presence of amphiphilic molecules. The oscillation was attributed to a periodic formation and destruction of the amphiphile monolayer at the oil/water interface based on the chemical potential difference of the amphiphile between the oil and water phases. They<sup>15,16</sup> also reported on an oscillation in the interfacial tension or macroscopic self-motion of oil-

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water two-phases system when water with an amphiphile is placed in contact with an oil. Such a phenomenon was first reported by Dupeyrat *et al.*<sup>12</sup> and is known as the Marangoni effect.<sup>18</sup>

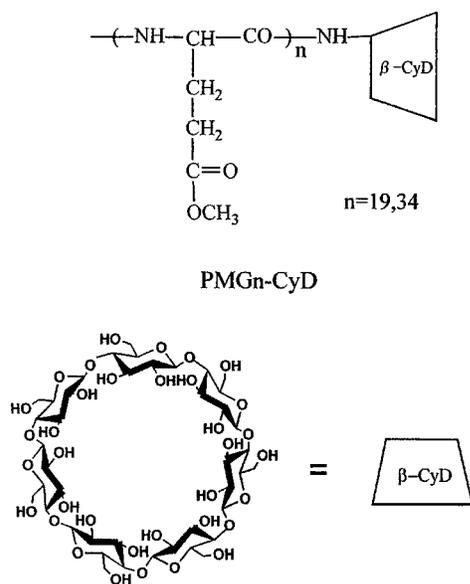
We<sup>19</sup> previously reported that a rod-like polymeric amphiphile, hydrophobic poly( $\gamma$ -methyl L-glutamate) containing  $\beta$ -cyclodextrin as a hydrophilic terminal group (PMGn-CyD;  $n$  is the degree of polymerization), formed a stable monolayer and showed a possibility for the regulation of the molecular orientation in the monolayer at the oil/water interface. On the other hand,  $\beta$ -cyclodextrin,<sup>20</sup> a typical host compound, is widely employed as a molecular recognition site, since it includes specific molecules in its cavity with a large binding constant.

In this study, we prepare a PMGn-CyD monolayer at the  $n$ -hexane/water interface, and introduced a guest molecule for the terminal CyD into the water phase beneath the monolayer. The oscillation in the interfacial tension of the monolayer could be observed in the presence of the guest molecule only when the helix rod of PMGn-CyD was vertically oriented in the monolayer. A possible mechanism for the guest-induced monolayer oscillation at the oil/water interface related to the polymer-polymer and host-guest interactions in the system was proposed.

## II. EXPERIMENT

### A. Materials

PMGn-CyD (Scheme I) was obtained by the polymerization of N-carboxy L-glutamic acid anhydride  $\gamma$ -methyl ester

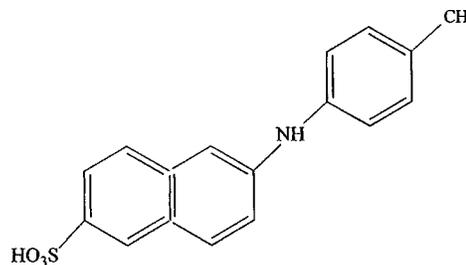


Scheme I.

with 6-amino-cyclodextrin<sup>21</sup> as an insulator. The molar ratio of anhydride to the initiator was 20 and 30, respectively, and the polymerization occurred at room temperature over 5 h. The PMG-CyD was precipitated by adding ether. The average degree of polymerization of PMG moiety in the PMGn-CyD was estimated to be 19 and 34, PMG<sub>19</sub>-CyD and PMG<sub>34</sub>-CyD, respectively, by high-resolution <sup>1</sup>H-NMR spectra (Varian XL-200 spectrometer) of the product in trif-

luoroacetic acid (10 mg/mL): i.e., these values were derived from the ratio of the peak area at around 3.9 ppm and 5.3 ppm associated with the main chain CH group of PMG moiety and the OH group of the terminal CyD, respectively.

A guest molecule for the terminal CyD, 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS, Scheme II), Sigma Chemical



2-*p*-toluidinylnaphthalene-6-sulfonate (TNS)

Scheme II.

Company, was used without further purification. Other chemicals were extrapure grade from Nacalai Tesque, Inc., Japan.

### B. Methods

The interfacial pressure ( $\pi$ ) of the PMG-CyD monolayer at the  $n$ -hexane/water interface was measured by the Wilhemy method using a Langmuir film balance (Nippon Laser & Electronics Lab., NL-LB240-MWA), as is shown in Fig. 1. A circular Teflon trough ( $\phi=5.6$  cm) was filled with Milli-Q treated water (50 mL), and then  $n$ -hexane (20 mL) was introduced above the water. 1.0 mg of PMG-CyD was dissolved in 10 mL dimethylformamide (DMF). A measured small amount of the DMF solution was put on the  $n$ -hexane surface from a Termo micro syringe. It could be seen that a drop of the solution was slowly down to the  $n$ -hexane/water interface and spread over to form the PMG-CyD monolayer. This procedure was repeatedly performed to increase the interfacial concentration of PMG-CyD. The area of the monolayer is fixed, through the measurement, to be equal to that of the cross-section of the circular trough. Therefore, the area occupied by one PMG-CyD molecule in the monolayer ( $A$ ) can be determined by the variable interfacial concentration of PMG-CyD and the fixed monolayer area. The interfacial pressure was determined with a precision of 0.1 mN/m.

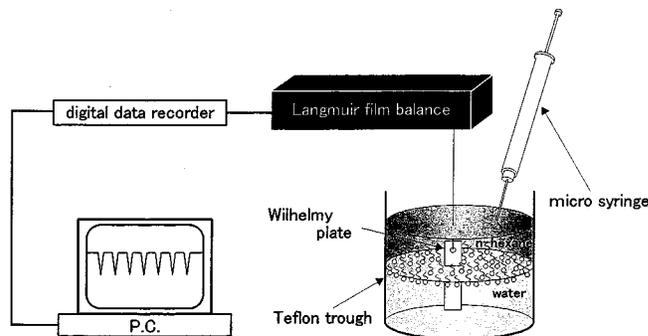


FIG. 1. Schematic representation of the apparatus for the measurement of interfacial pressure.

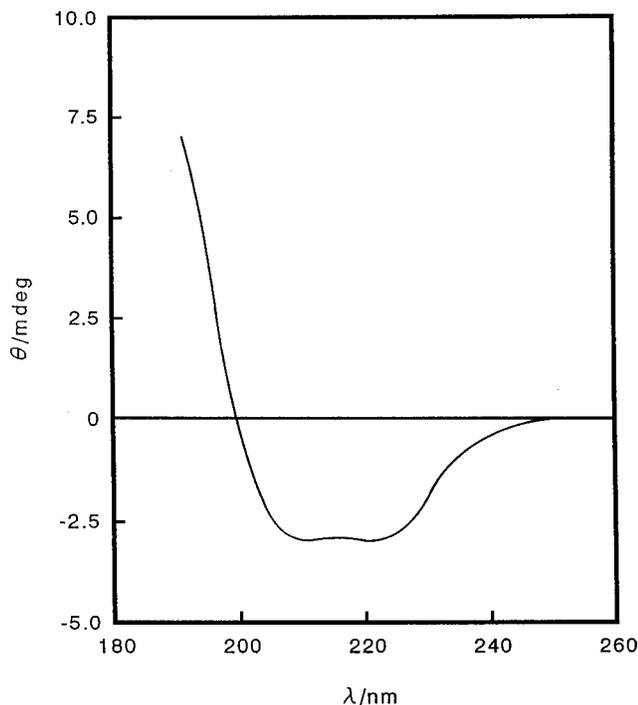


FIG. 2. Circular dichroism spectrum of a PMG<sub>19</sub>-CyD LB membrane (five layers) on a quartz plate.

The interfacial pressure–area per molecule ( $\pi$ - $A$ ) isotherms were thus obtained, and then a known amount of aqueous solution (100  $\mu$ L) of TNS ( $1.0 \times 10^{-3}$  M), a guest molecule for the CyD, was directly injected into the water phase beneath the PMG-CyD monolayer. The induced oscillation in the interfacial pressure of the monolayer was monitored by the Langmuir film balance and stored in a personal computer through a digital recorder (MacLab<sup>®</sup> ML740, AD-Instruments Co. Ltd.). The sampling rate was 100 Hz. Using the program “Spectrum” in this system, the Fourier Transform method was applied to convert the data from time to frequency domains. Any filters were not employed during the operation. These measurements were carried out at 25 °C.

In order to confirm the  $\alpha$ -helix structure of PMG-CyD, circular dichroism (CD) measurements were carried out. The PMG<sub>19</sub>-CyD monolayer was formed at an air/water interface in a L-B trough (Nippon Laser & Electronics Lab., NL-LB80-MT-M). When the surface pressure reached 20 mN/m, the monolayer was deposited on a quartz plate by the LB method. The deposition was repeated to form five layers of the PMG<sub>19</sub>-CyD on the plate. CD spectra of the LB film obtained were measured with a Jasco, J-600, spectropolarimeter.

The binding constant,  $K$ , between  $\beta$ -CyD and TNS in an aqueous solution and  $n$ -hexane, respectively, was determined by fluorescence spectroscopic measurements (Jasco, FP-777 spectrofluorometer). The excitation wavelength of TNS was 365 nm in water and 325 nm in  $n$ -hexane, respectively. The emission maximum of TNS, near 500 nm in pure water, shifted to 453 nm in the presence of  $\beta$ -cyclodextrin. The shift of emission maximum of fluorescence guest molecules is owing to their binding in the cavity of the CyD.<sup>22,23</sup> From the decrease in the fluorescence intensity at 500 nm and the

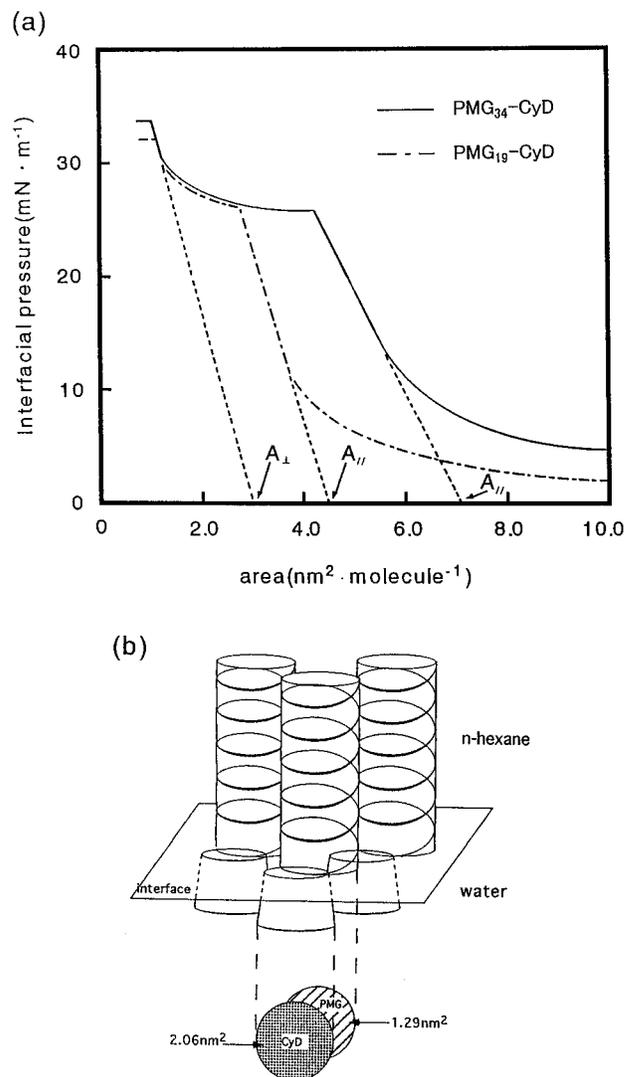


FIG. 3. (a) Interfacial pressure–area ( $\pi$ - $A$ ) isotherms of PMG<sub>19</sub>-CyD and PMG<sub>34</sub>-CyD at the  $n$ -hexane/water interface at 25 °C. (b) A schematic picture of the orientation of  $\alpha$ -helix rods of PMG $_n$ -CyD at the  $n$ -hexane/water interface.

calibration curve, fluorescence intensity–TNS concentration ( $10^{-8}$ – $10^{-6}$  M) relation, we can estimate the concentration of free and bound TNS,  $[\text{TNS}]_f$  and  $[\text{TNS}]_b$ , respectively, in the presence of a known amount of the CyD in water. The binding constant  $K$  was obtained from the next equation.  $K = [\text{TNS}]_b / ([\text{CyD}]_t - [\text{TNS}]_b) \cdot [\text{TNS}]_f$ , where  $[\text{CyD}]_t$  is the total concentration of the  $\beta$ -CyD introduced into the water. The  $K$  value in  $n$ -hexane was similarly obtained based on the decrease in the emission intensity of free TNS at 428 nm in the solvent by adding the known amount of CyD.

TABLE I. Limiting area,  $A_{||}$  and  $A_{\perp}$ , estimated from interfacial pressure–area isotherms of PMG $_n$ -CyD monolayers.

	$A_{  }(\text{nm}^2 \cdot \text{molecule}^{-1})$	$A_{\perp}(\text{nm}^2 \cdot \text{molecule}^{-1})$
PMG <sub>19</sub> -CyD	4.55	3.05
PMG <sub>34</sub> -CyD	7.20	3.05

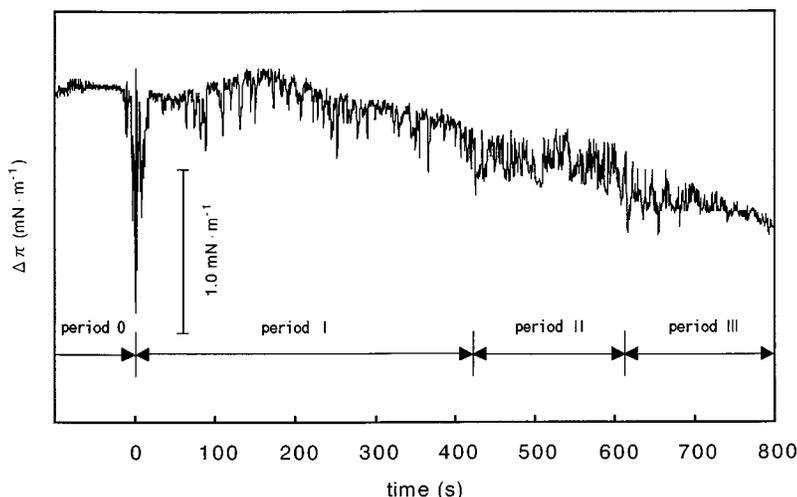


FIG. 4. An oscillation of the interfacial pressure of PMG<sub>19</sub>-CyD monolayer at the *n*-hexane/water interface induced by adding TNS into the water phase ( $2.0 \times 10^{-6}$  M) at time=0.  $A = 3.26$  nm<sup>2</sup>/molecule.

### III. RESULTS AND DISCUSSION

#### A. $\alpha$ -helix structure of PMG-CyD

Poly( $\gamma$ -methyl L-glutamate) (PMG) is known to form a stable  $\alpha$ -helix structure in the membrane. The secondary structure of PMG<sub>19</sub>-CyD was estimated from a circular dichroism (CD) spectrum of the LB membrane in Fig. 2. The molar ellipticity,  $[\theta]$ , could not be calculated from the ellipticity,  $\theta$ , in this figure, because of an uncertainty of the thickness of the PMG<sub>19</sub>-CyD LB membrane. Therefore, a quantitative estimation of the helix stability was impossible. The spectrum in Fig. 2 shows, however, a typical  $\alpha$ -helix CD pattern having the negative bands at 208 and 222 nm, respectively, associated with a stable right handed  $\alpha$ -helix. In addition, the band associated with random coil, 197 nm (negative), and  $\beta$ -structure, 217 nm (negative), could not be seen. It may say, therefore, that the introduction of the  $\beta$ -CyD to the main chain terminal position did not induce large changes in the  $\alpha$ -helix stability of the PMG.

#### B. Molecular orientation of PMG-CyD in the monolayer

The molecular orientation of PMG<sub>n</sub>-CyDs at the *n*-hexane/water interface was characterized by their  $\pi$ -A isotherms. The  $\pi$ -A isotherms for PMG<sub>19</sub>-CyD and PMG<sub>34</sub>-CyD monolayers in Fig. 3(a) showed two steep increase parts, respectively, suggesting that the monolayers can take two possible orders in the solid state monolayer at the oil/water interface. Extrapolations of the each steep parts of the isotherm to  $\pi=0$  gave two extrapolated values of area per PMG-CyD molecule,  $A_{\parallel}$  and  $A_{\perp}$  in Table I. As for the larger area,  $A_{\parallel}$ , there was a difference, 2.65 nm<sup>2</sup>/molecule, between PMG<sub>19</sub>-CyD and PMG<sub>34</sub>-CyD. X-ray analysis of the PMG solid film in the hexagonal packing of the helices showed that the diameter of the PMG  $\alpha$ -helix rods, including the side chain region, is 1.22 nm. Based on this diameter, we can calculate the difference in the area per molecule, owing to the difference in the degree of polymerization between the two PMG-CyDs to be *ca.* 2.75 nm<sup>2</sup>/molecule when the polypeptides lie down in the monolayer. This difference is almost equal to that obtained from the values of  $A_{\parallel}$ , thus confirming the parallel orientation of the  $\alpha$ -helices at the

larger monolayer area. On the other hand, the values of  $A_{\perp}$ , the smaller area, were found to be consistent with each other, even though the length of their helix rods is different from each other. One of the possible molecular arrangement is that  $\alpha$ -helix rod of PMG-CyD may be oriented perpendicular to the interface, as is shown in Fig. 3(b) by increasing the interfacial concentration of the polypeptide. However, the area, 3.05 nm<sup>2</sup>/molecule, is much larger than those of the cross sectional area, 2.06 nm<sup>2</sup>, of CyD and that, 1.29 nm<sup>2</sup>, for the rod of PMG. This difference may be explained in terms of the deviation of the center axis between PMG helix and CyD.

#### C. Guest-induced oscillation of a PMG-CyD monolayer at the *n*-hexane/water interface

A guest molecule for the CyD, 2-*p*-toluidinyl-naphthalene-6-sulfonate (TNS), was injected into the water phase beneath the PMG<sub>19</sub>-CyD monolayer at  $A = 3.26$  nm<sup>2</sup>, in which the  $\alpha$ -helix rod of PMG<sub>19</sub>-CyD is nearly perpendicular to the interface. The terminal CyD in the monolayer should face the water phase because of its hydrophilic character. The concentration of TNS added in the water phase is  $2.0 \times 10^{-6}$  M. Within a minute, the monolayer began to oscillate, resulting in the rhythmic response of the interfacial

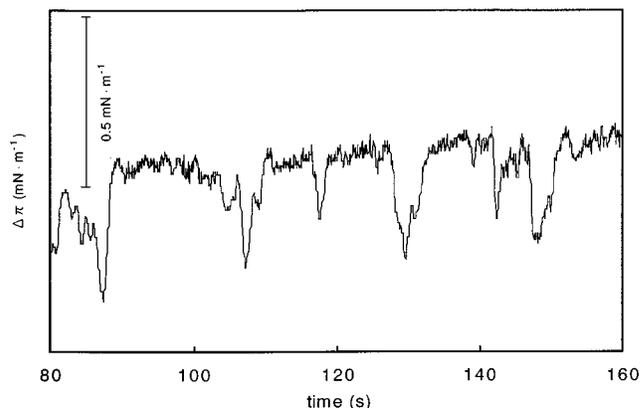


FIG. 5. Oscillation of the interfacial pressure in a part of period I in Fig. 4.  $A = 3.26$  nm<sup>2</sup>/molecule.

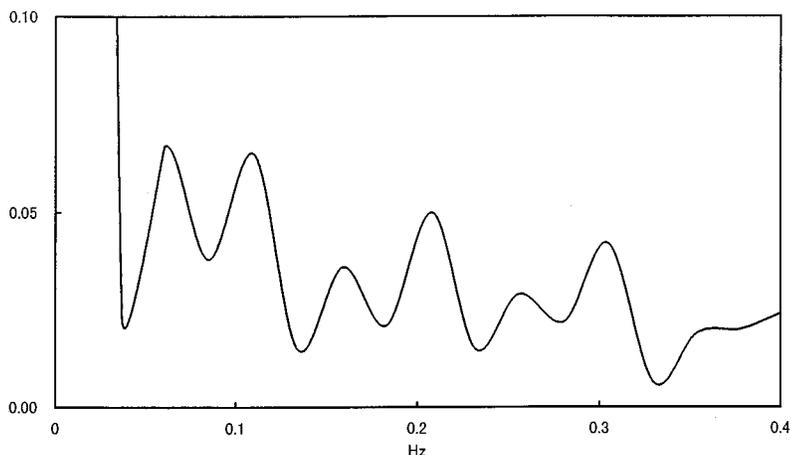


FIG. 6. The power spectrum of the oscillation of the interfacial pressure in period I in Fig. 4.

pressure. Figure 4 shows the oscillations of the interfacial pressure of the PMG<sub>19</sub>-CyD monolayer. It was found that the frequency and amplitude of the oscillations change with time. During the initial stage, period I in Fig. 4, about 30 s after the TNS injection, regular oscillation is generated and continued more than 400 s. The most striking characteristic is the clear and sharp reduction of the interfacial pressure resulting in the periodic negative peaks. Figure 5 shows a part of period I. The interfacial pressure more clearly indicates the periodic and negative peaks. The period between the main peaks is found to be *ca.* 18 s. The power spectrum of the oscillation in period I is shown in Fig. 6. A characteristic frequency at 0.055 Hz appears as the maximum peak in Fig. 6. Another frequency peak, at almost regular frequency intervals, may be attributed to higher harmonics of the characteristic frequency at 0.055 Hz.

Figure 7 shows a part of period II. The pattern of the oscillation induced is the opposite of that of the oscillation in period I, i.e., the oscillation in period II could be characterized by the sharp increase in the interfacial pressure yielding the positive peaks. The Fourier analysis of the oscillation in period II was shown in Fig. 8. The periodicity is not clearly seen in this figure. It is also noted that the interfacial pressure, accompanying the oscillations, is gradually decreased, as a whole, after the second half of period I in Fig. 4.

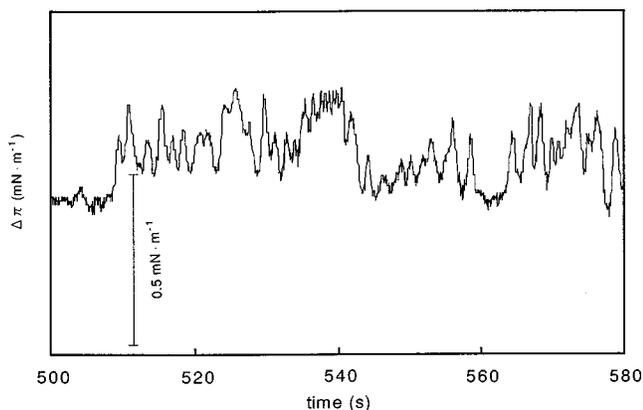


FIG. 7. The oscillation of the interfacial pressure in a part of period II in Fig. 4.  $A = 3.26 \text{ nm}^2/\text{molecule}$ .

The oscillations in period III in Fig. 4 were composed of positive and negative peaks in the interfacial pressure and became more irregular. The final stage of the oscillation in period III is shown in Fig. 9 with the trace of the interfacial pressure of the monolayer in the absence of the TNS in period 0 in Fig. 4. Their Fourier transform shown in Fig. 10 indicated the inert spectra.

For the oscillations in the oil–water systems, Yoshikawa *et al.*<sup>15,16</sup> showed that the interfacial tension changes in a rhythmic manner in the presence of an amphiphile. This oscillatory phenomenon has been explained in terms of the Marangoni effect,<sup>18</sup> i.e., the instability of the interfacial tension due to a concentration gradient of the amphiphile causes spontaneous agitation of the interface between the liquids. In our systems, the amphiphilic PMG-CyD formed a stable monolayer at the *n*-hexane/water interface and did not oscillate without TNS. This implies that the driving force of the oscillation must be the difference in the chemical potential of the guest TNS molecule between water and *n*-hexane phases. So the binding constant,  $K$ , between the CyD and TNS was measured. The value of  $K$  was estimated to be  $1.1 \times 10^5$  in water and  $8.1 \times 10^3$  in *n*-hexane, respectively. This means that the terminal CyD strongly interact with TNS in the water, whereas it is easy to release the guest in the *n*-hexane phase. Moreover, TNS is a well known hydrophobic fluorescence probe,<sup>23</sup> and the inclusion structures of the various naphthalene derivatives in the CyD cavity were also studied.<sup>24</sup> The inclusion of the hydrophobic TNS, therefore, may reduce the hydrophilic nature of the terminal CyD, resulting in the loss of the amphiphilic character of the PMG-CyD. In addition, the individual molecular events in the monolayer cannot be reflected as periodic and macroscopic oscillatory phenomena, i.e., the oscillations of the interfacial pressure of PMG<sub>19</sub>-CyD monolayers may be based on the movement of macroscopic domains of the monolayer. The two dimensional regular packing and stable domain formation of PMG at the air/water interface has been already confirmed and in the condensed monolayer domain  $\alpha$ -helices were found to be impossible to rotate each other because of strong interactions between the polypeptides.<sup>25</sup>

Under the considerations, a possible molecular mechanism of the oscillation is proposed in Fig. 11. This figure

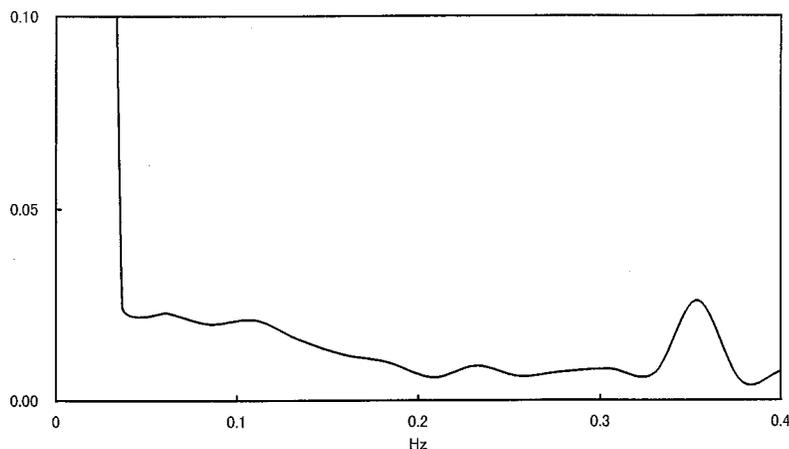


FIG. 8. The power spectrum of the oscillation of the interfacial pressure in period II in Fig. 4.

shows the changes in the location of the main domain of the PMG-CyD monolayer relative to the oil/water interface. At first, the terminal CyD, in contact with water, interacts with the guest TNS with a large binding constant, (a) in Fig. 11. The chemical energy of TNS is stored at the interface and the PMG-CyD loses its amphiphilicity based on the binding of the hydrophobic guest. During the process the interfacial tension,  $\gamma$ , is locked. When the interfacial concentration of the binding TNS reaches a critical value, the monolayer moves to the *n*-hexane phase, (b) in Fig. 11. As a result,  $\gamma$  approaches the interfacial tension of the water/*n*-hexane without contaminants,  $\gamma_0$ . Therefore, the interfacial pressure,  $\pi = \gamma_0 - \gamma$ , may be reduced. And then the terminal CyD in the oil phase releases the guest owing to the low binding constant and very low concentration of TNS in the new medium, (c) in Fig. 11. As a result, the monolayer may recover the original position and initial  $\pi$  value. This process gave the negative feedback loop essential for the oscillation in the PMG-CyD monolayer at the oil/water interface. The process (b)→(c)→(a) occurs immediately because the concentration of TNS in *n*-hexane is low in the initial stage. This is the reason why the interfacial pressure showed the sharp reduction in period I. The critical concentration of TNS at the interface may determine the frequency of the oscillation. On the other hand, when the concentration of TNS in the oil phase becomes higher, it is hard for the terminal CyD to

release the TNS. The monolayer gradually makes a longer stay in the oil phase. As a result, a temporary recovery of the original state, (c)→(a), induces a sharp increase in the interfacial pressure in period II. This is also the reason why the  $\pi$  value decreases as a whole after the second stage in period I. The TNS released from the terminal CyD penetrates through the inter-helices and/or more effectively interdomains of the monolayer to the *n*-hexane bulk phase. Such a fluctuation in the TNS concentration near the interface induces the irregular oscillation in period III.

It is also noted, here, that the oscillation could be induced above the critical concentration of TNS,  $10^{-8}$  M, and at the monolayer area around  $A_{\perp}$ .

#### IV. CONCLUSIONS

The guest-induced oscillation of the interfacial pressure of the host containing polymer monolayer could be observed at the oil/water interface. The driving force of the oscillation is the concentration gradient, between water and oil phases, of the guest molecule injected into the water. The binding of the guest molecules to the host sites in the monolayer effectively occurred when the monolayer is in contact with the water phase, and increased the chemical potential at the interface, which induced the movement of the monolayer to the oil phase. On the contrary, the monolayer released the guest molecules based on the low binding ability in the oil phase, and as a result, it recovered the original location at the oil/water interface. This cycle of the change in the location of the monolayer could be monitored by the oscillation in the interfacial pressure.

The cooperative movement of the monolayer, not an individual molecular event, may be based on the domain formation in the monolayer resulting from the interactions between PMG segments. This polymeric interaction may escalate the competition for CyD of PMG/water and PMG/*n*-hexane into the macroscopic monolayer wave measurable as the rhythmic changes in the interfacial pressure. On the other hand, the quick diffusion of TNS may effectively eliminate the oscillation. Therefore, the PMG layer at the interface is also useful for the maintenance of the concentration gradient of TNS to produce the oscillation having a longer lifetime. Thus, the oscillatory behavior cannot emerge

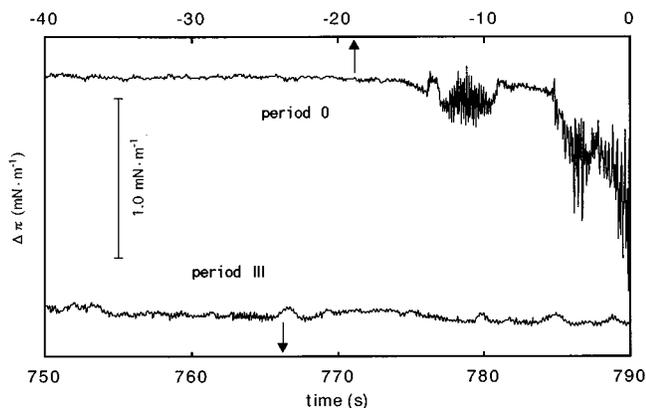


FIG. 9. The oscillation of the interfacial pressure in a part of periods 0 and III, respectively, in Fig. 4.  $A = 3.26$  nm<sup>2</sup>/molecule.

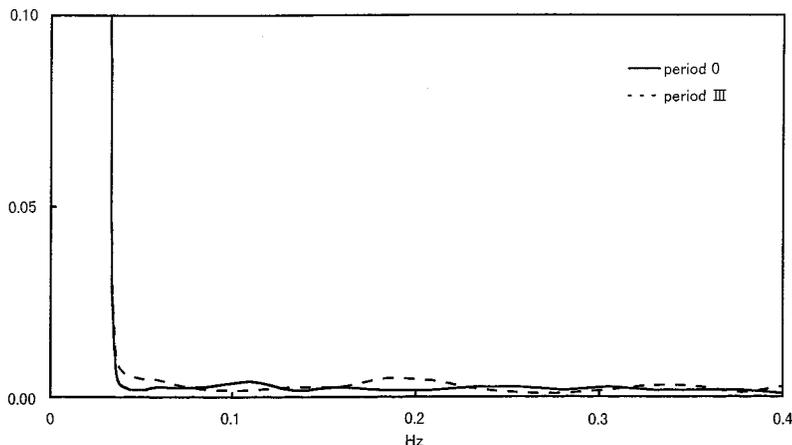


FIG. 10. The power spectra of the oscillation of the interfacial pressure in periods 0 and III in Fig. 4.

without the PMG chains and/or their interactions, i.e., the cooperativity based on the polymer-polymer interaction in the monolayer and the binding and releasing time required based on the host-guest interaction across the interface are found to be essential for the coupling between the chemical potential gradient and the periodic oscillation phenomenon at the oil/water interface.

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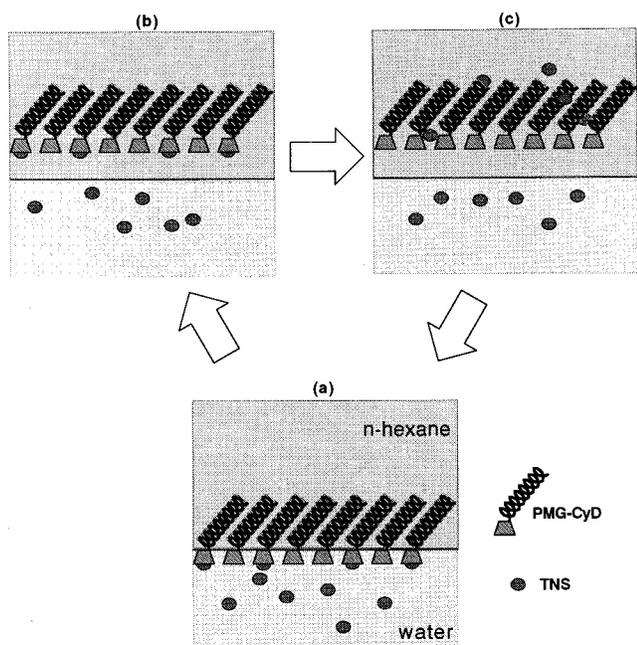


FIG. 11. A schematic picture of the change in the location of the PMG<sub>19</sub>-CyD monolayer resulting from the binding and releasing of the guest TNS across the *n*-hexane/water interface.

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