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Preparation of Mono- and Multilayer Films of Calmodulin Using Langmuir-Blodgett Technique

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Monolayer film of calmodulin using Langmuir-Blodgett technique was successfully deposited onto appropriate plates. The conformational study of calmodulin in Langmuir-Blodgett film was carried out using infrared spectra and circular dichroism spectra, and these results showed that α-helical conformation was predominant and its structure was very similar to that in aqueous solutions.

Many studies on the biochemical functions of the proteins in its multilayer films have been done.[1-4] Recently it has been tried to use the multilayer films of enzymes and the antigen-antibody as the biosensors or biological electronic components, so called the biochips, by means of their high selectivities for their substrates, and the biomolecular switch made by ordering or immobilizing the multilayer of the proteins will be feasible in future.[5] However, the main problem to use the multilayer of the proteins for such applications is that the proteins are denaturated and forming the multilayers of proteins without the surface denaturation seems to be impossible; thus few works were reported.[6]

Calmodulin(CaM), a heat-stable and the multifunctional Ca^{2+}-binding protein responsible for the Ca^{2+} stimulation of cyclic nucleotide phosphodiesterase(PDE), is now known to play a central role in the Ca^{2+}-dependent regulation of eukaryotic cells.[7] This Ca^{2+} receptor is a single 148-amino acid polypeptide containing four Ca^{2+}-binding sites and low molecular weight(ca.17 000) monomeric protein. In general, CaM works in the cells as a regurating protein for various reactions dependent on the Ca^{2+} concentrations, and from this CaM is thought to be a sensor of Ca^{2+} ions in the body. From the points mentioned above, the use of CaM, which is heat-stable and is also resistive for surface denaturation, as a Ca^{2+} sensor seems to be feasible and hopeful. In this paper, we describe the first successful preparation of mono- and multilayer films of CaM obtained with Langmuir-Blodgett technique.

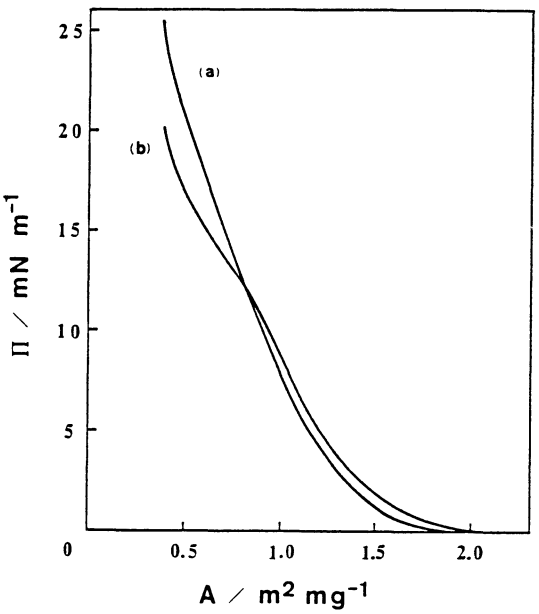

Fig. 1. Surface pressure-area curves of calmodulin : (a) on 1.5 M KCl + 1 mM $CaCl_2$; (b) on 1.5 M KCl + 1 mM EGTA solution.

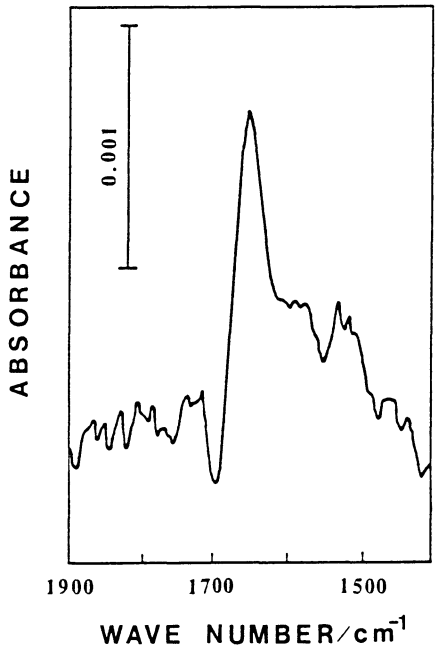

Fig. 2. IR spectrum of transferred monolayers of calmodulin.

CaM was prepared from porcine brain by the method of Ishioka et al.[8] The purity of the protein was checked by the SDS-polyacrylamide gel electrophoresis. CaM was dissolved in distilled water to about 0.04 wt% and spread on potassium chloride(KCl) solution of some concentration.

Figure 1 shows surface pressure-area isotherms for CaM spread on the subphase of 1.5 M KCl + 1 mM ethyleneglycol bis(2-aminoethylether) tetraacetic acid(EGTA) and of 1.5 M KCl + 1 mM $CaCl_2$. Isotherms were measured using a barrier speed from 5 mm min^{-1} after 30 min. The films was dilute films, because it was spread on the initial area of 2.26 m^2mg^{-1}. The compressibility coefficient of the surface film is expressed by $\delta = -dA/A \cdot d\Pi$, where A is the area in m^2mg^{-1} of protein and Π is the surface pressure in mN m^{-1}. The area when the compressibility coefficient was minimum value is believed to the limiting area,[3] and this minimum value gives around 1.1 m^2mg^{-1}. Extrapolation of this curves to zero pressure give about 1.2-1.5 m^2mg^{-1}, which is resemblance with the area of other globular proteins[3] on the water surface. The surface pressure-area curves due to Ca^{+2} ions altered at more than 12 mN m^{-1} as shown in Fig.1, which indicates that the conformation of CaM which binds Ca^{2+} ions in subphases resembles to that in aqueous solutions.[9]

Deposition of CaM carried out at a constant surface pressure of 16 mN m^{-1} onto glass, CaF_2 and quartz plates by drawing down- and upward through the liquid-air interface at a rate of 10 or 20 mm min^{-1} at 15 °C. The transfer ratio was almost 1 in a first downward and upward motion, but it was gradually unsteading from the next motion.

IR spectroscopy provides an alternate method for determing the conformation of proteins in LB films. Therefore, we employed the difference IR spectra by ratio method to study the CaM conformation in LB films. The difference IR spectra was measured using by the plate of CaF_2 with 5 layers of the stearic acid and 4 layers of CaM molecules were deposited on this plate. Figure 2 shows an IR spectrum by JASCO IR-810 spectrophotometer of CaM LB film in the characteristic amide bands region. As can be seen in Fig.2, amide I absorption band at 1650 cm^{-1} suggests that the α-helix conformation is preferred and predominant one.[10]

In Fig.3, is shown the CD spectra of CaM LB films with eight layers deposited on the quartz plate for CD measurement. It is clear from the typical negative peaks at 208 and 221 nm that CaM molecules in LB films exist predominantly in the α-helix and that these peaks are similar to those for CaM in aqueous solutions.[9] In order to calculate the molar ellipticity, we made assumptions that the LB films consisted of only CaM molecules and the shape of CaM molecule was ellipsoidal, and by this assumption, 25 Å, the shorter diameter of CaM molecule, was used as the thickness of CaM monolayer.[11] In the absence of Ca^{2+} (a in Fig.3) the molar ellipticity at 208 and 221 nm gave about -11000 and -9500, respectively, and about -12500 and -9300 in the presence of Ca^{2+} (b in Fig.3). These results suggest that CaM molecules are much more stabilized when Ca^{2+} ions bind to CaM and that

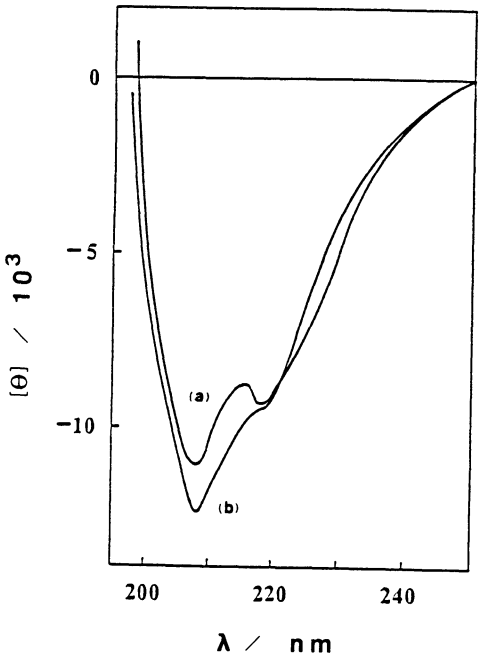

Fig. 3. CD spectra of transferred monolayers of calmodulin : (a) Ca^{2+}(-); (b) Ca^{2+}(+).

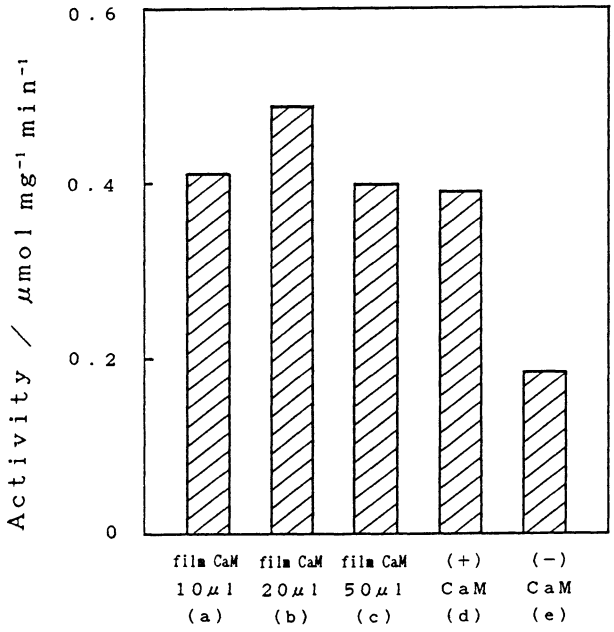

Fig. 4. The activation of PDE activity by CaM : (a) — (c) in LB films ; (d) in solution ; (e) control.

the conformational changes of CaM by the binding of Ca^{2+} ions seems to take place in a similar way as that in aqueous solutions.

Retaining the activity of CaM in LB films, on the other hand, is very important for the practical use of this film as a biosensor, so the activation of the PDE activity by CaM in LB films was measured in the presence of Ca^{2+}. CaM in LB films was dissolved in a minimum amount of 10 mM Tris-HCl buffer (pH 7.5), and then measured.[12] The result showed that CaM in LB films was retained the activation of the PDE activity(a,b,c, in Fig.4), same as a native CaM solution(d in Fig.4).

From the results shown above, it is concluded that CaM molecules, which are heat-stable, resistive to the surface denaturation, and receptors for Ca^{2+} ions, can retain their secondary or higher structure and also the activity of PDE even in LB films, and the use of this film as the Ca^{2+} sensor will be feasible in future. Regarding the application of CaM LB films to such sensors, it is necessary to study the orientation and the heterogenity of CaM molecules in LB films, especially the detailed conformation of CaM on the surface of LB films, by IR, X-ray, and ESCA. Further details of the preparation and the properties of the LB films of CaM will be presented in a near future.

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