NOTE

폴리(γ-벤질 L-그루타메이트)/폴리에테르 블록 공중합체 단분자막에의 단백질의 흡착

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Adsorption of Proteins to Poly(γ-benzyl L-glutamate)/ Polyether Block Copolymer Monolayers

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INTRODUCTION

Adsorption of proteins to membrane surfaces and their behaviour at interfaces as well as interactions with lipids are of particular interest in relation to cell membrane phenomena and physicochemical processes in biological systems.[1] Monolayers are convenient experimental model system to investigate lipid-protein interactions since they provide controlled conditions.[2]

Microphase separated structures are found out in a living cell membrane. Block copolymers containing two kinds of polymer chains having dissimilar properties, for example, hydrophilicity and hydrophobicity, undergo a microphase separation in the casting process. The combination of hydrophobic and hydrophilic components may result in high quality monomolecular assemblies displaying a high degree of structural order.

In the previous studies,[3~6] it was found that monolayers of the triblock copolymers consisting of poly(γ-benzyl L-glutamate)(PBLG) as the hydrophobic group and polyether as the hydrophilic one could be formed. Also monolayer behavior of PBLG/polyether block copolymer is affected by the content of polyether and rigidity of polyether segment in the block copolymer. The blcok copolymers transferred from the water surface onto a solid support by the Langmuir-Blodgett(LB) technique showed the layered structures with a well-defined order or orientation of the molecules. Furthermore, much more platelets were adhered onto the LB surface than cast one due to the difference of microstructure of the surface of the block copolymer.

In this study, we wish to report the adsorption of proteins to PBLG/polyether block copolymer monolayers at the air/water interface as a note.

The adsorption of proteins can be related to the thrombogenicity of polymers.[7]

EXPERIMENTAL

The PBLG/poly(ethylene glycol)(PEG) and PBLG/poly(propylene glycol)(PPG) block copolymers were prepared by polymerization of γ-benzyl

L-glutamate *N*-carboxyanhydride(γ-BLG NCA) initiated with amine-terminated PEG and amine-terminated PPG, respectively, in methylene dichloride by the same method as that described previously.[8,9]

Proteins : Human albumin was purchased from Sigma(A-9511). Human fibrinogen was purchased from IMCO of Sweden (F-149).

Surface pressure was measured by the Wilhelmy hanging plate method[10] and the sensor plate was placed on the end position at 3cm from the wall of the trough. The compression of the monolayer was made by a moving barrier by the same procedure as that described previously.[11] Isotherms were measured using a barrier speed of 15 mm/min after 10 min.

10 mM phosphate solution was used as a buffer (pH=7.4) and chloroform as the spreading solvent. Deionized water from a Milli-Q was used for preparing the subphase.

Apparatus : The apparatus used for spreading monolayers and for measuring surface pressure was as described by Fromherz.[12]

Adsorption of Protein : Proteins were dissolved in the phosphate buffer solution(10mM $NaH_2PO_4 \cdot 2H_2O$+10mM $Na_2HPO_4 \cdot 12H_2O$, pH 7.4) to give a concentration of 0.04 wt%. A monolayer was spread on PBS buffer at 20℃. The monolayer was moved to the protein solution by sliding it between two barriers kept the same distance apart to avoid any pressure changes in the film during transposition. The surface pressure was monitored during adsorption of proteins to monolayer.

RESULTS AND DISCUSSION

Fig. 1 shows the F-A (F ; surface pressure, A ; surface area) isotherm curve of the PBLG homopolymer and PBLG/PEG block copolymers spread on the water surface from chloroform solution. The curve of PBLG has the apparent plateau which is a region of very high compressibility while block copolymers gave the curves of expanded types which are entirely different from those of PBLG. Also, the plateau regions which are attributed to the passage from a monolayer to a bilayer,[13] disappeared in the block copolymer because the PEG component is in a random, liquid-like conformation and has a plasticizing effect on the block copolymer main chains.

Fig. 2 is the F-A isotherm curves of the PBLG homopolymer and PBLG/PPG block copolymers spread on the water surface from chloroform solution. The trend of the curves of the block copolymers is almost similar to that of the PBLG. It may be regarded that the methyl group of the side chain in PPO remains at the surface when the film of the block copolymer is compressed.

Fig. 3 shows the interaction of albumin with monolayers of PBLG/PEG block copolymers at initial pressure of 3 dyne/cm and 7 dyne/cm, respectively. Change in surface pressure increased with time and reached a constant level at 60 min in every monolayers. Surface pressure change is usually attributed to the adsorption of the protein molecule to the monolayer.[1] Rapid surface pressure change took place with decreasing content of

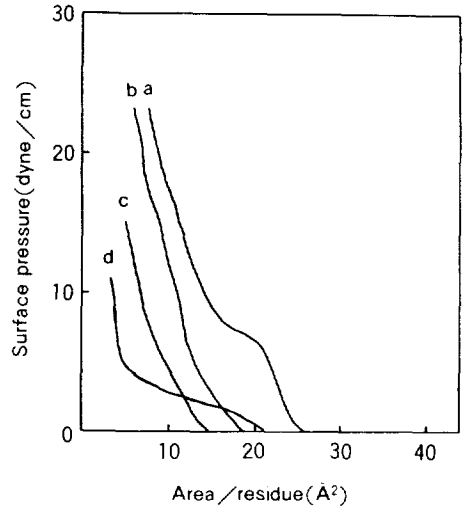

Fig. 1. Surface pressure-area isotherms of PBLG homopolymer and PBLG/PEG block copolymers : curve a, PBLG ; curve b, PBLG/PEG-1(PEG 24.4 mol.%) ; curve c, PBLG/PEG-2(PEG 35.6 mol.%) ; curve d, PBLG/PEG-3(PEG 73.8 mol.%).

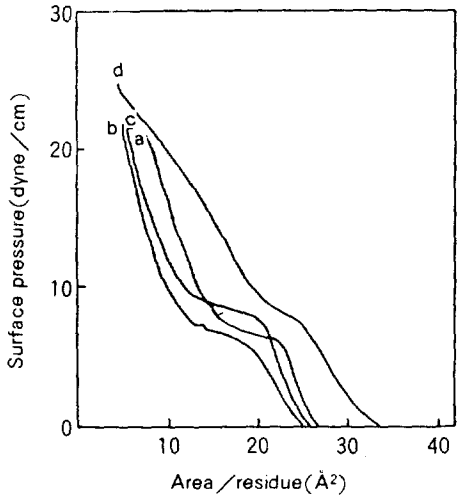

Fig. 2. Surface pressure-area isotherms of PBLG homopolymer and PBLG/PPG block copolymers : curve a, PBLG ; curve b, PBLG/PPG-1(PPG 17.0 mol.%) ; curve c, PBLG/PPG-2(PPG 26.0 mol.%) ; curve d, PBLG/PPG-3(PPG 60.0 mol.%).

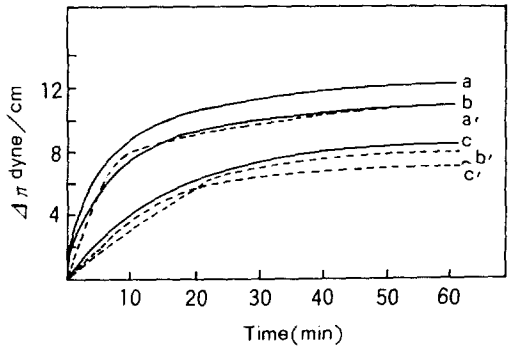

Fig. 3. Interaction of albumin and PBLG/PEG monolayers at 3 dyne/cm and 7 dyne/cm, respectively. Subphase, 10mM-phosphate buffer(pH7.4). Surface pressure changes, $\Delta\pi$. curve a, PBLG ; curve b, PBLG/PEG-1 ; curve c, PBLG/PEG-3. Straight line, 3 dyne/cm ; dotted line, 7 dyne/cm.

PEG in the block copolymers. Also, much change in surface pressure was shown in PBLG/PPG monolayers than PBLG/PEG ones (not shown in Fig.). These results were ascribed to hydrophobic interaction between albumin and block copolymer monolayers mainly.[14] Much change in surface

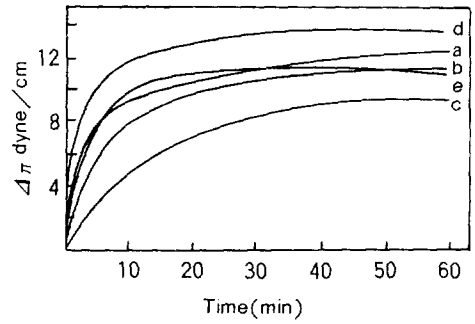

Fig. 4. Interaction of fibrinogen and PBLG/polyether monolayers at 3 dyne/cm. Subphase, 10mM-phosphate buffer(pH 7.4). curve a, PBLG ; curve b, PBLG/PEG-1 ; curve c, PBLG/PEG-3 ; curve d, PBLG/PPG-1 ; curve e, PBLG/PPG-3.

pressure happened in 3 dyne/cm than 7 dyne/cm. This result indicated that proteins could easily be interacted with expanded type monolayers than compressed type one. Mohwald et al. reported that cytochrome C preferentially binds to lipid monolayers in the liquid phase.[15]

Fig. 4 shows the interaction of fibrinogen with monolayers of PBLG/polyether block copolymers at initial pressure of 3 dyne/cm. The surface pressure change increases with time as same tendency of albumin. But much more changes in surface pressure were found than albumin. These results are attributed to the more adsorption of fibrinogen on the hydrophobic surface than hydrophilic one.[16] Also, much more change in surface pressure occurred in PBLG/PPG monolayer than PBLG/PEG at same content of polyether in the block copolymers. This may be caused by the methyl group of the side chain in the PPG.[14]

In conclusion, the adsorption of proteins to PBLG/polyether block copolymer monolayers is dependent on the content of polyether, initial surface pressure and kinds of proteins. The interaction between proteins and block copolymer monolayers may be regarded as the hydrophobic bonding mainly. The desorption of proteins from the PBLG/polyether block copolymer monolayers will be reported.

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