2P252 固体NMRによるコレステロール－酸性混合脂質二重膜系とACTHの相互作用変化の解析
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Different interaction of ACTH with acidic mixed lipid bilayers in the presence and absence of cholesterol as studied by Solid state NMR.
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Adrenocorticotropin hormone (ACTH) consists of 39 amino acid and mainly causes the promote of glucocorticoid secretion. In this work, ¹H and ¹³C solid state NMR spectra were observed for ACTH(1-24) reconstituted in to an acidic membrane in the presence and absence of cholesterol. The aim of this study is to clarify biological function of ACTH by analyzing the interaction of ACTH with the membrane. [1- ¹³C]Gly¹⁰ and [1-¹³C]Val¹³:ACTH(1-24) peptide were synthesized using a peptide synthesizer. The crude peptides were purified by a HPLC. The obtained peptides were reconstructed to in the MLVs (hydrated by Tris buffer). The molar ratio of peptide:lipids was adjusted to be 1:20. The lipids components prepared for the experiments were 1)DMPG:DMPC=1:3 2)DMPG:DMPC:cholesterol=1:3:1. ¹³C DD-MAS NMR spectra were measured and the results indicated that ACTH of Gly¹⁰and Val¹³ forms α-helical structure in the both systems at 40 °C. The intensity analysis of ¹³C DD-Static NMR spectra at 40 °C showed that Gly¹⁰ and Val¹³ bonded tightly to lipid bilayer in the absence of cholesterol. By contrast, they were released from the bilayer in the presence of cholesterol. Gel-to-liquid crystalline phase transition temperature(T_b) can be determined by using ¹H MAS NMR. We found that T_b of 2) with ACTH increases about 3 °C as compared to that without ACTH. Hence,it is stressed that the interaction of ACTH with cholesterol-doped bilayer is stronger than that with cholesterol free bilayer.

2P254 脂肪酸上に積層したジミリストイルフォスファチジルコリンとバクテリオロドプシンの複合薄膜
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Composite film of dimyristoyl-phosphatidylcoline and bacteriorhodopsin fabricated on fatty acid film
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To investigate and create a bio molecular device unit with a surface that is highly similar to native cellular membrane surface, we fabricated a novel patterned dimyristoyl-phosphatidylcoline (DMPC) film on stearic acid (SA) or retinoic acid (RetA) film using Langmuir-Blodgett method and compared. The fabricated film on RetA had a curved bio mimetic structure with a diameter of 200 nm and a height of 9nm. On the contrary, the fabricated film on SA had a plane surface structure and a difference in level like a crater. These structures were basically different from each other, and have different physical adsorption features between film structure of DMPC and fatty acid. AFM images of fabricated DMPC film on SA film or RetA film revealed them. Next we constructed a composite film of DMPC and bacteriorhodopsin (BR) in several molecular ratios and compared FTIR reflection absorption spectrums of DMPC / BR composite film on SA film or RetA film. We constructed several films with several layers and analyzed FTIR reflection absorption spectrums and UV absorption spectrums to characterize the detail of surface structure of films. The analysis revealed the surface entirely had been different in structural change on two fatty acids.

2P253 脂肪酸上に積層した二種のフォスファチジルコリン複合膜の比較解析
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Comparative analysis on two composite films of phosphatidylcoline on fatty acid film
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To construct a bio molecular device in which protein or macro bio molecule used, high precision of bio mimetic molecular film structure is a good candidate to fulfill their functions. We fabricated a novel patterned dimyristoyl-phosphatidylcoline (DMPC) film on retinoic acid (RetA) film using Langmuir-Blodgett method. The fabricated film had a curved bio mimetic structure with a diameter of 200 nm and a height of 9nm. This structure was periodically continuous all over the RetA film. AFM images of fabricated DMPC film on RetA film showed several structural features. For comparison of structural difference of phosphatidylcoline we fabricated a different film. It was a hydrogenated soy phosphatidylcoline (h-soy-PC) film on stearic acid (SA) or RetA film. We analyzed the surface structure compared with DMPC film on fatty acids. Further, we constructed several films with several layers and analyzed FTIR reflection absorption spectrums and UV absorption spectrums to characterize the detail of surface structure of films. The spectroscopic data exhibited specific surface structure with layer by layer.

2P255 DSC およびX線回折によるセラミドを含むモデル生体膜の物理化学的研究
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Physicochemical studies of model membrane containing ceramide by calorimetry and x-ray diffraction
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Lipid raft domain which is mainly made from cholesterol and sphingolipids plays a key role in intracellular signal transductions. Ceramide is a base of sphingolipids and is also found in the raft domain. To clarify the mechanism of raft formation, it is necessary to know interactions between various lipid molecules found in the raft domain. In this study, we investigated interactions between ceramide and other lipid raft components in model systems, using a synthetic ceramide((2S,3R)-2-Octadecanoylaminoctadecane-1,3-diol ((2S,3R)-Cer2)) synthesized by Takasago Corp. (2S,3R)-Cer2/cholesterol, (2S,3R)-Cer2/DPPC and (2S,3R)-Cer2/Cholesterol/DPPC mixed systems were examined by means calorimetry and X-ray diffraction.

(2S,3R)-Cer2/cholesterol mixed system formed a lamellar liquid crystal phase with periodicity of 4.22nm at physiological temperature in molar ratio 1/1, agreeing with [1]. (2S,3R)-Cer2/DPPC system formed a lamellar gel phase with periodicity of 7.02nm from 30°C to 75°C. In addition to the lamellar phase, another new lamellar phase with a shorter periodicity was observed from 75°C to 95°C. Pn3m cubic phase and hexagonal II (HII) phase were coexisted above 95°C. (2S,3R)-Cer2/Cholesterol/DPPC mixed system formed two lamellar phases with periodicity of 4.05nm and 6.72nm from 20°C to 90°C. This system transformed into HII phase at 90°C. From these results, we will discuss the role of ceramide in the raft formation.

[1] K.Ishida, Oleoscience, Vol. 4, No. 3, 105-116 (2004) (Japanese)