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モデル生体膜の構造に対する重水効果

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Effect of deuterium oxide on the structure of model biomembranes

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Deuterium oxide (D₂O) is toxic for many living things and inhibits cell proliferation for some organisms. To elucidate the origin of these D₂O effects, detailed knowledge of interactions between D₂O and biological molecules is indispensable. From this viewpoint, we have studied the physical properties of lipid membrane in D₂O and H₂O. Here, we report our results that suggest that the substitution of H₂O by D₂O affects the lateral molecular packing of bilayer membranes. We estimated the intermolecular distance for dipalmitoylphosphatidylcholine (DPPC) membranes in H₂O and D₂O, from fluorescence measurements. The estimated distance for DPPC in D₂O was about 0.01 nm smaller than that in H₂O. Lamellar repeat distances of PCs in a liquid-crystalline phase in D₂O were about 0.1 nm smaller than those in H₂O, agreeing with [1]. This result has been interpreted as indicating a following mechanism: D₂O strengthens the interactions between PC molecules. As a result, the undulation force is reduced, resulting in the decrease of the thickness of water layer between PC bilayers [1]. Our results described above support this interpretation. To confirm that this mechanism works for other lipid systems, we investigated the effect of D₂O on the lattice constants of other various lipid membrane systems using X-ray diffraction. The results suggest that there are different mechanisms for the lattice constant changes induced by the substitution of H₂O by D₂O, depending on the kinds of lipids.[1] Kobayashi, Y. and Fukada, K. (1998) *Chem. Lett.* 1105-1106

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ガングリオシド／コレステロール／ジオレオイルホスファチジルコリン混合系の構造に及ぼすポリビニルピロリドンの影響

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Effect of polyvinylpyrrolidone on the structure of ganglioside/cholesterol/dioleoylphosphatidylcholine mixtures

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Polyvinylpyrrolidone (PVP) is known to show various properties such as water solubility and low toxicity, therefore PVP is widely used for the medicine, the cosmetic, and the other industrial products. In the previous studies the effects of PVP on phospholipids bilayer was investigated by other authors. However, there is little information about the vesicle containing glycosphingolipids. In the present study using synchrotron small-angle X-ray scattering, we have focused to clarify the structure of small unilamellar vesicle (SUV), composed of monosialoganglioside (GM1)/ cholesterol/ dioleoylphosphatidylcholine (DOPC), with varying PVP content. The X-ray wavelength used was 1.499Å, the sample-to-detector distance were 80cm and 200cm. For the binary mixtures (cholesterol/DOPC), we have found that the uni-lamellar vesicle structure change to the multilamella one above the PVP concentration of ~20 %w/w. In addition, the lamellar peak becomes to be more evident with increasing the cholesterol content. This tendency would be reasonable as a well-known effect of cholesterol on lipid bilayer. In the case of the ternary mixture (GM1/cholesterol/DOPC), the above change does not appear. SUV structure is kept in spite of the increase PVP content (0~25 %w/w). It suggests that the presence of GM1 suppresses the deformation of SUV structure caused by PVP.

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WAXS および SAXS によるガングリオシド混合脂質と変性蛋白質との相互作用の研究

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Interacting of ganglioside-contained lipid mixtures with denatured protein clarified by SAXS and WAXS

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Functions of mammalian plasma membrane microdomains being rich in glycosphingolipids (GSL), so-called rafts, are now one of the current hot topics in cell biology from the intimate relation to cell adhesion and signaling. Gangliosides, major components of GSLs, are rich in lipid microdomains with other particular lipids and proteins in neuronal cells. Previous spectroscopic studies showed that amyloid β proteins (A β) interact strongly with monosialogangliosides (GM1) to promote the structural transition of A β from helix to sheet and that the GM1-A β complexes form seeds in the course of A β polymerization as amyloid fibril. On the other hand, even ordinary globular proteins, such as apomyoglobin, were shown to form amyloid fibrils under some condition where a native state of the protein is unstable. Then we have carried out the small-angle Xray scattering (SAXS) and wide-angle X-ray scattering (WAXS) experiments to clarify a specificity of the interaction between the amyloid protein and GM1 and lipid mixture containing GM1, cholesterol, and PC by varying lipid compositions.

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パターン化脂質膜に固定されたチトクロム P450 を用いた酵素活性アッセイ法

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Immobilization of cytochrome P450 on micropatterned lipid bilayer substrates for the parallel assay of their activities

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We report on a methodology for immobilizing cytochrome P450 on the surface of micropatterned lipid bilayer membranes and measuring the enzyme activity. The patterned bilayer comprised a matrix of polymeric lipid bilayers and embedded fluid lipid bilayers. The polymeric lipid bilayer domains act as a barrier to confine fluid lipid bilayers in defined areas and as a framework to stabilize embedded membranes. The fluid bilayer domains, on the other hand, can contain lipid compositions that facilitate the fusion between lipid membranes, and are intended to be used as the binding agent of microsomes containing rat CYP1A1. By optimizing the membrane compositions of the fluid bilayers, we could selectively immobilize microsomal membranes on these domains. Enzyme activity of immobilized P450, as measured by the dealkylation of a fluorogenic substrate (7-ethoxyresorufine), was significantly higher on lipid bilayer substrates compared with direct adsorption on glass. This result indicates that fluid lipid bilayers act as a spacer between glass surface and cytochrome P450 and prevent inactivation of cytochrome P450 because of nonspecific adsorption. Furthermore, competitive assay experiment between two fluorogenic substrates demonstrated the feasibility of bioassays based on immobilized P450 enzyme.