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論文内容の要旨

The combination of silicon technology with the cell biological functions is to be an attractive research field for the development of scientific and technological applications including the *in vitro* study of the fundamental properties of biological membranes and medical diagnosis and the screening for the new drug discovery. Supported membranes are generally prepared by two methods; Langmuir-Blodgett (LB) method and vesicle fusion method. The former is useful for the formation of the hybrid type lipid bilayer comprised of a different type of lipid monolayers. The latter, more convenient and prevail, has several advantages with respect to Langmuir-Blodgett method in a viewpoint of easy deposition, high area selectivity, and defect free high quality membrane formation in a limited area. In this method, Ca^{2+}, as a facilitating material, is used to form the lipid bilayer by the vesicle fusion. However, addition of Ca^{2+} gives rise to the limitation to utilize supported membranes for the application areas in relation to cell physiological phenomena. In this thesis, he focused on the addition effects of cholesterol in LB method and the Ca^{2+} free vesicle fusion method for the lipid bilayer formation.

In LB method, the addition effects of cholesterol on the dipalmitoylphosphatidylcholine (DPPC) monolayer have been investigated by AFM and IRRAS, since Cholesterol, one of the major components in cell membranes, is a regulator to maintain biological and physical properties of the membranes including permeability, fluidity and mechanical strength as well as enhances the resistivity of a supported membrane on a silicon substrate.

In the analysis of pressure (Π) - area (A) isotherms for monolayers with different molar cholesterol concentrations (C_{chol}) (10, 20, 30, and 35%) at the air-water interface, with addition of cholesterol, the shift of the isotherms toward the left side (small area per molecule region) and the disappearance of plateau region indicate the condensing effects governed by heterogeneous hydrophobic interactions between DPPC and cholesterol.

For AFM images of the DPPC/cholesterol monolayers varied with C_{chol} (0~35 %) transferred onto mica surfaces at the 10 mN/m deposition pressure, there are two

stages in the transformation from pure DPPC (liquid crystalline (LC) + liquid expanded (LE)) to DPPC/cholesterol mixture (liquid ordered (LO)) in the C_{chol} range of 0 ~ 35 %: at C_{chol} from 0 % to 10 %, ν_{LC} only slight decreases in spite of the drastic change in the morphology and at C_{chol} between 10 % and 35 %, ν_{LC} decreases accompanied with the appearance of the depletion areas, which are cholesterol rich domains. At the initial stage of the cholesterol addition (5%), cholesterol molecules probably distribute at the boundary of the LC domains. After the boundary region is saturated with cholesterol, excess cholesterol gathers at the interface with LE, which is observed as the depletion area or diffuses into the inside of the LC domain.

In the IRRAS spectra of the DPPC, DPPC/cholesterol mixtures (10, 20 and 35 %) and cholesterol monolayers prepared at the 10 mN/m surface pressure, addition of 10 mol % of cholesterol caused the shift of the CH_2 vibrational modes from 2917 to 2922 cm^{-1} for $\nu_{as}(CH_2)$ and from 2848 to 2852 cm^{-1} for $\nu_s(CH_2)$, and also caused the appearance of $\nu_{as}(CH_3)$ at 2964 cm^{-1}. The blue-shifts of $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ indicate the disordering of the conformation of the DPPC acyl chains due to the weakened lateral hydrophobic interaction and the reduction of the trans population, whereas the position and the shape of the newly appeared $\nu_{as}(CH_3)$ indicates that terminal methyl groups are ordered similarly to that in the solid-like gel phase. The shoulder of $\nu_{as}(CH_2)$ at 2937 cm^{-1} is assigned to the Fermi-resonance between $\nu_s(CH_3)$ and the overtone of the asymmetric deformation $\nu_{as}(CH_3)$. Addition of cholesterol also caused the broadening of the $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ peaks. The broadening at C_{chol} = 10 % will be due to the heterogeneous distribution of cholesterol in the LC domains between the edge and the center. At the higher cholesterol concentration (C_{chol} > 25 %), at which the mobility of DPPC molecule increases, the fluidity is a major contribution to the peak broadening. On the basis of above results, I suggest that the transformation from the co-existing LC and LE phases on the pure DPPC monolayer to the homogenous LO phase on DPPC/cholesterol proceeds through two stages: initial drastic changes in the surface morphology and the conformation of the DPPC acyl chains below 10 % cholesterol, and the gradual homogenization of the morphology

towards the liquid ordered phase up to 35 % cholesterol.

In vesicle fusion method, the effect of the electrostatic attractive force between vesicles and the substrate surface on Ca^{2+} free supported lipid bilayer formation has been investigated by using atomic force microscopy and fluorescence microscopy.

In a typical of fluorescence images of giant vesicles (GUVs), GUVs containing neutral lipids were aggregated each other, while GUVs including negative-charged lipids were individually segregated. The aggregation is due to the non-charge inter-vesicle attractive interaction. The segregation is from the inter-vesicle charge repulsion induced by the negative-charged lipids, which interrupts the vesicle - vesicle aggregation. The positive-charged surface was prepared by the monolayer deposition of 3-aminoprophyldimethylethoxysilane (APS) on the SiO_2 surface. The observed value of water contact angle (WCA) for the bare SiO_2 surface was ~ 10^0 and it changed to ~51^0 after the surface modification by APS.

When the negative-charged GUVs were incubated without Ca^{2+}, extremely low surface coverage of lipid bilayer was observed on the bare SiO_2 surface. In the presence of Ca^{2+}, the high surface coverage of lipid bilayer was observed when Ca^{2+} was added before incubation. The remarkable difference in the coverage of the lipid bilayer on the SiO_2 surface is explained in terms of the adsorption of GUVs. As to the formation of the lipid bilayer by vesicle fusion method, the adsorption is an initial step. In the absence of Ca^{2+}, the electrostatic repulsion between the surface and the vesicles was induced during incubation, which results in very low surface coverage of the lipid bilayer. In case of the positive-charged surface modified by the APS monolayer deposition, the high surface coverage of the lipid bilayer was obtained through the electrostatic attractive force between vesicles and the surface. The strong electrostatic attractive force between vesicles and the surface enhances the stable adsorption of the negative-charged GUVs, which promotes the lipid bilayer formation. The rupture of GUVs is induced by the interaction between GUVs and the SiO_2 surface, almost without influence of the supported GUV-GUV interactions. And, in AFM observation, the thickness of the water layer between SLB and the surface decreases by the surface APS

modifications.

論文の審査結果の要旨

Kim Yong-Hoon君の博士論文はサポーテッドメンブレンバイオセンサーの製作に必須とも言える、固体表面上への平面脂質膜の形成に関する問題を扱っており、その中で特に重要な、丈夫な脂質二重膜を製作するために添加したコレステロールの添加効果についてと、二重膜をベシクルフュージョンで形成する際、Ca^{2+}イオンを添加しないで形成する方法を開発する目的で、脂質分子に電荷を持たせた場合の影響についての二つの問題を系統的に調べている。前者については、コレステロールを添加した場合の膜の構造変化を赤外吸収スペクトルとAFMの併用により分子のレベルで詳細に説明することに成功しており、後者については、水溶液のイオン強度を変え脂質二重膜の形成速度とイオン強度の関係を水中AFM観察により調べ、基板表面と脂質分子の静電相互作用により二重膜が形成されることを、証明することに成功している。全体で五章から構成されている。

第一章の序論ではサポーテッドメンブレンバイオセンサーの説明、サポーテッドメンブレンの形成法、固液界面にできる電荷二重層の性質など基礎知識について記述している。第二章では本研究で用いる実験系についてまとめて説明がなされている。

第三章では、本研究の主題の一つである、コレステロール添加の効果について記述している。 DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) にコレステロールを0%から35% の範囲で濃度を変えて添加し、Langmuir-Blodgett 法により脂質一重膜をマイカやSiO_2表面に形成し、膜の表面構造をAFMで調べた。また、同時に埋め込み金属層基板赤外反射吸収スペクトルを測定し、 AFMの結果と比較して構造の解析を進めた。コレステロールを数パーセント添加するだけで、顕著な相分離が観測され、30%位添加すると、均一な相となる。AFM観察によると、高さの異なる三つの相の存在が示唆されるが、赤外スペクトルから、これが、最初からある、liquid expanded phase, とliquid condensed phase に加え、あらたに、liquid ordered phase と呼ばれる新しい相が形成されることによるとして、全体の相転移の様子を統一的に説明することに成功している。

第四章では、本研究のもう一つの主題である、ベシクルフュージョンにおける静電相互作用の影響について調べた結果が記述されている。 基板表面をアミノプロピルジメチルエトキシシランにより表面がアミノ基で終端されるように化学修飾し、一方中性である脂質分子DPPCにマイナスの電荷を持つPOPS (Palmitoyl-2-oleoyl-sn-3-[phosphor-L-serin]) を添加し、ベシクルを含むKCl水溶液のイオン強度を変化させてベシクルが二重膜に展開する効率のイオン強度依存性を、蛍光顕微鏡と水中AFMとにより詳細に調べた。このように電荷を持たせると、Ca^{2+}イオンを添加しなくても二重膜に展開することから、静電相互作用によりフュージョンが起こっている事が示唆される。一方この展開速度が、イオン強度の増大とともに減少することから、電荷二重層の厚みがイオン強度の増大とともにどのように変化するかを計算し、展開速度の減少が電荷二重層の厚みの減少と相関があることを示した。これにより、二重膜への展開が静電相互作用によることを明解に証明す

ることに成功している。

以上いずれも独創性の高いアイデアと粘り強い実験により世界に先駆けて重要な技術の達成と反応機構の分子レベルでの解明に成功しており、非常に高いレベルの研究成果である。公開発表もきちんとしており、審査委員会は出願論文が博士（理学）の授与に値すると全員一致で判断した。