1P201

浸透圧を加えた条件下におけるガングリオシド GM1 / コレステロール混合系の X 線回折

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ガングリオシドは主として細胞外表面に存在するスフィンゴ脂質の一種であり、コレステロールと共に脂質ドメインを形成することによって、細胞同士の接着や認識、情報伝達に関与すると共に、様々な細菌、ウイルス等のレセプターとしても機能することが示唆されている。これらの機能を理解するためには、ガングリオシド/コレステロール混合系の構造や糖鎖-糖鎖間の相互作用の詳細な知見が必要であり、我々は、その構造や相互作用を明らかにすることを目指して研究している。

巨大な親水性頭部を持つガングリオシドは単独では二重膜を形成できず、水中でミセルを形成するが、ガングリオシド GM1 / コレステロール混合系においては、モル比で GM1 : コレステロール = 1 : 1 以上のコレステロールが存在するとベシクルを形成し、モル比 1 : 1 までは、ベシクルにコレステロールが収められることが、既に早川と平井によって報告されている (1)。本研究では、GM1 : コレステロール = 1 : 1 の混合系ベシクルに浸透圧を加え、放射光 X 線を用いた X 線回折によって詳細な膜構造を得ることを試みた。

浸透圧を加えた結果、ガングリオシド GM1 / コレステロール混合系膜は、ラメラ状にスタックした状態となり、さらに、浸透圧の上昇にしたがい、室温付近で、ラメラ間隔は 89 Å から 80 Å まで変化した。また、7 次までのシャープな反射ピークが得られたので、電子密度分布の再構成を行った。その結果、浸透圧を高くすると二重膜の中心から 40 Å 付近に糖鎖同士が重なったと解釈されるピークがみられた。これらの結果をもとに、GM1 / コレステロール混合系の構造から糖鎖-糖鎖間の相互作用を考察したい。また、80 °C でも測定を行ったので、その結果との比較も予定している。

(1) T. Hayakawa and M. Hirai (2003) J. Appl. Cryst. 36, 489-493

S. Abe, H. Takahashi : Osmotic stress-X-ray diffraction study of ganglioside GM1/cholesterol mixture

1P203

電氣的に中性の脂質膜界面と相互作用するペプチドが MO 膜のキュービック相の構造安定性に及ぼす効果の pH 依存性

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Electrostatic interactions between negatively-charged lipids and positively-charged domain of proteins are known to play important roles in structure and functions of biomembranes. However, interaction of proteins with lipid membranes of electrically neutral lipids are not well understood. Recently, we have designed a new peptide (WLFLKKKK) with a segment (WLFL) which can be partitioned into the electrically neutral membrane interface. This peptide greatly changed the intermembrane distance of POPC-MLV and induced various shapes of PC-GUV (1). The stability of cubic phase of neutral monoolein (MO) membrane also changed with the peptide concentration. As the peptide concentration increased, the most stable phase changed in order of: Q224 → Q229 → L α (2).

In this study, we designed a peptide, Ac-WLFLLEE (peptide-3), which would induce phase transitions in the MO membrane during pH change. As peptide-3 concentration increased, a phase transition from Q224 to Q229 occurred at R=0.011 (R: molar ratio of peptide to MO) at pH 7.0. As NaCl concentration increased in MO/peptide-3 (R=0.020) membrane, a Q229 to Q224 phase transition occurred at 25 mM NaCl. On the other hand, as pH decreased, a Q229 to Q224 phase transition occurred in the MO/peptide-3 membrane (R=0.020) at pH 6.4, indicating that at low pH (pH ≤ 6.4) the Q224 phase was stable. The protonation of peptides-3 at low pH is a main reason of this pH-induced phase transition in the MO membrane.

(1) Langmuir, 18, 9638, 2002 ; (2) Langmuir, 19, 4745, 2003

S.M.Masum, T.S.Awad, M.Yamazaki : PH Dependence of Effect of Peptides Interacting with Electrically Neutral Membrane Interface on Stability of Cubic Phases of MO Membrane

1P202

MO/DOPA 一混合膜のキュービック相の安定性に対する正電荷を持つペプチドの効果

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To elucidate the stability and phase transition of cubic phases of biomembranes is indispensable from biological and physicochemical aspects. Recently, we found that as the electrostatic interactions in the membrane interface increased by either the increase in surface charge density of the membrane or by the decrease in salt concentration, the most stable phase of these lipid membranes changes as follows; Q224 → Q229 → L α [1-3]. In this report, we investigated the effect of short peptides containing positive charges on monoolein (MO) membranes containing negatively charged DOPA (i.e. DOPA/MO membranes) in cubic phases.

At first, we investigated peptide-3K (LLKKK) on 10%DOPA/90%MO membrane in the Q229 phase. At 3.4 mM peptide-3K, a Q229 to Q230 phase transition occurred, and at ≥ 3.4 mM peptide-3K, the membrane was in the Q230 phase. We also investigated the effect of peptide-3K on MLV of 25%-DOPA/75%-MO membrane in L α phase. In the absence of peptide, the spacing of MLV was very large (11.3 nm), but at ≥ 8 mM peptide-3K, it greatly decreased to 5.2 nm, which was constant irrespective of the peptide concentration. The effect of peptide-3K is different from that of osmotic stress on 10%DOPA/90%MO membrane. With an increase in PEG-6K concentration, the most stable phase changed as follows; Q229 (P surface) → Q224 (D) → Q230 (G). Based on these results, we discuss the mechanism of the effects of peptide-3K on the stability of cubic phases.

[1] BBA, 1461, 96, 1999, [2] Biophys. J. 81, 983, 2001 [3] Langmuir, 19, 4745, 2003

S.M.Masum, T.S.Awad, S.J.Li, M.Yamazaki : Effect of Positively-Charged Short Peptide on Stability of Cubic Phases of Monoolein-Dioleoylphosphatidic Acid Mixtures

1P204

Ca²⁺ が誘起する MO/DOPG-混合膜の一枚膜ベシクル (LUV) からキュービック相への構造転移

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Recently, we have systematically investigated the effects of electrostatic interactions due to surface charges on the structure and stability of cubic-phase membranes, and have found that electrostatic interactions due to surface charges induce transitions between cubic phase and L α phase, and between different IPMS cubic phases [1-3].

In this report, we have investigated possibility of ion-induced transformation of LUVs of lipid membrane into cubic phase membranes, and succeeded it for the first time. We consider the use of a negatively charged-lipid, dioleoylphosphatidylglycerol (DOPG) to give negative charges on the MO membrane instead of DOPA. At first, we investigated effect of DOPG on structures and phase stability of the cubic phase of the MO membrane by SAXS. We have found a similar result as for the effect of DOPA on it [2]; as increasing the DOPG concentration, most stable phase changed as follows, Q224 → Q229 → L α. As Ca²⁺ concentration in the bulk phase increases, the phase of the 25%DOPG/75%MO membrane changed from L α to Q229 phase, and then to Q224 phase at low concentrations, although Ca²⁺ did not induce these phase transitions in DOPA/MO membranes. Then, we investigated effect of Ca²⁺ on LUVs of DOPG/MO membranes. Low concentrations of Ca²⁺ transformed these LUVs into cubic phases (Q224 or Q229 phase depending on Ca²⁺ concentration) rapidly. In this conference, we will report a detailed data on this transformation. [1] BBA, 1461, 96, 1999, [2] Biophys. J. 81, 983, 2001 [3] Langmuir, 19, 4745, 2003

T.S.Awad, S.M.Awad, R.Sano, M.Yamazaki : Formation of Cubic Phases from Large Unilamellar Vesicles of Monoolein/ Dioleoylphosphatidylglycerol Membranes Induced by Ca²⁺