Structural Characterization of Phosphatidylcholine-Diacylglycerol System by Neutron Scattering and X-ray Diffraction

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Received: date / Revised version: date

Abstract Diacylglycerol (DAG) is recognized one of the most important lipids for biological functions of cell membranes. In order to understand the functions of DAG, it is indispensable to study the effect of DAG on phosphatidylcholine (PC) which is a main lipid component of biomembranes. Here we report neutron scattering data of sonicated PC/DAG vesicles and X-ray diffraction data of oriented PC/DAG multilamellar systems. These data imply that addition of DAG induces the change in the tilt angle of lipid molecules and that, as a result, the increase of the membrane thickness is induced.

PACS number: 87.14.Cc, 87.16.Dg

1 Introduction

Including the activation of protein kinase C [1], diacyl-glycerol (DAG) plays an important role in various biological processes taken place on cell membranes [2]. To understand the biological functions of DAG at molecular level, it is important to study on the physical properties of DAG and its effects on membrane lipids. A large number of biophysical studies, therefore, has been conducted for the binary systems composed of DAG and phosphatidylcholine (PC) which is a main lipid component of biomembranes [3–5].

Recent X-ray diffraction measurements on PC/DAG multilamellar vesicles have shown that addition of DAG to PC bilayers causes the increase of the lamellar spacings [6]. In multilamellar systems, the lamellar spacing is the sum of the thickness of lipid bilayer and that of interlamellar water layers. Hence, from the change of lamellar spacing, it is impossible to determine which layer (lipid or water layer) is affected by addition of DAG. In the

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previous study [6], to make clear this point, the analysis of X-ray intensity data has been performed by assuming a model of electron density distribution of the system. As a result, it has been suggested that addition of DAG thickens the membrane bilayer.

In this study, using sonicated vesicle and oriented multilamellar samples, we reexamined the PC/DAG systems by means of neutron scattering and X-ray diffraction, paying attention to the effect of DAG on the PC bilayer structure.

2 Materials and Methods

Powders of L- α -dipalmitoylphosphatidylcholine (DPPC) and L- α -dipalmitoylglycerol (DPG) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA) and Sigma (St. Louis, Mo, USA), respectively. The purity of these lipids was checked by thin layer chromatography and then they were used without further purification.

Vesicle samples were prepared as follows: DPPC and DPG were dissolved in chloroform and mixed to achieve the molar fraction of DPPC: DPG = 1:1. Pure DPPC chloroform solution was also prepared in. The chloroform solution containing DPPC or DPPC/DPG was taken into a small test tube. The solvent was evaporated under a stream of dry nitrogen. Residual solvents were removed by storing for 16 h in vacuum. The dried lipid samples were hydrated in 100 % D_2O by shaking on a Vortex mixer. To get unilamellar vesicles, the lipid dispersion was subjected to further sonication using a Branson-type sonicator (10 min for DPPC, 30 min for DPPC/DPG). The final total lipid concentration was 10 mg/ml.

Oriented multilamellar samples were as follows: DPPC and DPG were dissolved in the mixture of chloroformmenthol (1:1v/v) and mixed to achieve the molar fraction of DPPC: DPG = 1:1. Pure DPPC solution was also prepared in. The lipid concentration was about 100 mg/ml. A small amount of the lipid-chloroform-methanol

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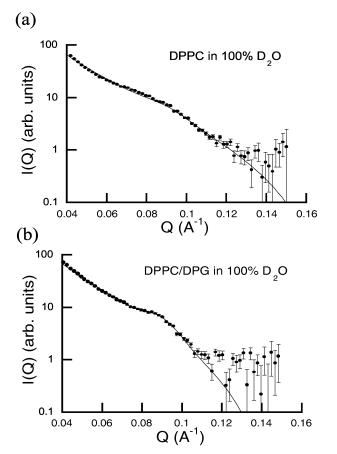


Fig. 1 Small angle neutron scattering spectra from the DPPC vesicles (a) and the DPPC/DPG vesicles (b) in 100 % D₂O recorded at 25 °C.

solution (about 200 μ l) was deposited on a thin polyethylene film (thickness 10 μ m). The solvent was evaporated for 2-3 h and then the film was given convex curvature by winding around a glass rod with diameter of 3 mm. Further, residual solvents were removed by storing for more than 20 h in vacuum. The specimen was mounted in a controlled humidity and temperature chamber on a point focused X-ray camera. The humidity was changed using various saturated salt solutions.

X-ray diffraction measurements were carried out using a Ni-filtered CuK_{α} radiation source (RU200EBH, Rigaku, Tokyo, Japan) and a two-dimensional area detector (Imaging plate, Fuji Photo Film, Tokyo, Japan). The detail of them has been reported elsewhere [7].

Small angle neutron scattering measurements were performed with the SANS-U spectrometer at JRR-3M reactor of the Japan Atomic Energy Research Institute (Tokai, Japan)at neutron wavelength of 7 Å and a sample-to-detector distance of 2 m. The detail of the spectrometer has been reported elsewhere [8].

All measurements were done at 25 °C. At this temperature, the both DPPC and DPPC/DPG systems are in the gel phase [6].

3 Results and Discussion

Neutron scattering was used to estimate the membrane thickness of DPPC and DPPC/DPG sonicated vesicle systems. For various systems, the thickness of lipid bilayers has been determined with an accuracy about 1 Å, by analyzing the neutron scattering data [9–12].

To estimate the membrane thickness, we assumed that the neutron scattering length density distribution of the lipid bilayer membrane in D_2O is homogeneous. In other words, we neglected the detailed internal structure. Thereby, the scattering from the lipid bilayer in D₂O can be regarded as that from a single slit. The assumption is based upon following two grounds: (1) Small angle scattering data do not contain basically detailed internal structural information. (2) The difference of the relative contrast between the polar headgroup and hydrocarbon regions $(1.7 \times 10^{-14} \text{ cm/Å}^3)$ is small as compared with the large contrast between the hydrocarbon region and D_2O (6.4 x 10^{-14} cm/Å³). These values are calculated from the absolute scattering length densities of phospholipid components which have been estimated by Knott and Schoenborn [13]. Incidentally, the above second ground is not satisfied for X-ray. It is, therefore, hard to use the above assumption in the analysis of Xray scattering data from the lipid bilayers.

Figure 1 (a) and (b) show the neutron scattering intensity data from DPPC and DPPC/DPG vesicles subjected to sonication as a function of the magnitude of the scattering vector (Q), respectively. Weak diffraction peaks appear around $Q = 0.09 \text{ Å}^{-1}$. For pure DPPC, the diffraction peak is very weak. For DPPC/DPG, the diffraction peak is clearly observed at $Q = 0.088 \text{ Å}^{-1}$. At the same Q positions, lamellar diffraction peaks were observed for the multilamellar vesicle samples prepared without sonication (data not shown). From this fact, we judged that, owing to insufficient sonication, our samples are mainly unilamellar vesicles but containing a small number of multilamellar vesicles. In addition, from the analysis of neutron data of multilamellar vesicle samples, we found that the shape of lamellar diffraction peak is well expressed by one Gaussian function.

Then we analyzed the neutron scattering data by least squares fitting using the scattering function of a single slit and a Gaussian function. As a result, the membrane thicknesses of DPPC and DPPC/DPG vesicle systems were estimated to be 39.4 ± 2.1 Åand 43.3 ± 2.4 Å, respectively. This result indicates that addition of DAG increases the membrane thickness of the lipid bilayers.

Next, we performed X-ray diffraction measurements for the oriented multilamellar samples in order to investigate the effect of DAG on PC membrane thickness. Typical two-dimensional X-ray diffraction patterns are presented in Fig.2. In the small angle regions, four to nine lamellar diffraction peaks were observed along to the meridian line. As increasing relative humidity, observed values of the lamellar spacing increases for the

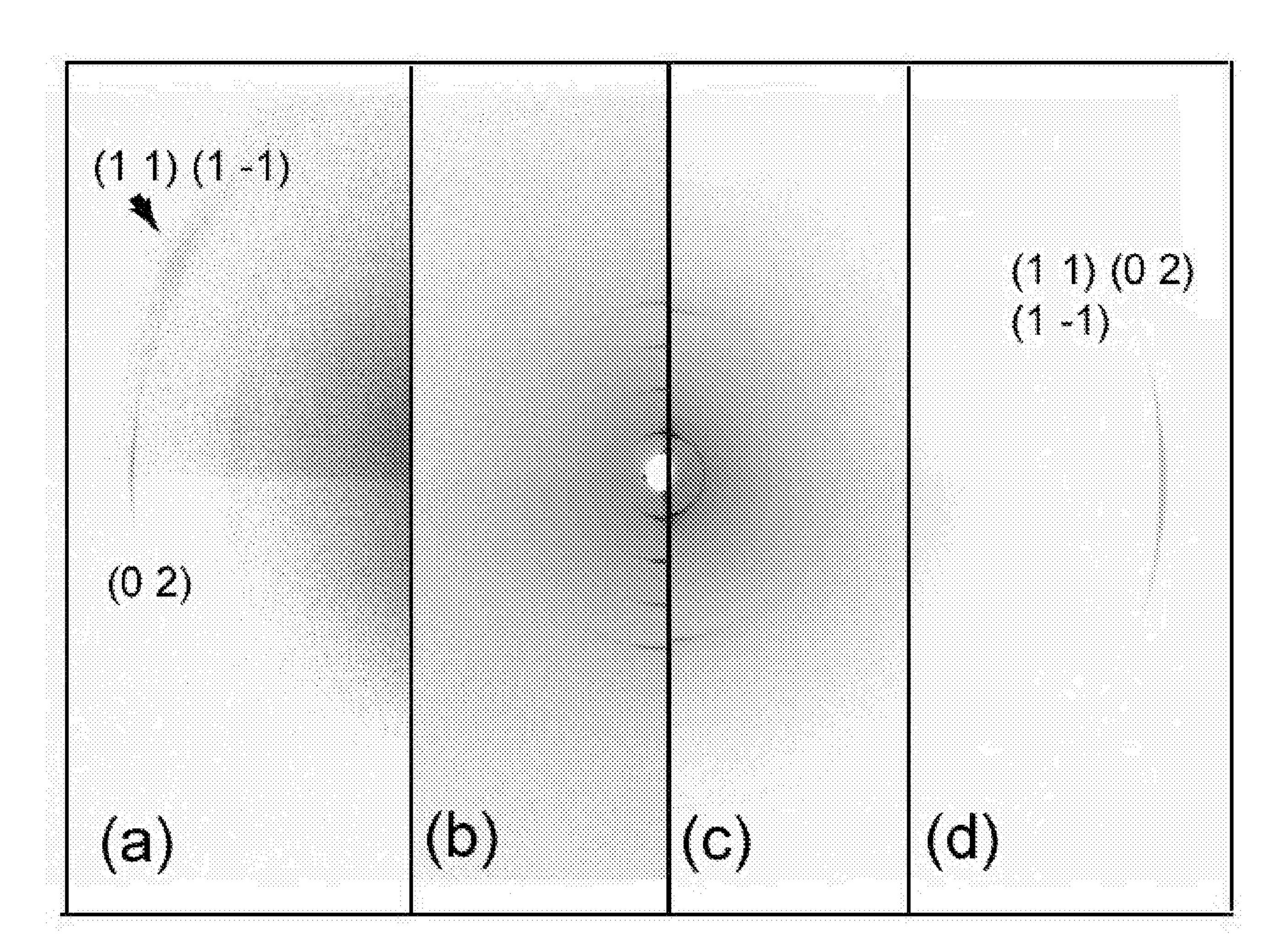


Fig. 2 X-ray diffraction patterns of the oriented multilamellar DPPC (a, b) and DPPC/DPG (c,d) samples recorded at 100 % relative humidity. Gray level in the patterns is logarithmic with intensity. The patterns are divided into wide angle (a,d) and small angle (b,c) regions and the gray level is changed for each column in order to visualize the diffraction patterns clearly. The directions of meridian and equatorial lines coincide in each column.

both samples. In the relative humidity range from 100 % to about 10 %, the lamellar spacings were changed from 60.7 Å to 56.5 Å for DPPC systems and form 66.2 Å to 56.4 Å for DPPC/DPG systems.

Diffraction arcs reflected from an ordered lattice of hydrocarbon chain packing were observed in the wide angle regions. For DPPC samples, the diffraction arcs were observed not only on equatorial but also off equatorial regions. Referring to several previous works [14–16], we indexed these diffraction arcs as shown in Fig. 2 (a). The positions of the diffraction arcs observed off equator approach to the equator, as decreasing relative humidity. This result indicates that the hydrocarbon chains are tilted to the bilayer normal and that the tilt angle decreases according to lowering relative humidity. This agrees with the previous work [15]. From the diffraction pattern, the tilt angle of the chain was estimated to be 36.1 ° at 100 % relative humidity.

On the other hand, for DPPC/DPG sample, diffraction arcs appeared only on equatorial region and the positions were independent on humidity. This result implies that the hydrocarbon chains of DPPC/DPG bilayers are parallel to the bilayer normal in the humidity range investigated in this study. If the length of one CH₂-CH₂ distance in all-trans conformation is 1.25 Å, the change of tilt angle from 36.1 ° to 0 ° induces to increasing the hydrocarbon region thickness of about 7.7 Å. This value is good agreement with the difference of membrane thickness between DPPC and DPPC/DPG systems estimated above by neutron scattering. We conclude, therefore, that the change of tilt angle of the hydrocarbon chains can be assumed as the origin of increasing membrane thickness by addition of DAG.

4 Conclusions

From the analysis of neutron scattering data from sonicated samples, we estimated the membrane thickness of DPPC and DPPC/DPG samples. Using oriented multilamellar sample, we directly determined the chain tilt angle from two-dimensional X-ray diffraction patterns. Based upon these results, we conclude that addition of DPG to DPPC bilayer membrane induce to change the tilt angle of hydrocarbon chains, and that as a result, the presence of DPG in DPPC membrane induce to increase the membrane thickness. Furthermore detailed studies are required to clarify the relation between the effect of DAG revealed in this study and DAG's biological functions.

Acknowledgements. We would like to thank to Mr. Y. Wakui in the machine shop of Department of Applied Physics, Nagaya University for making the controlled humidity and temperature chamber used in the present X-ray diffraction study. This work is supported in part by a grant from Gunma University Foundation for Science and Technology

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