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Study on Monolayer of Vitamin E and Phosphatidylcholines

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Abstract The surface pressure-area isotherms of mixed monolayers at the air/water interface have been determined for systems: d-α-, d-β-, d-γ-, and d-δ-tocopherol (TOC) with dipalmitoylphosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC); d-α-TOC and cholesterol (CH) with dilinoleoylphosphatidylcholine (DLPC); and d-α-TOC with DPPC/DLPC (1:1/mol:mol). The effect of the chromanol methyl groups of vitamin E(VE) on its physicochemical behaviors in these monolayers had been discussed. It was observed that (1) TOC caused the DPPC monolayer to expand in the order of $\alpha > \beta \approx \gamma > \delta$, and the DOPC monolayer to condense in reverse order; (2) The collapse pressure of monolayer for DOPC and DLPC was not changed by TOC, while that of DPPC decreased; (3) Addition of d-α-TOC into the monolayer of equimolar mixture of DPPC and DLPC did not change the collapse pressure but produced an expanding effect; (4) Contrary to the generally accepted view, the physicochemical function of VE in mixed monolayer of phospholipid was obviously different from that of CH.

Keywords: Monolayer, Vitamin E, Phosphatidylcholine, Tocopherol, Cholesterol, Surface pressure

1 Introduction

It is generally belived that vitamin E(VE) i.e. TOCs (Fig.1) are indispensable components of biological membranes[1,2], and can protect the membranes from damages induced by lipid peroxidation through their chemical antioxidation[3]. On the other hand, it has been suggested that the TOCs can physically stabilize a biomembrane through the interactions with membrance lipids[4]. One of the model membranes used for studies was the monolayer. Some investigations on mixed monolayers of α-TOC with lipids have been reported[5,6]. In this work, the mixed monolayers of d-α-, d-β-, d-γ-, and d-δ-TOC with DPPC and DOPC were studied in order to examine the effect of the chromanol methyls on physicochemical role of TOC, and to clarify the difference of the interactions of TOC with

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saturated and unsaturated lipids in the monolayers. Considering the fact that biomembranes usually contain both saturated and unsaturated phosphatidylcholine (PC), the mixed monolayers of d-α-TOC with equimolar mixture of DPPC and DLPC were also studied. Since it was disputed if the physical roles of VE and CH in lipid membranes were similar to each other as previously proposed[7,8], the roles of TOC and CH in DPPC and DLPC monolayers have been compared.

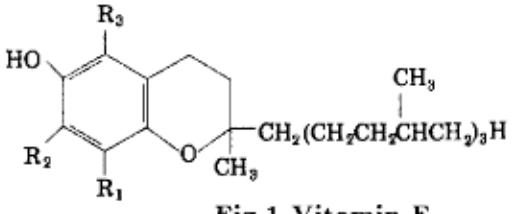

Fig.1 Vitamin E

α-Tocopherol: $R_1=R_2=R_3=CH_3$
β-Tocopherol: $R_1=R_3=CH_3$, $R_2=H$
γ-Tocopherol: $R_1=R_2=CH_3$, $R_3=H$
δ-Tocopherol: $R_1=CH_3$, $R_2=R_3=H$

2 Experimental

2.1 Materials

d-α-, d-β-, d-γ-, and d-δ-TOC (>99%) were kindly supplied by Eisai CO. (Tokyo). DPPC was isolated and purified from egg yolk by the previous method[9-11]. DOPC and DLPC were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). CH was purchased from Wako Pure Chemical Industries Ltd. (Osaka) and used after recrystallizing from ethanol.

2.2 Method

The surface pressure was measured by the Wilhelmy method with a digital electrobalance. Both surface pressure measurement and film compression were controlled by a personal computer. Twice distilled chloroform was used as a solvent for spread. Monolayers were prepared by spreading the solutions on the subphase by means of a syringe. A time interval of 10 minutes was allowed for complete evaporation of the solvent before the film was compressed. The rate of compression was $1\text{Å}^2\cdot\text{molecule}^{-1}$ per 10 seconds. The subphase water was deionized and twice distilled. All the experiments were repeated two or three times at 21 ± 0.1°C. Reproducibilities in the surface pressure-area isotherms were within $\pm0.5\text{Å}^2\cdot\text{molecule}^{-1}$ for surface area, and $\pm1.0\text{mN}\cdot\text{m}^{-1}$ for collapse pressure.

3 Results and Discussion

3.1 Mixed Monolayers of TOCs with DPPC

The isotherms for mixed monolayers of d-α-TOC/DPPC are given in Fig.2. The isotherms for d-β, d-γ-, and d-δ-TOC/DPPC mixed monolayers are similar to Fig.2. It was found that addition of TOC into DPPC monolayer decreased the collapse pressure, and caused to form an inflexion on isotherms for the monolayers with higher molar ratio of TOC (>40%). It is of interest to note that the part of isotherm above the inflexion does not have the character of a liquid condensed state. This showed that the inflexion does not reflect the phase transition from liquid expanded state to liquid condensed state.

More likely, it means the formation of complexes between TOCs and DPPC as suggested by Erin and co-workers[12,13]. The complex between TOCs and DPPC can be produced by forming hydrogen bond between the chromanol hydroxyl with the carboxyl of DPPC or by interaction between the fatty acyl chains in DPPC with the chromanol methyls in TOC. In either case, it is necessary that the TOC molecules are able to leave water surface easily. Cushley, *et al.*, according to their bilayer study, have indicated that α-TOC, because of its extreme hydrophobic nature, can not penetrate so far as into the region of the lecithin head group[14]. A fluorescence investigation upon α-TOC in phospholipid vesicles also indicated that although α-TOC might have its chromanol group relatively close to the polar part of the bilayer, it could not be exposed sufficiently[15]. Our monolayer study supported these propositions. However, decrease in the collapse pressure of TOC/DPPC monolayers with increasing molar ratio of the TOC showed that some of the TOC molecules were in contact with the water surface when the monolayer was compressed. Because of the intercalation of TOC molecules, the interaction between the polar head groups of DPPC, which is possibly of the hydrogen bond type, may be broken. This accelerates the collapse of the monolayer and hence leads to the decrease in collapse pressure. From Fig.2 it was found that the collapse pressure of the monolayer with 80 mol% of TOC was the same as the pure TOC monolayer. This showed that TOC was not miscible completely with DPPC, that was in agreement with the bilayer study of Lai *et al.*[16]. The excess TOC would separate from the mixed monolayer and form multilayer on the water, namely the phase separation would occur when the surface pressure is higher, so that the isotherm above the inflexion had a linear character and extended infinitely to zero area as the pure TOC monolayer did.

From the isotherms of TOC/DPPC monolayers the deviations of the mean area per molecule from additivity rule[17] were calculated. The results showed that TOCs caused the expansion of DPPC monolayer in the order of $\alpha > \beta \approx \gamma > \delta$, when the surface pressure was higher than $5 mN \cdot m^{-1}$. The TOC molecule has a longer hydrocarbon side chain with 3 methyls, hence, has a greater degree of freedom of intramolecular rotation. Due to the thermal motion and unmatched structure, TOC and DPPC molecules mixed together in a monolayer require a larger space per molecule than they would if they were in a pure component monolayer. As the surface pressure increases, DPPC molecules tend to come into an orderly arrangement, and the intermolecular spaces become smaller, so the space requirement of the TOC molecules is no longer satisfied. In this case, the TOC molecules would disrupt the orderliness of the arrangement and the orientation of DPPC molecules. Thereby, the monolayer expands. The expanding effect is strengthened in proportion to the number of the chromanol methyls, what can be contributed to the increase in the degree of freedom of intramolecular rotation with the increasing number of the methyls. It is notable that order of the expanding effect mentioned above is in agreement with their bioactivity order[18].

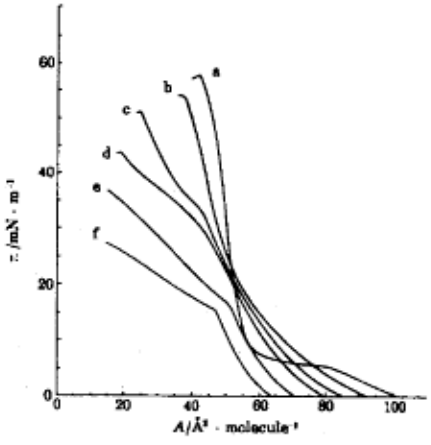

Fig.2 Surface pressure-area isotherms of mixed monolayers for d-α-TOC with DPPC at 21°C and different molar ratios of TOC (mol%)
(a) 0, (b) 20, (c) 40, (d) 60, (e) 80, (f) 100

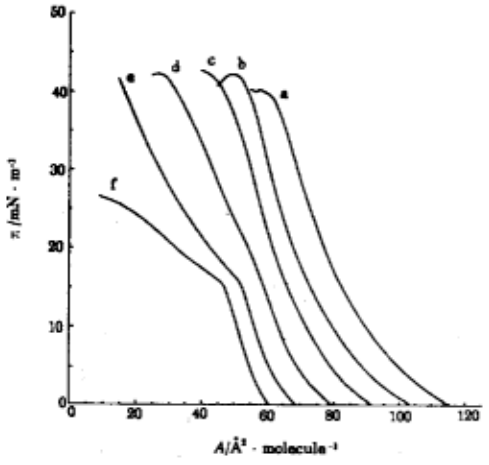

Fig.3 Surface pressure-area isotherms of mixed monolayers for d-α-TOC with DOPC at 21°C and different molar ratios of TOC (mol%)
(a) 0, (b) 20, (c) 40, (d) 60, (e) 80, (f) 100

3.2 Mixed Monolayers for TOCs with DOPC and DLPC

Fig.3 gives the surface pressure-area isotherms of mixed monolayers of d-α-TOC with DOPC. Isotherms of d-α-TOC/DLPC monolayers are similar to Fig.3. It was found that the collapse pressure of mixed monolayers of TOC with unsaturated PC was essentially independent of the molar ratio of TOC. This was clearly different from TOC/DPPC mixed monolayers, and implied that addition of TOC into unsaturated PC monolayer did not affect the interactions between the polar region of monolayer and water molecules. The monolayer collapse pressure of saturated PC was lowered by TOC, while that of unsaturated PC was not, and the main difference between the two kinds of PC studied lay in whether their hydrocarbon chains had double bonds. This suggests that the double bond evidently affects the character of the TOC/PC mixed monolayer. According of Erin, *et al.*, a stronger interaction between chromanol methyls in TOC and the double bonds of fatty acyl groups in unsaturated PC may occur[12,13]. Owing to the interaction, TOC molecules are able to leave water surface hence the monolayer collapse pressure depends only on the interactions between the polar head groups of the unsaturated PC and water molecules.

The isotherms of mixed monolayers of d-β-, d-γ-, and d-δ-TOC/DOPC are similar to Fig.3. From the isotherms the deviations of the mean area per molecule in the monolayers from the additivity rule were calculated. The results showed that TOCs made the DOPC monolayer condensed in the order of $\alpha < \beta \approx \gamma < \delta$. The condensing effect may be due

to the fact that in unsaturated PC, the hydrocarbon chain is bent and has a lower degree of orientation, thereby there are greater intermolecular cavities which can be occupied by the TOC molecules, and make the mean area per molecule decreased. The decrease in the number of chromanol methyls in a TOC molecule leads to the decrease in volume requiried by the rotation of the molecule, which in turn can cause a stronger condensing effect. Therefore, the condensing effect order mentioned above was observed.

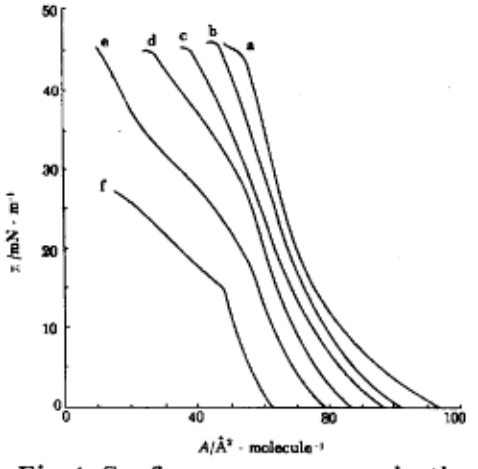

Fig.4 Surface pressure-area isotherms of mixed monolayers for d-α-TOC with equimolar mixture of DPPC and DLPC at 21° and different molar ratios of TOC (mol%)
(a) 0, (b) 20, (c) 40, (d) 60, (e) 80, (f) 100

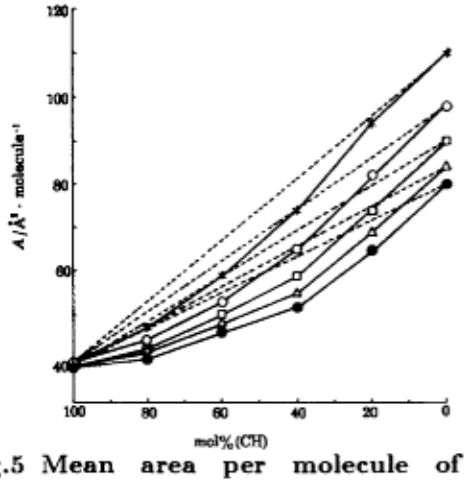

Fig.5 Mean area per molecule of CH/DLPC mixed monolayers at 21°C and different surface pressure: 5mN·m^{-1}(×), 10mN·m^{-1}(○), 15mN·m^{-1}(□), 20mN·m^{-1}(△), and 25mN·m^{-1} (●)
The solid and dashed lines represent respectively estimated values from experimental results and those calculated by additivity rule

3.3 Role of d-α-TOC in Mixed Monolayers Containing both Saturated and Unsaturated PC

Fig.4 shows the surface pressure-area isotherms of the mixed monolayers of d-α-TOC with equimolar mixture of DPPC and DLPC. It can be seen that the collapse pressure of the monolayers does not vary with the molar ratio of d-α-TOC within the range of 0~80 percent. This phenomenon resembles that occurring in the d-α-TOC/DLPC monolayers. However, the calculated results showed that the mean area per molecule in d-α-TOC/DPPC-DLPC mixed monolayers positively deviated from additivity rule, which was clearly similar to the behaviour of d-α-TOC/DPPC monolayers. It may be supposed that d-α-TOC molecules interact not only with DLPC but also with DPPC, they bind their head groups with double bonds of fatty acyl side chains in DLPC and at the same time expand the DPPC-DLPC mixed monolayer through the thermal motion of their tails. In

consideration of the mechanism of the lipid peroxidation[19], it can be suggested that the interaction between the head group of TOC and the double bond of fatty acyl side chains in unsaturated PC is in favor of antioxidation of TOC in a biomembrane, since the double bonds of fatty acyl side chains can be screened by the interactions, and so may be protected from attack by peroxyl radical. However, further investigation is required.

3.4 Comparison between Roles of TOC and CH in PC Monolayer

The mean areas per molecule as function of molar ratio for CH/DLPC mixed monolayers are given in Fig.5. Those for CH/DPPC are in agreement with results reported previously[20]. Comparing with the results for d-α-TOC (not shown) it may be concluded that the roles of TOC and CH in PC monolayer are different from each other. CH always causes PC monolayer to condense, whether the PC is saturated or unsaturated. While the TOC expands saturated PC monolayer, and the condensation of unsaturated PC monolayer induced by TOC is not so obvious as by CH. From the cavity-filling theory[21,22], the thermal motions of the fatty acyl chains lead to the formation of intermolecular cavities in a lipid monolayer, CH molecule can occupy these cavities, thereby the mean area per molecule is decreased. TOC, however, is quite different from CH in molecular structure, in particular, it has a longer tail with 3 methyls. Therefore, TOC molecule cannot easily enter the intermolecular cavities in PC monolayer, so that its role in the monolayer is clearly different from CH, which is in contrast to the generally accepted view.

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维生素 E 与磷脂酰胆碱的单分子膜研究

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摘要 测定了下列气水界面单分子膜的表面压 - 平均分子面积等温线：(1) d-α-, d-β-, d-γ-, 和 d-δ- 生育酚等 4 种维生素 E 与 DPPC, DOPC 及 DLPC 的混合物；(2)d-α- 生育酚等摩尔比的 DPPC 和 DLPC 的混合物；(3) 胆固醇与 DPPC, DLPC 的混合物. 讨论了维生素 E 色满环上甲基对其在 PC 单分子膜中物理化学作用的影响. 实验结果表明：(1) 维生素 E 以 d-α->d-β-≈d-γ->d-δ- 的次序引起 DPPC 单分子膜的膨胀，以相反的次序使 DOPC 单分子膜凝缩. (2) 维生素 E 可降低 DPPC 单分子膜的崩裂压，但不引起 DOPC 和 DLPC 单分子膜崩裂压的改变. (3) 向等摩尔比的 DOPC 和 DLPC 的混合单分子膜中混入生育酚不改变膜的崩裂压，但引起膜的膨胀. (4) 与一般认为生育酚和胆固醇在磷脂膜中的物理化学作用彼此类似的看法不同，它们有着明显的差别.

关键词：单分子膜，　维生素 E，　磷脂酰胆碱，　生育酚，　胆固醇，　表面压