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不同固醇与 DPPC 二元体系的液态有序相

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摘要: 应用同步辐射 X 射线衍射和差示扫描量热法研究了由不同结构的固醇(胆固醇、脱氢胆固醇、豆固醇、谷固醇、麦角固醇以及固醇核)和二棕榈酰磷脂酰胆碱(DPPC)二元体系形成的液态有序相. 研究表明, 胆固醇比植物固醇(豆固醇和谷固醇)和真菌固醇(麦角固醇)能更有效地与 DPPC 形成液态有序相($\mathbf{L}_o$); 有胆固醇或者脱氢胆固醇参与的液态有序相能够在较宽的温度范围内保持稳定, 而由植物固醇和真菌固醇参与的液态有序相对温度有较强的依赖性, 在 DPPC 主相变温度附近有明显的热致相变过程, 因此这一液态有序相应该进一步区分为 $\mathbf{L}_{o\beta}$ 和 $\mathbf{L}_{o\alpha}$ 相. 研究结果有助于阐明固醇尾链在液态有序相以及脂筏中的作用, 也有助于理解在进化过程中动物细胞膜为何选择胆固醇作为主要固醇.

关键词: 固醇; DPPC; 脂筏; X 射线衍射; 量热技术
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Liquid Ordered Phase of Binary Mixtures Containing Dipalmitoylphosphatidylcholine and Sterols

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Abstract: The effect of cholesterol, desmosterol, stigmasterol, sitosterol, ergosterol, and androsterol on the phase behavior of aqueous dispersions of dipalmitoylphosphatidylcholine (DPPC) was studied to understand the role of the side chain in the formation of ordered phases of the type observed in membrane rafts. Thermotropic changes in the structure of mixed dispersions and transition enthalpies were examined by synchrotron X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The observations indicated that cholesterol was more efficient than phytosterols (stigmasterol and sitosterol) or ergosterol in its interaction with DPPC to form the liquid ordered phase ($\mathbf{L}_o$). The $\mathbf{L}_o$ induced by cholesterol or desmosterol was stable over a wide temperature range, whereas, the liquid ordered phase containing phytosterols or ergosterol was profoundly dependent on temperature, which should be distinguished as $\mathbf{L}_{o\beta}$ and $\mathbf{L}_{o\alpha}$, representing the phases below and above the main transition temperature. The characteristics in forming ordered structures of cholesterol and other sterols imply that the evolution may have selected cholesterol as the most efficient sterol for animals to form rafts in their cell membranes.

Key Words: Sterol; DPPC; Rafts; X-ray diffraction; Calorimetry

Sterols are important for the structure and dynamic properties of cell membranes or artificial membranes[1–5]. Cholesterol is found as a key integral component of membrane microdomains (rafts)[6]. These domains are believed to be involved in numerous cellular processes, including signal transduction, protein sorting, cellular entry by toxins and viruses, and viral budding[7–11]. It is

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now well accepted that lipid rafts are composed of lipids in the liquid ordered phase ($\mathbf{L}_o$)[12]. The liquid ordered phase is characterized by tight packing, similar to that in the gel state, but also with fast lateral motion. The tight packing characteristic of lipids in the $\mathbf{L}_o$ state is considered to be the reason that rafts resist to cold nonionic detergents, such as, Triton X-100, named detergent-resistant membrane fractions (DRMs). Besides animal cells, DRMs have also been isolated from plant cells, where the dominant sterols are the cholesterol analogs, phytosterols such as stigmasterol and sitosterol[13–15].

Molecular structures of a few common sterols are presented in Fig.1. As can be seen in the figure, cholesterol has (i) a hydrophobic and planar fused tetracyclic ring structure with two-oriented methyl groups at C10 and C13, (ii) an isooctyl side chain at C17, and (iii) a hydrophilic-oriented hydroxyl group at C3. Desmosterol, phytosterol (stigmasterol and sitosterol), fungi sterol (ergosterol), and androsterol differ from cholesterol on the degree of unsaturation of the ring system or the side chain, and the size or the branch of the alkyl side chain. Compared to cholesterol, desmosterol has a double bond between C24 and C25. Stigmasterol and sitosterol have an ethyl group at C24, and stigmasterol contains an additional *trans* double bond between C22 and C23. Ergosterol has a methyl group at C24, and contains two more double bonds at C7 and C22, respectively. Androsterol has precisely the same structure and stereochemistry of the rigid planar fused ring system as the parent cholesterol molecule, but lacks the C17 alkyl side chain.

To better understand the essential structural features of sterols that are required to support mammalian cell growth, mutant strains of Chinese hamster ovary cells, defective in sterol biosynthesis, have been cultured with various sterols[16]. It has been found that sterols with minor modifications of the side chain such as desmosterol, supported long-term growth of mutant cells, but sterols with marked modifications of the side chain or the sterol nucleus itself (androsterol) did not. Although there have been numerous studies on the structure-function relationship of sterols, the secret of the structural requirements for the selection of cholesterol as the predominant sterol in animal membranes is still not completely clear[16,17].

Dipalmitoylphosphatidylcholine (DPPC) is a well-known model raft lipid because its saturated acyl chains and phosphorylcholine head group duplicate some of the important structural features of sphingomyelin (SM), and DPPC does form the $\mathbf{L}_o$ phase when mixing with adequate amounts of cholesterol[18,19].

The focus of the present study is on characterizing the effects of those sterols, as shown in Fig.1, on the thermotropic properties of the model membrane with either moderate sterol concentration (17% (molar fraction, x) sterol) or high sterol concentration (33% (x) sterol). The aim is to answer questions if the phytosterol or fungi sterols can play an identical role as cholesterol in forming liquid ordered phase in the DPPC bilayer, and whether these states have identical properties. This information may help to further understand the structure requirement of the hydrocarbon chain of cholesterol in promoting the formation of highly ordered domains such as rafts, and unravel the relationship between sterol functions and molecular structure.

1 Materials and methods

1.1 Materials

1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was purchased from Sigma Chemicals (St. Louis, MO, USA). Cholesterol and androsterol were obtained from Steraloids Inc.(Rhode Island, USA). Ergosterol, stigmasterol, sitosterol, and desmosterol were bought from MP Biomedicals, Inc. (Germany). All chemicals were used without further purification.

Mixtures of phospholipid and sterol, with designated mole ratios, were dissolved in chloroform, dried under a stream of oxygen-free dry nitrogen, and stored in vacuum overnight to remove any remaining traces of solvent. The lipid films were hydrated with excess Tris-HCl buffer (pH 7.2) consisting of 50 mmol·L^{-1} Tris-HCl, 150 mmol·L^{-1} NaCl, 0.1 mmol·L^{-1} $CaCl_2$, and dispersed by repeated vortex mixing and thermal cycling between

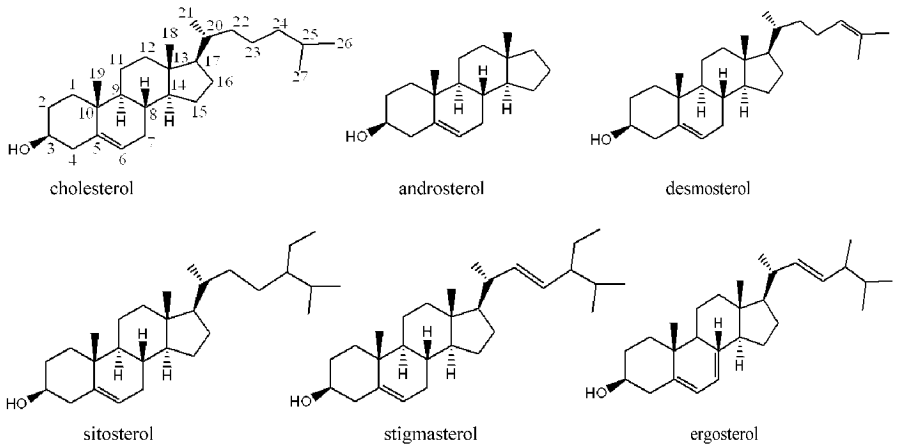

Fig.1 Molecular structures of cholesterol (Chole), androsterol (Andro), desmosterol (Desmo), sitosterol (Sito), stigmasterol (Stigma), and ergosterol (Ergo)

−20 ℃ and 65 ℃.

1.2 Synchrotron X-ray diffraction (XRD)

Synchrotron X-ray diffraction experiments were performed at Station BL40B2 of SPring-8, Japan and the Beijing Synchrotron Radiation Facility. The XRD data were recorded with an image plate detector. The detected angles (2θ) were in the range of 0.02° to 30°. A standard silver behenate sample was used for calibration of diffraction spacings. X-ray scattering intensity patterns were recorded during 30 s exposure of the sample to the synchrotron beam. A Linkam thermal stage (Linkam Scientific Instruments, UK) was used for temperature control (±0.1 ℃). Samples were heated from 30.0 to 57.5 ℃ (20.0 to 50.0 ℃ for DPPC/androsterol system) at a heating rate of 0.5 ℃·min^{-1}. Static X-ray powder diffraction intensity data were analyzed and integrated by a computer program Fit2D (http://www.esrf.eu/computing/scientific/FIT2D/).

1.3 Differential scanning calorimetry (DSC)

Calorimetric investigation was performed using a Mettler-Toledo DSC821^e differential scanning calorimeter with a high-sensitivity sensor (HSS7). Samples were examined using a heating rate of 0.5 ℃·min^{-1} and at least three scans were performed to verify reproducibility.

2 Results and discussion

2.1 Thermal analysis of DPPC bilayers with different sterols

DSC thermograms of DPPC dispersions, with or without sterol, recorded at a heating rate of 0.5 ℃·min^{-1} are presented in Fig.2. In the absence of sterol, DPPC dispersions display a low enthalpic pretransition ($\mathbf{L}_{\beta'}$ to $\mathbf{P}_{\beta'}$ phase) at 35.5 ℃ and a sharp main transition ($\mathbf{P}_{\beta'}$ to $\mathbf{L}_{\alpha}$ phase) at 42.0 ℃, which are identified as the peak temperatures of the DSC curves according to the viewpoint of McMullen and McElhaney[20]. The phase transition temperatures are in good agreement with the published data[21,22].

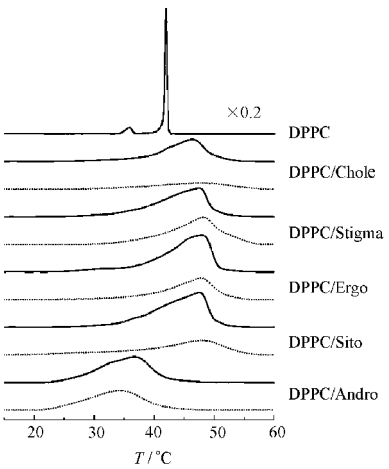

Fig.2 DSC thermograms of mixtures of DPPC dispersions containing different sterols with DPPC/sterol molecular ratios of 5:1 (solid line) and 2:1 (dot line)

Samples equilibrated at 10 ℃ were heated to 60 ℃ at a heating rate of 0.5 ℃·min^{-1}.

Generally, the endothermic peak of the main phase transition of the phospholipids becomes broad in the presence of sterols. In Fig.2, all the five sterols, cholesterol, ergosterol, stigmasterol, sitosterol, and androsterol broaden the endothermic peak of the main transition of DPPC with both 5:1 and 2:1 molar ratios of DPPC/sterol dispersions. Moreover, a clear shoulder peak feature appears when the molecular ratio of DPPC to sterol is 5:1. This suggests that these dispersions may be comprised of more than one phase. As previously described, this could have resulted from two components, representing the melting of sterol-poor and sterol-rich domains, respectively[22–27]. By contrast, the thermograms of the samples containing 33% (x) sterols are relatively symmetric and may probably originate from a single phase transition.

To the sterols with a hydrocarbon chain (cholesterol, stigmasterol, sitosterol, and ergosterol), they induced the shift of the endothermic peak of the main transition of DPPC to a higher temperature. Furthermore, the transition temperature of the thermograms of DPPC with 33% (x) sterol is higher than that of mixtures with 17% (x) sterol, which indicates that phospholipids in this phase state are more stable and orderly, therefore the melting temperature of hydrocarbon chains moves to a higher temperature. Conversely, the sterol nucleus (androsterol) induces the shift of the main transition temperature of DPPC to a lower temperature, and a higher concentration of androsterol is more effective.

It is interesting to note that the enthalpy of DPPC dispersion with 33% (x) cholesterol is the smallest in all these binary systems of DPPC and sterols. Furthermore, the main phase transition peak is found to be diminished when the molar ratio of DPPC to cholesterol reaches 1:1 (data not shown). This is in agreement with McMullen′s results that increasing proportions of cholesterol cause a progressive broadening of the gel to liquid crystal phase transition and a decrease in the enthalpy change of transition[23]. It suggests that the binary system of DPPC and cholesterol above the molecular ratio of 2:1 reaches a stable phase.

2.2 Characteristics of in-plane packing of DPPC bilayers with different sterols

Wide-angle X-ray scattering (WAXS) was employed to measure the in-plane packing within the lipid bilayer. Fig.3 shows the WAXS patterns of mixtures of DPPC and different sterols with the temperature range from 30.0 to 57.5 ℃ (20.0 to 50.0 ℃ for DPPC/androsterol system).

In the binary systems of DPPC and sterols with molecular ratio of 5:1, the WAXS pattern, centered on 0.423 nm (S=2.36 nm^{-1}; S is the reciprocal spacing of d-spacing), is relatively sharp below the main transition temperature of DPPC (42 ℃), which is a characteristic of the gel phase. Then the maximum of WAXS is broadened when the temperature increases near the main transition temperature of DPPC. With the assistance of the DSC results, the characteristic of the broad pattern is attributed to the liquid-crystal phase state, with higher fluidity.

When the molar ratio of the binary systems changes to 2:1, the WAXS pattern is relatively broad over the entire temperature

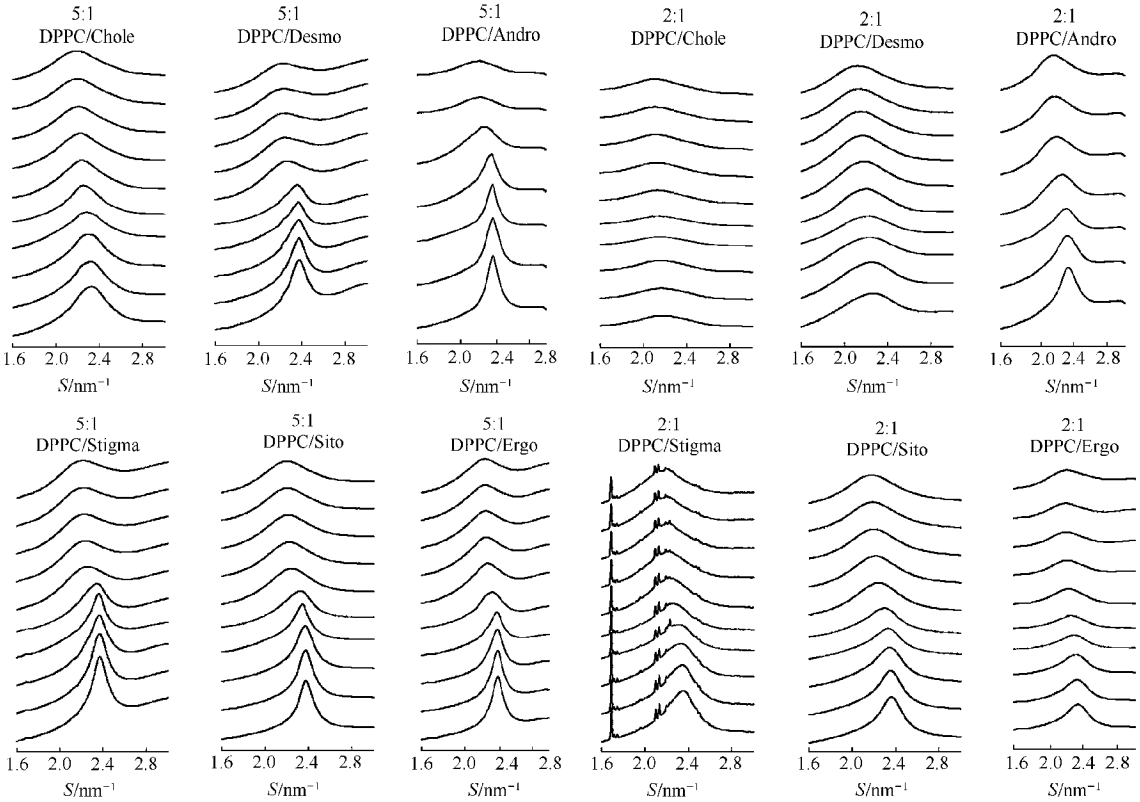

Fig.3 Wide-angle X-ray diffraction patterns of DPPC/sterol dispersions at molecular ratios of 5:1 and 2:1 in the temperature range from 30.0 to 57.5 ℃ (20.0 to 50.0 ℃ for DPPC/androsterol) compiled from bottom to top with equal intervals

S is the reciprocal spacing of *d*-spacing.

range. This is because that higher sterol concentration induces the mixtures to form the liquid ordered phase. In a recent article, Clarke *et al*.[28] reported that the introduction of sufficient cholesterol into a gel phase formed the $\mathbf{L}_o$ phase, causing disruption of the phospholipid packing and thus a fast lateral-motion like in the case of liquid crystal phase. This, in turn, results in a broad wide-angle diffraction peak. In addition, a number of sharp diffraction peaks can be seen in the WAXS pattern of the stigmasterol system (Fig.3). This is because the sterol is saturated in forming the $\mathbf{L}_o$ phase and is separated out as crystals.

The shifts in the position of the WAXS peaks of the binary mixtures of DPPC and sterols as a function of temperature are summarized in Fig.4. For pure DPPC, the sigmoid shape of the curve shows a clear gel to liquid-crystal phase transition. For binary DPPC/sterol (5:1 in molar ratio) mixtures, similar results are observed, indicating that the phase transition is still reserved. Two differences, however, can be noticed. The first one is that, for the cholesterol mixture, the phase transition is not very sharp. Moreover, at low temperatures, the *d*-spacing is significantly greater than that of other systems. The other one is about the androsterol system, which gives a much lower transition temperature and greater *d*-spacing, indicating less ordered structure in this mixture.

For binary DPPC/sterol (2:1 in molar ratio) mixtures, all mix-

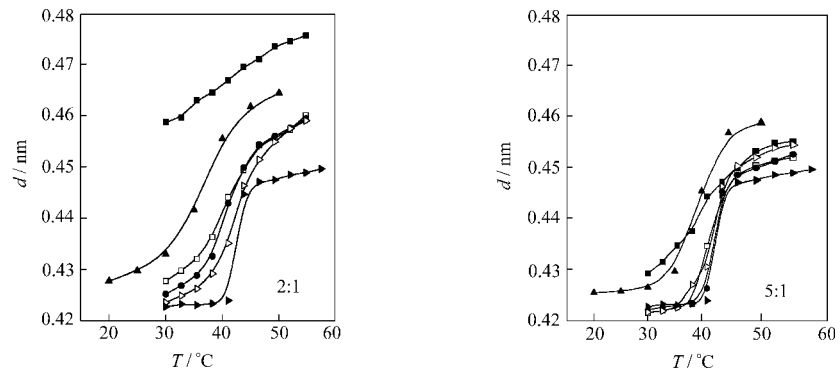

Fig.4 In-plane spacing (d=1/S) of DPPC and DPPC/sterol dispersions at 5:1 and 2:1 molar ratios

–■– Chole, –□– Ergo, –●– Stigma, –▷– Sito, –▲– Andro, –▶– DPPC

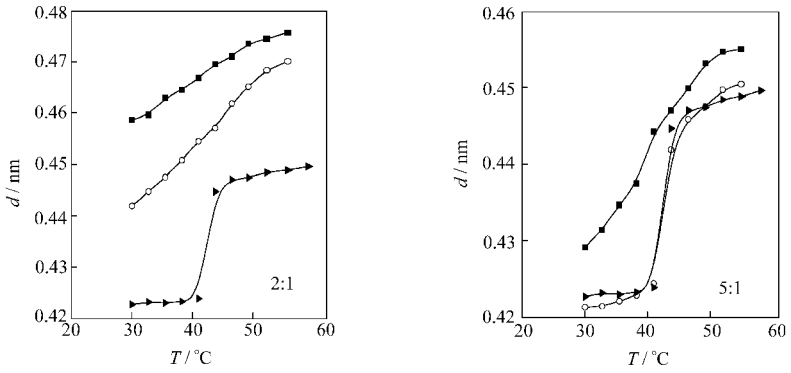

Fig.5 Comparison of the temperature dependence of in-plane spacing of DPPC and binary mixtures of DPPC/desmosterol and DPPC/cholesterol with different molar ratios

—▪— Chole, —◦— Desmo, —▸— DPPC

tures, except cholesterol, show sigmoid dependence of *d*-spacing on temperature, indicating a non-negligible phase transition in each system. In comparison with the pure DPPC dispersion, the phase transitions are much more gradual, and the *d*-spacings increase more or less. It is interesting to note that the DPPC/cholesterol mixture dismisses phase transition completely. The *d*-spacing shows a nearly linear relationship with temperature. This is consistent with the DSC results, where the enthalpic event almost disappears (Fig.2).

For DPPC/desmosterol system, when the molar fraction of desmosterol is low (17% (*x*)), the phase behavior resembles that of pure DPPC (Fig.5). When the molar fraction of desmosterol increases to 33% (*x*), the phase transition disappears and desmosterol behaves similar to cholesterol except the somewhat smaller *d*-spacing.

The above experimental results show that, first, among the sterols investigated in this study, cholesterol is the most effective in influencing the phase behavior of DPPC, followed by desmosterol, which is the precursor in the synthesis of cholesterol with two less hydrogen atoms than the latter. Both cholesterol and desmosterol dismiss the main phase transition of DPPC, forming a stable liquid-ordered phase when the molar ratio is 2:1. This property of desmosterol may be the reason that it can replace cholesterol in keeping mice alive[29,30]. Both molecules have streamline shape structures and are flexible to change their chain conformations to adapt the surrounding DPPC chains. As a result, they can be accommodated comfortably into the DPPC assembly to form a stable $\mathbf{L}_o$ phase.

Second, with minor modifications on the structure of cholesterol, sterols (stigmasterol, sitosterol, and ergosterol) can only exert limited influence on the properties of DPPC. At a molar ratio of 2∶1, a phase transition can still be seen. However, the broad WAXS patterns (Fig.2) and longer *d*-spacings (Fig.4) at low temperatures support the understanding that the phases are close to the $\mathbf{L}_o$ phase. The authors, therefore, distinguish the phases before and after the phase transition as $\mathbf{L}_{o\beta}$ and $\mathbf{L}_{o\alpha}$, following the study of McMullen and McElhaney[20]. However, different from their study, the results here demonstrate that such distinguishment in the DPPC/cholesterol mixture is not as typical as that in the mixtures involving stigmasterol, sitosterol, and ergosterol.

Third, with major modifications on the structure of cholesterol, androsterol has no side chain at all. Its chain length mismatches that of DPPC, so the surrounding DPPC alkyl chains have more space to fill in. This causes a very severe disorder in the arrangement of hydrocarbon chains of DPPC, especially at high temperatures. The main transition temperatures in these androsterol mixtures are about 5–10 ℃ lower than other sterols with side chains. Therefore, the alkyl chain at C17 plays a necessary role in keeping the order degree of surrounding lipid chains and restricting movement. The phytosterols and fungi sterol are less effective than cholesterol in forming the $\mathbf{L}_o$ phase with DPPC. This can be explained by the addition of the ethyl or methyl group at position C24. The more bulky alkyl moiety may cause a steric hindrance for highly ordered arrangement of the binary lipid systems. The effect of double bonds, however, is very limited. For example, sitosterol differs from stigmasterol only in a double bond at position C22. They behave almost identically in interactions with DPPC. Similarly, desmosterol has one more double bond than cholesterol, but both of their mixtures with DPPC (33% (*x*) sterol) can form a stable $\mathbf{L}_o$ phase. These studies illuminate the concept that the stability and fluidity of liquid ordered phase may be a key point in explaining the selection of cholesterol in cells of animals.

3 Conclusions

On the basis of the results obtained in this study, several conclusions can be made. (i) Cholesterol was more efficient than phytosterols and ergosterol in the interactions with DPPC; (ii) Phytosterol or fungi sterol weaken, but did not fully abolish, the ability to promote the formation of an ordered state in the DPPC bilayer; (iii) Cholesterol and desmosterol could promote a stable ordered phase, little dependent on temperature, whereas, the ordered phase containing phytosterol or ergosterol was strongly dependent on temperature.

The different abilities of various sterols in forming ordered structures with phospholipids imply that evolution may have selected cholesterol as the most efficient sterol species for animals to form rafts in their cell membranes.

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