Differential Properties of the Sterols Cholesterol, Ergosterol, β-Sitosterol, *trans*-7-Dehydrocholesterol, Stigmasterol and Lanosterol on DPPC Bilayer Order

C. Bernsdorff and R. Winter*

University of Dortmund, Department of Chemistry, Physical Chemistry I, Otto-Hahn Str. 6, D-44227 Dortmund, Germany

Received: April 8, 2003; In Final Form: July 22, 2003

Cholesterol, its precursor lanosterol, the plant sterols β-sitosterol and ergosterol, as well as three further sterols were added up to 50 mol % to vesicles of the phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC). The aim of this study was to investigate the influence of the sterol side chain and ring structure on the acyl chain orientational order of the lipid bilayer by measuring the steady-state fluorescence anisotropy r_{ss} of the fluorophore 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) and to establish the molecular basis underlying the changes in order parameter of the lipid bilayer system. Experiments were carried out in the temperature range from 30 to 60 °C, i.e., at temperatures below and above the gel to liquid-crystalline phase transition temperature T_m of DPPC bilayers. In general, the incorporation of the different sterols into the DPPC bilayer enhances the orientational order of TMA-DPH in the liquid-crystalline state of DPPC, whereas the orientational order in the gel state is slightly reduced. We found that the molecular interactions between DPPC and sterols distinctly depend on the structure of the side chain and nucleus of the sterols. Compared to cholesterol, the presence of the additional ethyl group on the alkyl side chain of β-sitosterol markedly reduces the effectiveness of the sterol on ordering the fluid DPPC bilayers over the whole concentration range of the sterol up to 50 mol %. As can be seen from the comparison with the other sterol mixtures, an increase in alkyl chain volume has the most drastic effect on the condensing capacity of sterols. Introduction of a double bond in the side chain counteracts this effect, owing to the decreased volume fluctuations in the steroid alkyl chain region. Interestingly, also introduction of a double bond in the sterol ring system leads at low sterol levels to a drastic increase in conformational order of the lipid acyl chains, which can even be substantially higher than that induced by cholesterol. Additional methyl groups in the ring system of the sterol markedly counteract this rigidifying effect. Hence, the lipids in the lanosterol-containing membrane are significantly less ordered than those in the cholesterol-containing membrane. Sterols with the bulkiest unsaturated side chains or sterol nuclei (stigmasterol, β-sitosterol, and lanosterol) induce the smallest order parameter increase of the fluid bilayer at high sterol concentrations (> 30 mol %), and hence become less potent rigidifiers at high sterol levels. At the highest sterol levels, cholesterol and the plant sterol ergosterol have the most profound ordering effect on fluid DPPC bilayers.

I. Introduction

Sterols such as cholesterol (β-cholestanol) are important for the structural and dynamical properties of cell membranes.[1−3] Sterols are found in high concentrations (up to about 50 mol %) in plasma membranes of animal and higher plant cells, but in markedly lower concentrations in intracellular membranes where they are synthesized. A considerable amount of research has been devoted to elucidate the different biological functions of sterols, such as cholesterol, in cells, to understand the physical basis for the biological functions by investigating the role of sterols in modulating physical properties of artificial and biological membranes, and to unravel the relationship between their functions and molecular structure. One intriguing question, the question of the origin of cholesterol in the context of cell evolution, is still unanswered.[4−7] One approach to the question of the biological role of sterols in membranes is to examine the structural specificity of various sterols differing in configuration. This approach is also pursued in this work.

Cholesterol (see Figure 1) has a hydrophobic and planar fused tetracyclic ring structure with two β-oriented methyl groups at positions 10 and 13, a branched extended iso-octyl side chain at position C17, and a hydrophilic β-oriented hydroxyl group at position C3. The sterol ring orients itself parallel with the acyl chains of membrane phospholipids, with its 3 β-OH group in proximity to the phospholipid ester carbonyl oxygen at the lipid−water interface. The van der Waals interactions between the lipid acyl chains and the sterol ring as well as its branched side chain seem to be the most important contributions stabilizing cholesterol−phospholipid interactions.

It has been demonstrated by a variety of physical techniques[1] that cholesterol has a "condensing" (ordering) effect on the packing of phospholipids in their liquid-crystalline state, because the rigid ring structure of the sterol limits the possibility for cis-trans isomerizations of neighboring lipid chains, and a disordering effect below the chain-melting transition, i.e., in the gel state of the lipid bilayer. However, the extent of these effects depends on the detailed molecular structure of the lipid, but probably also on the sterol molecule.[5−22] The factors controlling the physical state of natural membranes at a molecular level are difficult to understand due to their structural complexity. To gain information about specific lipid−sterol interactions, it is thus needed to study simple well-defined model biomembrane

10.1021/jp034922a CCC: $25.00
Published on Web 08/29/2003

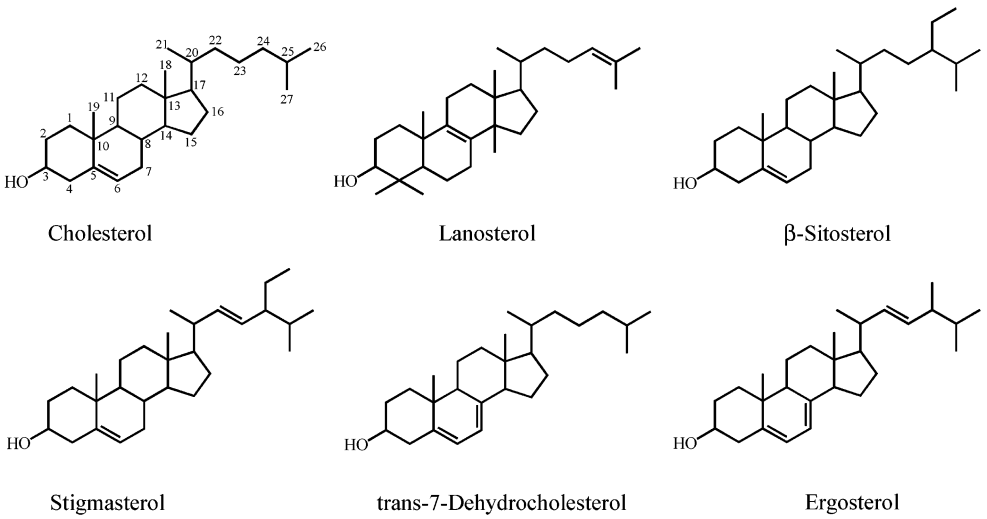

Figure 1. Chemical structures of the sterols used in this study. The formula of cholesterol includes the numbering of the carbon atoms.

systems. In this study, we investigated structural properties of lipid vesicles of the common phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), containing different amounts of various sterols up to 50 mol %.

DPPC phospholipid bilayers exhibit two principal thermotropic lamellar phase transitions, corresponding to a gel to gel ($L_{\beta'} - P_{\beta'}$) pretransition and a gel to liquid-crystalline ($P_{\beta'} - L_\alpha$) main transition at $T_m \approx 41.5$ °C.[23] In the fluidlike L_α phase, the hydrocarbon chains of the lipid bilayer are conformationally disordered, whereas in the gel phases, the hydrocarbon chains are more extended and relatively ordered. For the sterols examined, incorporation of more than about 5 mol % is sufficient to suppress the pretransition.

In general, sterol molecules are, in contrast to phospholipids, essentially rigid and relatively smooth in their hydrophobic parts. They prefer to have next to them lipid acyl chains that are ordered as in the *so* (solid ordered), gellike phase, thereby inducing chain ordering. On the other hand, because the sterols have different molecular shapes from conformationally ordered lipid chains, they tend to break the lateral packing of the *so* phase. This can lead to the emergence of a physical state of lipid-sterol membranes, which has characteristics intermediate between solid ordered and liquid disordered (*ld*), the liquid-ordered (*lo*) state. In recent years, cholesterol-rich liquid ordered (*lo*) structures have received much attention in membrane biophysics. Based on studies of detergent-resistant membrane fragments (DRMs), a hypothesis has been put forward that *rafts*, which are domain structures enriched in cholesterol and sphingolipids, exist in cell membranes and have potential functional importance in processes such as intracellular membrane sorting and signal transduction at the cell surface (see ref 22 and citations therein). This observed phenomenon is rationalized by a stronger cohesive interaction between cholesterol and the saturated lipids. Possibly, the structural properties of cholesterol are one of the key parameters in such domain formation: the better a sterol can form tight packing with lipids with saturated acyl chains, the more strongly it promotes the formation of these domains. Hence, also in this regard, a detailed study of sterols differing in their molecular configuration is very desirable.

The focus of our study is to identify and characterize the differential effects of various sterols including cholesterol, ergosterol (which is essential in some fungi or protozoan cells), the plant sterol β-sitosterol, stigmasterol, *trans*-7-dehydrocholesterol, and lanosterol, the evolutionary precursor of cholesterol on equilibrium structural properties of the DPPC bilayer membrane and to relate these effects to the difference in their molecular chemistry. We use the fluorescence depolarization techniques to determine the physical state (molecular orientational order) of the DPPC−sterol mixtures.[24−27] These techniques utilize the fluorescence anisotropy elicited from probe molecules embedded at very low concentration in the lipid bilayers. In this study, fluorescence measurements using 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (see Figure 2) as fluorescence probe were performed. The amphiphilic probe molecule TMA-DPH is located in the bilayer with its positively charged trimethylamino group being anchored in the headgroup region of the lipid bilayer.[25,28] Therefore TMA-DPH detects the interaction between the rigid ring system of the sterols with the upper acyl chain region of the phospholipids.

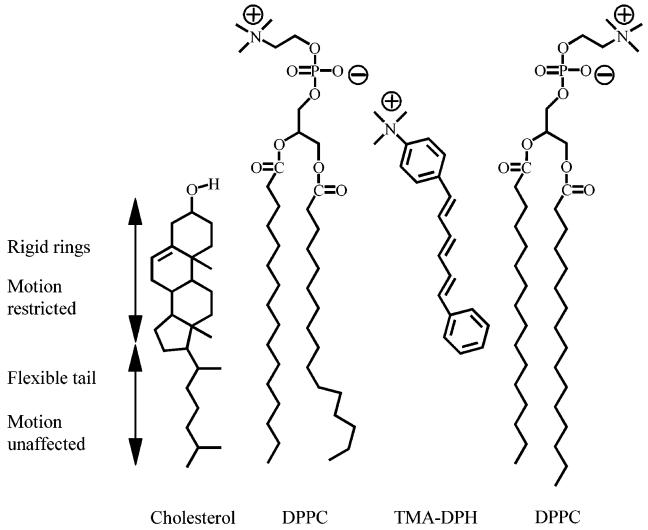

Figure 2. Schematic representation of the structure and location of cholesterol and the cationic fluorescence probe TMA-DPH in the DPPC lipid bilayer.

II. Theoretical Background

Since the absorption and the emission of light depend on the orientation of the transition dipole moments, the introduction of polarized excitation light can provide information on the rotational motion of a fluorophore. After creation of a dis-

tribution of excited fluorophores (photoselection), the excited fluorophores will relax to a uniform random set. This process can be followed in order to determine the rotational motion of the fluorophores, if the rotational correlation time lies within the lifetime of the fluorescence radiation. One observes this motion by measuring the state of emission anisotropy with time, which can generally be described as a multiexponential decay given by[24]

$$r(t) = \frac{I_{||}(t) - I_{\perp}(t)}{I_{||}(t) + 2I_{\perp}(t)} = r_0 e^{-t/\phi_{rot}} \quad (1)$$

where $r(t)$ is the anisotropy at time t after excitation and r_0 is the limiting anisotropy in the absence of rotational diffusion. Maximum values for r_0 are observed when the absorption and emission dipole moments are collinear ($r_{0,max} = 0.4$). The subscripts of $I(t)$ indicate the relative orientation, parallel (||) and perpendicular ($\perp$), of the polarizers, respectively. ϕ_{rot} is the rotational correlation time (assuming that only one rotational correlation time is present).

The motion of a fluorescence probe in a lipid bilayer is generally not isotropic but restricted by a potential cage determined by the surrounding lipid acyl chains. It is often described by a wobble-in-cone model.[29−32] Hence, $r(t)$ does not decay to zero but to a finite value, r_∞. Equation 1 can often be simplified to

$$r(t) = r_\infty + (r_0 - r_\infty)\, e^{-t/\phi_{rot}} \quad (2)$$

An excitation with vertically polarized light of constant intensity yields the steady-state fluorescence anisotropy r_{ss}. From the time dependence of $r(t)$, r_{ss} can be determined by integration

$$r_{ss} = \frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}}$$

where

$$r_{ss} = \langle r(t) \rangle = \frac{\int_0^\infty r(t) I(t)\, dt}{\int_0^\infty I(t)\, dt} \quad (3)$$

Here, r_{ss} is thus defined as the difference between the parallel ($I_{||}$) and the perpendicular ($I_{\perp}$) emission components relative to the total fluorescence intensity.

The extent of depolarization of the emission of a fluorophore in the membrane thus reflects the degree to which a population of photoselected excited fluorophores loses its initial selective orientation and becomes randomized. Several studies revealed that r_{ss} is mainly determined by the degree to which the fluorophore rotations are restricted by the molecular packing of the lipids, i.e., the acyl chain orientational order, rather than by its rotational rate. According to this interpretation, r_{ss} can be resolved into a static part, the residual or limiting anisotropy r_∞, and a dynamic part, the dynamic anisotropy r_f:[31,32]

$$r_{ss} = r_\infty + r_f \quad (4)$$

The dynamic part r_f is related to the rotational rate, i.e., the rotational correlation time ϕ_{rot} of the fluorophore:

$$r_f = \frac{r_0 - r_\infty}{1 + \tau_F/\phi_{rot}} \quad (5)$$

where τ_F is the fluorescence lifetime. By contrast, r_∞ describes the static or structural part that is found to be proportional to the square of a structural order parameter, S, for fluorophores with cylindrical symmetry. With absorption or emission transition dipole moments, being parallel to the long axis of the fluorophore, one obtains

$$r_\infty = r_0 S^2 \quad (6)$$

with $0 \le S \le 1$, which thus reflects the average order parameter of the lipid bilayer at the position of the fluorophore. S is given by the second Legendre polynomial $\langle P_2 \rangle$:

$$S_2 = \langle P_2 \rangle = \frac{1}{2}\langle 3\cos^2\theta - 1 \rangle \quad (7)$$

The angle brackets denote an ensemble average. θ is the angle between the molecular axis of the probe and the z axis, the main axis of rotation. Hence, the orientational order parameter is $S = 1$ if the fluorophore is parallel to the normal of the membrane, and $S = 0$ for a statistical orientational distribution. It should be emphasized that the order parameter is probe specific, and comparisons of order parameters obtained by fluorescence and NMR or ESR techniques result in similar values only in very special cases. By comparing steady-state fluorescence anisotropy values with those in an isotropic reference oil, one obtains for fluorophores, embedded in lipid bilayers, equations of the form

$$r_\infty = \frac{r_0 {r_{ss}}^2}{\left(r_0 r_{ss} + \frac{(r_0 - r_{ss})^2}{m} \right)} \quad (8)$$

where the parameter m expresses the difference between the rotational diffusion of the fluorophore in the membrane (or another anisotropic matrix) and that in an isotropic reference oil.[31] van der Meer et al.[32] have fitted a number of measured r_{ss} and r_∞ data to eq 8 and listed values of m for different probes embedded in a series of model membrane systems. For TMA-DPH in DPPC vesicles the fitted data yield a value for m of 0.63 ± 0.1. Together with the initial anisotropy for TMA-DPH, $r_0 \approx 0.39$,[25,17,18] the order parameter S can then be calculated for each steady-state anisotropy value using eq 8.

III. Materials and Methods

A. Sample Preparation. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids Inc. (Alabastar, AL), and the sterols cholesterol, lanosterol, *trans*-7-dehydrocholesterol, and ergosterol from SIGMA (Deisenhofen, Germany). β-Sitosterol was a generous gift from Hoffmann-LaRoche (Basel, Switzerland). All chemicals were used without further purification. TMA-DPH as a *p*-toluenesulfonate salt was purchased from Molecular Probes Inc. (Eugene, OR). Lipid and sterol stock solutions were prepared in chloroform at concentrations of 10 mmol·L^{-1}. TMA-DPH was dissolved in ethanol at a concentration of 1 mmol·L^{-1}. DPPC vesicles containing the desired amounts of sterol (0−50 mol %) were prepared by cosonication. The solvent chloroform was removed by a flow of nitrogen gas. The remaining film was then resuspended in Tris buffer (50 mM, pH 7.4), vortexed, and sonicated for 5 min at a low power level (Labsonic U, Braun). After sonication, the vesicle solutions were centrifuged for 3 min. The fluorescence probe TMA-DPH was not cosonicated with the vesicle solution, because TMA-DPH binds rapidly to phospholipid membranes.[33] TMA-DPH stock solution was added to the final vesicle solutions to yield a 1:500 fluorophore

to lipid mixture on a molecular basis. The samples were measured after preparation in a 3 mL quartz cuvette. The final concentration of the lipid vesicles in the sample used for fluorescence measurements was 0.3 mmol·L^{-1} and that of the fluorescent probe was 0.6 μmol·L^{-1}.

B. Steady-state Fluorescence Polarization Measurements. Measurements of the temperature dependence of the steady-state fluorescence anisotropy r_{ss} were carried out in a Perkin-Elmer LS50B spectrofluorometer, aligned to the "L"-format light path and equipped with polarizers. Excitation and emission wavelengths were 338 and 446 nm, respectively. Background fluorescence was measured by using identical samples without probe and was found to be negligibly small. The corrected steady-state fluorescence anisotropy values

$$r_{ss} = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \tag{9}$$

were calculated using the software program of Perkin-Elmer, where $G = (I_{HH}/I_{VH})$ represents an optical correction factor for the slightly unequal H and V polarized excitation intensities (compare with eq 3, where, ideally, $I_{VV} = I_{II}$, $I_{HV} = I_{HH} = I_{VH} = I_{\perp}$). The temperature of the lipid dispersion was controlled to within ± 0.1 °C by use of a circulating thermostated water bath connected to the sample chamber. The data presented are the average of at least three independent runs on different preparations.

IV. Results and Discussion

Cholesterol analogues were selected on the basis of simple geometrical changes in the nucleus or side chain, or both, of cholesterol. Modification in cholesterol structure involving (i) the introduction of a double bond at position 22, (ii) the addition of an alkyl group at position 24, or (iii) the combination of these alterations make the sterol side chain less flexible, bulkier, or both. Additional modifications in the sterol nucleus also change the interactions of the sterol ring system with the phospholipid molecules (Figure 1). The different sterols were added to DPPC vesicles in the same concentration range as that corresponding to cholesterol.

The steady-state fluorescence data were taken in the temperature range between 30 °C and 60 °C, i.e., at conditions, where DPPC is either in the gel or the liquid-crystalline (L_α) state. The steady-state fluorescence anisotropy data of TMA-DPH in the different DPPC−sterol mixtures as a function of temperature and sterol concentration are presented in Figures 3−8, respectively. The sterol concentrations vary from 0 to 50 mol % in steps of 10 mol %. All measurements were carried out at least three times. The error in the r_{ss} values are smaller than ± 0.005.

At first sight, the corresponding r_{ss} values as a function of temperature and sterol concentration show no dramatic alterations to the system DPPC−cholesterol. However, there are distinct differences in the increase or decrease of r_{ss} in the different DPPC−sterol vesicles depending on the particular sterol structure. The probe TMA-DPH mainly detects the influence of the sterol nucleus on the upper acyl chain orientational order, since the probe resides in the interfacial region of the phospholipid bilayer. However, the side chain of the sterols also influences the acyl chain orientational order in the upper region of the lipid bilayer.

As can be clearly seen in Figure 3, the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in the liquid-crystalline phase is significantly lower than that in the gel phase of DPPC, indicating that greater static and/or dynamic molecular disorder

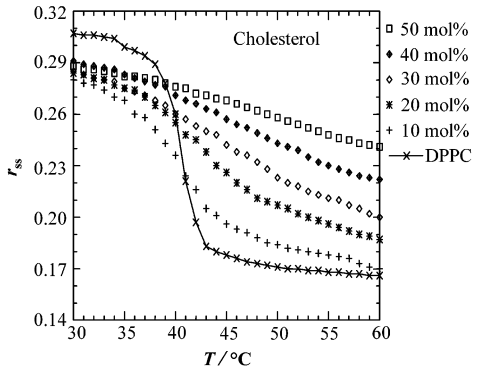

Figure 3. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−cholesterol vesicles at different sterol concentrations.

is present in the liquid-crystalline phase of the bilayer. We found an average r_{ss} value of about 0.30 for TMA-DPH in pure DPPC in the gel phase, and of 0.17 in the liquid-crystalline phase, corresponding to a marked difference in the order parameter S of TMA-DPH of 0.83 and 0.45, respectively. The chain melting transition occurs around 41 °C, in accordance with literature data.[23]

As expected, the incorporation of cholesterol into the DPPC bilayer reduces the disorder in the liquid-crystalline state, as can be deduced from the observed larger steady-state anisotropy values. Contrary to the behavior for $T > T_m$, the r_{ss} values slightly decrease for $T < T_m$, i.e., in the gel phase, at the most for the lower cholesterol concentrations. For example, r_{ss} increases about 60% in the fluid phase and decreases about 7% in the gel phase by addition of 50 mol % cholesterol. Hence, the rigid ring system of cholesterol significantly enforces the orientational ordering of the acyl chains in the fluidlike state. In the gel phase, the orientational order of the lipid system is slightly disturbed. These results are in good agreement with results obtained from a variety of other spectroscopic methods on DPPC−cholesterol mixtures [1,7].

The spreading of the gel to liquid-crystalline phase transformation of DPPC increases with increasing cholesterol concentration. The phase transition as detectable by fluorescence anisotropy seems to be almost smeared out above a cholesterol level of about 30 mol %. The steady-state fluorescence anisotropy and hence the conformational order of the lipid bilayer containing concentrations higher than 30 mol % cholesterol decreases only modestly with increasing temperature, i.e., these high cholesterol concentrations can stabilize the acyl chain orientational order in the bilayer over a wide range of temperatures.

The influence of structural changes in the sterol side chain on the steady-state anisotropy of TMA-DPH can be discussed by comparing the r_{ss} of the DPPC−cholesterol system with that of the 24-ethyl cholesterol derivatives β-sitosterol and stigmasterol (Figures 4 and 5). β-Sitosterol contains a 24-ethyl group in the side chain, whereas stigmasterol contains an additional *trans*-22-double bond as well as a 24-ethyl group. We first compare the r_{ss} data of DPPC − cholesterol (Figure 3) with those of DPPC−β-sitosterol (Figure 4). The overall features of the anisotropy data as a function of temperature and sterol concentration are similar for both sterols. However, there are distinct differences visible in the rate that r_{ss} increases with sterol concentration. In the fluid phase, the r_{ss} values obtained for all β-sitosterol levels are lower than those observed for the corresponding cholesterol levels. In the gel phase, a different behavior is observed. Whereas in DPPC−cholesterol mixtures,

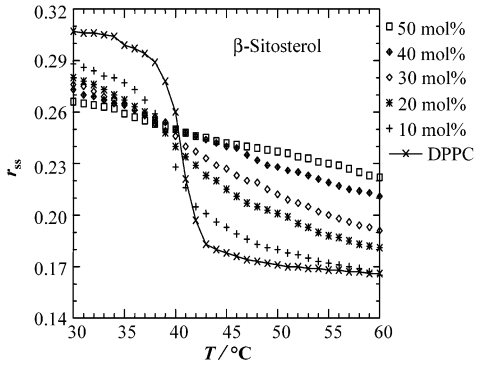

Figure 4. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−β-sitosterol vesicles at different sterol concentrations.

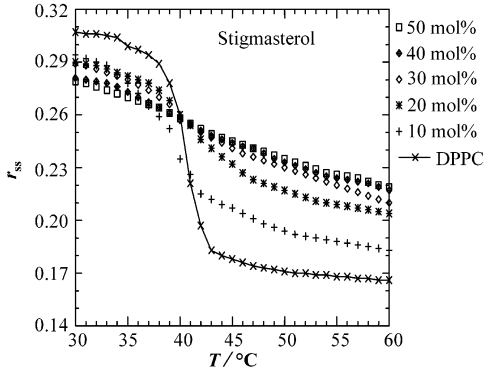

Figure 5. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−stigmasterol vesicles at different sterol concentrations.

the orientational order is essentially reduced at lower cholesterol concentrations, in DPPC−β-sitosterol, r_{ss} values decrease continuously with increasing sterol concentration and the overall disturbance of the lipid bilayer gel phase structure appears to be greater.

These data thus clearly indicate that β-sitosterol is less effective than cholesterol in ordering the lipid acyl chains in their fluidlike state, which appears to correlate with the greater volume of the sterol side chain and thus, due to steric hindrance, with a lesser interaction with neighbored phospholipid molecules than the interaction of cholesterol. Compared to cholesterol, high levels of β-sitosterol disturb the acyl chain packing of DPPC in the gel phase to a greater extent.

The temperature dependence of r_{ss} of TMA-DPH in DPPC−stigmasterol vesicles resembles that of DPPC−β-sitosterol vesicles at high sterol levels; $r_{ss}(x_{sterol})$ follows the same order in the gel state and is thus different from the system DPPC − cholesterol. The r_{ss} values and thus the conformational order at low cholesterol levels (10, 20 mol %) of the system DPPC−stigmasterol containing a *trans*-22 double bond, which reduces the flexibility of the sterol's alkyl chain, are higher in the fluid phase, however. The state of DPPC vesicles containing 10 mol % stigmasterol is similar to that of DPPC vesicles containing 20 mol % β-sitosterol in the liquid-crystalline state. Both 24-ethyl sterols are also able to order membrane lipids, albeit less effectively than that of cholesterol at high sterol levels. The similarity of both 24-ethyl sterols in the effectiveness of phospholipid −sterol interaction appears to correlate with the greater volume of their side chain and thus with a less effective interaction with neighbored fluid phospholipid molecules than the interaction of the more streamline cholesterol molecule with the DPPC molecules in the lipid bilayer.

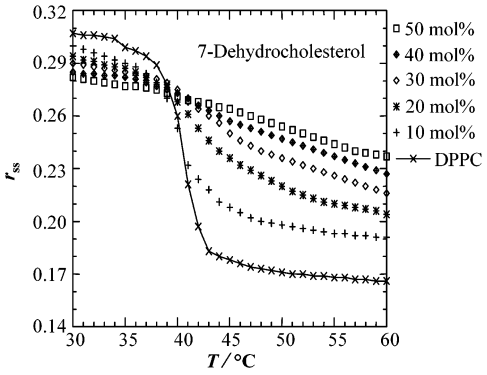

Figure 6. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−*trans*-7-dehydrocholesterol vesicles at different sterol concentrations.

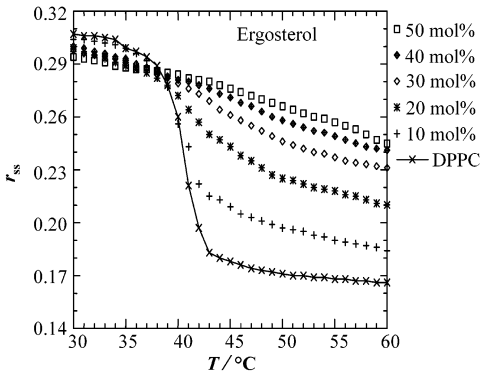

Figure 7. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−ergosterol vesicles at different sterol concentrations.

The differential influence of the fungal sterol ergosterol (24-ethyl-*trans*-7, 22-dehydrocholesterol, Figure 1), is more difficult to discuss as both the side chain and the nucleus are changed in comparison to cholesterol. The differences in r_{ss} values are considerable in the liquid-crystalline phase in the lower sterol concentration range (Figure 7). Incorporation of 10 and 20 mol % ergosterol leads to a marked increase in the r_{ss} value, of about 17% and 36% when compared to the system DPPC−cholesterol in the liquid-crystalline phase of the lipid bilayer. This can be explained by the conjugated double bond system in ring 2 of the ergosterol nucleus, which probably increases the interaction with the neighboring acyl chains caused by an enhancement of van der Waals forces and an increased smoothness of the ring system. Hence, the disordering effect in the gel phase of DPPC is much smaller.

trans-7-Dehydrocholesterol (Figure 1) contains the same conjugated system in the sterol nucleus, whereas its side chain is identical with that of cholesterol. The comparison of both systems, DPPC−ergosterol and DPPC−*trans*-7-dehydrocholesterol (Figure 6), to DPPC−cholesterol thus reflects the influence of an additional double bond and methyl group in the alkyl chain region on the orientational order of the acyl chains of the lipid bilayer. The effect is a less disordering effect on the gel-phase lipid structure and a slightly greater ordering effect of ergosterol in the fluid phase at high sterol levels.

To study also the influence of significant changes in the structure of the sterol nucleus on the acyl chain order parameter of DPPC vesicles, we measured the changes in r_{ss} by incorporation of lanosterol (Figure 8), which has three additional methyl groups in the sterol nucleus (Figure 1). Lanosterol is a natural precursor of cholesterol. As one can see in Figure 8, the changes in r_{ss} in the liquid-crystalline phase of DPPC are comparable

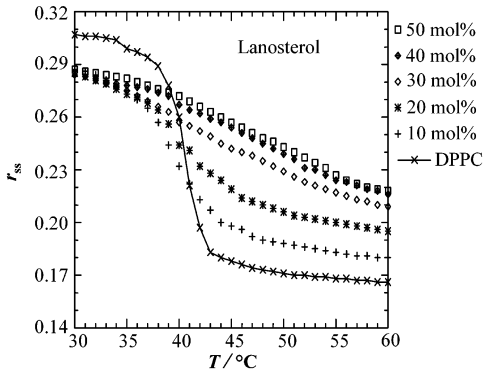

Figure 8. Temperature dependence of the steady-state fluorescence anisotropy r_{ss} of TMA-DPH in DPPC−lanosterol vesicles at different sterol concentrations.

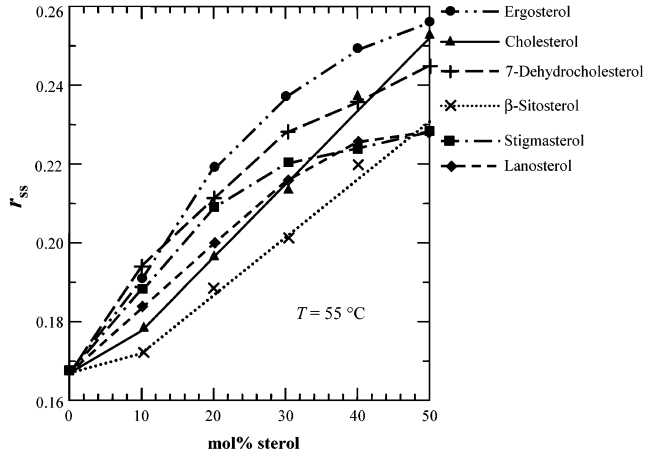

Figure 9. The effect of various sterols on the steady-state anisotropy r_{ss} of TMA-DPH embedded in DPPC vesicles as a function of sterol concentration at $T = 55$ °C.

to those in DPPC−cholesterol vesicles up to a sterol level of 30 mol %. Only a modest change in r_{ss} of DPPC−lanosterol mixtures is observed above 30 mol %, however. The r_{ss} values for 40 mol % and 50 mol % lanosterol content are almost indistinguishable. The additional three methyl groups in the sterol nucleus of lanosterol thus impose a smaller condensing effect on the acyl chains of DPPC at high sterol levels, probably owing to increasing packing problems of the bulkier sterol rings. In the gel-phase, the $r_{ss}(T,x_{sterol})$ values of the lanosterol system are similar to those of DPPC−cholesterol.

The comparison of the sterols with alterations in the sterol nucleus, lanosterol, *trans*-7-dehydrocholesterol, and ergosterol, incorporated into DPPC bilayers thus reveals remarkable changes in r_{ss} in the fluid phase of DPPC upon incorporation of lower sterol concentrations only, while these changes in r_{ss} almost obliterate at sterol levels above ∼30 mol %. Hence, major variations in the structure of the sterol nucleus seem to limit the interaction and condensing capacity of these sterols in the DPPC bilayer. A similar limitation of the condensing effect is observed for the stigmasterol system having a rather bulky alkyl chain.

V. Summary

The objective of our study was to establish the systematics of structural behavior of phospholipid bilayer systems with different sterols in terms of the molecular basis underlying the order parameter changes of the lipid bilayer system. To summarize our findings, Figure 9 elucidates the influence of the sterol side chain or nucleus structure on the static fluorescence anisotropy r_{ss} of TMA-DPH in DPPC vesicles at a constant temperature of 55 °C, i.e., 8 °C above the gel to fluid phase transition. The following observations can be made.

The comparison of, e.g., the cholesterol and β-sitosterol data reveal that, with increasing side-chain volume, the conformational order of the lipid acyl chains decreases over the whole concentration range of the sterol up to 50 mol %. As can be seen from the comparison with the other sterol mixtures, an increase in alkyl chain volume has the most drastic effect on the condensing capacity of sterols.

Introduction of a double bond in the side chain counteracts this effect, owing to the decreased volume fluctuations in the steroid alkyl chain region (compare β-sitosterol with stigmasterol).

Introduction of a double bond in the ring system leads at low and medium high sterol levels (up to ∼40 mol %) to a drastic increase in conformational order of the lipid acyl chains (compare, e.g., *trans*-7-dehydrocholesterol with cholesterol), which can indeed be substantially higher than that induced by cholesterol.

Additional methyl groups in the ring system of the sterol markedly counteract this rigidifying effect (compare lanosterol with ergosterol).

Sterols with the bulkiest unsaturated side chains or sterol nuclei (stigmasterol, β-sitosterol, and lanosterol) induce the smallest order parameter increase of the fluid bilayer at high sterol concentrations (> 30 mol %), and hence become less effective rigidifiers at high sterol levels. The difference might also be due to the different solubility/partitioning in the bilayer thus leading to a different lateral organization and thus phase diagram. This would be in accord with the different phase diagrams observed for the systems POPC−cholesterol and POPC−lanosterol, as observed recently.[22] Cholesterol is known to partition preferentially into bilayers with saturated acyl chains. The plant sterols β-sitosterol and stigmasterol are known to partition into membranes with a higher degree of unsaturated lipid acyl chains. Lanosterol is a different case as it is a sterol precursor. Owing to its additional methyl groups in the ring system, its partitioning is much less effective than that of cholesterol at high sterol levels. An important conclusion is that cholesterol, with its streamlined molecular structure, interacts more effectively with lipid chains and stabilizes the liquid-ordered state of the lipid bilayer more effectively than lanosterol (minimized steric crowding). Dahl et al.[34] also showed that cholesterol increases the "microviscosity" of membranes much more effectively than lanosterol, and they demonstrated that cholesterol reduces the membrane permeability more effectively than lanosterol. Also, Yeagle[35] and Urbina et al.[36] showed that cholesterol has a stronger ability than lanosterol to induce conformational order in lipid chains. Furthermore, recently, a molecular dynamics simulation study was carried out by Smondyrev et al.[37] on lipid−lanosterol and lipid−cholesterol bilayers, showing that at already relatively modest sterol concentrations (∼10%), subtle differences in sterol location and mobility in the bilayer can be identified, which indicate that lanosterol is more mobile than cholesterol as a bilayer constituent. At a sterol concentration of 50%, cholesterol was observed to have an overall stronger condensing effect on the bilayer than lanosterol, which is in agreement with our experimental data. We observed a similarly large condensation capacity of fluid DPPC only for the plant sterol ergosterol.

Acknowledgment. Financial support from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

(1) Finegold, L. *Cholesterol in Membrane Models*; CRC Press: Boca Raton, Florida, 1993.
(2) Kritchevsky, D. In *Comprehensive Biochemistry*; Florkin, M., Stoltz, E. H., Eds.; Elsevier: New York, 1963; Vol. 10, pp 1−22.
(3) Bloom, M.; Mouritsen, O. G. *Can. J. Chem.* **1988**, *66*, 706.
(4) Bloch, K. *CRC Crit. Rev. Biochem.* **1983**, *14*, 47.
(5) Bloom, M.; Evans, E.; Mouritsen, O. G. *Q. Rev. Biophys.* **1991**, *24*, 293.
(6) Bloom, M.; Mouritsen, O. G. In *Handbook of Biological Physics*; Lipowski, R., Sackmann, E., Eds.; Elsevier Science: Amsterdam, 1995; Vol. 1, pp 65−95,
(7) Vance, D. E. *Cholesterol in the year 2000*. *Biochim. Biophys. Acta*, special issue (dedicated to the memory of Konrad Bloch), van den Bosch, H. (Eds.), **2000**, *1529*, 1.
(8) Demel, R. A.; de Kruyff, B. *Biochim. Biophys. Acta* **1976**, *457*, 109.
(9) Yeagle, P. L. *Biochim. Biophys. Acta* **1985**, *822*, 267.
(10) Jain, M. K. *Curr. Top. Membr. Transp*. **1975**, *6*, 1.
(11) Finean, J. B. *Chem. Phys. Lipids* **1990**, *54*, 147.
(12) Dahl, C. E. *Biochemistry* **1981**, *20*, 7158.
(13) Yamauchi, H.; Takao, Y.; Abe, M.; Ogino, K. *Langmuir* **1993**, *9*, 300.
(14) McMullen, T. P. W.; Lewis, R. N. A. H.; McElhaney, R. N. *Biophys. J.* **1994**, *66*, 741.
(15) Slotte, J. P.; Jünger, M.; Vilchèze, C.; Bittman, R. *Biochim. Biophys. Acta* **1994**, *1190*, 435.
(16) Slotte, J. P. *Biochim. Biophys. Acta* **1995**, *1237*, 127.
(17) Bernsdorff, C.; Winter, R. *Ber. Bunsen-Ges. Phys. Chem.* **1995**, *99*, 1479.
(18) Bernsdorff, C.; Winter, R. *Z. Phys. Chem*. **1996**, *193*, 151.
(19) Bernsdorff, C.; Winter, R. *Biophys. J.* **1997**, *71*, 1264.
(20) Reis, O.; Winter, R.; Zerda, T. W. *Biochim. Biophys. Acta* **1996**, *1279*, 5.
(21) Endress, E.; Bayerl, S.; Prechtel, K.; Maier, C.; Merkel, R.; Bayerl, T. M. *Langmuir* **2002**, *18*, 3293.
(22) Miao, L.; Nielsen, M.; Thewalt, J.; Ipsen, J. H.; Bloom, M.; Zuckermann, M. J.; Mouritsen, O. G. *Biophys. J.* **2002**, *82*, 1429.
(23) Cevc, G.; Marsh, D. *Phospholipid Bilayers*; John Wiley & Sons: New York, 1987.
(24) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.
(25) Lentz, B. R. *Chem. Phys. Lipids* **1989**, *50*, 171.
(26) van der Meer, B. W. *Biomembranes − Physical Aspects*; Shinitzky, M., Ed.; VCH: Weinheim, 1993; pp 98−158.
(27) Lakowicz, J. R. *Topics in Fluorescence Spectroscopy*; Gryczynski, I., Ed.; Plenum Press: New York, 1991; Vol. 1−3.
(28) Straume, M.; Litman, B. J. *Biochemistry* **1987**, *26*, 5113.
(29) Kinosita, K.; Kawato, S.; Ikegami, A. *Adv. Biophys.* **1984**, *17*, 147.
(30) Engel, L. W.; Prendergast, F. G. *Biochemistry* **1981**, *20*, 7338.
(31) van Blitterswjik, W. J.; van Hoeven, R. P.; van der Meer, B. W. *Biochim. Biophys. Acta* **1981**, *644*, 323.
(32) van der Meer, B. W.; van Hoeven, R. P.; van Blitterswjik, W. J. *Biochim. Biophys. Acta* **1986**, *854*, 38.
(33) Trotter, P. J.; Storch, J. *Biochim. Biophys. Acta* **1989**, *982*, 131.
(34) Dahl, C.; Dahl, J.; Bloch, K. *Biochemistry* **1980**, *19*, 1462.
(35) Yeagle, P. L. *Biochim. Biophys. Acta* **1985**, *815*, 33.
(36) Urbina, J. A.; Pekerar, S.; Lee, H. B.; Patterson, J.; Montez, B.; Oldfield, E. *Biochim. Biophys. Acta* **1995**, *1238*, 163.
(37) Smondyrev, A. M.; Berkowitz, M. L. *Biophys. J.* **2001**, *80*, 1649.