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Soybean phosphatidylcholine vesicles containing plant sterols: a fluorescence anisotropy study

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The typical plant sterols (sitosterol, stigmasterol and campesterol) were compared with respect to their ability to regulate membrane fluidity of soybean phosphatidylcholine (PC) vesicles. Fluidity changes were monitored by the steady-state fluorescence anisotropy with 1,6-diphenyl-1,3,5-hexatriene as a probe and assigned to a measure of the acyl chain orientational order. Sitosterol and campesterol appear to be the most suitable sterols in ordering the acyl chains of soybean lecithin bilayers, even more efficient than cholesterol, the standard of reference for sterol effects on membranes, suggesting that they play a significant role in the regulation of plant membrane properties. Stigmasterol is shown to be much less active. Cycloartenol, a biosynthetic precursor of plant sterols, increases the acyl chain order with the same efficiency as cholesterol. We also investigated the effects of two unusual sterols, 24-methylpollinastanol and 14α,24-dimethylcholest-8-en-3β-ol, which were shown to accumulate in plants treated with fungicides belonging to two important classes, N-substituted morpholines and triazoles, respectively. These two sterols exhibit a behavior very similar to that of stigmasterol. The results are discussed in terms of sterol effects on the molecular packing of soybean PC bilayers.

Introduction

Whereas animal and fungal cells mainly contain one major sterol (cholesterol (4) and ergosterol (8), respectively), in most higher plants, sterols are present as complex mixtures in which sitosterol (1), stigmasterol (2) and 24-methylcholesterol (3) often predominate [1]. Plant sterols differ from cholesterol by the presence of an additional alkyl group at C-24 of the side-chain (Fig. 1). Major compounds are 24-ethylsterols (sitosterol and stigmasterol), with a unique stereochemistry at C-24; 24-methylcholesterol is present as a mixture of two epimers (24*R*)- (campesterol) (3a) and (24*S*)- (22-dihydrobrassicasterol) (3b) [2]. Cholesterol has been also found in many higher plant species. In most cases, it was reported to occur only in trace amounts [3], but it has been also identified as a major compound [3–6].

In living organisms, sterols play at least two types of functions. First of all, they are membrane components. It is now well established that as well as in animal [7] and fungal [8] cells, sterols of plant cells are concentrated in the plasma membrane [9]. In animal cells, cholesterol is believed to regulate the plasma membrane fluidity and the activity of many membrane-bound enzymes. Such a role would be played by significant amounts (so-called bulk) of sterol(s). But a few sterol molecules may be also involved in some metabolic, non-structural functions [10].

In plants, structural and functional roles of sterols

Abbreviations: PL, phospholipids; PC, phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; PG, phosphatidylglycerol; FA, fatty acids; LUV, large unilamellar vesicles; SUV, small unilamellar vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene; r_s, steady-state fluorescence anisotropy; r_0, limiting fluorescence anisotropy.

Chemical nomenclature: sitosterol (1), (24*R*)-24-ethylcholest-5-en-3β-ol; stigmasterol (2), (24*S*)-24-ethylcholest-5,22E-dien-3β-ol; 24-methylcholesterol (3), (24ξ)-24-methylcholest-5-en-3β-ol; campesterol (3a), (24*R*)-24-methylcholest-5-en-3β-ol; 22-dihydrobrassicasterol (3b), (24*S*)-24-methylcholest-5-en-3β-ol; cholesterol (4), 5-cholesten-3β-ol; 24-methylpollinastanol (5), 14α,24ξ-dimethyl-9β,19-cyclo-5α-cholestan-3β-ol; 24-ethylpollinastanol (6), 14α-methyl-9β,19-cyclo-5α-stigmastan-3β-ol; (7), 14α,24ξ-dimethylcholest-8-en-3β-ol; ergosterol (8), (24*R*)-methylcholesta-5,7,22E-trien-3β-ol; cycloeucalenol (9), 4α,14α-dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol; obtusifoliol (10), 4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol; cycloartenol (11), 4,4,14α-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol; lanosterol (12), 4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol.

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are far from being understood. Given the structural complexity of lipids in biological membranes, the identification of specific factors controlling the membrane fluidity at the molecular level is difficult. To gain more insight into phospholipid (PL)–sterol interactions, it was needed to use simple well-defined model membranes. Most of the studies have been devoted to interactions between cholesterol and either DPPC or egg PC. It has been demonstrated by a variety of physical techniques that cholesterol has a condensing (ordering) effect on the packing of PL in the liquid-crystalline (fluid) state, i.e., above their phase transition temperature and a fluidizing one below this temperature [11]. This condensing effect has been shown to depend on the molecular species of the lipids involved [12,13], the degree of fatty acyl (FA) unsaturation and the distribution of *cis*-double bonds between the acyl chains [13,14]. Thus, a possible role of cholesterol would be to control an intermediate-state fluidity in natural membranes.

Plant membranes differ from animal and fungal membranes by their higher content in polyunsaturated FA [15], which is probably an important feature for a good fit between plant PL acyl chains and alkylated plant sterols. In order to investigate whether plant sterols are able to regulate membrane fluidity as well as cholesterol does, we prepared model membranes constituted of soybean PC and sterol(s) in different molar ratios. Soybean PC has been chosen as a major ubiquitous PL of plant membranes [15]. The present work deals with effects of different plant sterols on the membrane fluidity as monitored by changes in the steady-state fluorescence anisotropy of the lipidic probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Effects induced by two unusual sterols, 24-methylpollinastanol (5) and 14α,24-dimethylcholest-8-en-3β-ol (7), were also investigated. These two sterols were previously shown to accumulate in plants treated with morpholine [16–18] and triazole [19–21] fungicides, respectively. Whereas N-substituted morpholines mainly block the cycloeucalenol-obtusifoliol isomerase [22], thus inducing an accumulation of 9β,19-cyclopropyl sterols like cycloeucalenol (9) and 24-methylpollinastanol (5), triazoles inhibit the obtusifoliol-14α-methyl demethylase [21], a cytochrome-*P*-450 monooxygenase, leading to an accumulation of sterols with a 14α-methyl group and a Δ8(9) such as obtusifoliol (10) and 14α,24-dimethylcholest-8-en-3β-ol (7). Under certain conditions, these unusual sterols can replace almost completely the normally occurring Δ5-sterols (sitosterol, stigmasterol and 24-methylcholesterol). The membrane response to these sterols is therefore of special interest.

Materials and Methods

Materials. Soybean L-α-phosphatidylcholine (PC) and phosphatidylglycerol (PG) were purchased from Sigma Chemical Co. (St Louis, MO) and used without further purification. Egg PC was isolated from egg yolks according to Nielsen [23]. Sitosterol (1) and stigmasterol (2) were from Fluka and campesterol (3a), cholesterol (4) and ergosterol (8), from Sigma. All sterols from commercial sources were checked for purity by GC as described previously [9]. Sitosterol was found to contain 8% 24-methylcholesterol (3) and stigmasterol, 3% sitosterol and 1% 24-methylcholesterol; campesterol and cholesterol were 99% pure; cycloartenol (11) was a gift from Dr. A. Milon and found to be 98% pure; ergosterol was recrystallized two times in methanol before use; 24-methylpollinastanol (5) was isolated from maize roots treated with fenpropimorph (20 mg/l) according to Bladocha and Benveniste [16] and was found to contain 2% 24-ethylpollinastanol (6). The sterol 14α,24ξ-dimethylcholest-8-en-3β-ol (7) was isolated from maize roots treated with LAB 170250F (5 mg/l); it was found to be 95% pure and to contain 2.5% 24-methylcholesterol and 2.5% 14α-methyl-stigmast-8-en-3β-ol. Fenpropimorph and LAB 170250F were kindly provided by BASF (F.R.G.). The fluorescent probe DPH was purchased from Sigma.

Preparation of vesicles. Large unilamellar vesicles (LUV) were prepared in 0.1 M NaCl buffered with 5 mM Hepes (pH = 7.4) by the reverse-phase evaporation procedure of Szoka and Papahadjopoulos [24] from soybean PC, PG and sterol(s) in different molar ratios. PG was present at 10 mol% in all the assays. Sterol-free vesicles were also prepared. To avoid the oxidation of PL fatty acyl chains, 1 mol% of butylated hydroxytoluene was systematically introduced in the assays and all the steps involved in the preparation of vesicles were performed under argon atmosphere. Sterol and phospholipid (PL) stock solutions were prepared in chloroform and in chloroform/methanol (98 : 2, v/v), respectively and stored at −20 °C in sealed vials.

Lipid phosphorus as well as sterol content of vesicles were determined after LUV recovery, i.e., after the last step consisting in a centrifugation at 10 000 × *g* for 4 min. Non-incorporated sterols were generally eliminated as precipitates. Thus, values given in the text correspond to PL and sterol amounts readily incorporated into vesicles and not to amounts added initially.

Lipid analysis. Sterols were extracted from liposomal preparations three times with 3 vol of hexane and quantified by GC with cholesterol or cholesteryl acetate as an internal standard as previously described [9]. Briefly, sterols were analyzed on a WCOT glass capillary column (25 m × 0.25 mm) coated with OV-1. The temperature program used included a fast rise from 60 to 230 °C at 30 C°/min, a slow rise from 230 to 280 °C at 2 C°/min and finally a plateau at 280 °C for 5 min. Standard errors were estimated to be ±5%. Total PL were determined by phosphate analysis according to Rouser et al. [25]. PL were checked for peroxidation of unsaturated FA by measuring the absorbance ratio $A_{233\,nm}/A_{215\,nm}$ [26] and for the presence of lyso compounds. No hydroperoxide was detected. Soybean and egg PC were found to contain 2% and 1% lyso PC, respectively. The fatty acid composition of soybean and egg PC was determined. FA methyl esters from both lecithins were prepared by transesterification using 0.1 M KOH in anhydrous methanol at 37 °C for 30 min and analyzed by GC on a Chromoptic glass capillary column (30 m × 0.32 mm) packed with DB wax. The column temperature was held at 50 °C for 5 min, then raised successively from 50 to 150 °C at 10 C°/min, from 150 to 192 °C at 3 C°/min and from 192 to 220 °C at 1 C°/min, and finally held there for 10 min.

Steady-state fluorescence anisotropy measurements. All the data were obtained on an SLM 8000 SC spectrofluorimeter in the T-format. The steady-state anisotropy (r_s) is defined as the ratio $(I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities measured parallel and perpendicular to the vertically polarized exciting beam. The excitation wavelength was 350 nm and the fluorescence light was detected by using 435-nm Schott interference filters. A home-built device ensured automatic rotation of the excitation polarizer. Measurements were performed at 20 °C. For all the assays, the turbidity measured by the OD at 350 nm was adjusted to 0.1 in order to limit light-scattering depolarization effects on fluorescence anisotropy [27]. Vesicles were labelled with 10^{-6} M DPH (from a 10^{-3} M stock solution in tetrahydrofuran) to give a final total lipid (PL + sterol) to probe molar ratio of 150–200 : 1. Suspensions were incubated for 30 min in the dark at room temperature just prior to fluorescence measurements.

Data analysis. By application of Perrin's formalism for rotational depolarization of a fluorophore, the fluorescence anisotropy r_s of DPH in membranes can be correlated with the apparent 'microviscosity' $\bar{\eta}$ [28]. More precisely, the fluorescence anisotropy parameter $[(r_0/r_s) - 1]^{-1}$, where r_0 is the limit anisotropy value in a rigid medium ($r_0 = 0.362$ [28]), is proportional to $\bar{\eta}$ and for comparative purposes can be used as a useful relative scale [28]. This microviscosity (or inversely the fluidity) is an average term which describes the bulk properties of the lipid bilayer.

Data were expressed as plots of the anisotropy parameter $[(r_0/r_s) - 1]^{-1}$ versus the sterol content (in mol%) of vesicles and analyzed with a linear least-squares curve fitting using a SAS procedure [29] that was applied to different theoretical models. The reliability of fitting was tested by means of a statistical analysis (correlation coefficients and F-test). In all the cases, the best fit ($r^2 > 0.94$ and $P < 0.001$) was given by the following exponential equation:

$$[(r_0/r_s) - 1]^{-1} = A\,e^{Bx}$$

The parameter B and its standard error were calculated after linear transformation, with the restriction that A was a fixed constant equal to either 0.323 or 0.38, which corresponded to the anisotropy parameter of sterol-free soybean and egg PC vesicles, respectively. Each determination of r_s resulted to the mean of eight readings of the fluorescence anisotropy and all experiments were performed in triplicate. Deviations between replicates were found not to exceed ±0.002.

According to Pottel et al. [30], an acyl-chain order parameter S can be estimated from DPH fluorescence anisotropy data using the following equation:

$$S = \frac{[1 - 2(r_s/r_0) + 5(r_s/r_0)^2]^{1/2} - 1 + (r_s/r_0)}{2(r_s/r_0)}$$

Calculations were done for vesicles containing 20 mol% sterol.

Results

Solubility of the different sterols in soybean PC vesicles

LUV were prepared from soybean PC, PG and sterol in different molar ratios. PL and sterol content of vesicles were systematically determined following LUV recovery. The yield of PL incorporation was generally high, around 80%. The sterol recovery was found to be very dependent on the sterol structure. The incorporation of the typical plant sterols: sitosterol (1), stigmasterol (2) and campesterol (3a) was investigated in comparison to that of cholesterol (4), the main animal sterol, and to that of ergosterol (8), the main yeast sterol. The incorporation of two other unusual sterols, 24-methyl-pollinastanol (5) and 14α,24-dimethylcholest-8-en-3β-ol (7), was also studied. The structures of all these sterols are shown in Fig. 1.

Among plant sterols, sitosterol (24-ethylcholesterol) and campesterol (24-methylcholesterol) were found to be those best incorporated into soybean PC vesicles (Fig. 2). The solubility limit of these two sterols is 30 and 34 mol%, respectively. Stigmasterol, i.e., 24-ethylcholesterol with an additional double bond at C-22, was incorporated into soybean PC with an efficiency two times lower (15 mol%).

Fig. 2 also indicates that cholesterol is incorporated into soybean PC with a relatively high efficiency (23 mol%), lower than that of sitosterol and campesterol, but higher than that of stigmasterol. Cycloartenol (11), the first biosynthetic intermediate with a steroid nucleus, is also very well solubilized, exhibiting a solubility limit of 34 mol%. In contrast, ergosterol has a very low solubility into soybean PC; whatever the concentration at which it was initially introduced (from 5 to 25 mol%), it was not incorporated with a yield higher than 3 mol%.

Concerning the two unusual sterols, it was found that 24-methylpollinastanol, a 9β,19-cyclopropyl sterol, was incorporated with an efficiency identical to that of

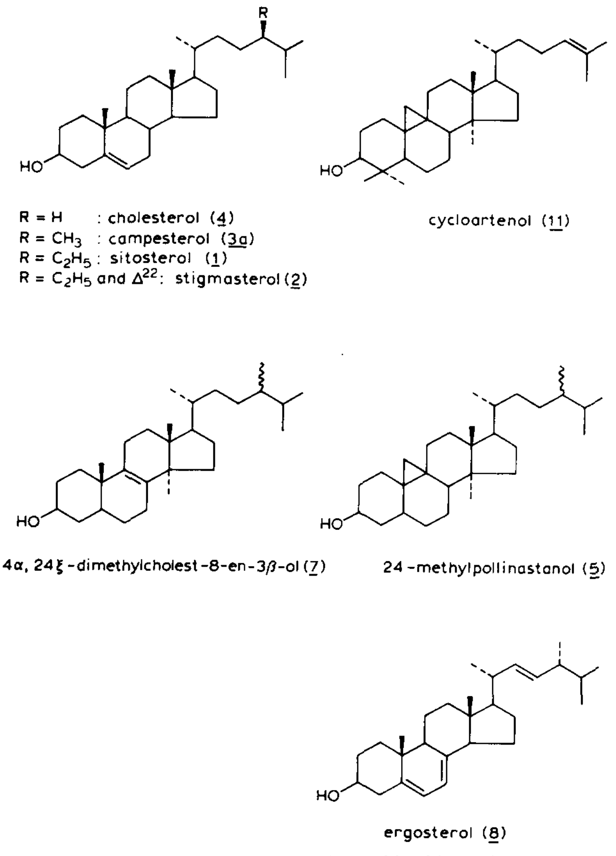

Fig. 1. Structures of sterols used in this study.

sitosterol (30 mol%) and 14α,24-dimethylcholest-8-en-3β-ol, with an efficiency (18 mol%) similar to that of stigmasterol (Fig. 2).

Steady-state fluorescence anisotropy measurements

Effects of the different sterols on the steady-state fluorescence anisotropy of DPH embedded into soybean PC vesicles were investigated at 20 ° C. Because the egg PC/cholesterol model system has been often taken as a reference, it was interesting first to compare the behavior of cholesterol incorporated into either egg PC or soybean PC bilayers. The FA composition of both kinds of lecithins was determined by GC of the corresponding FA methylesters as described in Materials and Methods. Table I clearly indicates that both lecithins are very different as far as their content in polyunsaturated FA is concerned, whether it is expressed as the unsaturation index (i.e., the sum of the mol% unsaturated FA multiplied by the number of double bonds per acyl chain) or as the mole percentage of unsaturated FA. Fig. 3 shows that, whatever the cholesterol content of vesicles, the DPH anisotropy parameter values are considerably higher for egg PC bilayers than those for soybean PC bilayers. This result is in agreement with the previously reported lower ability of cholesterol to condense bilayers of polyunsaturated PL than bilayers of saturated PL [13,14,30].

In Fig. 4 are shown the effects of different plant sterols on the anisotropy parameter of DPH embedded into soybean PC vesicles. Whatever the structure of the sterol incorporated, an increase in the sterol content of vesicles was found to correlate with an increase in the

TABLE I

Fatty acid composition of egg and soybean PC

Fatty acid	Egg PC	Soybean PC
16:0	31.5[a]	13.5[a]
18:0	13	4
18:1	30	12
18:2	16.5	63
18:3	0.2	6.5
Others	8.8	1
U.I.[b]	0.9	1.58
UFA/SFA[c]	1.25	4.71

[a] Percentage of total PL acyl chains.

[b] U.I., unsaturation index = [1·(% monoenes) + 2·(% dienes) + 3·(% trienes)]·100.

[c] UFA/SFA, unsaturated FA/saturated FA.

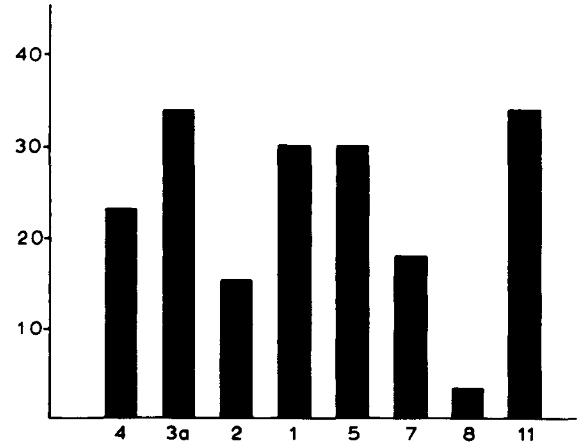

Fig. 2. Comparative limit solubilities of different sterols in soybean PC LUV.

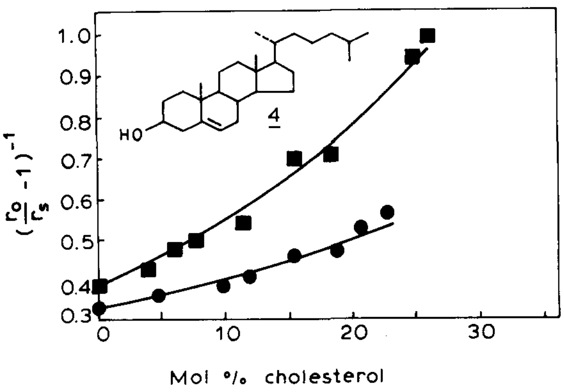

Fig. 3. Comparative effects of cholesterol incorporated into either egg or soybean PC vesicles on the DPH anisotropy parameter. Egg PC (■——■) ($r^2 = 0.9904$) and soybean PC (●——●) ($r^2 = 0.9538$).

fluorescence anisotropy parameter, indicating that they are all able to regulate the fluidity of soybean bilayers, with, however, very different efficiencies. It can be seen that sitosterol and campesterol are the most efficient sterols and stigmasterol, the least efficient. Cholesterol exhibits an intermediate behavior as cycloartenol does. Both unusual sterols, 24-methylpollinastanol and 14α,24-dimethyl cholest-8-en-3β-ol, appear to behave rather similarly to stigmasterol.

The experimental data given in Figs. 3 and 4 were found to fit best with an exponential relationship between the DPH anisotropy parameter and the sterol content of vesicles. If we assume that the probe motion closely reflects the extent of surrounding acyl chain motion, then it should be directly related to an acyl-chain order parameter. As reported previously [30,32], the order parameter S can be estimated from DPH steady-state fluorescence anisotropy data. Table II lists the S values calculated for vesicles containing 20 mol% sterol by using the equation described in Materials and Methods. Although the solubility limit of stigmasterol was found to be 15 mol%, an extrapolation of experimental results was done only with the purpose of comparing the behavior of this sterol with that of other sterols. As expected, the highest S value was obtained for cholesterol-containing egg PC vesicles. The incorporation of sterols into soybean PC vesicles is accompanied by an increase in the order parameter, i.e., a decrease of the amplitude of acyl chain motion. Sitosterol and campesterol are the most efficient sterols. Cycloartenol exhibits a behavior very similar to that of cholesterol. Stigmasterol, 24-methylpollinastanol and 14α,24-dimethylcholest-8-en-3β-ol present very low efficiencies for ordering soybean PC bilayers.

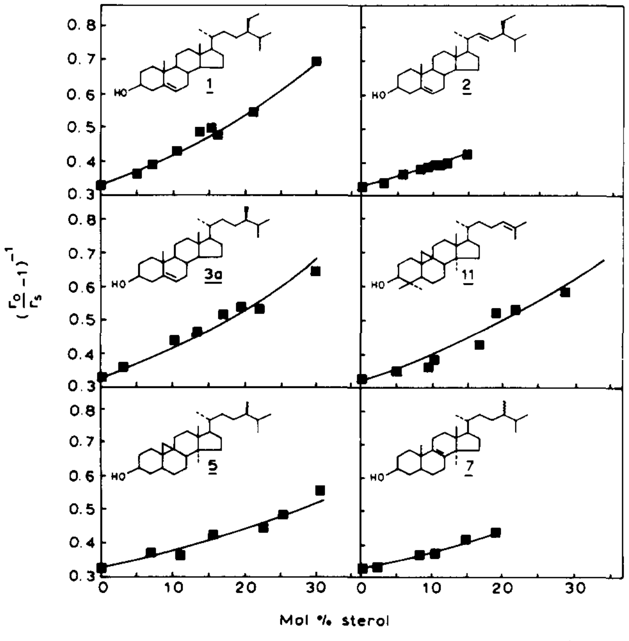

Fig. 4. Effects of different plant sterols on the DPH anisotropy parameter of soybean PC vesicles. (1) $r^2 = 0.9899$; (2) $r^2 = 0.9918$; (3a) $r^2 = 0.9655$; (11) $r^2 = 0.9442$; (5) $r^2 = 0.9535$; (7) $r^2 = 0.9706$.

TABLE II

Order parameter values calculated from DPH fluorescence anisotropy data for soybean PC vesicles containing 20 mol% of each sterol

Lipid composition	Sterol content (mol%)	r_s/r_0	S
Egg PC	0	0.275 [a]	0.337 [b]
Egg PC/cholesterol (4)	20	0.435	0.543
Soybean PC	0	0.244	0.295
Soybean PC/sitosterol (1)	20	0.350	0.436
Soybean PC/campesterol (3a)	20	0.346	0.430
Soybean PC/cycloartenol (11)	20	0.334	0.415
Soybean PC/cholesterol (4)	20	0.329	0.409
Soybean PC stigmasterol (2)	20	0.312	0.386
Soybean PC/24-methyl-pollinastanol (5)	20	0.306	0.379
Soybean PC/14α,24-dimethyl-cholest-8-en-3β-ol (7)	20	0.301	0.371

[a] r_s/r_0 values were calculated from curves of Figs. 3 and 4 ($\Delta r_s = \pm 0.002$).

[b] Standard deviations $\Delta S = \pm 0.007$.

Discussion

In order to gain more insight into the ability of the different plant sterols to interact with PL, we undertook a systematic study using model systems constituted of soybean lecithin, a well-represented plant PL, and sterols in different molar ratios. Because of the high polyunsaturated FA content of soybean PC (Table I), special attention was paid to avoid the presence of oxygen as much as possible during the preparation of vesicles. Polyunsaturated FA are indeed very susceptible to oxidation. For instance, they can generate hydroperoxides which have been shown to affect the DPH fluorescence anisotropy [33].

First, our results clearly show that the typical plant sterols (sitosterol (1), stigmasterol (2) and campesterol (3a)) can be incorporated into soybean PC LUV. The significantly lower incorporation of stigmasterol (15 mol%) compared to those of sitosterol and campesterol (30–34 mol%) (Fig. 2) indicates that the introduction of a double bond in the side-chain greatly affects the sterol solubility. Cholesterol (4) exhibits an intermediate solubility (23 mol%). Ergosterol (8), the typical yeast sterol, is shown to be incorporated into soybean PC vesicles to a very low extent (3 mol%) (Fig. 2). The side-chain of ergosterol differs from that of campesterol by both the stereochemistry at C-24 and the presence of a double bond at C-22. The introduction of this double bond probably also affects the sterol solubility as in the case of stigmasterol. The maximal incorporation of the two unusual sterols, 24-methylpollinastanol (5) and 14α,24-

dimethylcholesta-8-en-3β-ol (7), into soybean PC vesicles was found to be similar to those of sitosterol and stigmasterol, respectively.

To investigate whether plant sterols can regulate the plasma membrane fluidity of higher plant cells as well as cholesterol does in animal cells, the typical plant sterols were tested for their effects on the DPH steady-state fluorescence anisotropy. Studies were performed with varying sterol structures and contents of soybean PC vesicles.

All the sterols tested were shown to be able to increase the DPH fluorescence anisotropy parameter of sterol-free vesicles (Figs. 3 and 4). The best fit between the DPH anisotropy parameter data and the sterol content of vesicles was found to be an exponential relationship rather than a linear one as reported previously [34]. We impose on all the curves the same DPH anisotropy parameter value for sterol-free vesicles, i.e., 0, 38 and 0.323 for egg and soybean PC vesicles, respectively. It should be kept in mind that in the absence of sterol, the acyl chain terminal region of PL is quite disordered. Thus, a significant fraction of DPH molecules might be oriented parallel to the plane of the bilayer [35], with as a consequence a possible modification of the DPH fluorescence anisotropy [35].

If we assume that the DPH fluorescence anisotropy reflects the average extent of acyl chain motion within the bilayer, then it should be directly related to an acyl-chain order parameter and taken as a measure of the molecular packing of acyl chains. The study of the comparative behavior of cholesterol in egg and soybean PC bilayers emphasizes the importance of the unsaturation state of PL acyl chains. Fig. 3 and Table II indicate that the ordering influence of cholesterol in soybean PC is greatly reduced as compared to that in egg PC, a result which is in complete agreement with other data [13,14,31]. It is commonly accepted that the introduction of *cis*-double bonds into the FA chains of PL induces an increase of the area per molecule [14], resulting in a marked decrease of the molecular order of acyl chains in the liquid-crystalline state [13,31]. Cholesterol was shown to reverse this effect by limiting the probability of *trans-gauche* isomerization in the PL acyl chains. All the data so far reported show a preference for a saturated FA at the *sn*-1 position of the glycerol backbone and a preference for an unsaturated FA at the *sn*-2 position. Sterol-PL interactions mainly involve Van der Waals interactions. According to the model proposed by Huang et al. [36], the two angular methyl groups of the β-surface of the cholesterol molecule are thought to be accommodated by unsaturated acyl chains through a hydrophobic pocket generated by a *trans-gauche* kink adjacent to the $\Delta 9$ double bond. Thus, the cholesterol nucleus should fit between the carbonyl group of the acyl chain and this kink. The α-face of the cholesterol molecule is thought to interact with the saturated acyl chain of the adjoining PL [36]. In cases where the PL is highly unsaturated, it is clear that unsaturated FA will be found at the *sn*-1 position. Taking into account the above considerations, we can expect that plant sterols will induce weaker ordering effects on 'plant' lipids than cholesterol on more saturated 'animal' lipids. This is precisely what we have found. All the order parameters values listed in Table II are lower than that found for the cholesterol/egg PC reference model. At 20 mol%, sitosterol and campesterol are the most efficient sterols for increasing the order parameter of soybean PC bilayers, even more efficient than cholesterol, the standard of reference. As the order parameter S is believed to reflect the lipid packing of bilayers, our results indicate that sitosterol and campesterol could play a significant role in the regulation of membrane properties of higher plant cells. In contrast, stigmasterol exhibits a relatively low efficiency to order soybean PC bilayers. Such a behavior might appear surprising. This is a major sterol in many plant organs like corn roots [16]. It differs from sitosterol only by the double bond at C-22. According to a recent computer conformational study of the sterol side chain (Milon and Hartmann, unpublished results), the introduction of this $\Delta 22$ double bond clearly induces an increase in the amplitude of motion of the stigmasterol side chain terminal part. This might be a critical factor for a good fit with the terminal part of the acyl chain. Thus, a minor structural modification in the sterol molecule can lead to considerable changes in its ordering properties. Because of its low ordering efficiency, stigmasterol seems not to behave as a membrane reinforcer. Brassicasterol, a sterol with a side chain similar to that of ergosterol, also interacts with unsaturated lecithins more poorly than both epimers of 24-methylcholesterol [37]. A better interaction of stigmasterol with other PL species should not be excluded. This sterol was indeed recently found to induce effects similar to those of sitosterol and cholesterol for increasing the order of dioleoyl PC bilayers [38]. Thus, both the number and the position of the *cis*-double bonds in the acyl chains are probably of importance.

Cycloartenol exhibits an ability similar to that of cholesterol for ordering soybean PC bilayers. These two sterols also order the acyl chains of egg PC to the same extent, as evidenced by spin-label studies [39]. In contrast, lanosterol (12), an isomer of cycloartenol and the precursor of cholesterol in mammalian cells, behaves in a quite different way [39]. 24-Methylpollinastanol, another 9β,19-cyclopropyl sterol, was found to have a significantly smaller effect than cycloartenol (Table II). The rationale for such a difference remains to be elucidated.

Since the hypothesis proposed by Bloch [40], several works have been recently devoted to the possible conformations of cyclopropyl sterols [41,42], but no information about the conformation of these compounds embedded in membranes is available at this time. Anyway, it is clearly established that the cyclopropane ring of cyclopropyl sterols gives some flexibility to these molecules at the level of the ring C, affecting somewhat the angular orientation of the 14α-methyl group. Such a behavior is clearly different from that exhibited by compounds like obtusifoliol (10) and 14α,24-dimethylcholest-8-en-3β-ol. The presence of the Δ8(9) double bond makes the molecule more rigid and planar. As a consequence, the 14α-methyl group projects from the α-face. Several authors have emphasized the importance of a planar sterol α-face for interactions with PL acyl chains [40]. The lower efficiency of lanosterol for ordering egg PC bilayers compared to those of cholesterol and cycloartenol was suggested to be attributable primarily to the 14α-methyl group. However, whatever the respective conformation of 24-methylpollinastanol and 14α,24-dimethylcholest-8-en-3β-ol, these unusual sterols, which accumulate in plants treated with morpholine [16–18] or triazole [19–21] fungicides, exhibit a low efficiency for ordering soybean PC bilayers (Table II), suggesting that their presence in plant membranes in place of Δ5-sterols probably induces a looseness of the molecular packing of PL acyl chains and consequently an alteration of membrane functions.

In conclusion, our results clearly demonstrate that the different plant sterols, which have been tested in this study, do not interact with soybean PC with the same efficiency. Whereas sitosterol and campesterol probably play a significant role in regulating membrane properties of higher plant cells, the specific role of stigmasterol still remains to be elucidated. Further studies are planned to investigate in more detail the structural features of the sterol molecule needed for an optimal interaction with main PL species, and also their involvement in membrane functions (for instance, the membrane permeability).

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