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Original Article

Interactions among phospholipids of guinea pig rough microsomes, effect of fat deficiency

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Summary

The fatty acid composition and the steady-state fluorescence anisotropy (r_s) of 1,6-diphenyl-1,3,5-hexatriene (DPH) were determined for each of the five major phospholipid (PL) classes present in the liver rough microsomes (RM) of guinea pigs fed with control and fat-deprived diets. In order to obtain information about PL-PL interactions and their contribution to the overall membrane fluidity the experimental r_s of phospholipid mixtures were compared to the molar weighed average values of the individual phospholipid r_s values. The PL ratios in the mixtures were the same to those found in the RM membranes. Binary mixtures of phosphatidylcholine (PC) with phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM) show higher values of r_s than those estimated from the individual component parameters indicating a 'rigidizing' interaction. The rigidizing effect of PE was also observed when this phospholipid was sonicated with mixtures of PC with PS and PI. However, no rigidizing effect of PE was observed in the PC bilayers when SM was included in the composition suggesting that PE-SM interactions prevent 'rigidizing' effects of PE. Besides, in spite that PC-PI and PC-PS mixtures have 'rigidizing' interactions, the incorporation of PI and PS to PC-PS and PC-PI mixtures, respectively, have a 'fluidizing' effect. In consequence, phospholipid polar head groups interact in RM membranes modifying the molecular packing and/or the rotational diffusion of acyl chains. The complexity and variety of constituent phospholipids could prevent major changes in the fluidity. The comparison of results obtained with PL mixtures of control and fat-deficient animals indicate that a change in the number of double bonds does not evoke a significant difference between either the 'rigidizing' of 'fluidizing' effects. However, there is a general tendency indicating that phospholipids with higher double bond index evoke lower 'rigidizing' and 'fluidizing' interactions. Moreover, PL of animals fed a fat-deficient diet have less fluidity than those of control animals.

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Introduction

The reasons for the great variety of lipids found in biological membranes and the relationship between lipid composition and membrane functions pose major unsolved problems in membrane biology. Lipid diversity can be rationalized on the basis that integral protein functions may be modulated by the fluidity of the bilayer matrix and/or by specific compositions of the boundary lipids at the protein-lipid interface. Boundary lipids rapidly exchange with membrane bulk lipids [1, 2] but, this event not necessarily prohibits that specific lipids can constitute microenvironments surrounding the proteins. Membrane fluidity has been considered important in regulating some membrane functions [3, 4] and it has been designated 'viscotropic regulation' [5]. Moreover, evidence exists that differences in the conformation or disposition of membrane proteins in the presence of different phospholipids [6] are due to altered fluidity [7].

The lipid and fatty acid composition of membranes is unique for each type of membrane and it is, to a great extent, species independent [8] suggesting a specific role for many lipids or particular combinations of lipids. Although physical studies have been done on individual phospholipids isolated from natural sources, no studies have been carried out in phospholipid mixtures derived from a single membrane. In order to determine how each phospholipid component contributes to the overall membrane properties, it is necessary to know the individual phospholipid properties and the effect of their interaction on the physical properties. In these experiments, we have determined the 'fluidity' of the five major phospholipid classes present in the guinea pig liver rough endoplasmic reticulum membrane and the corresponding physical properties of a set of phospholipid mixtures. In order to determine the fatty acid composition effect on the phospholipid-phospholipid interaction, the same study was done with phospholipids isolated from guinea pigs fed on a control or a fat free diet.

In this research, the term 'fluidity' is used to indicate the dynamic-structural properties checked by steady-state fluorescence anisotropy (r_s) of membranes labeled with 1,6-diphenyl-1,3,5-hexatriene (DPH).

Materials and methods

Animals and diets

Male guinea pigs, fourty to fifty days old, weighing 150–200 g were divided in two groups. One group was fed a semi-synthetic diet according to Reid and Briggs [9] and the other the same purified diet where sucrose substituted corn oil. Water was given ad libitum. Animal were killed by decapitation on 26 days after the administration of the diets.

Membrane preparation

Rough endoplasmic reticulum membrane was obtained according to the technique of Blobel *et al.* [10] with several minor modifications of Ramsey *et al.* [11]. Effective purification of the membranes was indicated by the following ratios of membrane-bound RNA [12] to protein [13] (mg/mg): 0.21 and 0.22 for control and fat deficient membranes, respectively. Since no cardiolipin was found, contamination with mitochondria could be excluded.

Lipid composition

Lipids were extracted by Folch *et al.* [14] procedure. Phosphorus was measured by the method of Chen *et al.* [15]. Phospholipids were separated by two-step thin-layer chromatography (TLC) procedure on Silica gel H plates [16]. The solvents used were chloroform:methanol:ammonia:water (70:25:3.5:1.5) and chloroform:methanol:acetic acid:water (80:10:2:0.75). They were visualized by exposure to iodine vapors. Unexposed spots were scrapped and eluted with methanol. The methyl esters were prepared by transesterification with 3 M HCl in methanol (5 h at 64°C). The fatty acid composition of individual phospholipids was determined by GLC-FID in a Hewlett-Packard model 5840-A Chromatograph equipped with the 5840-A GC terminal and using a 6-ft column filled with 10% SP 2330 on 100/200 mesh Chromosorb WAW.

Steady-state fluorescence anisotropy determinations

Fluorescence anisotropy (r_s) was determined according to the fluorescence polarization technique of Shinitzky and Barenholz [17] using the lipid-soluble fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene. Measurements were carried out in an Aminco Bowman spectrofluorometer equipped with two glan prism polarizers. Temperature was monitored by a thermistor placed in the sample just above the excitation beam and controlled by a circulating water bath. Vertical and horizontal emission values were taken at 440 nm with excitation at 362 nm in the vertical plane. The polarization ratio P is defined as I_{II}/I_I $G \cdot I_{II}$ and I_I refer to intensities emitted, parallel and perpendicular, to the vertically polarized excitation and G is a factor for instrumental correction [18]. The polarization alignment was verified using fluorescein in alkaline glycerol at 10°C [19]. The maximum reached by P in this system was 2.75 ± 0.05. The steady-state fluorescence anisotropy of DPH was calculated using the following equation:

$$r_s = \frac{P - 1}{P + 2}$$

Unlabeled samples with the same concentration were used as reference blanks which served to correct for the excitant light scattering and other contributions to the fluorescence signal. 2.0 M $NaNO_2$ solution placed between the emission monochromator and the photomultiplier was used as a cut-off filter for the wavelengths below 390 nm. Light scattering was less than 5%.

Preparation of phospholipid dispersions and DPH labeling of samples

Individual phospholipid fractions or phospholipid mixtures used for fluorescence measurements were separated by two-step TLC procedure previously described [16] and extracted from the silica with methanol. To detect possible contaminations in the phospholipid fractions, an aliquot of the methanolic extract was rechromatographed on TLC. Contaminated mixtures were discarded. The phospholipid molar ratios of the phospholipid mixtures used, were always those naturally found in the membranes.

Chloroform solutions of membrane phospholipids were dried under N_2 in tubes and then dispersed in 50 mM, pH 7.2, potassium phosphate buffer, by sonication for 10 min in a Heat System Sonicator at 40°C. In addition Mg^{+2} 0.8 mM derived from the Florisil of the TLC plates was detected in all the lipid dispersion using atomic absorption spectroscopy. Dispersions were centrifuged for 10 min at 7,000 × g in a SS34 Sorvall rotor and the supernatant collected for fluorescence measurements. In each case, 0.35–0.45 mg of phospholipids were dispersed in 4.0 ml of buffer.

Deoxigenated lipid dispersions were labeled by adding 3–5 ul of DPH in tetrahydrofuran, and incubating during 30 minutes in dark with slight agitation. In each case, the DPH:PL ratio was 1:200.

Results

Lipid composition

Table 1 shows the main features of the fatty acid composition of guinea pig rough microsomes (RM) phospholipid fractions from animals fed on a fat-free and control diets. Data evidence a decrease of linoleic and arachidonic acids and an increase of oleic and palmitoleic acids in all glycerophosphatides after 26 days of fat deprivation except for phosphatidylethanolamine that showed an increase of arachidonic acid. Sphingomyelin fraction showed the absence of arachidonic acid, the lowest percentage of unsaturated acids, and a high proportion of long chain saturated fatty acids mainly 22:0 acid.

Data in Table 1 also demonstrate a decrease in the double bond index ratio in all the phospholipid fractions after 26 days of essential fatty acid deficiency. No significant changes in the phospholipid distribution was found in these experiments.

Phospholipid-phospholipid interactions

The order of the hydrocarbon chains as sensed by DPH may be thought of as an average order for the membrane bilayer. The rod-shaped molecule of DPH inserts into the hydrocarbon portion of the membrane, and the fluorescence anisotropy is dependent on the motional freedom of the molecule in this environment. The motional freedom of DPH in a phospholipid (PL) dispersion depends on the phospholipid-phospholipid polar head group interactions and on the fatty acyl chain interactions. Then, if we have two PL dispersions, A and B with r_s^A and r_s^B fluorescence anisotropies, ideal mixtures of these phospholipids in which r_s values will be intermediate between r_s^A and r_s^B, will have a fluorescence anisotropy given by:

$$r_s^{A/B} = r_s^A x^A + r_s^B x^B$$

where x^A and x^B are the molar fractions of the components A and B in the mixture. If r_s is the fluorescence anisotropy of the A-B phospholipid dispersion, (interacting mixture) with the same molar fractions than the phospholipids in the ideal mixture, the ratio:

$$\gamma = r_s^{A\text{-}B}/r_s^{A/B}$$

is a parameter that may be used to estimate approximately PL-PL interactions. The γ-value will be higher, lower or equal to unity depending on the nature of the phospholipid-phospholipid polar head group interactions, and fatty acyl chain interactions. Attractive forces between A-B molecules larger than in A-A or B-B molecules would lead to values of γ-higher than 1 and attractive forces between A-B molecules less than between A-A or B-B molecules produce values of $\gamma<1$. Phospholipid mixtures with γ-values higher than unity will be called 'rigidizing' mixtures and those values lower than unity will be designated 'fluidizing' mixtures.

Fluorescence anisotropy data of DPH for phospholipid fractions or phospholipid mixtures em-

Table 1. Composition of principal fatty acids of phospholipid fractions obtained from control and fat-deficient guinea pig rough microsomes

Fraction		Fatty acids						
	% P_i[a]	16:0	18:0	16:1	18:1	18:2	20:4	DBI[b]
PC								
C	52.7	10.2	32.1	0.2	8.9	42.8	4.1	1.17
FD	48.6	11.7	34.9	0.9	11.6	36.1	3.3	1.02
PE								
C	22.3	8.5	28.3	1.2	7.8	34.0	13.5	1.62
FD	23.7	12.1	31.3	3.1	11.1	20.6	15.8	1.42
PI								
C	11.2	4.3	42.3	1.0	8.5	19.0	18.7	1.43
FD	13.1	2.4	47.6	2.8	12.8	10.0	14.0	1.27
PS								
C	7.0	7.6	40.5	1.4	8.1	25.1	10.6	1.25
FD	7.0	7.3	44.3	2.0	12.7	17.8	7.8	1.05
SM[c]								
C	6.8	10.0	40.9	0.7	7.8	14.7	0.0	0.44
FD	7.6	10.0	34.7	2.5	8.6	7.8	0.0	0.33

[a] Percent of P_i in total membrane phospholipids.

[b] DBI: number of double bonds per fatty acyl chain.

[c] 22:0 comprise a 16% of the control-SM fatty acids and about a 20% of the FD-SM fatty acids.

C: control; FD: fat deficient.

ployed in these physical studies, are shown in Table 2. The r_s values of the different pure lipids show a decrease in the fluidity (higher r_s) following the order PC>PE>SM>PI>PS. Besides, the corresponding fluidity of the lipids in fat deficient animals is lower than in control animals. A similar result is shown when the phospholipid mixtures are compared.

Figure 1 shows the γ-values obtained for different mixtures of phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI) derived from normal and fat-deficient guinea pig rough microsomes. Data evidence that binary mixtures of PC-PI and PC-PS have larger values of DPH fluorescence anisotropy than those obtained from the addition of the independent r_s values. Incorporation of PI to PC have a lower 'rigidizing effect' than PS incorporation.

Results in Fig. 1 also indicate that phospholipid mixtures of PC and PI or PC and PS from fat-deficient animals, that is with DBI smaller than those phospholipids of control animals, have higher values of γ than those mixtures obtained from control animals. Since phospholipid proportions are practically the same in the control and fat-

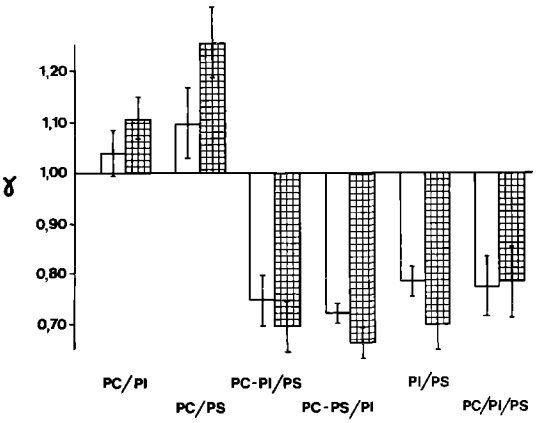

Fig. 1. Ratios of experimental to calculated steady-state fluorescence anisotropy of DPH in phospholipid dispersions of PC, PI and PS obtained from control (open bars), and fat-deficient guinea pig rough microsomes (filled bars). Bars separating components of a mixture indicate which components or component mixture were employed to estimate the γ-values (i.e. PC/PI/PS indicates that γ-value was obtained from the anisotropies of these three individual phospholipids, while PC-PI/PS indicates that the r_s of the PC-PI mixture and the r_s of the PS fraction were used to calculate the γ-value). The r_s values and the molar fractions of the phospholipid mixtures or fractions were the same indicated in Table 2. Vertical bars correspond to limit error of γ calculated from the standard deviations of r_s values and the molar fractions used.

Table 2. Steady-state fluorescence anisotropy of DPH in phospholipid mixtures at 37°C

Phospholipid dispersions	Control		Fat-deficient	
	r_s	Molar ratio	r_s	Molar ratio
PC	0.072		0.079	
PE	0.082		0.087	
PC+PE	0.083	70.3:29.7	0.103	67.2:32.8
SM	0.129		0.132	
PC+SM	0.090	88.6:11.4	0.099	86.5:13.5
PC+PE+SM	0.081	64.4:27.3:8.3	0.084	60.8:29.7:9.5
PC+PS	0.096	88.3:11.7	0.125	87.4:12.6
PS	0.205		0.240	
PI	0.173		0.188	
PC+PI	0.093	82.5:17.5	0.113	78.8:21.2
PI+PS	0.144	61.5:38.5	0.144	65.2:34.8
PC+PI+PS	0.078	74.3:15.8:9.9	0.091	70.7:19.1:10.2
PC+PI+PS+PE	0.125	56.5:12:7.5:24	0.172	52.6:14.2:7.6:25.6
PC+PS+PI+SM	0.083	67.8:14.4:9:8.8	0.102	63.7:17.2:9.2:10
PC+PI+PS+SM+PE	0.081		0.107	

Molar ratios of phospholipid mixtures are the same than those found in membranes. Standard deviation of r_s values ranging from 2×10^{-3} to 4×10^{-3}.

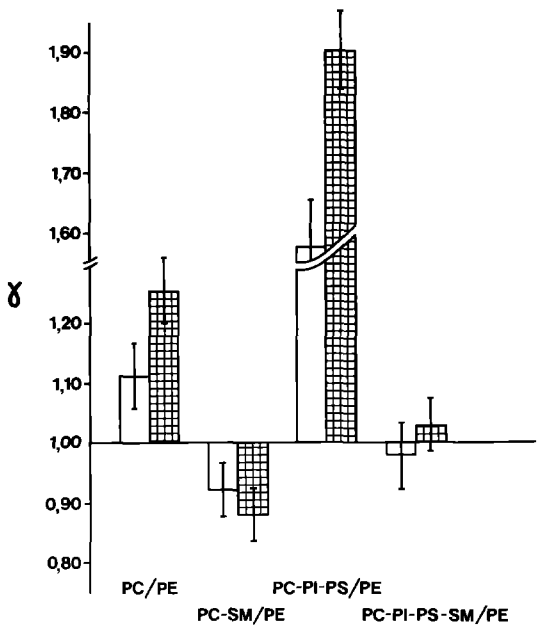

Fig. 2. Gamma ratios of experimental to calculated steady-state fluorescence anisotropies of DPH in phospholipid mixtures containing PE fractions. Explanations are detailed in Fig. 1.

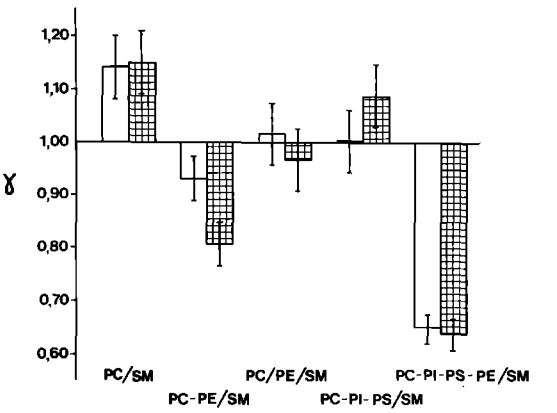

Fig. 3. Gamma ratios of experimental to calculated steady-state fluorescence anisotropies of DPH in phospholipid mixtures containing SM fraction. Explanations are detailed in Fig. 1.

deficient samples, the differences mentioned above would reflect the fatty acid composition effect on the phospholipid-phospholipid interactions.

Figure 1 also shows that, inversely to that observed on binary mixtures of PC-PI or PC-PS, the addition of PI to the PC-PS mixtures or the incorporation of PS into the PC-PI mixtures, have a 'fluidizing' effect since the values obtained were lower than unity. Similarly, γ-values of PI and PS mixtures (γ-PI/PS) indicate that there is a 'fluidizing' effect produced by PI-PS interactions.

Comparison of γ-values of normal and fat-deficient samples shows that, the phospholipid samples with higher DBI have in general a smaller 'fluidizing' effect. However, the results are not statistically significant. Finally, γ-values obtained from the anisotropies of PC, PI and PS (γ-PC/PI/PS) isolated from both, normal and fat-deficient animals, show that ternary mixtures of these three phospholipids constituted 'fluidizing' mixtures.

Figure 2 shows the γ-values of the phospholipid mixtures obtained by addition of phosphatidylethanolamine to PC mixtures from control and fat-deficient guinea pig rough microsomes. PC/PE γ-values higher than unity indicate a 'rigidizing' effect of the PC/PE interactions, while the γ-value lower than unity found after the addition of PE to a PC-SM mixture indicates a 'fluidizing' effect. Therefore, the presence of SM prevents the 'rigidizing' effects of PE.

Similarly, data in Fig. 2 also show that the incorporation of PE to a PC-PI-PS mixture has a 'rigidizing' effect and, this effect is even higher when compared to that observed when PE is mixed with PC. Correspondingly, the presence of SM in the mixture eliminates the 'rigidizing effects' of PE.

The comparison of membranes from normal and fat-deficient guinea pigs revealed an approach of fat-deficient samples (less unsaturated) to major 'fluidizing' and 'rigidizing' effects than those from control animals.

In Fig. 3 the γ-values obtained by the incorporation of SM into membranes of PC, PC-PE, PC-PI-PS and PC-PI-PS-PE, are represented. Results for PC-SM mixture indicate that there is a 'rigidizing' interaction between these two phospholipids. In binary mixtures of PC-PE or in quaternary mixtures of PC-PI-PS-PE, the addition of SM causes a 'fluidizing' effect, lowering the 'rigidizing' effect of these two independent lipids over PC. Only 'rigidizing' effects of SM fraction were observed when it was added to a mixture of PC-PI-PS from fat-deficient animals.

Discussion

Phospholipids are necessary constituents of biological membranes and their physico-chemical properties are important factors that determine their structure and functions. Physical properties of phospholipids or phospholipid mixtures depend on the nature of the polar head group and on the fatty acyl chain compositions. Thus, the molecular packing of phospholipids in membranes depend on space requirements and interactions of the polar head groups, and on the space requirements and interactions of the hydrocarbon chain. The presence of both, negatively and positively charged moieties and hydrogen bond donating and accepting groups in the polar heads of phospholipids, allows them to interact with each other as well as with proteins [20]. Heteromolecular interactions among lipids are increased as long as suitable hydrogen bond donating and accepting groups are present [21].

In the present experiment steady-state fluorescence anisotropies of heteromolecular mixtures of natural phospholipids of guinea pig liver microsomes labeled with DPH were used to investigate the effect of polar head group interactions and fatty acid chains on the fluidity of the hydrophobic zone.

Time-resolved fluorescence anisotropy decay measurements have revealed that the fluorophore rotations are restricted by a static factor [22, 23] related to the molecular packing of lipids and a dynamic factor which is proportional to the microviscosity [24]. The steady-state fluorescence anisotropy r_s can be resolved in a static part r_{∞} and a dynamic part, r_f [25, 26], and if the total fluorescence intensity is assumed to follow a simple exponential decay the r_s of a phospholipid mixture A-B is:

$$r_s^{A\text{-}B} = r_f + r_\infty = \frac{r_0 - r_\infty^{A\text{-}B}}{1 + \tau^{A\text{-}B}/\varphi^{A\text{-}B}} + r_\infty^{A\text{-}B}$$

where r_0 is the value of r_s in the absence of rotation, τ the fluorescence lifetime and φ the rotational correlation time.

In order to obtain further information upon the phospholipid-phospholipid interactions and on the effect of the different phospholipid fractions over the membrane properties, we have compared the $r_s^{A\text{-}B}$ value of a phospholipid mixture with the r_s values of their individual components by the parameter γ (equations 1 and 2). Since the r_s of a membrane depends on both, the rate and range of wobbling motion, as well as on the fluorescence lifetime, the validity of the γ-values as interaction coefficients will be restricted to the variation of τ. Thus, values of γ higher than unity will imply larger $r_\infty^{A\text{-}B}$ and/or $\varphi^{A\text{-}B}$ values than the respective molar weighed average values of r_∞^A, r_∞^B and φ^A, φ^B, only if the value of $\tau^{A\text{-}B}$ is not lower than the molar weighed average value of the lifetimes of the A, B components. However, as the lifetimes are shorter in fluid phases than in more solid phases [27] and, in the systems studied the τ values of PL mixtures were nearly always the molar weighed average value of the lifetimes of the individual phospholipid [28, 29], the γ-parameter will be a proper estimation of the PL-PL interaction. Only some underestimation of both, 'rigidizing' or 'fluidizing' interactions, would be expected to be produced by changes in the lifetimes of DPH.

Binary mixtures of lipids have been already used to study the interactions among different lipid species. However, mixing PC with PE [30–31], PC with PS and SM with PC [32] in the gel state was found to be less than ideal. Although a better mixing occurs in the liquid-crystalline state, simulation of phase diagrams for different PC-PE mixtures indicates that mixing is not ideal in the liquid-crystalline phase, either [33, 34].

Presently, studies using fluorescence anisotropies indicate that in the four binary mixtures of PC: PC-PE, PC-PI, PC-PS and PC-SM under the effect of heteromolecular interactions, the 'fluidity' of the mixture was decreased more than it was expected in accordance to the properties of the individual components. These results would indicate that heteropolar interactions are stronger than the homopolar interactions of PC for all the phospholipids studied and that the interactions of the polar heads increase the packing modifying the freedom of movement in the hydrophobic zone. These effects might be interpreted on the basis that, while all the phospholipids mixed with PC have donating and

accepting hydrogen groups, PC has only accepting groups [20, 35]. Then, in isolated PC bilayer the hydrogen bonds among the polar head groups do not exist [36], while in the binary mixtuires, PC could form part of a hydrogen-bond network through the donor groups of the other incorporated phospholipids.

Despite the 'rigidizing' nature of our mixtures of PC-PI and PC-PS, we found that PC-PI-PS membranes constitute fluidizing mixtures of phospholipids. These observations can be explained firstly, by the increase in the negative charge density produced by the incorporation of PI or PS into the binary mixtures of PC-PS or PC-PI respectively. This interpretation may be supported by the work of Okhi *et al.* [37] in DPPC-PI liposomes, who found that the increase in the liposome negative charge density from 10 to 25% eliminates the phase transition. Secondly, the 'fluidizing' interaction of PI-PS (Fig. 1) may also be affecting the PC-PI-PS membrane's properties.

Following this rationale it is possible to explain the larger 'rigidizing' effect of PE mixing with PC-PI-PS than with PC. The incorporation of zwitterionic PE into PC-PI-PS bilayer lowers the negative charge density reducing the repulsive negative charges and favouring the intermolecular hydrogen bond formation. Moreover, the 'fluidizing' interactions between PI-PS were diluted by the addition of PE which would also produce PC-PE rigidizing interactions.

Although it was observed a high rigidizing effect of PE when it was incorporated into PC-PI-PS bilayers, no 'rigidizing' effect was found when it was incorporated into PC-PI-PS-SM membranes. The presence of SM would apparently impair the 'rigidizing' effects of PE over PC-PI-PS. In addition, we have observed that, while 'rigidizing' effects due to the PC-PE or PC-SM interactions exist in PC-PE and PC-SM mixtures, these effects disappear when these three phospholipids interact altogether in the PC-PE-SM membrane. Hence, these results suggest that PE-SM interaction produces a 'fluidizing' effect in the PC-PE-SM membrane.

When discussing these results we have not taken into account the possible asymmetry in the membrane phospholipid distribution. Evidence for transmembrane lipid assymetry [38] or for the heterogeneity of lateral organization]27] has been proved in a variety of studies. We have found that the effect of one particular phospholipid over the membrane properties depends on the whole phospholipid composition of the membrane. Thus, there are 'fluidizing' or 'rigidizing' phospholipid mixtures depending on the presence or not of a particular lipid class. In some cases, it is possible to explain these differences assuming an homogeneous phospholipid distribution, but in some other cases, i.e. effects of PE over the PC-PI-PS or the PC-PI-PS-SM membranes, it is necessary to consider that the fluidity changes produced by one particular phospholipid class could be evoked at least partially by changes in the lateral or tranversal phospholipid distribution.

Figures 1 and 2 show that the differences between the 'rigidizing' effects of phospholipid mixtures obtained from normal and fat-deficient animals, were not significant. Nevertheless, these results indicate a general tendency where the more fluid (larger DBI) phospholipi mixtures present less 'rigidizing' effects than those mixtures of phospholipids with lower double bond index. This tendency could be explained by the effect of the fatty acyl chain-packing density over the molecular area and thus, over the head group network. In the liquid-crystalline state the acyl chains are considered to be disordered in spite of showing an average chain orientation perpendicular to the bilayer surface [39]. This implies that the molecular area required by a lipid with two melted chains will depend, in a certain degree, on the number of double bonds [35, 40, 41]. Therefore, when the packing of the fatty acyl chains is decreased by an increase of cis double bonds, the effect of attractive head group interactions over the membrane fluidity will be smaller due to the increased space requirements of fatty acyl chains. In an opposite way, expanding phospholipid-phospholipid interactions will also be less notorious in more fluid phospholipid mixtures.

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