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Interaction of retinol and retinoic acid with phospholipid membranes. A differential scanning calorimetry study

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The influence of retinol and retinoic acid, two retinoids of major interest, on the main gel to liquid-crystalline phase transition of different phospholipid membranes has been studied by means of differential scanning calorimetry. Both compounds exerted perturbing effects on the phase transition of membranes composed of dipalmitoylphosphatidylcholine or dipalmitoylphosphatidylethanolamine. At concentrations up to 42.5 mol% of retinoid in the membrane, the ΔH was not much affected with respect to the pure phospholipid, indicating a rather slight interaction. As the concentration of retinol was increased the T_c transition temperature decreased. A fluid-phase immiscibility was observed for the system DPPC/retinol at concentrations between 0 and 33 mol%. Almost ideal phase diagrams were obtained for the mixture DPPE/retinol. At concentrations of 33 mol% and higher retinol was able to induce phase separations in DPPC membranes, but not in DPPE. The effect of retinoic acid was much weaker, the T_c and ΔH remaining almost unaltered and equal to that of the pure phospholipid up to concentrations of 30 mol%, at neutral pH. Retinoic acid exerted a pH-dependent effect. As the pH decreased, and therefore increased the extent of protonation of retinoic acid, the perturbation of the membrane induced by this compound was less. A strong effect, both on T_c and ΔH, was observed at pH 10, where the retinoic acid moiety will be mainly unprotonated and the negative charge will generate repulsive forces thus destabilizing the membrane. The mixture DPPC/retinoic acid presents a region of fluid-phase immiscibility. At low pH, when the retinoic acid moiety was fully protonated, this fluid-immiscibility region extended from 0 to 36 mol% of retinoic acid, but its size decreased with increasing pH, and at pH 10 it was only found from 0 to 3 mol%. These results are discussed in terms of the possible retinoid/phospholipid interactions and the disposition of the retinoid moiety in the bilayer.

Introduction

Retinoids (vitamin A and its derivatives) are essential compounds for the maintainance of health in an organism. The term retinoids comprises a number of naturally occurring compounds such as retinol, retinal and retinoic acid, as well as a large list of synthetic analogs with or without biological activity. Their structure is very similar and it only differs at the polar end (Fig. 1), however, their physiological functions are markedly different. All-*trans*-retinol, apart from being a metabolic precursor of 11-*cis*-retinal, involved in the mechanism of vision in animals, is essential for reproduction while, retinoic acid is active in the promotion of growth [1,2]. All of them are highly hydrophobic compounds which preferentially partition into non-

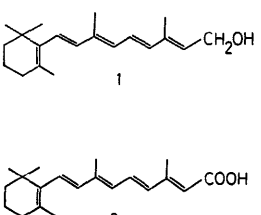

Fig. 1. The chemical structures of all-*trans*-retinol (1) and all-*trans*-retinoic acid (2).

Abbreviations: DMPC, 1,2-dimyristoylphosphatidylcholine; DPPC, 1,2-dipalmitoylphosphatidylcholine; DPPE, 1,2-dipalmitoylphosphatidylethanolamine; DSC, differential scanning calorimetry; ΔH, enthalpy change of the gel to liquid-crystalline phase transition; PE, phosphatidylethanolamine; retinoic acid, all-*trans*-retinoic acid; retinol, all-*trans*-retinol; T_c, onset temperature of the gel-to-liquid crystalline phase transition.

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polar phases. Because of its amphiphylic nature (Fig. 1) they may display a detergent-like effect. Retinol and retinoic acid have been shown to disrupt the membranes of erythrocytes and lysosomes [3]. This fact, together with its marked hydrophobicity points to biological membranes as one of their physiological sites of action.

Protein kinase C activity is also modulated by retinoids [4]. Since retinoids do not compete for the phorbol ester-binding site [4], it is possible that they associate with the plasma membrane lipids affecting the activity of protein kinase C associated to the membrane.

Several studies have correlated the membrane effects of retinoids and their biological activity. Furthermore, the ability of retinoids to fluidize membranes has been correlated with their toxic effects [5,6].

Some biophysical studies have dealt with the interaction of retinoids and model membranes. The effect on the phase transition temperature of DMPC and DPPC has been studied by following changes in water permeability and liposome size [7]. EPR studies on the interaction of retinoids and DPPC bilayers have reported a retinoid-induced restriction of the lipid side-chain motions in the lower portion of the chain [8]. NMR data indicated a large perturbation of the lipid bilayer structure induced by retinol and retinoic acid, as well as the presence of lateral phase separations [9].

In the this paper we present a detailed study on the interaction of all-*trans*-retinol and all-*trans*-retinoic acid with DPPC and DPPE bilayers by means of differential scanning calorimetry. The incorporation of either retinoid into the membrane has been measured and a limited incorporation has been observed for some mixtures at high concentrations of retinoids. Calorimetric data are analyzed by constructing partial phase diagrams. We find that both retinol and retinoic acid perturb the phospholipid bilayer, but each compound has its own peculiarities.

Materials and Methods

DPPC and DPPE were from Avanti Polar Lipids, Birmingham, AL. All-*trans*-retinol was obtained from Fluka and all-*trans*-retinoic acid was from Eastman-Kodak. All the other reagents were of the highest purity available. Water was twice-distilled in an all-glass apparatus and deionized in a Milli-Q system from Millipore. Retinoids were always handled in the dark and under inert atmosphere to prevent light-induced decomposition and peroxidation. Occasionally samples were analyzed by thin-layer chromatography to monitor stability and it was found that there was no appreciable alteration as a consequence of the experiment.

The lipid mixtures for calorimetry measurements were prepared by combination of chloroform solutions containing 5 μmol of the phospholipid and the appropriate amount of retinoid when indicated. The organic solvent was evaporated under a stream of dry N_2, free of O_2, at a temperature above the highest transition temperature of the phospholipid present in the mixture and the last traces of solvent were removed by a further 1–2 h evaporation under high vacuum. After the addition of 1 ml of the appropriate buffer, multilamellar liposomes were formed by mixing, using a bench-vibrator, always keeping the samples at a temperature above the highest phase transition temperature of the mixture. Three buffers were used: 0.1 mM EDTA, 100 mM NaCl, 10 mM Hepes (pH 7.4); 0.1 mM EDTA, 60 mM NaCl, 50 mM glycine-HCl (pH 3.0) and 0.1 mM EDTA, 60 mM NaCl, 50 mM glycine-NaOH (pH 10.0). Mixing was continued until a homogeneous and uniform suspension was obtained. The suspensions were centrifuged at high speed in a bench microfuge, the pellets were collected and placed into small aluminium pans. Pans were sealed and scanned in a Perkin-Elmer DSC-4 calorimeter, using a reference pan containing buffer. The heating and cooling rates were 4 C°/min in all the experiments. The DSC instrument was set at a sensitivity of 2 mcal/s. Peak areas were measured by weighing paper cut-outs of the peaks. For the determination of the total phospholipid contained in a pan, this was carefully opened, the lipid was dissolved with chloroform/methanol (1:1, v/v) and the phosphorus contents were determined using the method of Böttcher et al. [10]. The instrument was calibrated using indium as standard.

Phase diagrams were constructed as previously described [11]. The pretransitions have been omitted from the phase diagrams for the sake of simplicity.

The incorporation of retinol or retinoic acid into liposomes was assayed by *n*-pentane extraction as described before for ubiquinone or vitamin K-1 [12–14], to remove any retinoid remaining in the aqueous phase. The incorporation of retinol or retinoic acid into either DPPC or DPPE liposomes was in general higher than 90% of the initial amount. Only for the mixture DPPE/retinoic acid at pH 7.4, incorporations of 50% were obtained for the samples containing the two highest concentrations of retinoic acid. In any case, in all the following results, the depicted retinoid concentrations are the actual measured concentrations in the membrane.

Results

The samples examined in this work were always prepared to give homogeneous mixtures. Incorporation of retinoids into the membrane was checked for each sample (see Materials and Methods) and only in the mixture of retinoic acid with DPPE at neutral pH a limited incorporation was found. Therefore, for the

sake of comparison, the results presented here were obtained always for mixtures containing a retinoid mol fraction lower than 0.5, which, on the other hand, is the most significant range from the physiological point of view.

The DSC profiles obtained for pure DPPC and mixtures with retinol are shown in Fig. 2. For the pure phospholipid a T_c for the main gel to liquid-crystalline phase transition of 41°C is observed, this value being in good agreement with those reported before [15]. The presence of low concentrations of retinol make the pretransition to disappear and the main transition is broadened and shifted to lower temperatures. Increasing the concentration of retinol induces a further broadening and shift of the transition peak to lower temperatures. The samples containing 33 and 42.5 mol% of retinol show a lateral phase separation, as two well-resolved peaks are observed in the thermograms. The presence of low concentrations (mol fraction 0.009) of retinol produces a decrease in ΔH, but increasing retinol concentration does not cause further changes (Table I).

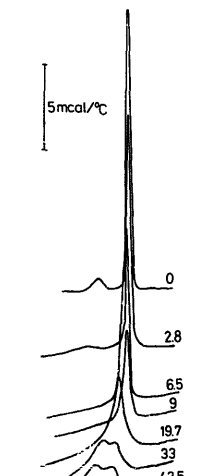

Fig. 2. DSC thermograms for the mixture DPPC/retinol at pH 7.4. Mol% of retinol is expressed on the curves. The profiles correspond to heating scans.

The partial phase diagram corresponding to the DPPC/retinol system is shown in Fig. 3. The solidus line displays a near ideal behaviour, the temperature decreasing as the retinol mol fraction increases. A fluid-phase immiscibility is observed, since the fluid line keeps horizontal at a temperature value of 41°C in a concentration range from 0 to 0.33 retinol mol fraction. At higher concentrations (0.42 mol fraction) the fluidus line seems to start to decline.

Since retinoic acid is a carboxylic acid, its protonated and unprotonated forms will be in an equilibrium which will depend on the pH of the medium. Fig. 4 shows the DSC profiles obtained for mixtures of retinoic acid and DPPC at three different pH values. At pH 3.0 (Fig. 4a) in the pure phospholipid thermogram the pretransition is abolished and the main transition is broadened with respect to neutral pH, according to previous results [16]. The incorporation of increasing concentrations of retinoic acid produces a slight broadening of the transition. The ΔH of the transition is not changed, as can be observed in Table I. At neutral pH the scenario is completely different (Fig. 4b). The pretransition is affected by the presence of small concentrations of retinoic acid, but it is still present at 6.1 mol%. Higher concentrations completely abolish the pretransition and the main transition is broadened and shifted to lower temperatures. The ΔH of the main phase transition is slightly decreased (Table I). The effect of retinoic acid on the transition parameters of DPPC at pH 10.0 (Fig. 4c), where the retinoic acid moeity should be fully unprotonated, is markedly more prominent than at lower pH values. It can be seen that the pretransition already disappears at 2.7 mol% of retinoic acid. Increasing the concentration of this retinoid gives rise to a considerable broadening of the main transition, which also shifts to lower temperatures. The enthalpy change of the main transition is now clearly affected, decreasing to values near 5 kcal/mol, as compared to 8.8 kcal/mol for the pure phospholipid.

The partial phase diagrams for the system DPPC/retinoic acid at different pH values are shown in Fig. 5. The main feature of these diagrams is the presence of a fluid-phase immiscibility, comprising a retinoic acid concentration range which depends on the pH, and therefore on the protonation degree of the molecule. At pH 3.0 (Fig. 5a) this fluid immiscibility region extends over the whole concentration range studied (up to a retinoic acid mol fraction of 0.36). The extent of the immiscibility region decreases as the pH increases, ranging from 0 to 0.044 retinoic acid mol fraction at pH 7.4 and from 0 to only 0.027 mol fraction at pH 10.0 (Figs. 5b and c, respectively). The solidus line also displays a complex behaviour. A

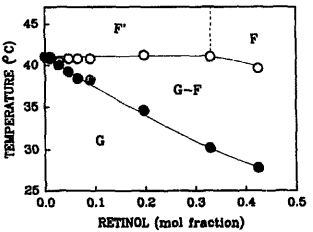

Fig. 3. Partial phase diagram for the mixture of DPPC with retinol at pH 7.4. Black circles correspond to the solidus line and open circles to the fluidus line. F indicates a fluid phase and G a gel phase. F' indicates that different fluid phases coexist. Dashed line separates different regions of the diagram.

solid-phase immiscibility is observed along a concentration range which is displaced to higher retinoic acid concentrations as the pH is raised. Since these are partial phase diagrams, these solid immiscibility regions cannot be fully defined at pH 7.4 and 10.0.

The study of the effect of retinoids on phospholipids was extended to cover DPPE. This phospholipid was chosen because PE's are also of great importance in biological membranes. Fig. 6 illustrates the influence of retinol (Fig. 6a) and retinoic acid (Fig. 6b) at pH 7.4, on the gel to liquid-crystalline phase transition of DPPE. For the pure phospholipid a T_c of 63°C is obtained in good agreement with values reported in the literature [17]. Both compounds induce a slight broadening and shift of the transition to lower temperatures. The effect on the T_c shift is markedly more prominent for retinol than for retinoic acid. No phase

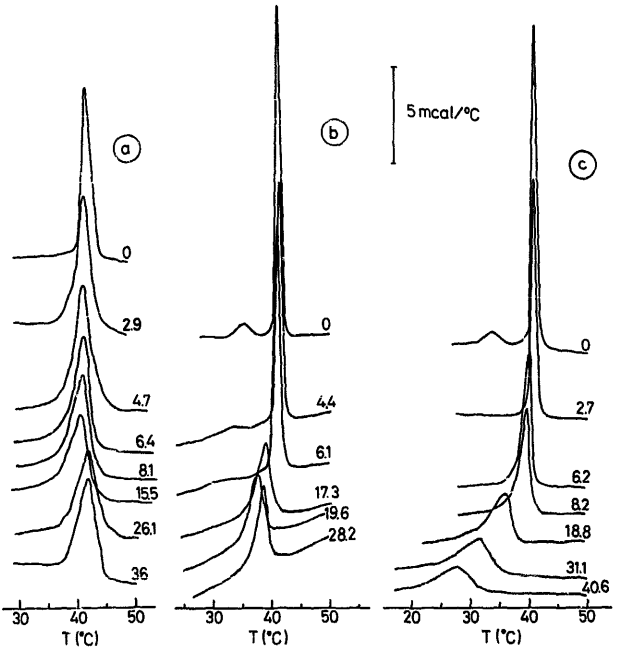

Fig. 4. DSC themograms for the mixture of DPPC and retinoic acid as a function of pH. (a) pH 3.0, (b) pH 7.4 and (c) pH 10.0. Mol% of retinoic is expressed on the curves. The profiles correspond to heating scans.

TABLE I

The enthalpy changes for the gel to liquid-crystalline phase transition of mixtures of DPPC / retinol at neutral pH and DPPC / retinoic acid at three different pH values

X represents the actual measured mol fraction of retinoic acid in the membrane.

Retinol		Retinoic acid					
		pH 3.0		pH 7.4		pH 10.0	
X	ΔH	X	ΔH	X	ΔH	X	ΔH
0	8.5	0	5.8	0	8.5	0	8.8
0.009	6.9	0.009	6.9	0.009	8.0	0.009	9.1
0.028	7.1	0.029	5.8	0.027	8.0	0.027	8.0
0.047	7.8	0.047	6.4	0.044	6.9	0.045	8.3
0.065	6.8	0.064	6.1	0.061	8.8	0.062	7.3
0.092	7.0	0.081	6.1	0.085	8.0	0.082	7.9
0.197	6.5	0.155	6.0	0.173	6.1	0.188	6.2
0.329	7.4	0.261	6.1	0.196	7.5	0.311	4.8
0.425	7.2	0.359	6.7	0.282	7.0	0.406	5.2

separation is observed. Interestingly, the presence of retinoic acid decreases the ΔH of the transition to a higher extent than retinol (Table II).

In the partial phase diagrams for the mixtures of retinol and retinoic acid with DPPE at neutral pH,

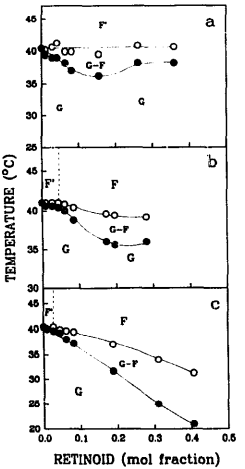

Fig. 5. Partial phase diagrams for mixtures of DPPC with retinoic acid as a function of pH. Panel a, pH 3.0; panel b, pH 7.4 and panel c, pH 10.0. Black circles correspond to the solidus line and open circles to the fluidus line. F indicates a fluid phase and G a gel phase. F′ indicates that different fluid phases coexist. Dashed lines separate different regions of the diagram.

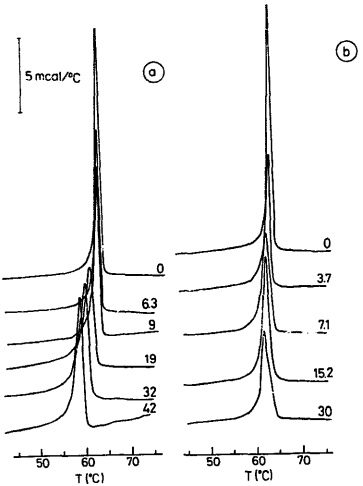

Fig. 6. DSC thermograms for the mixtures of DPPE with retinol (panel a) and retinoic acid (panel b) at pH 7.4. Mol% of retinoid is expressed on the curves. The profiles correspond to heating scans.

shown in Figs. 7a and 7b, respectively, it is observed that the region of coexistence of solid and fluid phases is very narrow for concentrations up to approx. 0.1 retinoid mol fraction. The system DPPE/retinol displays a near ideal behaviour (Fig. 7a). The mixture of DPPE and retinoic acid (Fig. 7b) gives rise to a phase

TABLE II

Values for the enthalpy changes (ΔH, kcal / mol) of the gel to liquid-crystalline phase transiton of mixtures of DPPE with all-trans-retinol and retinoic acid at neutral pH

X represents the actual measured mol fraction of retinoid in the membrane.

Retinol		Retinoic acid	
X	ΔH	X	ΔH
0	8.8	0	8.8
0.009	8.0	0.008	7.6
0.028	7.6	0.022	6.9
0.046	7.1	0.037	5.5
0.063	6.3	0.052	6.7
0.090	7.0	0.071	6.0
0.190	6.9	0.152	6.2
0.320	8.8	0.180	6.0
0.420	7.6	0.300	5.7

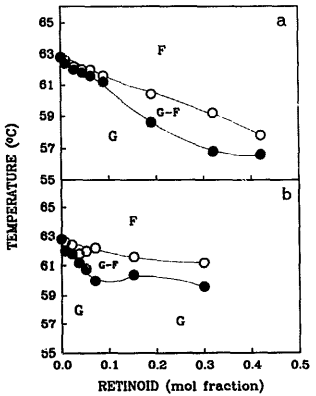

Fig. 7. Partial phase diagrams for mixtures of DPPE with retinol (panel a) and retinoic acid (panel b) at pH 7.4. Black circles correspond to the solidus line and open circles to the fluidus line. F indicates a fluid phase and G a gel phase. Dashed lines separate different regions of the diagram.

diagram qualitatively similar to that shown for DPPC/retinoic acid (Fig. 5b). From our data it is not possible to conclude whether or not a fluid-phase immiscibility at very low retinoic acid concentrations is taking place, as it was observed for DPPC. A solid phase immiscibility qualitatively similar to that observed for DPPC/retinoic acid (Fig. 5b) is also observed here.

Discussion

The molecule of a retinoid consists of three main sections, a lipophilic part at one end, connected via a polyunsaturated chain as a spacer to a hydrophilic group at the other end of the molecule. This amphiphylic nature makes biological membranes one of the most likely sites of retinoids action. The aim of this work is to provide information on the interaction of two retinoids of major relevance, namely retinol and retinoic acid, with lipid bilayers made of DPPC and DPPE. We have performed the study of the interaction of retinoids with membranes by using DSC, in order to characterize the influence of retinoids on the thermotropic properties of phospholipids.

The profile of a DSC thermogram of a phospholipid phase transition is determined by the transition temperature and the enthalpy change. Determining the temperatures of the transitions allows the construction of phase diagrams, which provide information regarding the equilibrium between gel and liquid-crystalline phases.

We will discuss first the interaction of retinol and retinoic acid with DPPC bilayers to follow with the effect of these retinoids on DPPE. For retinol/DPPC systems (Fig. 2) we observe a progressive broadening of the transition peak and a shift of the T_c to lower temperatures (27.8°C for the sample containing 42.5 mol% retinol). These results are indicative of the stablishment of a molecular interaction between the phospholipid acyl chains and the retinol molecule, perturbing the cooperative behaviour of the phospholipid and can be explained by the intercalation of the retinol molecule between the DPPC molecules. Therefore these results are compatible with the retinol molecule aligning itself principally with the prevailing direction of the phospholipid acyl chains, the hydroxyl group of retinol would be placed at the lipid/water interface where it could form hydrogen bonding with water and it may also establish other interactions with the polar part of the phospholipids. These interactions will keep the retinol molecule in the upper part of the phospholipid palisade which would explain the small effect on the enthalpy change observed (Table I). A similar location for retinol in DPPC bilayers has been previously suggested on the basis of EPR [8] and NMR [9] measurements. The latter authors found a 21°C decrease of the T_c of DPPC when 33 mol% of retinol were incorporated. We find retinol, at this concentration, to decrease the T_c of DPPC by as much as 11°C, this discrepancy must be undoubtly due to the higher accuracy of DSC in determining transition temperatures. At concentrations of 30 mol% and higher a second peak appears in the thermograms. This is attributed to a lateral phase separation of a retinol-rich domain. Nevertheless, enough retinol seems to remain in the bulk phase so that there is a further shift of T_c. In any case both peaks correspond to phospholipid organized in lamellar structures as it has been reported that DPPC containing up to 33 mol% of retinol remains in a bilayer organization [9].

In the partial phase diagram corresponding to retinol/DPPC (Fig. 3) the fluidus line keeps horizontal in a concentration range from 0 to 0.33 retinol mol fraction, indicating a fluid phase immiscibility. This is an interesting observation which has been reported before for unsaturated fatty acids [11] and diglycerides [18]. This type of immiscibility was predicted on the basis of theoretical calculations for mixtures of DPPC and anesthetics [19], where a relatively strong interaction between the anesthetic was supposed, so that clusters were formed. Our results can also be explained by a process of formation of retinol-rich domains when retinol is incorporated into DPPC membranes. In the

region F', DPPC and retinol-rich domains in the fluid phase should be present. When the temperature decreases below 41°C the system enters a region of coexistence of fluid and solid phases (region G-F). When the temperature is further decreased below the solidus line, which displays near-ideal behaviour, the system enters the G region, where a solid phase is present.

The interaction of retinoic acid with DPPC was carried out at different pH values, since this molecule posseses an ionizable group. Inclusion of increasing concentrations of retinoic acid into DPPC bilayers gives rise to a broadening of the peak and a shift of T_c to lower temperatures (Fig. 4) as well as a decrease of the ΔH of the transition (Table I). These effects are pH-dependent, being much more pronounced at pH 10.0 where the retinoic acid moiety should be fully unprotonated. At variance with retinol-containing systems no lateral phase separation is observed in retinoic acid/DPPC mixtures. At pH 3.0 the retinoic acid molecule is fully protonated and this must be the reason why it has a minor effect on T_c and ΔH of the phase transition, possibly because this protonated form is organized in a different way to that of the ionized form. At pH 10.0 the molecule of retinoic acid is totally ionized, producing a spreading of the polar headgroups of DPPC as a consequence of the strong electrostatic repulsions between the negatively charged carboxyl group of the retinoid and the negatively charged phosphate group of the phospholipid, giving rise to the pronounced changes observed in the transition parameters.

At neutral pH we find retinol to perturb DPPC bilayers more efficiently than retinoic acid, in good agreement with previous results by NMR [9]. This might be explained by the higher lipid solubility of retinol due to its less polar terminal group [20]. According to this, retinoic acid would position also aligned with the phospholipid acyl chains but closer to the polar region of the phospholipid. Results by EPR [8] are in conflict with DSC and NMR, the discrepancy can be explained by the perturbation produced in the bilayer by the spin label probe.

The partial phase diagrams of mixtures of retinoic acid with DPPC (Fig. 5) show a pH-dependent behaviour. Fluid-phase immiscibility is observed along a retinoic acid concentration range which decreases with increasing pH. From these diagrams it can be concluded that only the protonated form of retinoic acid is able to produce fluid-immiscibilities, this could be explain by the same rationale of domain formation given above for retinol. The strong repulsive forces between ionized retinoic acid molecules would prevent formation of domains at high pH. Under physiological conditions (pH 7.4) only a fraction of the retinoic acid population should be protonated, giving rise to a very small region of fluid-immiscibility. Although the pK of retinoic acid in membranes has not been published to the best of our knowledge, it can be assumed to be similar to that of unsaturated fatty acids in membranes, with values of approx. 7.5 [21]. At pH 3.0 (Fig. 5a) the solidus line shows a bell-shape region indicative of a gel-immiscibility along a certain range of retinoic acid concentration. The possible explanation would be that the reticular structure formed by the DPPC molecules below the T_c will have a very limited capability of accommodating protonated molecules of retinoic acid. Ionized molecules of retinoic acid seem to be more easily accommodated in the gel phase of DPPC, since near-ideal solidus lines are observed in the phase diagrams of Figs. 5b and 5c. This gel-immiscibility has been reported for other systems like DPPC/diglycerides [18].

If the polar group of retinoids is located near the polar headgroup region of the bilayer, one might expect retinoids to differently interact with different types of phospholipids. This is indeed the case. As shown in Fig. 6 both retinol and retinoic acid perturb DPPE bilayers to a lesser extent than DPPC bilayers.

The system DPPE/retinol corresponds to a molecule which intercalates between the acyl chains of the phospholipid, decreasing T_c (Fig. 6a) and having a negligible effect on ΔH (Table II). This effect may be due to the perturbation of the intermolecular hydrogen bonds present in phospholipid vesicles made of DPPE [22]. The partial phase diagram (Fig. 7a) shows near-ideal behaviour both in the solidus and fluidus lines. The region of coexistence of solid and fluid phases is very narrow between 0 and 0.1 retinol mol fraction. Neither lateral phase separation nor phase immiscibilities are observed in this system. In this respect De Boeck and Zidovetzki [9] reported lateral phase separations upon incorporation of retinol into equimolar mixtures of DPPC and DPPE. They show a preferential interaction of retinol with DPPE leading to the formation of retinol-enriched DPPE domains. Our DSC results on the interaction of retinol with DPPC and DPPE also support this observation, since we show that while retinol might form domains in DPPC bilayers a near-ideal behaviour is observed for its mixtures with DPPE.

The effect of retinoic acid on the T_c of DPPE (Fig. 6b) is weaker than that of retinol, T_c being only slightly altered. On the other hand, ΔH is markedly decreased. These observations can be explained by assuming that only a small fraction of retinoic acid is homogeneously distributed among the DPPE bilayer, whereas most of it would be forming retinoic acid-enriched domains within the bilayer. In the corresponding phase diagram (Fig. 7b) both the solidus and fluidus lines decrease with increasing concentrations of retinoic acid. The fraction of unprotonated retinoic acid present at pH 7.4, responsible for the fluid immiscibility observed in mixtures with DPPC, does not seem to show here the same behaviour. Nevertheless a gel

immiscibility similar to that described and discussed above for DPPC is observed in this system.

To conclude, we find retinol to perturb phospholipid bilayers more effectively than retinoic acid and this is consistent with the more apolar nature of the former, allowing retinol to localize deeper into the membrane, while retinoic acid seems to position closer to the lipid/water interface.

Retinoids interact differently with phospholipids than free fatty acids, nevertheless it is worth mention that there are certain analogies. Protonated fatty acids and fatty alcohols of the same chain length have a similar type of interaction with phospholipids [23] and we are describing in this paper a qualitatively similar behaviour for retinol and protonated retinoic acid. However, protonated fatty acids and their corresponding sodium salts behave quite differently in their interactions with phospholipids [24], and we show here that the protonated and unprotonated forms of retinoic acid display a different interaction with DPPC bilayers.

Although retinoic acid has been described to act by modulating the genetic expression of the cell [25], the incorporation of retinoids into membranes can be of biological relevance. It has been described that retinoic acid may activate protein kinase C [4], acting in a way similar to that of phosphatidylserine [26]. This is not surprising since also unsaturated free fatty acids are able of doing so [27,28].

All-*trans*-retinol is found to be the metabolic precursor of 11-*cis*-retinal which is implicated in the mechanism of vision. The biological transformation of retinol into retinal is carried out by the enzyme retinol dehydrogenase in rod outer segments of retina. This enzyme has been found to be bound to membranes [29] and it has been very recently purified [30]. Therefore the disposition of retinol in membranes must be of great importance in order to determine the control of the enzymatic activity of retinol dehydrogenase. On the other hand, retinol has been found to inhibit peroxidation processes in membranes [31]. This antioxidant role, which could be similar to that of β-carotenes or α-tocopherol, may be also important for the cell. Apart from that, retinol has been also shown to inhibit the oxidation of arachidonic acid, by influencing both the cyclooxygenase and lipooxygenase pathways [32], and these are processes that also take place in membranes. A much less extensive study using also DSC, previously presented, reported in some aspects behaviour of retinoids qualitatively similar to that described here [33].

It is very interesting when considering the possible actions of retinoids in membranes, that, as shown in this paper, they present immiscibilities, giving place to the formation of domains, where their concentration will be specially high. This will facilitate their activity even when they are at very low concentrations in the cell. It should be particularly remarked that they present fluid-phase immiscibilities which is very relevant given the normal fluid condition of biological membranes.

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