Biochimica et Biophysica Acta, 946 (1988) 244–252
Elsevier

BBA 74236

NMR study of the interaction of retinoids with phospholipid bilayers

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(Received 31 March 1988)
(Revised manuscript received 15 August 1988)

Key words: NMR; Lipid membrane; Vitamin A; Retinoid

The interaction of three vitamin A derivatives or retinoids: all-*trans*-retinoic acid, 13-*cis*-retinoic acid and retinol with multilamellar phospholipid bilayers was studied using a combination of ^{2}H- and ^{31}P-NMR measurements. The following model membrane systems were used: (1) dipalmitoylphosphatidylcholine (DPPC) bilayers; (2) bilayers composed of a mixture of DPPC and bovine heart phosphatidylcholine (PC); (3) mixed PC/phosphatidylethanolamine (PE) bilayers. Only a weak interaction was observed between 13-*cis*-retinoic acid and DPPC membranes. Addition of all-*trans*-retinoic acid at a molar ratio of 1:2 to the lipid causes a small decrease (5 C°) in the gel to liquid crystalline phase-transition temperature of DPPC, a small increase in the order parameters of the lipid side-chains of single component bilayers and no measurable effect in the other lipid systems studied. Considerably larger perturbation in the lipid bilayer structure is introduced by addition of retinol which, at a molar ratio of 1:2 to the lipid, lowered the gel to liquid crystalline phase-transition temperature of DPPC by 21 C° and caused a decrease of order parameters of the lipid side-chains in all three lipid bilayer systems. These effects are consistent with intercalation of retinol molecules into the bilayer interior. The results for the mixed PC/PE bilayers indicate that the presence of retinol caused lateral separation of PE- and retinol-enriched regions.

Introduction

Vitamin A and its derivatives, the retinoids, are known to be fundamental mediators of proliferation and differentiation of numerous cell types [1,2]. A large number of studies have shown that retinoids exhibit diverse and sometimes opposite effects on cells, depending on cell type, concentration of the retinoid and incubation conditions. So far, the mechanism of action of retinoids has not been deciphered. The two most likely cellular targets for retinoid action are the nucleus and the plasma membrane. Retinol and retinoic acid binding proteins were identified in several tissues [3] and it was suggested that their mode of action is similar to that of the steroid receptors. The initial correlation between the presence of the retinoid-binding proteins and the action of retinoids on cell growth [4] broke down when several cell lines were tested and it was found that retinoids may have similar biological effects on cells with or without expressed retinoid-binding proteins [5]. Thus, it is not possible to state that the binding proteins play an obligatory role in the mechanism of action of retinoids. Recently, a unifying mecha-

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DPPC, dipalmitoyl PC; DPPC-d_{62}, diperdeuteropalmitoyl PC; DPPE, dipalmitoyl PE; DPPE-d_{62}, diperdeuteropalmitoyl PE; DMPC, dimyristoyl PC; BH, bovine heart; PK-C, protein kinase C.

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nism of retinoid action has been proposed which involves the Ca^{2+}- and phospholipid-dependent protein kinase C (PK-C) signal transduction system [6]. Retinoids were shown to modulate PK-C activity [7] and the relative effectiveness of different retinoids to inhibit PK-C activity correlated with their ability to induce differentiation in cultured F9 mouse teratocarcinoma cells [8]. The retinoids, however, are not competing for the phorbol ester-binding site on the enzyme [7]. It is possible that retinoids associate with plasma membrane lipids and thus affect the conformation and activity of membrane-associated PK-C. Interestingly, retinoic acid was reported to have opposite effects on the activity of PK-C when assayed in the absence or presence of diacylglycerol, an endogenous ligand for PK-C and a product of receptor-mediated phosphatidylinositol hydrolysis [9].

Lipophilicity of retinoids and their toxic effects at higher concentrations make it likely that the plasma membrane is one of the targets for retinoid action. Moreover, structurally, since the retinoids have some similarity with detergents, it is possible that their toxicity is due to a detergent-like effect. Retinol and retinoic acid were shown to disrupt the membranes of erythrocytes and lysosomes [10]. Similarities between the effects of some retinoids and detergents were indeed observed in a study on the activity of the membrane form of galactosyl transferase [11]. In contrast to this finding, the comparison of the effects of retinoids and detergents on 'microviscosity' of rat erythrocyte membranes showed that the retinoids were effective in reducing the microviscosity at concentrations two orders of magnitude lower than the detergents [12]. The importance of the effects of retinoids on membranes was demonstrated by the finding that the effectiveness of the retinoids to increase the fluidity of the membranes paralleled their toxicity, as determined in bioassays [13,14].

Several studies reported a correlation between membrane effects of retinoids and their biological activity. The retinoid-induced differentiation of embryonal carcinoma cells was accompanied by an increase in lipid microviscosity, with only biologically active analogs showing the membrane effect [15]. No correlation between membrane and biological effects was, however, observed with fibroblasts [16]. Retinol and retinoic acid are the two most commonly compared analogs. In general, retinoic acid appears to be more biologically potent, often 100–1000-times more active than retinol in many in vivo and in vitro systems [17–19]. Several studies on the structure–activity relationship of these and other retinoids have been carried out [13,20,21]. The general conclusion is that the polar terminal group of a retinoid molecule is particularly important in determining its activity, toxicity, metabolism and tissue distribution. Biophysical studies on the interactions of retinoids with model membranes are scarce. Retinoid-induced increases in membrane permeability to water, ions and glucose were measured by osmotic swelling, ion-specific electrodes and colorimetric tests, respectively [22]. Retinoid-induced lowering of the phase-transition temperature of DMPC and DPPC liposomes was monitored by the temperature dependence of liposome permeability to water and of liposome size [22]. The observed effects are larger for retinoic acid than for retinol [22]. ESR studies of the interactions of retinoids with DPPC bilayers reported that both retinol and all-*trans*-retinoic acid restrict lipid side-chain motions in the lower portion of the chain. The effects of the two retinoids on the upper part of the lipid chains differed: all-*trans*-retinoic acid decreased the order parameter, while retinol had little effect [23].

In the present work we report the study of the interaction of three retinoids with bilayers of different compositions, using a combination of ^{31}P- and ^{2}H-NMR. The first step involved DPPC or DPPC-d_{62} bilayers, which provided a system with a clearly defined phase transition. The complexity of the model bilayers was then increased stepwise, to include PC and PE extracts. We found that each of the retinoids studied has a distinct effect on the lipid bilayer structure.

Materials and Methods

The retinoids were obtained from Aldrich Chemical Co. (all-*trans*-retinoic acid) and from Eastman Kodak Co. (13-*cis*-retinoic acid and retinol); they were handled under subdued light. DPPC and DPPE were purchased from Calbiochem, San Diego, CA. The ^{2}H-labeled lipids DPPC-d_{62} and DPPE-d_{62}, as well as the unlabeled

lipid extracts from bovine heart (BH), PC and PE, were obtained from Avanti Polar Lipids, Birmingham, AL.

Multilamellar dispersions of the lipids were prepared as follows: the lipids or the lipid/retinoid mixture were dissolved in methanol and/or chloroform. The solvent was then evaporated off with dry nitrogen and the NMR tube was subsequently kept under vacuum (< 1 mtorr) for at least 8 h. The hydration buffer was a 25 mM Tris buffer solution (pH 7.4) either in ^2H_2O for ^{31}P-NMR experiments or in ^{2}H-depleted H_2O (Sigma) for ^{2}H-NMR samples. The samples were kept in a sand bath at 45°C and vortexed until a uniform suspension was obtained. Besides single-component DPPC multilayers, we used a mixture of DPPC and bovine heart PC extracts at a molar ratio of 1:2. ^{2}H-NMR experiments were also performed using multilayers composed of DPPC, BH-PC, DPPE and BH-PE; the molar ratio of PC:PE was 2:1, while the molar ratio of synthetic to extracted lipids was kept at 1:3. Either DPPC or DPPE was substituted with DPPC-d_{62} or DPPE-d_{62}, respectively, as a ^{2}H-NMR probe.

^{31}H-NMR spectra were acquired at 11.74 T (202.49 MHz ^{31}P-NMR frequency) on a General Electric GN 500 spectrometer, using a spectral width of 50 kHz, a 75° pulse, a relaxation delay of 1.5 s and gated broadband proton decoupling (10 W). ^{2}H-NMR spectra were acquired at 11.74 T (76.87 MHz ^{2}H-NMR frequency) on a Bruker AM 500 spectrometer, using a high-power home-built probe [24] and the standard quadrupole echo sequence [25]. The spectral width was 166 kHz, the refocusing time 50 μs, and 90° pulse was 4.1 μs.

Results

^{31}P-NMR study

Fig. 1 shows typical ^{31}P-NMR spectra of DPPC multilayers with or without retinol. Both spectra show the same spectral pattern, which is characteristic of the multilamellar bilayer phase [26] and which was also observed in the presence of all-*trans*-retinoic acid or 13-*cis*-retinoic acid (not shown). The chemical shift anisotropy $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$ can be determined from the edges of the spectrum as shown in [26]. Since $\Delta\sigma$ depends on molecular motions and orientation of the phospholipid headgroups and since the motional state of the lipids depends on temperature with an abrupt change at the gel to liquid crystalline phase-transition temperature (T_c), $\Delta\sigma$ can be used to estimate T_c and to monitor the effect of the retinoids on T_c and on the orientation of the phospholipid headgroups [27].

The temperature-dependences of $\Delta\sigma$ of DPPC in the absence and presence of all-*trans*-retinoic acid or 13-*cis*-retinoic acid, both at a retinoid : lipid molar ratio of 1 : 2, are summarized in Fig. 2. The addition of these two retinoids causes only a small decrease in the phase-transition temperature of DPPC, 2 C° in the presence of 13-*cis*-retinoic acid and 5 C° in the presence of all-*trans*-retinoic acid. No change of $\Delta\sigma$ above T_c was observed in the presence of either of these two retinoids. Comparison of the $\Delta\sigma$ values below T_c at the same reduced temperature, $T_r = (T - T_c)/(273.15 + T_c)$, shows that 13-*cis*-retinoic acid, but not all-*trans*-retinoid acid, causes a small decrease in the absolute value of $\Delta\sigma$ (Fig. 2). This can be visualized by sliding

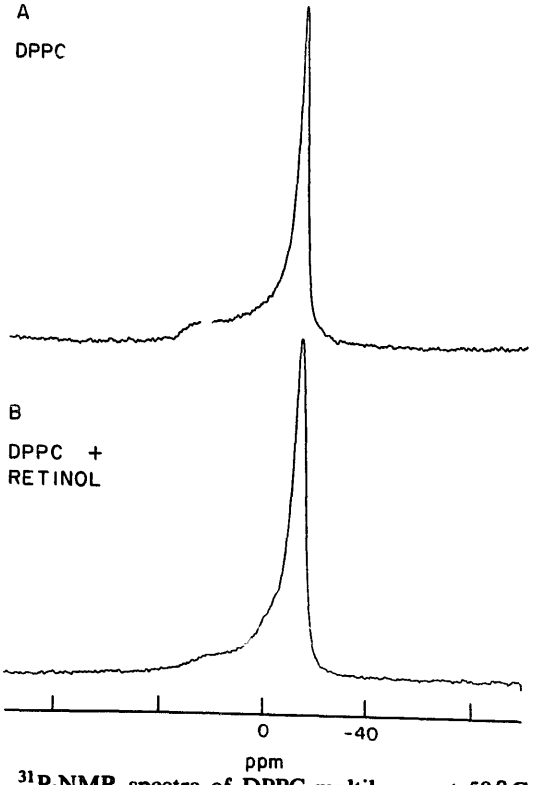

Fig. 1. ^{31}P-NMR spectra of DPPC multilayers at 50°C. (A) DPPC only; (B) 1 : 2 retinol : DPPC molar ratio.

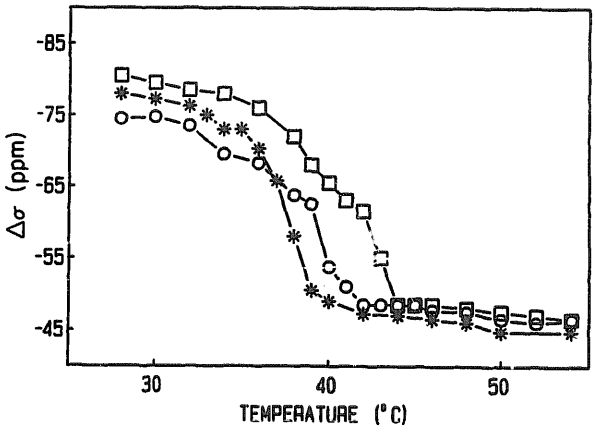

Fig. 2. Plot of $\Delta\sigma$ vs. temperature for aqueous dispersions of retinoid/DPPC mixtures (1:2 mol/mol) □, DPPC; ○, DPPC with 13-*cis*-retinoic acid; *, DPPC with all-*trans* retinoic acid.

the curves for the 13-*cis*-retinoic acid and for the all-*trans*-retinoic-acid-containing sample to the right, so that the sharp rises in $|\Delta\sigma|$ (which correspond to T_c) are superimposed with the corresponding point for the control DPPC curve (Fig 2). Thus, the ^{31}P-NMR results showing retinoid-induced decreases in T_c and changes in $\Delta\sigma$ indicate that both all-*trans*-retinoic acid and 13-*cis*-retinoic acid interact with DPPC bilayers.

The temperature-dependence of $\Delta\sigma$ of DPPC in the presence of different concentrations of retinol is shown in Fig. 3. The effect of retinol on the phase-transition temperature of DPPC is considerably larger than that of the other two retinoids. Retinol, in a molar ratio of 1:2 to DPPC lowers T_c of DPPC by 21 C°. Lower concentrations of

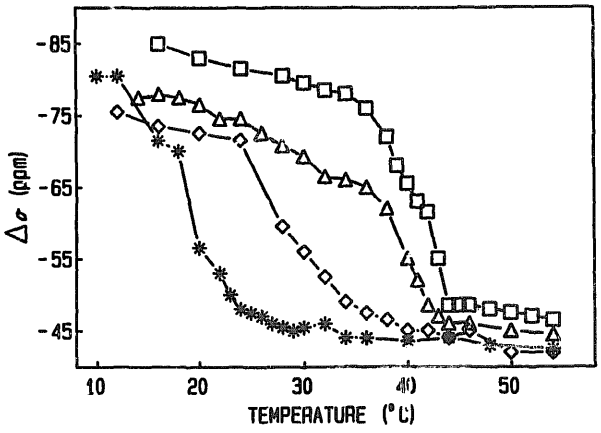

Fig. 3. Plot of $\Delta\sigma$ vs. temperature for aqueous dispersions of retinol/DPPC mixtures at different molar ratios. □, no retinol; △, 1:20 retinol:DPPC molar ratio; ◇, 1:6 retinol:DPPC molar ratio; *, 1:2 retinol:DPPC molar ratio.

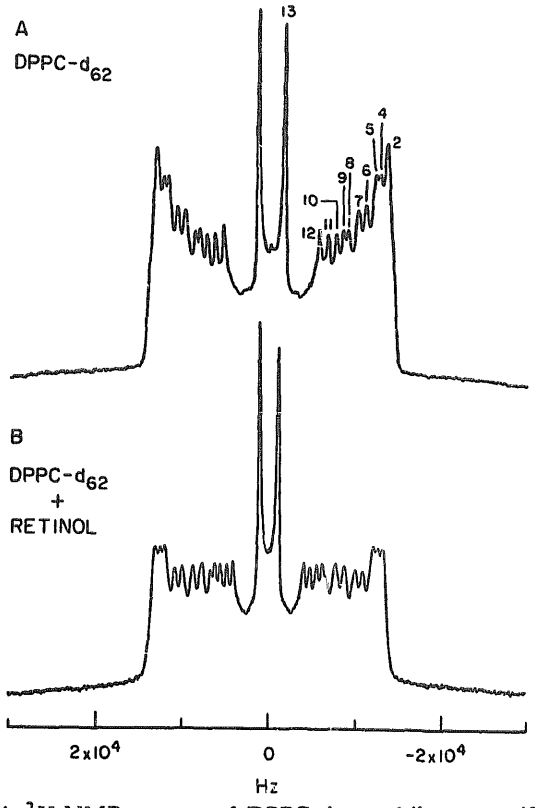

Fig. 4. ^{2}H-NMR spectra of DPPC-d_{62} multilayers at 40 °C. (A) DPPC-d_{62} only, (B) 1:2 retinol:DPPC-d_{62} molar ratio.

retinol induce smaller decreases in T_c (Fig. 3). Above T_c, there is a small decrease in the absolute value of $\Delta\sigma$ in the presence of retinol. Below T_c there is no change in the absolute value of $\Delta\sigma$ in the presence of retinol when compared at the same reduced temperatures.

^{2}H-NMR study

^{2}H-NMR spectra of DPPC-d_{62} above the phase-transition temperature in the absence and in the presence of retinol are shown in Fig. 4. These spectra represent the superposition of axially averaged powder patterns arising from the different deuterons for the various C^2H_2 segments along the acyl chains. $S^i_{C^2H}$, the C^2H bond-order parameter for the ith segment, can be deduced from the quadrupole splitting at the perpendicular orientation, $\Delta\nu$, using

$$\Delta\nu^i_D = 3/4(e^2qQ/h)S^i_{C^2H}$$

where (e^2qQ/h) is the C^2H deuteron quadrupole coupling constant (170 kHz for aliphatic C-^{2}H bonds [28]).

Comparison of ^{2}H-NMR spectra of perdeuterated DPPC-d_{62} with those of specifically labeled samples [29] has led to a tentative assignment of the resolvable peaks to specific chain positions [30]. These works established a characteristic order parameter profile along the hydrocarbon chains of a phospholipid molecule in a multilayer. The methylene segments near the glycerol backbone show a characteristic plateau due to coinciding order parameters. The methylene segments in the lower parts of the chains are progressively more disordered and their quadrupole patterns show well-resolved peaks at the perpendicular orientation. In the present work we resolved up to eleven quadrupole splittings of DPPC-d_{62} above T_c (Fig. 4). The ^{2}H-NMR spectra of DPPC-d_{62} with or without the retinoids have very similar shape confirming the ^{31}P-NMR data about the maintenance of the bilayer phase, even at high concentrations of the retinoids (Fig. 4). The different appearance of Fig. 4B as compared to Fig. 4A is not due to the presence of a broad component in Fig. 4B, but is probably related to the decrease in T_c by 21 C° in the presence of retinol. A spectrum of DPPC-d_{62} by itself at the same reduced temperature (experimental temperature of 61°C) looks similar to the one in the presence of retinol at 40°C. The effects of the three retinoids, each at a molar ratio of 1 : 2 to the lipid, on the quadrupole splittings of DPPC-d_{62} are shown in Fig. 5. This figure shows the quadrupole splittings obtained from ^{2}H-NMR spectra in the absence and in the presence of a retinoid at 40°C, and also gives the quadrupole splittings for DPPC-d_{62} at the same reduced temperature as the retinoid-containing sample. The reduced temperatures used here are based on the change in T_c obtained from ^{31}P-NMR measurements. We additionally checked retinoid-induced changes in T_c by ^{2}H-NMR measurements of the corresponding DPPC-d_{62} retinoid samples. In all cases studied, the difference between such ^{2}H- and ^{31}P-measurements was about 5 C°, corresponding to the T_c difference between DPPC and DPPC-d_{62}. Furthermore, we have measured T_c of DPPC-d_{62} retinoid samples using ^{31}P-NMR and found good agreement with the ^{2}H-NMR results.

Only a very small decrease in the quadrupole splittings of DPPC-d_{62} is observed in the presence of 13-*cis*-retinoic acid (Fig. 5A). This effect becomes even smaller and insignificant upon comparison with DPPC-d_{62} at the same reduced temperature, corresponding to measurement of DPPC-d_{62} at 42°C. The presence of all-*trans*-retinoic acid (Fig. 5B) induces a small but significant

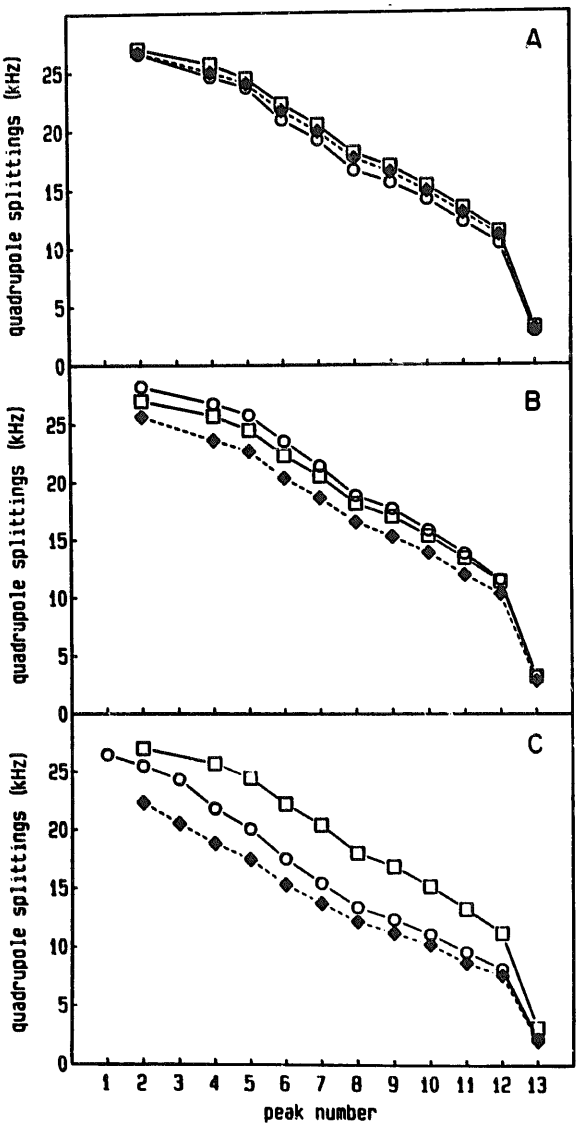

Fig. 5. Plots of the quadrupole splittings of DPPC-d_{62} vs. peak number in the presence and absence of the retinoids at a molar ratio of retinoid-to-lipid 1:2. (A) □, DPPC-d_{62} at 40°C; ○, DPPC-d_{62} with 13-*cis*-retinoic acid at 40°C; ◆, DPPC-d_{62} at 42°C (same reduced temperature as the 13-*cis*-retinoic-acid-containing sample). (B) □, DPPC-d_{62} at 40°C; ○, DPPC-d_{62} with all-*trans*-retinoic acid at 40°C; ◆, DPPC-d_{62} at 45°C (same reduced temperature as all-*trans*-retinoic-acid-containing sample). (C) □, DPPC-d_{62} at 40°C; ○, DPPC-d_{62} with retinol at 40°C; ◆, DPPC-d_{62} at 61°C (same reduced temperature as the retinol-containing sample).

increase in the quadrupole splittings of DPPC-d_{62}. The comparison with DPPC-d_{62} at the same reduced temperature enhances this effect. The effect of retinol (Fig. 5C) on the quadrupole splittings of DPPC-d_{62} is considerably stronger than that of the other two retinoids. Retinol induces a large decrease in the quadrupole splittings of DPPC-d_{62}. Comparison with DPPC-d_{62} at the same reduced temperature (corresponding to measurement of DPPC-d_{62} at 61°C) shows that the presence of retinol increases the order parameter of the side-chains of DPPC-d_{62}. This is the opposite of the effect observed at the same temperature of measurement (40°C).

In addition to the study of DPPC multilayers, ^{2}H-NMR experiments were performed using a mixture of DPPc-d_{62} with BH-PC extracts. The quadrupole splittings of DPPC-d_{62} in such mixed bilayers, in the absence or presence of retinol or all-*trans*-retinoic acid, are represented in Fig. 6. The data for 13-*cis*-retinoic acid are not shown in Fig. 6 because of complete overlap with the $\Delta\nu$ values for DPPC-d_{62} with and without all-*trans*-retinoic acid. In this lipid system only retinol caused significant disordering of the lipid side-chains, with the other two retinoids exhibiting no effect.

We also studied the interactions of retinoids with phospholipid multilayers composed of a mixture of BH-PC extracts and BH-PE extracts at a PC/PE ratio of 3 : 1, which corresponds to that of

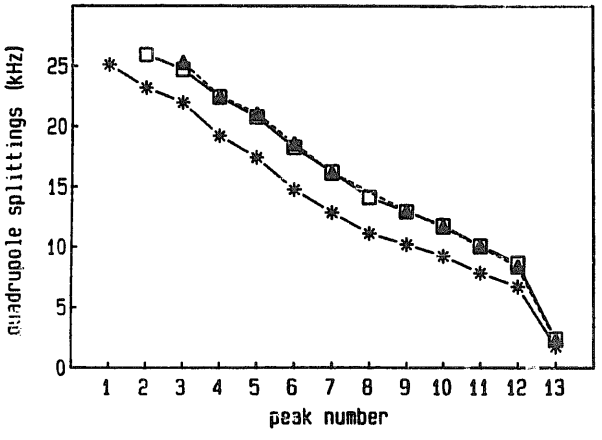

Fig. 6. Plot of the quadrupole splittings vs. peak number for DPPC-d_{62}/BH-PC multilayers in the absence and presence of retinoids at a molar ratio of retinoid-to-lipid of 1 : 2 at 37°C. □, DPPC-d_{62}/BH-PC only; ▲, DPPC-d_{62}/BH-PC with all-*trans*-retinoic acid; *, DPPC-d_{62}/BH-PC with retinol.

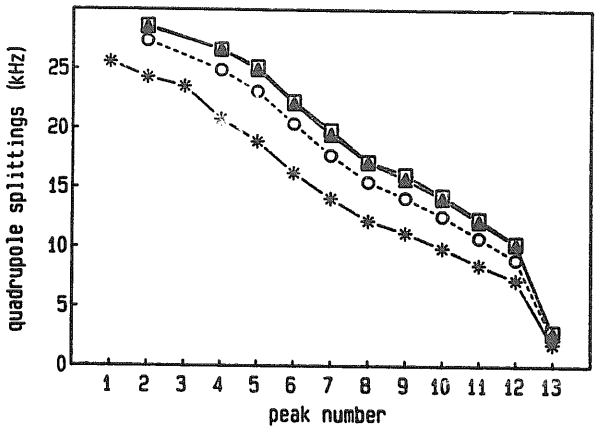

Fig. 7. Plot of the quadrupole splittings vs. peak number of PC/PE mixtures in the absence and in the presence of retinol (retinol-to-lipid molar ratio 1 : 2) at 37°C. □, PC/PE mixture with DPPC-d_{62}; ▲, PC/PE mixture with DPPE-d_{62}; ○, PC/PE mixture with DPPC-d_{62} and with retinol; *, PC/PE mixture with DPPE-d_{62} and with retinol.

many natural membranes. The ^{2}H-NMR probe was either DPPC-d_{62} or DPPE-d_{62}. As can be seen in Fig. 7, both control mixtures, with no retinoids added, have identical quadrupole splittings. This indicates that the components of these mixtures are well mixed. Neither all-*trans*-retinoic acid nor 13-*cis*-retinoid acid had any effect on the quadrupole splittings of either DPPC-d_{62} or DPPE-d_{62} (results not shown). Retinol, on the other hand, again affects the quadrupole splittings significantly. This effect is considerably stronger when the monitored perdeuterated probe is DPPE-d_{62} (Fig. 7).

Discussion

This work was initiated to provide information on the interaction of three retinoids with multilamellar lipid bilayers of different composition. The ^{31}P-NMR spectra of DPPC show that the presence of any of the three retinoids at a retinoid-to-lipid molar ratio of 1 : 2 does not disrupt the bilayer phase of DPPC. Each retinoid lowers the gel to liquid crystalline phase-transition temperature of DPPC, albeit to a different extent. For 13-*cis*-retinoic acid and for all-*trans*-retinoic acid, only small decreases in T_c of 2 and 5 C°, respectively, were noted at a molar ratio of 1 : 2 to the lipids. The meaning of these small effects is discussed below, together with the ^{2}H-NMR results.

The presence of retinol at a molar ratio of 1 : 2 to the lipids lowers T_c by 21 C°. Such a large decrease of the phase-transition temperature is a feature indicative of molecules which intercalate into the bilayer. Further confirmation of intercalation of retinol into the interior of DPPC bilayers comes from ^{2}H-NMR measurements, where decrease of the quadrupole splittings of all segments of the DPPC-d_{62} side-chains indicates retinol-induced perturbation of the lipid side-chain packing (Fig. 5C). It was previously pointed out that such an overall decrease of the lipid side-chain order parameters reduces Van der Waals forces in the bilayer, resulting in the decrease of T_c [31–33]. In order to deduce the position of the intercalated retinol molecules relative to the surrounding lipids, it is useful to compare ^{2}H-NMR spectra of DPPC-d_{62}, with or without retinol, at the same physical state by applying the reduced temperature scale, which, in our case, closely corresponds to an equal $\Delta T = T - T_c$, where T is the temperature of measurement. Fig. 5C shows that, at the same reduced temperature, the presence of retinol causes a uniform increase of the order parameters of the DPPC-d_{62} side-chains. A similar effect was found in the case of intercalation of *n*-octanol into DMPC bilayers [34]. The interpretation of our results basically follows that of Pope and Dubro [34], indicating that the hydroxyl terminals of the retinol molecules are anchored at the polar-group region of the bilayer and their polyene chains extend into the bilayer among the lipid chains, thereby restricting their motions and increasing the average bilayer order. This positioning of the retinol molecules agrees with the conclusion reached in recent work by Wassall et al. [23]. Our observation that the absolute value of $\Delta\sigma$ of DPPC above T_c decreases in the presence of retinol indicates changes in the average orientation of the phosphorus moiety of the DPPC headgroups [27] and is consistent with the location of retinol molecules as described above. Furthermore, if the hydroxyl terminal group of retinol is indeed located at or near the polar-headgroup region of the bilayer, one might expect that retinol would interact differently with different types of lipids. Our results indeed show that in mixed PC/PE bilayers retinol interacts preferentially with the PE component (Fig. 7). In these mixed bilayers, the order parameters of either DPPC-d_{62} or DPPE-d_{62} side-chains are identical prior to the addition of retinol, indicating ideal mixing of the lipids. Introduction of retinol decreases the order parameters of both the PC and PE deuterated probes, with the change being significantly larger in the case of PE (Fig. 7). Such behavior is consistent with the formation of laterally separated regions enriched with PE and retinol.

The perturbation of the DPPC bilayer structure by all-*trans*-retinoic acid is qualitatively somewhat similar to that of retinol, but the effects are much smaller. This difference between the effects of retinol and all-*trans*-retinoic acid indicates that retinol is more soluble in the lipids than all-*trans*-retinoic acid. Our results agree with observations by Dingle and Lucy [10], showing retinol as a powerful membrane-active agent and demonstrating that replacement of the terminal hydroxyl group by a carboxyl group (retinoic acid) diminished the ability to increase the area of a PC-cholesterol film at constant pressure. The higher lipid solubility of retinol can be correlated with its less polar terminal group which results in a more uniform distribution of electron densities along the polyene chain of retinol, making this molecule more soluble in the lipid phase than retinoic acid [35].

Based on ESR studies it was recently suggested that all-*trans*-retinoic acid intercalates only halfway into the bilayer [23]. Our results indicate that such a location of all-*trans*-retinoic acid molecules is unlikely, since it would produce a relatively larger decrease of the order parameters in the segments below those in direct contact with the all-*trans*-retinoic acid molecules. Such a situation was described by Pope and Dubro [34] upon incorporation of *n*-butanol into DMPC bilayers. In addition, positioning the all-*trans*-retinoic acid molecule with the bulky ring system halfway along the acyl chains would very likely induce a large effect on the order parameters, which is not observed here. Instead, we observed a small but uniform increase of the DPPC-d_{62} side-chain order parameters (Fig. 5B), indicating that all segments of the DPPC-d_{62} side-chains are directly in contact with all-*trans*-retinoic acid, leading to positioning of the hydrocarbon chain of this molecule along the acyl chains of the phospholipids, as in

the case of retinol. This leads to positioning of the bulky ring system close to the end of the acyl chains, where it is more plausible that no large effect is observed, since the order parameters are already quite low in this region. A figure illustrating possible positionings of the retinoids can be found in Ref. 23. The difference between our results and those of Wassall et al. [23] is probably due to employment of the bulky spin probe in the latter work, which is likely to cause additional perturbation of the conformation of surrounding lipids. Such a discrepancy between ^{2}H-NMR and ESR results has been observed before, e.g., by Wassall et al. [36] in the case of α-tocopherol–phospholipid interaction, and was explained by perturbation problems associated with the bulky nitroxide spin-label. Similar explanation for the discrepancies between ^{2}H-NMR and ESR order parameters is given in a review on ESR [37]. We did not observe any perturbation of lipid side-chains by all-*trans*-retinoic acid in lipid extract-containing bilayers. The reason for this lack of observable effect is not entirely clear, but it is possible that all-*trans*-retinoic acid molecules can fit better into the more loosely packed membranes which contain saturated and unsaturated lipid chains of different lengths.

The presence of 13-*cis*-retinoic acid in DPPC-d_{62} bilayers induces a small decrease in the quadrupole splittings all along the fatty acyl chains when measured at the same temperature (Fig. 5A). This effect can be attributed solely to a change in T_c, as substantiated by the fact that the effect is abolished upon comparison with DPPC-d_{62} at the same reduced temperature (Fig. 5A). No effects of 13-*cis*-retinoic acid on the ordering of the acyl side-chains were observed with the multicomponent bilayers. These observations and the previously mentioned small decrease in T_c of DPPC lead to the conclusion that 13-*cis*-retinoic acid interacts only with the headgroups of the lipids at the bilayer/water interface. Since there is no evidence for intercalation of 13-*cis*-retinoic acid into the bilayer, it is possible that micellization occurs, which would keep the hydrophobic polyene chains away from water.

Studies on the biological activity and on the toxicity of retinoids have consistently reported that retinoic acid is significantly more active and toxic than retinol. This means that the interaction of retinoids with zwitterionic lipids studied here is not sufficient to fully account for the mechanism of biological action of retinoids on cells. The observed strong interaction of retinol with PC and PE should be considered when evaluating the mechanism of biological action of this compound.

Acknowledgements

This work was supported by grants from the International Union against Cancer and NATO (to HDB) and the American Chemical Society PRF No. 16919-G4 (to RZ). The ^{2}H-NMR work was done at the NSF Southern California Regional NMR Facility (NSF CHE-791634 and CHE-8440137) located at the California Institute of Technology. The ^{31}P-NMR work was done using a GN 500 spectrometer (NSF DMB 840491 and NIH BRSG 2 507). We thank Drs. J. Yesinowski and R. Lee for their assistance and Dr. S. Wassall for making his work available to us prior to publication.

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