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Interaction of the plant hormone, indole-3-acetic acid, with phosphatidylcholine model membranes: effects of acyl chain length

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The interaction between the plant hormone, indole-3-acetic acid (IAA), and phosphatidylcholines (PC) of varying acyl chain length has been studied by monitoring the IAA-induced changes in ^{1}H-NMR chemical shifts of lipid headgroup -$\overset{+}{N}(CH_3)_3$ protons. For PCs in both micellar and vesicle bilayer systems these shifts increase with chain length although for the latter the magnitude of the shifts decreases with an increase in chain unsaturation. In systems composed of mixtures of pure PCs the headgroup -$\overset{+}{N}(CH_3)_3$ resonance for each phospholipid is shifted by IAA to different extents, indicating that IAA is able to distinguish between individual PCs in mixtures. In di-C_{12}PC and di-C_{14}PC, but not di-C_{10}PC vesicle systems, the -$\overset{+}{N}(CH_3)_3$ resonance is split into two components reflecting differences in packing of the inside and outside lamellae. This splitting is altered by IAA indicating that IAA interacts differently with the inside and outside PC molecules.

Introduction

The hypothesis has been put forward that at least some physiological and/or biochemical responses to plant hormones arise through interactions of the hormone with phospholipid membrane receptors [1–3]. In exploring this possibility much background information was obtained by studying the interaction between the plant hormone, indoleacetic acid (IAA), and several homologous series of amphiphiles containing the trimethylammonium group and a single acyl chain of different lengths [4]. In addition, other parameters of the hormone-phospholipid interaction were delineated with membrane preparations of complex mixtures of soybean phosphatidylcholines (PC). In no case, however, has an attempt yet been made to describe the interaction between IAA and individual PCs with known acyl chain composition nor has any study been undertaken of interactions between IAA and mixtures of PCs with known acyl chains. Since our aim is to understand both the physical parameters and the biological implications of such interactions (between IAA and phospholipids) elaboration of these two points, inter alia, is essential.

The method relies upon the measurement of IAA-induced changes in ^{1}H-NMR chemical shifts of headgroup -$\overset{+}{N}(CH_3)_3$ protons. The chemical shift changes, in turn, are thought to arise from changes in conformation induced by the interaction of the headgroups and the hormone [5]. Similar IAA effects on chemical shifts have been ob-

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served with soybean PC vesicle membranes with which an alkaline shift of approximately 1.5 units in pK_a of the IAA carboxyl group was measured [5]. This pK_a shift suggests that the carboxyl group is preferentially located in the phospholipid hydrocarbon/headgroup interface region. Since the properties of the hydrocarbon region are a function of acyl chain packing which, in turn, is a function of both the nature of the acyl chains and the nature of the headgroup [6], it was of interest to determine any effects of chain packing on the interaction of IAA with PC micelles and membranes.

This is part of a continuing program to develop sufficient knowledge to challenge complex and sophisticated biologically-relevant membranes with IAA, and understand the responses generated as well as the physiological and biological consequences.

Materials and Methods

Phospholipids were purchased from Avanti Polar Lipids (U.S.A.) and used as received. Purity of the compounds was determined by thin-layer chromatography on silica gel plates developed with chloroform/methanol/acetic acid/water (50:25:7:4, v/v). In all cases the samples gave a single spot ($R_f = 0.26$) when sprayed with molybdate reagent. IAA (Sigma, U.S.A.) was recrystallized from 1,2-dichloroethane and stored under nitrogen at 4°C. ^2H_2O (99.75%) was obtained from AAEC (Australia).

Micelle and vesicle preparations

Stock solutions of phospholipids were made up in chloroform and stored at 4°C. Concentrations were determined by phosphorus assay using the method of Bartlett [7]. For micelle preparations with di-C_4, di-C_6 and di-C_8, accurate volumes of the stock solutions were pipetted into small flasks, the solvent removed in vacuo at 30°C and acetate buffer (200 mM, pH 3.85, ^2H_2O) or maleate buffer (200 mM, pH. 5.26, ^2H_2O) added to give a final lipid concentration of 65 mM. The effects of pH on the magnitude of the IAA-induced changes in chemical shift of the $-\overset{+}{N}(CH_3)_3$ resonances of di-C_6PC micelles were investigated further by carrying out the titrations at pH 4.75 (200 mM acetate), pH 6.00 (200 mM maleate), pH 6.83 (100 mM phosphate), pH 7.56 and pH 8.35 (200 mM Tris). Vesicle preparations were made from phospholipid dispersed in buffer at approx. 65 mM which was then sonicated at 4°C with a Branson B12 ultrasonicator equipped with a microtip, as reported previously [5]. 0.5 ml of these preparations was transferred to 5 mm NMR tubes to which was added 0.8 µl dioxane as internal reference. IAA, as a solution of the sodium salt, was added with a microlitre syringe to the tube in 5- or 10-µl increments.

NMR measurements

NMR spectra at 90 MHz were recorded on a JEOL FX-90Q Fourier-transform spectrometer using a spectral width of 1000 Hz accumulated into 8 K data addresses at a probe temperature of 24°C. Chemical shift reproducibility, using the dioxane reference as 0.0 ppm, was better than ±0.005 ppm. NMR spectra at 300 MHz were recorded on a Bruker CXP300 spectrometer using a spectral width of 4000 Hz and an aquisition time of 1.0 s.

Calculation of dissociation constants

Dissociation constants (K_d) and complex shifts (Δ) for the interaction between IAA and PC in acetate buffer, pH 3.85, (where IAA is assumed to be completely unionised) were determined from the $-\overset{+}{N}(CH_3)_3$ resonance positions observed with changes in IAA concentration. For 1:1 stoichiometry, under conditions of rapid exchange of IAA between free and bound lipid states, the observed chemical shift (δ_{obsd}) of the lipid (L) resonance is related to the change in chemical shift on complex formation (Δ) and the chemical shift of the free species (δ_{free}) by

$$\delta_{obsd} = ([HL]/[Lo])\Delta + \delta_{free} \quad (1)$$

[HL] and [Lo] are the concentrations of the IAA-lipid complex at equilibrium and the total concentration of lipid, respectively. An iterative procedure was then used to calculate theoretical curves for a range of K_d values and Δ values which were then compared with the experimental data. Since IAA is able to quickly traverse the phospholipid

bilayer (on the timescales and pH values used [5]) all the lipid in vesicle systems is available for interaction with IAA. Further, this rapid transverse movement also allows intravesicular and extravesicular aqueous concentrations of IAA to equilibrate.

For binary lipid systems, and for di-C_6PC/di-C_{14}PC mixtures, in particular, the following equilibrium was used:

$$2\,IAA + L_1 + L_2 \rightleftharpoons IAAL_1 + IAAL_2 \qquad (2)$$

in which lipids L_1 and L_2 are assumed to exist exclusively in the associated form. It is noted that the above equations cannot be used for pH values near the effective pK_a of IAA since contributions from both ionised and unionised IAA are present (we reported previously that these forms of IAA interact differently with PC[5]) and it was for this reason that the majority of experiments were conducted at pH 3.85.

Results

The addition of IAA to the PC systems caused upfield changes in the ^{1}H-NMR resonance positions of headgroup protons (most notably $-\overset{+}{N}(CH_3)_3$ protons) as observed previously with various trimethylammonium-containing amphiphiles [4]. Fig. 1 illustrates the effects on the chemical shifts of the choline group $-\overset{+}{N}(CH_3)_3$ protons, of the addition of IAA to di-C_4, di-C_6, and di-C_8PC in acetate buffer. Di-C_4PC showed only a small chemical shift change with added IAA (−0.026 ppm at 0.5 mole ratio IAA/PC) which is consistent with other short chain amphiphiles [4]. Nevertheless, an interaction between this phospholipid and IAA is evident since up to 0.5 mole ratio (32.5 mM) of IAA can be solubilized (in the absence of phospholipid, IAA, at the lowest level used (3.25 mM), precipitates from acetate buffer pH 3.85). Increased PC chain length is associated with increased IAA-induced changes in ^{1}H-$\overset{+}{N}(CH_3)_3$ chemical shifts (Fig. 1). Significant increases in ^{1}H linewidths of di-C_8PC, due to increased solution viscosity at IAA concentrations greater than 0.2 mole ratio, prevented further data from being obtained. It has been

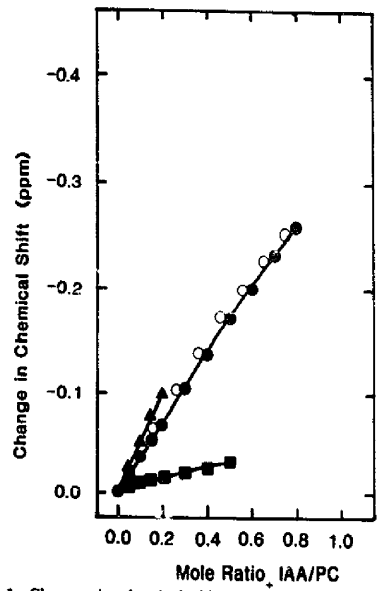

Fig. 1. Changes in chemical shift of $-\overset{+}{N}(CH_3)_3$ protons of di-C_4PC (■), di-C_6PC (●), and di-C_8PC (▲), 65 mM and di-C_6PC, 130 mM (○) in acetate buffer (pH 3.85) with increasing concentrations of IAA.

shown previously [4] that IAA-induced shifts of amphiphiles containing the trimethylammonium group are greatest when the amphiphiles are in an associated form (micelles or vesicles). Since the critical micelle concentration (cmc) of di-C_6PC in aqueous solution is approximately 14 mM [8,9] and changes with changes in salt concentration, it was possible that our results with this phosphatidylcholine, at 65 mM, reflected a mixture of monomers and micelles rather than an essentially homogeneous population of micelles [11,12]. This was tested by increasing the concentration of di-C_6PC to 130 mM at which a greater proportion of micellar species would be expected. Only very small differences in IAA-induced ^{1}H chemical shifts were observed between the two concentrations indicating that, in the present system, di-C_6PC at 65 mM lipid is principally in the form of micelles, as is di-C_8PC which has a cmc of < 4 mM [8,9]. Similar results were obtained with di-C_6PC and di-C_8PC micellar systems in maleate buffer at pH 5.26 (Fig. 2). The magnitude of the IAA-induced shifts are somewhat smaller at this

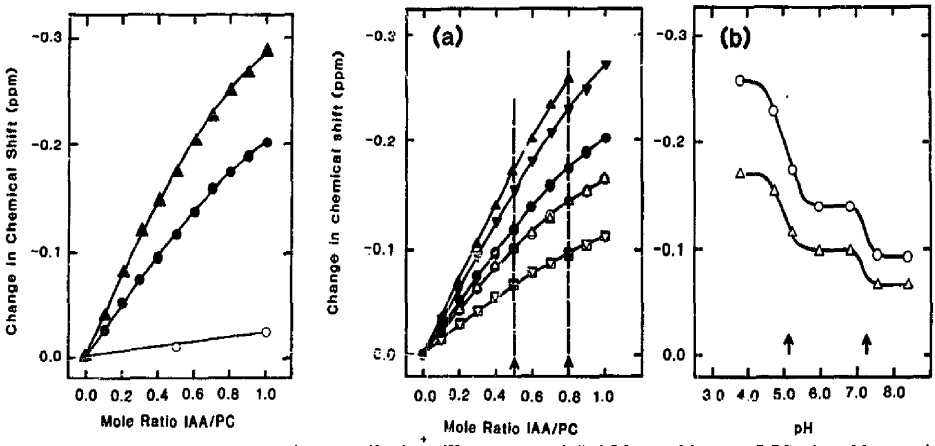

Fig. 2. (Left) Changes in IAA-induced chemical shift of $-\overset{+}{N}(CH_3)_3$ protons of di-C_6PC, 65 mM (●), di-C_6PC, 12.5 mM (○) and di-C_8PC, 65 mM (▲) in maleate buffer (pH 5.26).

Fig. 3. (Right) Effects of pH on the IAA-induced changes in chemical shift of $-\overset{+}{N}(CH_3)_3$ protons of di-C_6PC. (a) IAA titration curves carried out using: 200 mM acetate: pH 3.85 (▲), pH 4.75 (▼); 200 mM maleate: pH 5.26 (●), pH 6.00 (○); 100 mM phosphate: pH 6.83 (△) and 200 mM Tris: pH 7.56 (□), pH 8.35 (▽). (b) IAA-induced shift changes versus pH for 0.5 mole ratio (△) and 0.8 mole ratio (○) IAA/PC. The arrows indicate the approximate inflexion positions in the curves

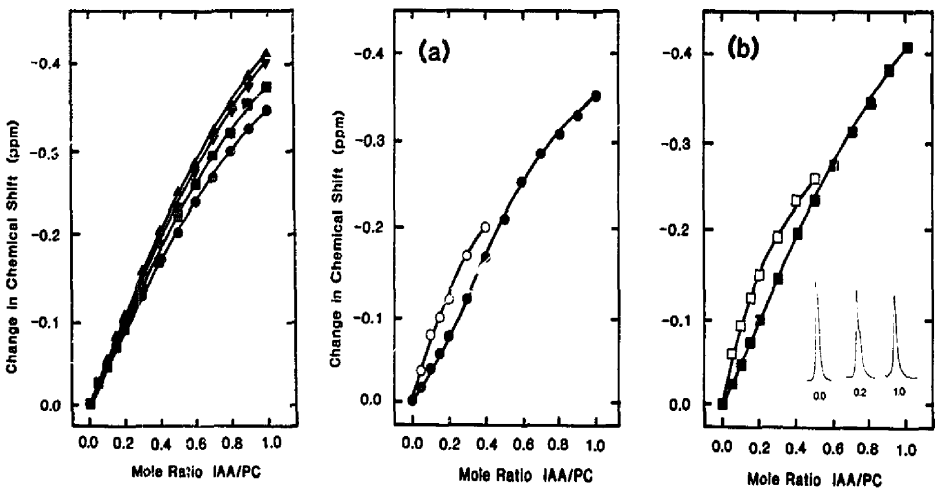

Fig. 4. (Left) Changes in chemical shift of $-\overset{+}{N}(CH_3)_3$ protons of di-C_{10}PC (●), di-$C_{18:1}$PC (▲), di-$C_{18:2}$PC (▼) and di-$C_{18:3}$PC (■) with increasing concentrations of IAA (65 mM in acetate buffer (pH 3.85)).

Fig. 5. (Right) IAA-induced changes in $-\overset{+}{N}(CH_3)_3$ proton chemical shifts for (a) di-C_{12}PC (●) and (b) di-C_{14}PC (■) at 65 mM lipid in acetate buffer (pH 3.85). The closed and open symbols refer to the downfield and upfield components of the peak, respectively. The ratio of the downfield and upfield components is approximately 2:1 in both cases. The inset shows the shape of the $-N(CH_3)_3$ resonance of di-C_{14}PC at three different IAA mole ratios.

pH than at pH 3.85 and this can be ascribed to the presence of ionised IAA. Furthermore, the increased solution viscosity of di-C_8PC with increasing concentrations of IAA was not observed. Note that when the concentration of di-C_6PC is reduced to below its cmc small IAA-induced shifts are observed in agreement with previous findings [4]. The magnitudes of the IAA-induced shifts are not a simple function of IAA ionisation however. Fig. 3a shows the IAA-induced shifts of di-C_6PC micelles in a range of buffers at different pH values. The plots of shift versus pH derived from the data at two different concentrations of IAA (Fig. 3b) show two inflexion points at approximately pH 5.1 and pH 7.2. It is not known if the two different pK_a values of IAA are caused by different IAA locations within the PC interface region of the micelle (as a function of pH), or by a pH-induced change in conformation of the PC headgroup, or a combination of these factors. With soy PC vesicles only one inflexion point, at pH 6.1, was observed [5].

The interactions of IAA with longer chain PCs in the form of vesicles are shown in Figs. 4 and 5. There is a small but significant increase in the IAA-induced shifts going from di-C_{10}PC to di-C_{14}PC, although a further increase to C_{18} acyl chains does not alter the IAA-induced shifts. Increased chain unsaturation appears to decrease the IAA-induced shifts. The K_d values and Δ values for the complexes formed between IAA and the lipids, computed using Eqn. 1, are given in Table I and range from 2.8 to 6.9 mM and −0.39 to −0.54 ppm, respectively. Di-C_{12} and di-C_{14}PCs are unusual in that their ^{1}H-$\overset{+}{N}(CH_3)_3$ resonances have a high-field shoulder in the absence of IAA. When titrated with IAA these shoulders become more pronounced up to IAA concentrations of approx. 0.25 mole ratio when they gradually merge with, and eventually disappear into the main peak (Fig. 5). The heights of these shoulders are approximately one half the height of the main peaks. The linewidths ($\nu_{\frac{1}{2}}$) of the -$\overset{+}{N}(CH_3)_3$ resonances at 0 and 1.0 mole ratio IAA are 5.5 and 4.5 Hz, respectively, for di-C_{12}PC and 8.1 and 6.5 Hz for di-C_{14}PC. Di-C_{10}PC does not show this splitting of the -$\overset{+}{N}(CH_3)_3$ peak, however, and the ^{1}H-NMR linewidths are 6.6 and 8.2 Hz in the presence of 0 and 1.0 mole ratio of IAA, respectively.

TABLE I

DISSOCIATION CONSTANTS (K_d) AND COMPLEX SHIFTS (Δ) DETERMINED FOR THE INTERACTION BETWEEN IAA AND VARIOUS DIACYLPHOSPHATIDYLCHOLINES [a] ASSUMING A 1:1 STOICHIOMETRY

PC	K_d (mM)	Δ(ppm)
di-C_6	3.7±0.8	−0.389±0.003
di-C_{10}	6.9±1.6	−0.489±0.004
di-C_{14}	4.0±1.0	−0.538±0.004
di-$C_{18:1}$	3.8±2.0	−0.518±0.005
di-$C_{18:2}$	3.4±2.4	−0.509±0.002
di-$C_{18:3}$	2.8±1.8	−0.465±0.005

[a] Curves could not be fitted to the data obtained for di-C_4PC, di-C_8PC and di-C_{12}PC.

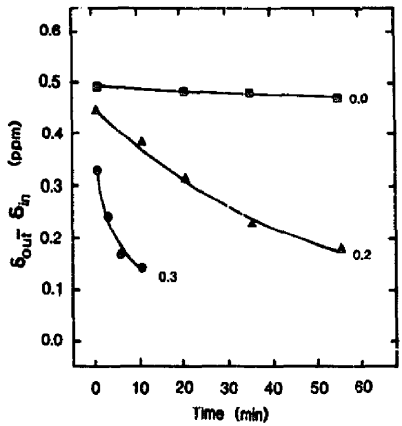

Fig. 6. Rates of movement of Pr^{3+} into di-C_{14}PC vesicles in acetate buffer (pH 3.85) as a functio. of IAA concentration in the presence of 10 mM Pr^{3+}. Lipid concentration was 65 mM. Mole ratios of IAA/PC are shown.

When paramagnetic ions such as Pr^{3+} or Nd^{3+} are added to long chain (> C_{14})PC vesicle dispersions after the sonication step, the inside and outside lamellae of the vesicle can be distinguished. This is due to the restricted permeability of the membrane to these cations such that only the outer PC lamellae 'see' the paramagnetic ions, resulting in the displacement of the outer headgroup NMR resonances [13]. The trans-membrane movement of the ions, enhanced by IAA and other auxins [14] and ionophores [15,16], can be observed and transport rates measured by

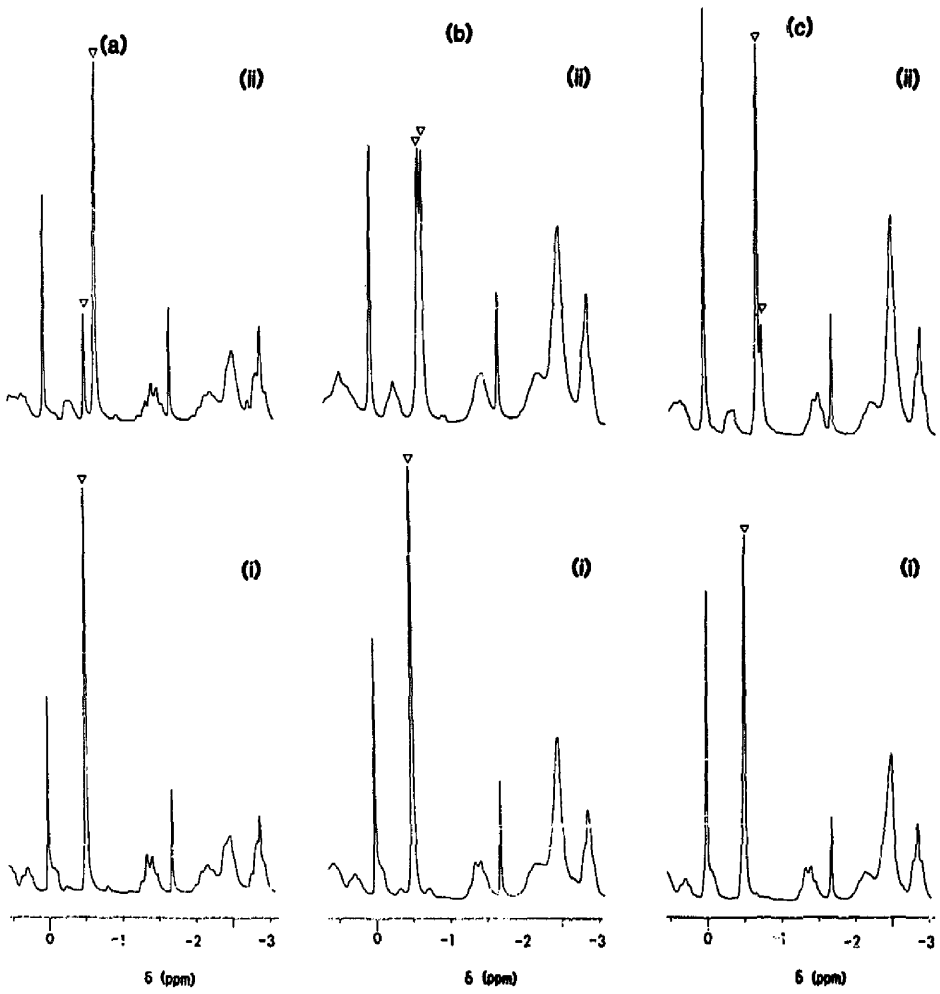

Fig. 7. ^{1}H-NMR spectra of mixtures of (a) di-C_4PC/di-C_6PC (20:80 mol %), (b) di-C_6PC/di-C_8PC (50:50 mol %) and (c) di-C_6PC/di-C_{14}PC (80:20 mol %), acetate buffer (pH 3.85) at 65 mM total lipid in the absence (i) and presence (ii) of 0.5 mole ratio IAA. Resonances marked by ▽ arise from -$N(CH_3)_3$ protons.

monitoring the displacement with time of the inside headgroup resonances As the acyl chain is shortened, however, the membranes become increasingly permeable. For example, Pr^{3+} movement into di-C_{14}PC vesicles is completed in approximately 24 h. This rate is substantially increased by adding IAA (Fig. 6) in agreement with previous findings using other PCs [17]. With di-C_{12}PC and di-C_{10}PC permeability rates, in the absence of IAA, are substantially faster with no differentiation between inside and outside signals for the latter on the timescale of the NMR measurements (approx. 1 min). These results are similar to those observed with Nd^{3+} [10].

Diacylphosphatidylcholine mixtures

In order to investigate further the effects of chain length on the phospholipid interaction with IAA, binary mixtures of PCs with chain lengths of 4, 6 and 8 carbon atoms were used. In addition,

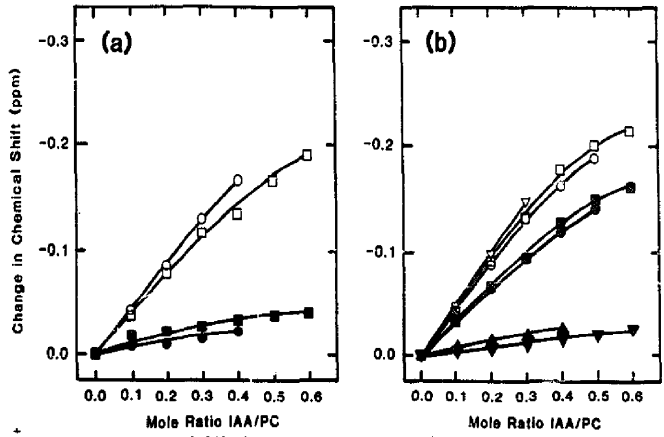

Fig. 8. Changes in $-\overset{+}{N}(CH_3)_3$ proton chemical shifts for mixtures of (a) di-C_4PC/di-C_6PC at mole ratios of 20:80 (●, ○) and 80:20 (■, □); (b) di-C_6PC/di-C_8PC at mole ratios of 20:80 (▽), 50:50 (■, □), 80:20 (●, ○) and di-C_4PC/di-C_8PC at mole ratios of 50:50 (▼) and 80:20 (▲) when titrated with IAA. All preparations were made in acetate buffer (pH 3.85) with a total lipid concentration of 65 mM. Where two symbols are shown for a particular lipid mixture the closed and open symbols refer to the downfield and upfield components, respectively.

di-C_{14}PC was mixed with di-C_6PC in the ratio 1:4. Short chain amphiphiles are readily incorporated into micelles formed from longer chain amphiphiles, and this incorporation also reduces the cmc of both the longer chain and shorter chain amphiphiles [12]. In the absence of IAA all preparations gave a single sharp $-\overset{+}{N}(CH_3)_3$ resonance and when IAA was added this peak moved upfield as previously observed. With a number of preparations, however, the $-\overset{+}{N}(CH_3)_3$ resonance was split into two components the ratios of the peak areas of which mirrored the mole fractions of the

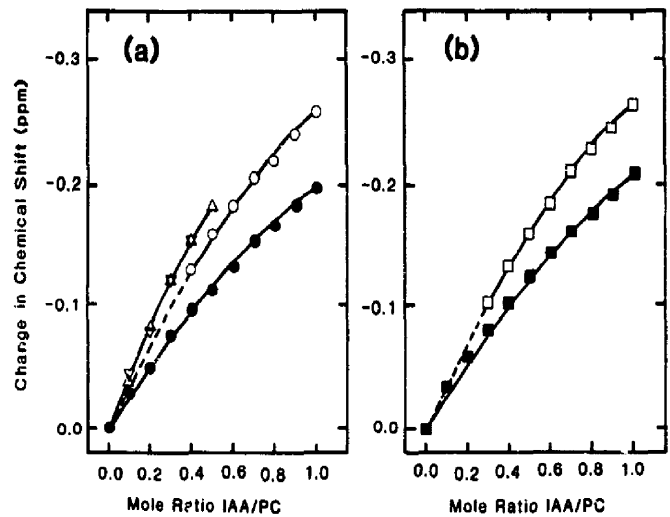

Fig. 9. Changes in IAA-induced $-\overset{+}{N}(CH_3)_3$ chemical shifts for mixtures of di-C_6PC and di-C_8PC in maleate buffer at pH 5.26. (a) 80:20 mol % di-C_6/di-C_8PC (●, ○), 20:80 mol (△) and 10:90 mol % (▽) and (b) 50:50 mol % diC_6/diC_8PC (■, □).

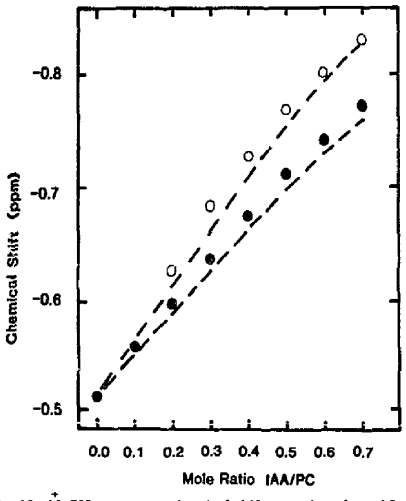

Fig. 10. $-\overset{+}{N}(CH_3)_3$ proton chemical shifts as a function of IAA concentration, for a mixture of di-C_6PC/di-C_{14}PC in the molar ratio of 80:20. Total lipid concentration was 65 mM. Solid and open circles show the experimental data whilst the broken lines were calculated using data in Table I.

individual phospholipids in the mixtures (Fig. 7). Fig. 8 shows the IAA-induced changes in $-\overset{+}{N}(CH_3)_3$ chemicals shifts for mixtures of (a) C_4/C_6, (b) C_6/C_8, and C_4/C_8 PCs at pH 3.85, and Fig. 9 shows the IAA-induced shift changes for mixtures of C_6/C_8 PC at pH 5.26. The magnitude of these shift changes appears to be similar to those of the individual compounds. Di-C_{14}PC, which forms multilamellar vesicular dispersions in aqueous buffer, can be incorporated into di-C_6PC micelles at 20 mol %. This system also demonstrated peak splitting of the $-\overset{+}{N}(CH_3)_3$ resonance, in the ratio of 4:1, on the addition of IAA (Fig. 9). The magnitudes of the IAA-induced upfield changes in chemical shift of these two components can be correlated with the values expected from the IAA-induced shifts of individual phospholipids using data from Table I. This is illustrated by the agreement between the theoretical values (broken lines) and the experimental points (Fig. 10, in which it is assumed that di-C_6PC is completely in micellar form). It is suggested that IAA interacts with phospholipids in binary systems as though the phospholipid molecules were discrete entities.

This was further explored by using mixtures of di-C_{10} and di-$C_{18:1}$ PCs. When vesicles made by sonicating a mixture of these two lipids in a molar ratio of 1:4, respectively, were titrated with IAA, the $-\overset{+}{N}(CH_3)_3$ proton resonance, which was narrow ($\nu_{\frac{1}{2}} = 6.5$ Hz) and symmetrical in the absence of IAA, moved upfield. Furthermore, at a proton frequency of 90 MHz, as the IAA concentration increased this resonance became progressively more asymmetric with a longer tail on the downfield side. At higher magnetic field strength (300 MHz proton frequency) this asymmetry was observed as a distinct low field shoulder on the $-\overset{+}{N}(CH_3)_3$ resonance, as illustrated in Fig. 11 where 0.5 mole ratio of IAA has been added to the lipid preparation. The positions of this shoulder and of the main peak are in agreement with the observed values for single lipid component di-C_{10}PC and di-$C_{18:1}$ PC systems (Fig. 4). The question of whether the results are derived from two different populations of vesicles (i.e., di-C_{10}PC vesicles and di-$C_{18:1}$ vesicles) or a single population of mixed lipid vesicles, was also examined by monitoring the permeability of these membranes to Pr^{3+}. As described earlier, di-$C_{18:1}$PC membranes are effective barriers to Pr^{3+} movement into the vesicles. However, when 20 mol % di-C_{10}PC is incorporated into the system the vesicles become very

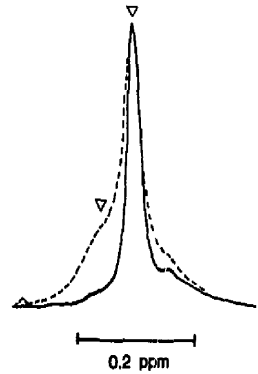

Fig. 11. Superimposed ^{1}H-NMR spectra at 300 MHz of a mixture of di-C_{10}PC and di-C_{18}PC (20:80) in acetate buffer pH 3.85 showing the shape of the $-\overset{+}{N}(CH_3)_3$ resonance in the absence (solid line) and presence of 0.5 mole ratio of IAA (broken line). Main component and low-field shoulder of the peak are marked ▽.

permeable, to the extent that Pr^{3+}-induced separation of outer and inner -$\overset{+}{N}(CH_3)_3$ resonances is not seen (data not shown). This indicates that di-C_{10}PC is incorporated into vesicles formed by di-$C_{18:1}$PC and that a single population of mixed lipid vesicles is formed as a result of sonication. It also indicates that IAA does, in fact, distinguish between the two components of a binary mixed lipid membrane system.

Discussion

In an earlier study with single acyl chain lipids it was found that IAA produced large changes in chemical shifts only when the lipids formed organized, membranous micellar or bilayer structures [4]. That most important finding is clearly true with diacyl chain PCs as well, since PC concentrations above the cmc are required before IAA can induce large shifts. In the single acyl chain lipid systems, with which micelles were the predominant species when concentrations exceeded the cmc, the IAA-induced shifts of the -$\overset{+}{N}(CH_3)_3$ protons were shown to be dependent on both the length of the acyl chain and the nature of the lipid headgroup [4]. It was also shown that an inverse relationship existed between the size of the IAA-induced shift and the headgroup volume. For IAA and lyso PCs the magnitude of the complex shifts were approx. −0.6 ppm compared to complex shifts of approx. −1.7 ppm calculated for IAA and trimethylammonium bromides. These values can be compared to complex shifts in the range of −0.35 to −0.54 ppm calculated for the interaction between IAA and diacyl PCs in the present study. The effects of acyl chain length on the IAA-induced shifts in single-chain systems, generally, had only minor significance, although the acyl cholines displayed large differences (up to 0.25 ppm at 1.0 mole ratio of IAA). With diacyl PC both micelle and vesicle systems show increasing IAA-induced shifts with acyl chain length, i.e. di-C_8 > di-C_6 > di-C_4 and di-C_{14} > di-C_{12} > di-C_{10}. The explanation for this in micellar systems probably lies in both the concentration and size of the micelles formed. The small IAA-induced shifts in di-C_4PC systems reflects the monomeric nature of this lipid at a concentration of 65 mM as used in the present experiments. However, since di-C_6PC and di-C_8PC at 65 mM lipid are principally in the form of micelles the differences in IAA-induced shifts of these lipids must be due to some other factor. A possible explanation resides in the proposal that small hydrophobic molecules like IAA are localized in the hydrocarbon region/headgroup region interface of both micelles and bilayers [18,19]. Furthermore, the degree of penetration of IAA into phospholipid bilayers has been suggested as the mechanism by which IAA causes the observed changes in headgroup ^{1}H-NMR chemical shifts [4]. The extent to which molecules such as IAA penetrate into the membrane is also a function of their molecular structure [13] in which the balance between the hydrophilic and lipophilic groups and the spatial orientation of these groups in the molecules, is of importance. Thus, both the interaction and the consequences of the interaction between a membrane and these hydrophobic molecules are determined not only by the structure of the membrane (which is, in turn, determined by the nature of the component lipid molecules), but by the characteristics of the hormone as well.

It is pertinent to note that the choline -$\overset{+}{N}(CH_3)_3$ signal of di-C_{10}PC does not shown any signs of splitting in the presence or absence of IAA in contrast to the effects observed with di-C_{12}PC and di-C_{14}PC. It is thought that the IAA-induced splitting of the -$\overset{+}{N}(CH_3)_3$ resonance in vesicle preparations of the latter phospholipids arises from a difference in interaction of IAA with the inside and outside phospholipid molecules as a result of differences in lipid packing on the inner and outer lamellar surfaces. At higher magnetic field strengths this difference in packing can be directly observed for egg PC vesicles [22]. The absence of IAA-induced splitting in di-C_{10}PC vesicles indicates that the interaction of IAA with PC in the inner and outer lamellae of these vesicles is the same, or that there is an averaging of the -$\overset{+}{N}(CH_3)_3$ signals from the inner and outer lamellae. For the latter to occur, however, the processes involved must be rapid (an upper limit for the lifetime of the species giving rise to the NMR signal, calculated from the fast exchange expression $\tau \ll 1/2\pi\Delta\nu$, is 8 mS (see Ref. 9)).

Using binary mixtures of short chain diacyl PCs, were have shown, by the differential magnitudes of the IAA-induced shifts, that IAA is able to distinguish between the different phospholipids in the mixture. Whilst we cannot completely rule out an IAA-induced separation of PC species into different acyl chain length micelles, this would appear unlikely. Furthermore, results with mixed micelles containing di-C_6PC and di-C_{14}PC, in which the latter does not form stable micelles except at very low concentrations, and where the ratio of $-\overset{+}{N}(CH_3)_3$ intensities corresponds to the mole fractions of the two components, suggest that only one type of micelle is formed with which IAA interacts. Similarly, using a mixture of di-C_{10}PC and di-$C_{18:1}$PC in the form of vesicular membranes it has been demonstrated again that IAA enters into specific interactions with the individual components that are a function of the fatty acid chain length of the phospholipids.

These findings are significant in that they demonstrate that IAA can interact with phospholipid membranes in a manner which is not only dependent on the nature of the phospholipid headgroup but also on the nature of the hydrocarbon region of the membrane. Additionally, since the charge density and charge distribution of the headgroup region of the membrane are changed as a result of IAA binding [14], the nature of the hydrocarbon region of the membrane will be a determinant of these changes and will confer on the membrane a specificity for other binding such as that of ions and macromolecules.

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