Complex Formation between Indole-3-acetic Acid and Phospholipid Membrane Components in Aqueous Media. 2. Interaction of Auxins and Related Compounds with Phosphatidylcholine Membranes†

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ABSTRACT: The interaction between indole-3-acetic acid (IAA) and soybean phosphatidylcholine (PC) in the form of small unilamellar vesicles has been studied with ^1H nuclear magnetic resonance. IAA induces upfield changes in chemical shifts of the protons of the choline head group, particularly $-N^+(CH_3)_3$ protons, which have been used to determine dissociation constants for the IAA–PC interaction. These effects are ligand specific in that compounds (auxins) closely related in chemical structure to IAA induce different chemical shift changes and give rise to different dissociation constants. The interaction appears to be determined by a sensitive balance between lipophilicity, charge density, and three-dimensional structure of the ligand. These IAA-related compounds are also able to promote changes in permeability of the PC model membrane to Pr^{3+} in a manner that is dependent on the nature of the ligand. These effects on permeability and the induced chemical shift changes of PC head group protons appear to be independent manifestations of the structural specificity of the auxins.

The plant hormone indole-3-acetic acid (IAA)[1] has been shown previously to interact with phosphatidylcholine (PC) in the form of micelles (Marker et al., 1978) and small unilamellar vesicles (Jones et al., 1984) by ^1H NMR techniques. As a means of elucidating the structural specificity of the interaction of IAA with PC vesicles, the abilities of compounds related to IAA (auxins) to induce PC head group ^1H chemical shift changes have been investigated. Furthermore, the effectiveness of these molecules in influencing cation movement through the membrane, previously demonstrated for IAA (Jones et al., 1984), was studied.

Experimental Procedures

Soybean PC vesicles were prepared in 200 mM acetate buffer (pH 3.85) at a concentration of ca. 65 mM as reported previously (Jones et al., 1984). All ligands were purchased from Sigma and used as received except IAA, which was recrystallized from 1,2-dichloroethane. Solutions of IAA and other carboxylic acids were made up as the Na^+ salts, at concentrations that were approximately isotonic with the PC vesicle systems, by titrating the free acids in D_2O with NaOD. Solutions of amine-containing compounds were made up as the hydrochlorides in D_2O.

Nuclear Magnetic Resonance. ^1H NMR spectra were recorded on a JEOL FX-90Q Fourier-transform spectrometer at 90 MHz with a spectral width of 800 Hz accumulated into 8K addresses at a probe temperature of 24 °C. Dioxane was used as an internal reference. Chemical shift reproducibility was better than ±0.005 ppm.

K_d Calculations. Values for the dissociation constants (K_d) and complex shifts (Δ) for the interaction between ligand and PC were determined from the concentration-dependent upfield changes in chemical shift of the PC $-N^+(CH_3)_3$ protons as reported previously (Jones et al., 1984). Occasionally, chemical shift changes of the choline group $N^+–CH_2–$ and $O–CH_2–$ protons were also used to obtain K_ds. In these cases, there was good agreement between the K_ds calculated for the three types of protons. Calculated parameters were obtained for all ligands by assuming a stoichiometry of 1:1 ligand to PC. Other simple stoichiometries failed to give satisfactory fits to the data.

Results

Ligand-Induced Changes in Chemical Shifts. The addition of IAA (as the Na^+ salt) to PC vesicles in acetate buffer (pH 3.85) produces upfield changes in chemical shifts of the protons of the choline head group. Of these protons, the $-N^+(CH_3)_3$ resonance is the most easily and accurately observed in ^1H spectra and gives rise to calculated parameters of the highest accuracy and precision. Other head group resonances such as the choline $N–CH_2$ and $O–CH_2$ methylene protons are broad complex multiplets and, in the case of the latter, are partially obscured by glycerol α- and γ-proton resonances. The glycerol β-proton resonance is obscured by olefinic protons of the acyl chains.

The abilities of IAA and the various ligands used in this study to induce changes in chemical shift of the $-N^+(CH_3)_3$ protons of PC are shown in Figures 1 and 2. Titration of PC vesicles with IAA produced the largest observed shift changes for which a K_d of 4.7 ± 0.6 mM and a Δ of −0.528 ± 0.005 ppm (Table I) were calculated.

Indole-3-propionic acid (IPA) produced smaller $-N^+(CH_3)_3$ shift changes than IAA although the K_d computed for this interaction is 3.9 ± 0.4 mM (Table I). The IPA complex shift is smaller, however. This trend is continued with indole-3-butyric acid (IBA) (Figure 1) for which a K_d of 2.0 ± 0.4 mM and a Δ of −0.317 ± 0.003 ppm were calculated.

Tryptophan, with an amine group in the side chain, is only weakly active in this system, producing a chemical shift change of approximately −0.04 ppm at a 1:1 mole ratio of tryptophan to PC. The presence of this amine group appears responsible

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[1] Abbreviations: NMR, nuclear magnetic resonance; IAA, indole-3-acetic acid; PC, soybean phosphatidylcholine; IPA, indole-3-propionic acid; IBA, indole-3-butyric acid; ICA, indole-2-carboxylic acid; α-NAA, naphthalene-1-acetic acid; β-NAA, naphthalene-2-acetic acid; PAA, phenylacetic acid; BA, benzoic acid; 2,4-D, (2,4-dichlorophenoxy)acetic acid.

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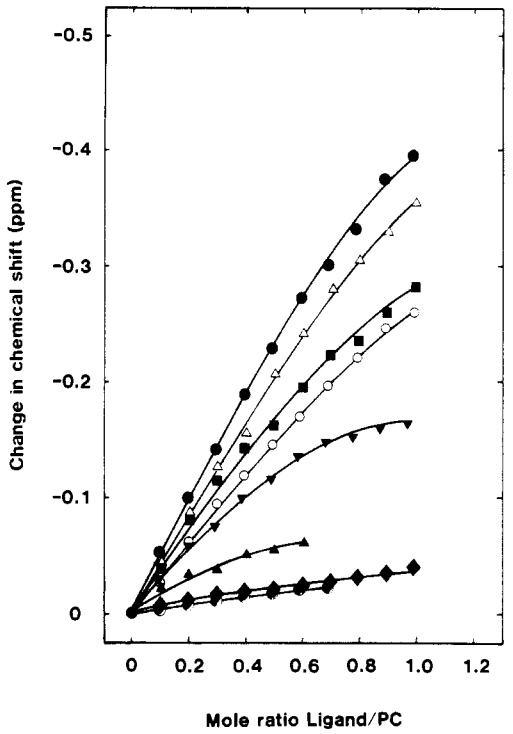

FIGURE 1: Changes in chemical shift of PC (66.4 mM) $-N^+(CH_3)_3$ protons as a function of incremental addition of IAA (●), IPA (Δ), IBA (○), α-NAA (■), β-NAA (▼), PAA (▲), BA (♦), and 2,4-D (◐). The solid lines drawn through the data points represent theoretical curves from which K_d and Δ values were obtained. Peak positions were measured relative to an internal dioxane reference.

Table I: Dissociation Constants (K_d) and Complex Shifts (Δ) Determined from Changes in Resonance Positions of $-N^+(CH_3)_3$ Protons

	K_d (mM)	Δ (ppm)
IAA	4.7 ± 0.6	−0.528 ± 0.005
IPA	3.9 ± 0.4	−0.465 ± 0.003
IBA	2.0 ± 0.4	−0.317 ± 0.003
tryptophan	22 ± 4	−0.100 ± 0.003
tryptamine	21 ± 3	−0.120 ± 0.004
5-hydroxy-IAA	32 ± 2	−0.672 ± 0.007
α-NAA	10.5 ± 0.5	−0.429 ± 0.003
β-NAA	24 ± 5	−0.328 ± 0.011
PAA	44 ± 11	−0.166 ± 0.011
BA	49 ± 14	−0.089 ± 0.008
2,4-D	66 ± 9	−0.088 ± 0.033

for the reduction in strength of the interaction since tryptamine also has comparable weak activity (Figure 2) with a K_d of 21 ± 3 mM and a Δ of −0.120 ± 0.004 ppm.

Smaller shift changes were observed with 5-hydroxy-IAA when compared with IAA although the Δ of −0.67 ppm is greater. Indole-2-carboxylic acid (ICA) displayed somewhat anomalous behavior. At low concentrations of this ligand, the PC $-N^+(CH_3)_3$ resonance moves upfield in a concentration-dependent manner as a single peak. However, at mole ratios greater than approximately 0.5, peak broadening was observed, which increased at higher concentrations of ICA. Eventually, the peak splits into two components that have integrated intensities in the ratio 1.7:1. The larger of these peaks moves upfield more rapidly than the smaller component (Figure 2). Significantly, the intensities of these components are the same as those obtained by using shift reagents to distinguish between outer and inner PC molecules in similar vesicle preparations (Jones et al., 1983), suggesting that ICA can be used to make this distinction. Unfortunately, theoretical K_d curves with the assumption of various simple stoichiometries for the interaction

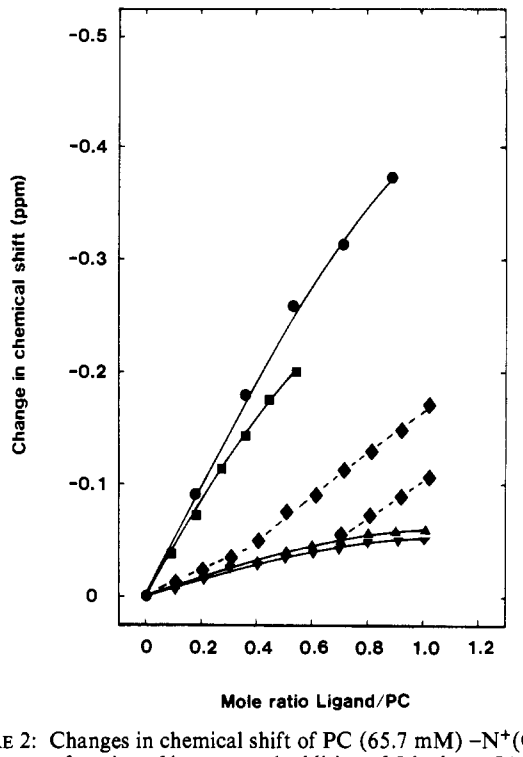

FIGURE 2: Changes in chemical shift of PC (65.7 mM) $-N^+(CH_3)_3$ protons as a function of incremental addition of 5-hydroxy-IAA (■), tryptamine (▲), tryptophan (▼), and ICA (♦). The data for IAA (●) have been included for comparison purposes. The solid lines drawn through the data points represent theoretical curves from which K_d and Δ values were obtained. The two sets of data for ICA are a result of the splitting of the $-N^+(CH_3)_3$ resonance. The broken lines through the data serve only as visual guides.

could not be fitted to the data for either component.

The effects of naphthalene-1-acetic acid (α-NAA) and of the 2-isomer (β-NAA) are different from those of IAA (Figure 1). Since the lipophilicity and the pK_a of the two NAA isomers are anticipated to be similar, the results demonstrate a high degree of structural specificity for the interaction with PC. Phenylacetic acid (PAA) is only weakly active in this system with a K_d of 44 mM and a Δ of −0.176 ppm, and benzoic acid (BA) and the herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) display even lower activities with K_ds of 49 and 66 mM and Δ's of −0.089 and −0.088 ppm, respectively (Figure 1, Table I).

Effects of Auxins on Vesicle Permeability to Pr^{3+}. In order to ascertain the ability of ligands to induce membrane permeability to Pr^{3+}, titration measurements were carried out in the presence of 10 mM Pr^{3+}. The induction of a downfield shift of the inner $-N^+(CH_3)_3$ resonance at a given concentration of ligand was used as an indication of its ability to induce Pr^{3+} influx into the vesicles. This approach is only valid, however, if the vesicles remain intact during the course of the titration experiments. In this context, we have previously demonstrated that IAA does not cause vesicle disruption in the presence of Pr^{3+} (Jones et al., 1984). Furthermore, during the present experiments the inner and outer $-N^+(CH_3)_3$ resonances remained symmetrical and showed no significant increases in line width, also indicating that vesicle structure remained intact [see Ting et al. (1981)].

In Figure 3, the changes in chemical shift of the inner $-N^+(CH_3)_3$ resonance are plotted against ligand concentration. As previously observed (Jones et al., 1983), the inner $-N^+(CH_3)_3$ resonance moves upfield with increasing concentrations of IAA until a mole ratio of ~0.5 IAA to PC is reached. At higher concentrations, the rate of upfield shift declines, and at a mole ratio of 0.7 the peak has moved downfield. We have

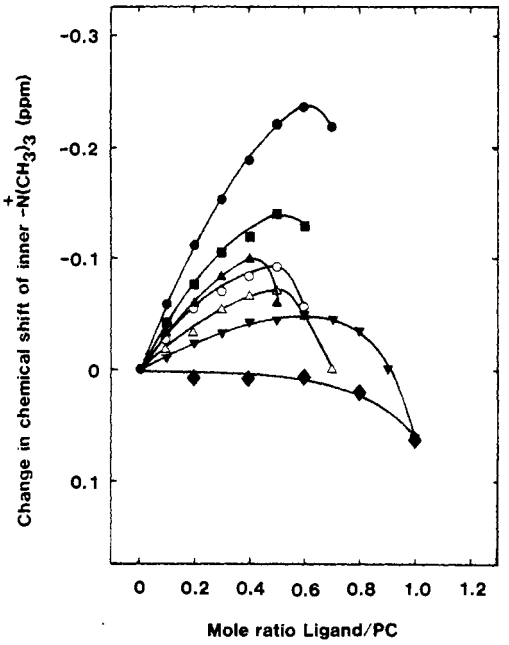

FIGURE 3: Changes in chemical shift of the inner facing PC $-N^+(CH_3)_3$ protons as a function of incremental addition of IAA (●), IPA (■), IBA (▲), PAA (▼), BA (♦), α-NAA (○), and β-NAA (△) in the presence of 10 mM extravesicular Pr^{3+}. Chemical shifts were measured relative to an internal dioxane reference. PC concentration is 65.1 mM.

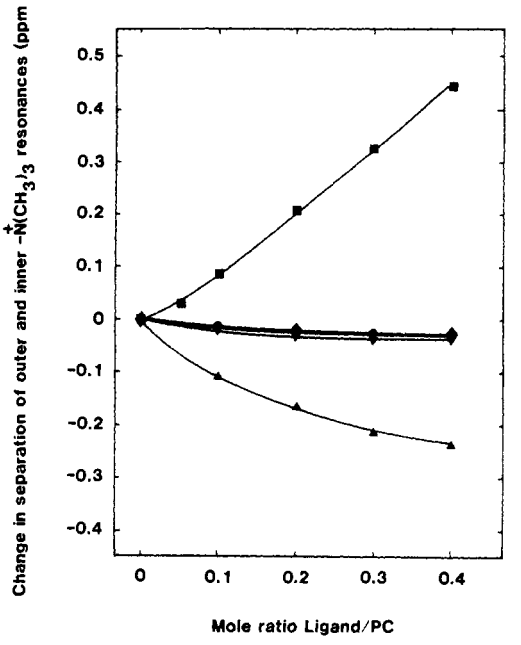

FIGURE 4: Effects of increasing concentrations of 2,4-D (■), IAA (●), PAA (▼), tetracaine (▲), and BA (♦) on the ability of extravesicular Pr^{3+} (10 mM) to influence the outer $-N^+(CH_3)_3$ proton resonance position of PC vesicles (65.0 mM). Changes in separation of outer and inner $-N^+(CH_3)_3$ group proton resonances are plotted against ligand concentration.

shown subsequently that the rate of this downfield movement is first order with respect to Pr^{3+} concentration and that at a mole ratio of 0.7 IAA to PC the half life of the process in the present system is 7.8 min (G. P. Jones and L. G. Paleg, unpublished data). Changes in membrane resistance to the movement of Pr^{3+} are also observed with other ligands. IBA is the most efficient ligand in promoting Pr^{3+} influx into PC vesicles (Figure 3) in that a mole ratio of 0.5 IBA to PC causes downfield movement of the inner $-N^+(CH_3)_3$ peak. Significantly, all of these carboxyl-containing ligands promote Pr^{3+} influx but at widely differing concentrations. Furthermore, the shapes of these curves suggest that the effects are not simply a function of ligand concentrations. In this respect, we have found that the rate of IAA-promoted Pr^{3+} influx into vesicles is proportional to the seventh power of the IAA concentration (i.e., rate $\propto$ [IAA]^7; G. P. Jones and L. G. Paleg, unpublished data), and it is plausible to assume that different rate equations apply for the various ligands. It is pertinent that in this system also, α-NAA and β-NAA show differential effects. Anomalous behavior is observed for 2,4-D, however. In the absence of Pr^{3+}, 2,4-D induces only a small change in the chemical shift of the PC $-N^+(CH_3)_3$ protons (Figure 1). Titration with 2,4-D in the presence of Pr^{3+} did not produce any significant changes in chemical shift of the inner $-N^+(CH_3)_3$ protons even at the highest concentrations used (1:1 mole ratio of 2,4-D to PC). However, a progressive downfield shift of the resonance associated with the outer $-N^+(CH_3)_3$ protons relative to the inner group resonance was observed, and this movement is dependent on the 2,4-D concentration (Figure 4). This effect is thought to occur through an enhanced binding of Pr^{3+} to the head group region of the bilayer, thereby promoting the ability of Pr^{3+} to induce downfield changes in chemical shift of protons in this region. Since 2,4-D does not appear to affect the resonance position of the inner $-N^+(CH_3)_3$ protons, this enhanced binding of Pr^{3+} to the head group is not instrumental in facilitating Pr^{3+} movement through the bilayer. Similar measurements obtained with IAA and the other arylacetic acids suggest that these compounds are able to reduce the binding of Pr^{3+} to the head group, in that small upfield displacements of the outer $-N^+(CH_3)_3$ resonance relative to the change in shift of the inner $-N^+(CH_3)_3$ are observed (Figure 4). The IAA and other arylacetic acid effects are small, however, when compared with that of the anesthetic tetracaine. Tetracaine has been shown to displace Pr^{3+} from the head group region of PC vesicles by reversing, in a concentration-dependent manner, the Pr^{3+}-induced downfield shift of outer $-N^+(CH_3)$ protons (Fernández & Cerbón, 1973). These findings were also obtained with the present system (Figure 4).

Discussion

The abilities of ligands to induce changes in chemical shift of the PC $-N^+(CH_3)_3$ protons in acetate buffer (pH 3.85) is a function of the molecular characteristics of the ligand. These concentration-dependent shift changes have been used to calculate K_ds and Δ's for the various ligands. The former is a measure of the strength of the ligand –PC interaction whilst Δ is a measure of the perturbation in the magnetic environment of the $-N^+(CH_3)_3$ group caused by the interacting ligand. Both of these parameters vary considerably with the nature of the ligand, and the variation indicates that the interaction has a high degree of specificity. The data suggest that this specificity is brought about by a sensitive balance between molecular geometry and lipophilicity of the ligand.

Whereas IAA produces the largest observed changes in chemical shift, it does not have the strongest binding or give rise to the largest complex shift of the compounds investigated. An increase in length of the carboxyl-containing side chain produces an increase in the strength of the ligand–PC interaction but a decrease in complex shift. The presence of a group capable of accepting a positive charge (at pH 3.85) reduces activity as is indicated by the weak activities of tryptamine and tryptophan.

The nature of the aromatic ring in the arylacetic acids is important in determining binding strength. This is not necessarily a direct function of ring lipophilicity, however, since

the activities in this system are IAA > NAA > PAA, whereas the oil/water partition coefficients (and hence lipophilicities) of the parent hydrocarbons are naphthalene > benzene > indole (C. Hansch, private communication). This is also illustrated by the differences in activities of α- and β-NAA. Nevertheless, a degree of lipophilicity is required since 5-hydroxy-IAA, which is less lipophilic than its parent compound, has substantially weaker binding. In this context it is important to note that the un-ionized form of IAA produces significantly larger changes in chemical shift than its ionized counterpart, with correspondingly stronger binding and a larger complex shift (Jones et al., 1983). Suppression of ionization of the carboxyl group is likely to enhance the lipid solubility of IAA and therefore enhance permeation of the molecule into the lipid bilayer.

The weak interaction between 2,4-D and PC may be explained by the inability of the former to penetrate deeply into the bilayer because of the charge on the carboxyl group at pH 3.85 (pK_a of 2,4-D is 2.8) although geometrical factors and the increased hydrophilicity of the oxyacetic acid side chain cannot be ruled out. Nevertheless, 2,4-D is associated with the head group region of the bilayer since it is able to increase the binding of Pr^{3+} to the head group of PC presumably by increasing the negative charge in the head group region. In this respect, its action is opposite to that of tetracaine for which repulsion of Pr^{3+} from the head group region is observed (Fernández & Cerbón, 1973).

IAA and related compounds have been shown to enhance the permeability of the bilayer to Pr^{3+}. These effects do not appear to be directly related to the strength of the interaction or to the complex shift produced although IBA, which gives the strongest binding with PC, appears to be the most effective ligand in promoting Pr^{3+} movement. In order for these changes in cation movement to occur, however, the Pr^{3+} must have a time-averaged location close to the head group region. The lack of effects of amine-containing ligands such as tryptamine, tetracaine, and tryptophan, at the pH studied, may be due to the repulsion of cations from this region. As noted earlier, the quantitative and qualitative nature of the titration curves obtained in the presence of Pr^{3+}, which are used as an indicator of Pr^{3+} movement across the bilayer, varies considerably from one ligand to another. A detailed discussion of these points must await a full kinetic analysis of the changes in ligand-induced membrane permeability phenomena (G. P. Jones and L. G. Paleg, unpublished results).

The interaction between IAA and related auxins and PC indicates that the ligands modify three different, apparently unrelated, features of the model membrane. The interaction of auxins with PC alters the magnetic and, hence, electronic environment of the head group as evidenced by the changes in ^1H chemical shifts. These changes are not, however, a simple function of head group charge density, since compounds that modify the overall charge in the head group region such as 2,4-D and the amine-containing ligands (which modify the ability of polyvalent cations to interact with the head group) produce only small ^1H chemical shift changes. Lastly, the auxins also influence the permeability of the membrane to cations in a ligand-specific manner, and these effects also do not appear to be directly related to their ability to induce changes in chemical shift of the head group protons. It is pertinent to note that these effects are observed with a single phospholipid component system.

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Registry No. IAA, 87-51-4; IPA, 830-96-6; IBA, 133-32-4; ICA, 1477-50-5; α-NAA, 86-87-3; β-NAA, 581-96-4; PAA, 103-82-2; BA, 65-85-0; 2,4-D, 94-75-7; Pr, 7440-10-0; 5-hydroxy-IAA, 54-16-0; tryptophan, 73-22-3; tryptamine, 61-54-1.

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