Interaction of Abscisic Acid with Phospholipid Membranes†

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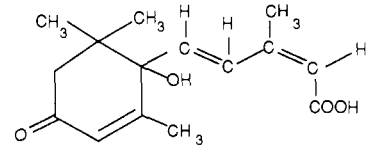
Received July 18, 1988; Revised Manuscript Received November 18, 1988

ABSTRACT: The plant hormone abscisic acid (ABA) is shown, under certain conditions, to greatly enhance the permeability of phospholipid bilayer membranes to the nonelectrolyte erythritol (followed spectrophotometrically by osmotic swelling) and the anion carboxyfluorescein (followed by fluorescence). The hormone is ineffective with single- and mixed-component phosphatidylcholine membranes in the liquid-crystalline or gel states. In contrast, substantial ABA-induced permeability is measured for two-component membranes containing lipids with different polar head groups or containing phosphatidylcholines with different acyl chains at temperatures where gel and liquid-crystalline phases coexist. Despite the large ABA-induced enhancement in bilayer permeability, no evidence for a substantial change at the molecular level was seen in the membranes by magnetic resonance techniques. ^{13}C NMR spin–lattice relaxation times, T_1, in sonicated unilamellar vesicles and ESR of spin-labeled fatty acids intercalated into membranes showed negligible effect on acyl chain order and dynamics within the bilayer, while ^{31}P NMR of sonicated unilamellar vesicles indicated negligible effect on molecular motion and conformation in the head-group region. We propose that, instead of causing a general nonspecific perturbation to the membrane, the hormone acts at membrane defects formed due to mismatch in molecular packing where two different head groups or acyl chain states interface. Increased membrane disruption by ABA at these points of membrane instability could then produce an enhancement in permeability.

The molecular mode of action has not been determined for any plant hormone. Attempts to identify specific receptors or binding proteins related to the hormones' physiological responses have resulted in conflicting or unconfirmed results or even in total failure (Venis, 1985). The hormone abscisic acid (ABA)[1] is believed to exert its initial influence at a target membrane altering the transmembrane distribution of ions (Van Steveninck & Van Steveninck, 1983). Two reports of specific proteinaceous binding sites isolated from membrane fractions have appeared. The initial paper of Hocking et al. (1978) was later shown to be incorrect when high specific activity [^{3}H]ABA was used. A subsequent paper from Hornberg and Weiler (1984) used photoaffinity labels to identify three ABA-binding proteins from *Vicia faba* guard cells. Despite the passage of almost five years, this potentially important work has not been confirmed. Furthermore, Smart et al. (1987), using a variety of binding assays, have not been able to detect any ABA-binding proteins. A real possibility exists that the interaction of ABA with membranes is not through specific proteinaceous receptors but instead may be with the lipid component of membranes.

Protein-free lipid bilayer membrane systems have been extensively employed to model various complex biological membranes. In our laboratory, we have studied the effect of ABA on phospholipid bilayers in an attempt to elucidate the possible role of the hormone in altering energy-independent, general membrane permeability (Stillwell & Hester, 1984a,b; Hester & Stillwell, 1984; Stillwell et al., 1985a,b, 1986, 1987; Wassall et al., 1985a,b; Schauf et al., 1987; Brengle et al., 1988). Utilization of these well-defined systems avoids many of the problems, which frequently lead to conflicting data (Glinka & Reinhold, 1971; Cram & Pitman, 1972), associated with the study of highly complex cell membranes that are responsive to ABA. We demonstrated that ABA enhances the permeability of bilayers composed of several types of natural and synthetic PCs to nonelectrolytes (urea and erythritol) and to ions (Pr^{3+} and Cl^-) but only when PE was incorporated as a minor membrane component. Lea and Collins (1979) showed that ABA increases electrical conductivity of egg PC planar bimolecular lipid membranes and suggested the hormone induces the formation of membrane channels. More recently, increased conductance to Na^+ or K^+ was measured by Bach (1986) in planar membranes formed from PC and PE. Harkers et al. (1986) reported ABA also enhances K^+ efflux from large unilamellar vesicles composed of soybean (mixed phosphatide) lecithin. In agreement with our own work (Stillwell & Hester, 1984b), these researchers found the protonated form of *cis-trans*-ABA is required for K^+ efflux. In contrast, no appreciable increase in permeability was detected at low hormone concentrations (10^{-4} M) by Parups and Miller (1978) for soy PC liposomes, while



cis-trans Abscisic Acid

† This research was supported, in part, by NSF Grants DMB 8607071 and DCB 8715558.

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[1] Abbreviations: ABA, abscisic acid; CF, carboxyfluorescein; CL, cardiolipin; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DPPA, dipalmitoylphosphatidic acid; DPPC, dipalmitoylphosphatidylcholine; IAA, indole-3-acetic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SA, stearyl amine; TMS, tetramethylsilane.

0006-2960/89/0428-2798$01.50/0

Hipkins and Hillman (1981) saw no effect for acid-loaded phospholipid vesicles. Markhart et al. (1979) found that ABA reduces the hydraulic conductivity of soybean root systems. They proposed that the elimination by ABA of a discontinuity in an Arrhenius plot of steady-state flow rate vs $1/T$ indicated an ABA-induced change in hydrocarbon chain mobility. Thus, although ABA appears to alter membrane-transport properties, it is apparent there is no consensus as to the mode of action for these processes.

From our prior experiments it is evident that ABA can dramatically increase the permeability of PC/PE lipid bilayers. Here we identify further important specific conditions under which substantial enhancement of permeability occurs for mixed phospholipid membranes and apply magnetic resonance techniques to search for hormone-induced changes in molecular ordering and dynamics within the membrane. These observations lead us to propose a hypothesis for the mechanism by which the hormone may alter membrane properties.

Experimental Procedures

Materials. Phospholipids were purchased from Avanti Polar Lipids, Birmingham, AL, and Sigma Chemical Co., St. Louis, MO. *cis-trans*-ABA and erythritol were also obtained from Sigma, while CF was supplied by Kodak, Rochester, NY. Molecular Probes, Eugene, OR, was the source of 5- and 12-doxyl spin-labeled stearic acids.

Sample Preparation. Aqueous dispersions of phospholipid multilamellar liposomes were prepared from the dried lipids by vortex agitation above the gel to liquid-crystalline phase transition temperature following addition of buffer. Sonication to produce small unilamellar vesicles was performed on ice or in a water-cooled jacket under nitrogen (5–10 min) with either a Tekmar Model VC 250 ultrasonic cell disruptor or Heat Systems Model W-220F cell disruptor.

Erythritol Permeability. Permeability of various PC membranes to erythritol was measured by osmotic swelling as described in Stillwell and Hester (1984b). Aqueous dispersions of 10 mM phospholipid multilamellar liposomes were made in 40 mM glucose and 10 mM sodium acetate, pH 5.0. After 1 h of incubation with appropriate amounts of ABA, the liposomes were equilibrated at the experimental temperature and then rapidly mixed in the swelling buffer (40 mM erythritol, 10 mM sodium acetate, pH 5.0). Initial liposome swelling rate, which is known to be proportional to erythritol permeability (De Gier et al., 1986), was determined spectrophotometrically on a temperature-controlled Beckman DU-8 computing spectrophotometer. Swelling rates, expressed as $d(1/A)/dt$ (%), where A is absorbance at 350 nm, are the averages of at least five determinations. Standard errors are always less than $\pm 0.5 \times 10^{-3}$ s^{-1}.

Carboxyfluorescein Leakage. Release of CF from small unilamellar vesicles was followed on a Perkin-Elmer MPF-66 fluorescence spectrophotometer. Excitation was at 490 nm and emission at 520 nm. Multilamellar liposomes were made in 60 mM CF and then were sonicated. Nonsequestered CF was removed on a Sephadex G-50 column. Vesicles were mixed at time zero with ABA dissolved in 10 mM sodium acetate, pH 5 buffer. At high concentrations CF is self-quenched, so that as it leaks out of the vesicles fluorescence intensity increases (Weinstein et al., 1977). CF release was recorded for 10 min, after which time the vesicles were disrupted with Triton X-100 to enable measurement of total fluorescence intensity. Results are expressed as the percentage of sequestered CF leaking out as a function of time.

ESR. ESR of nitroxide spin labeled (5- and 12-doxyl) stearic acids intercalated at low concentration (1 mol %) into 1% (w/v) aqueous dispersions of egg PC and egg PC (80 mol %)/egg PE (20 mol %) was employed to determine the influence ABA has on acyl chain order and fluidity within the membrane interior. Samples were prepared with the spin label (stored as a 5 mM solution in ethanol) codissolved with the phospholipid in chloroform prior to solvent removal and subsequent hydration in 20 mM acetate buffer (pH 5.0). An IBM/Bruker ER 200D X-band ESR spectrometer operating at 9.2 GHz was used. The spectrometer is interfaced to and controlled by a Hewlett-Packard 9816 computer system. Order parameters S at the 5-position and correlation times τ_c at the 12-position were obtained by direct computer calculation from spectra as previously reported (Wassall et al., 1988). Uncertainties in S and τ_c are less than ±1% and ±2%, respectively. Spectral parameters are as follows: center field, 3294 G; sweep width, 100 G (5-doxyl) or 80 G (12-doxyl); sweep time, 200 s (5-doxyl) or 160 s (12 doxyl); time constant, 500 ms; modulation amplitude, 2.0 G (5-doxyl) or 1.0 G (12-doxyl); microwave power, 12 dB; and dataset, 1000 (5-doxyl) or 2000 (12-doxyl) points.

NMR. The effects of ABA on acyl chain dynamics and head-group motion and conformation were investigated by ^{13}C and ^{31}P NMR. The spectra were recorded at 75.5 and 121.5 MHz, respectively, on a Nicolet NT-300 NMR spectrometer interfaced to a Nicolet 1280 computer. Dispersions of small unilamellar vesicles (pH 5.0), which give rise to high-resolution spectra, were always studied. Sonication was performed in the presence or absence of ABA. Broadband ^1H decoupling, gated on only during data acquisition to minimize sample heating problems, was applied. Spin–lattice relaxation times, T_1, were measured with the optimized inversion recovery pulse sequence and calculated from a fit to

$$M(\tau) = A - Be^{-\tau/T_1} \quad (1)$$

where $M(\tau)$ is the magnetization measured following a delay τ between inversion of the magnetization and the observation pulse and A and B are constants related to the equilibrium magnetization (Freeman et al., 1980; Canet et al., 1975). Line widths $\Delta\nu_{1/2}$ (resonance width at half-height) were measured directly from spectra. Uncertainty in T_1 and $\Delta\nu_{1/2}$ is ±10%. Chemical shifts (accurate to ±0.1 ppm) are relative to external TMS and H_3PO_4 (85%) for ^{13}C and ^{31}P NMR data, respectively, and assignments are made by comparison with previous work (Treleaven et al., 1983; Wassall et al., 1985a). Temperature regulation at 37 ± 1 °C was by a gas flow system. Spectral parameters are as follows: (^{13}C NMR) sweep width ±8 kHz, pulse width 32 μs (90° flip angle), data set 8K, lipid concentration 20% (w/v); (^{31}P NMR) sweep width ±2 kHz, pulse width 23 μs (90° flip angle), data set 4K, lipid concentration 10% (w/v).

Results

Previously we have measured ABA-induced permeability of lipid membranes to erythritol by light scattering (Stillwell & Hester, 1984a,b), Cl^- by ion-specific electrodes (Stillwell et al., 1987), and Pr^{3+} by ^{31}P (Wassall et al., 1985a) and ^1H NMR (Wassall et al., 1985b). These methods indicated that ABA has little effect on single-component bilayers made of PC but substantially enhances permeability upon incorporation of PE as a minor membrane component (5–20 mol %). Membranes composed of binary mixtures of PCs with different acyl chains were also unaffected by ABA when the phospholipids were both in the liquid-crystalline or gel states (Stillwell et al., 1987). Here we report the effect of ABA on isotonic erythritol swelling and carboxyfluorescein release for

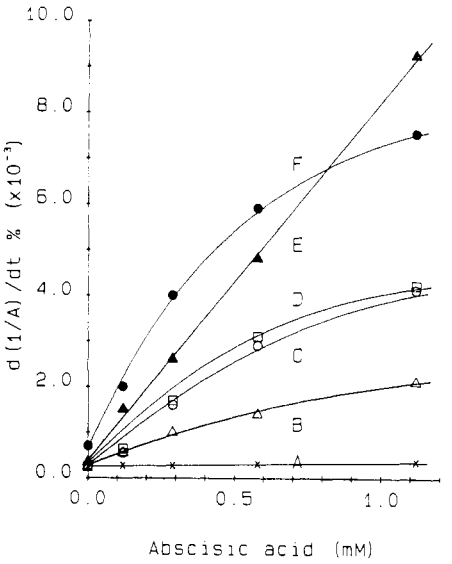

FIGURE 1: Effect of abscisic acid on the permeability rates to erythritol, expressed as $d(1/A)/dt$ (%), of liposomes composed of (A) 80 mol % egg PC/20 mol % cholesterol, (B) 80 mol % egg PC/20 mol % DMPG, (C) 80 mol % egg PC/20 mol % DPPA, (D) 80 mol % egg PC/20 mol % bovine heart CL, (E) 80 mol % egg PC/20 mol % brain PS, and (F) 80 mol % egg PC/20 mol % SA (37 °C, pH 5.0).

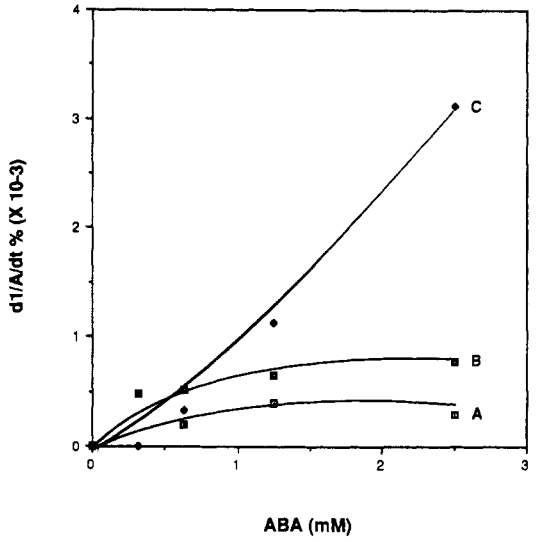

FIGURE 2: Effect of abscisic acid on the permeability rates to erythritol, expressed as $d(1/A)/dt$ (%), for liposomes (pH 5.0) composed of (A) 50 mol % DOPC/50 mol % DMPC, 50 °C, (B) 50 mol % DMPC/50 mol % DPPC, 15 °C, and (C) 50 mol % DMPC/50 mol % DPPC, 30 °C.

membranes containing several types of polar lipids as well as mixed acyl chain PC membranes where one phospholipid is in the liquid-crystalline state and the other in the gel state.

The isotonic erythritol permeability experiments reported in Figure 1 show the effect of ABA on bilayers composed of 80 mol % egg PC with 20 mol % PS, SA, CL, PA, PG, or cholesterol. Substantial ABA-induced increases in permeability to the neutral solute erythritol were measured with these mixed membranes where, in common with our earlier work on PC/PE bilayers, the two components possess different polar head groups. In contrast, ABA has no effect on permeability when cholesterol, which is known to reduce permeability of liquid-crystalline membranes (De Gier et al., 1968), is the second component. This observation, together with our previous work demonstrating no effect with mixed acyl chain PC membranes in the liquid-crystalline state (Stillwell et al., 1987), indicates that ABA permeability enhancement is not a general

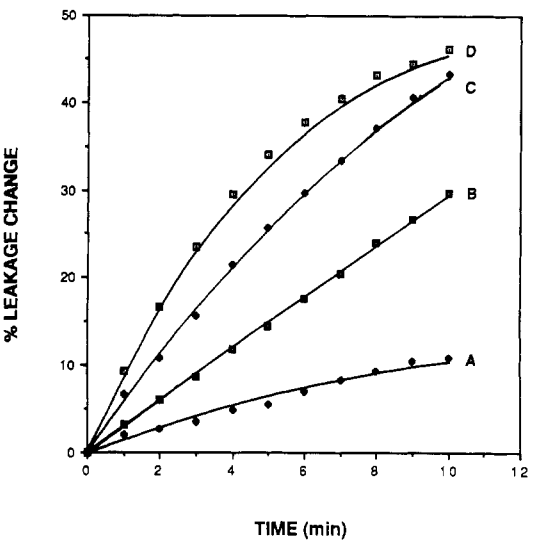

FIGURE 3: Effect of ABA on carboxyfluorescein release from vesicles composed of 90 mol % DOPC/10 mol % DOPE, 30 °C, pH 5.0. ABA/phospholipid molar ratio: (A) 0; (B) 0.25; (C) 0.50; (D) 1.0.

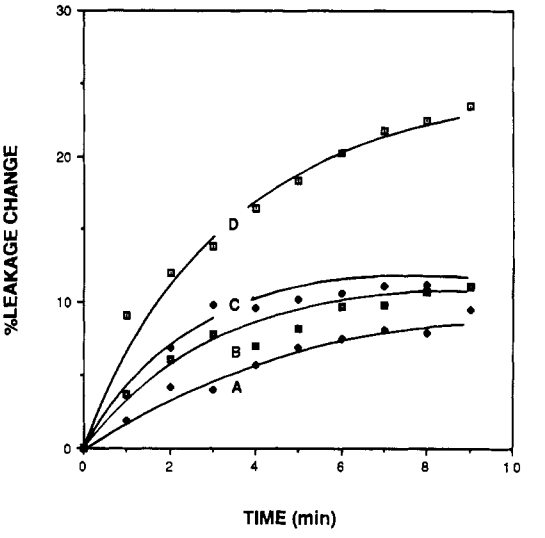

FIGURE 4: Effect of abscisic acid on carboxyfluorescein release from vesicles composed of 80 mol % DOPC/20 mol % DMPC at 10 °C, pH 5.0. ABA/phospholipid molar ratio: (A) 0; (B) 0.125; (C) 0.25; (D) 0.50.

feature of all mixed membranes but depends on the nature of the constituents.

Consistent with our previous studies, Figure 2 confirms that the permeability to erythritol of mixed PC membranes is insensitive to the presence of ABA in the liquid-crystalline (50 mol % DOPC/50 mol % DMPC, 50 °C) and gel (50 mol % DPPC/50 mol % DMPC, 15 °C) states. In marked contrast for 50 mol % DPPC/50 mol % DMPC membranes at 30 °C in the region of the phase transition where gel and liquid-crystalline lipids coexist (McElhaney, 1982), a significant enhancement in permeability is produced by the hormone (Figure 2).

Carboxyfluorescein leakage was used to follow the influence of ABA on phospholipid bilayer permeability to an anion. ABA was found to have almost no effect on CF permeability for liquid-crystalline-state DOPC membranes (30 °C) (results not shown), but upon incorporation of 10 mol % DOPE the membranes became responsive to the hormone (Figure 3). These results are in agreement with those we have previously reported for erythritol (Stillwell & Hester, 1984a,b), Cl^- (Stillwell et al., 1987), and Pr^{3+} (Wassall et al., 1985a)

Table I: Effect of ABA on ESR-Determined Acyl Chain Order Parameter S and Correlation Time τ_c for 5- and 12-Doxylstearic Acids, Respectively, Intercalated into Egg PC and 80 mol % Egg PC/20 mol % Egg PE Bilayers[a]

		S	
membrane composition	spin-label position	no hormone	+ABA
egg PC	5	0.429	0.442
80 mol % egg PC/20 mol % egg PE	5	0.435	0.444
		$\tau_c \times 10^{10}$ (s)	
membrane composition	spin-label position	no hormone	+ABA
egg PC	12	20.17	20.29
80 mol % egg PC/20 mol % egg PE	12	20.25	20.03

[a] The samples were multilamellar liposomes of 10 mM phospholipid (0.1 mM spin label) in 20 mM acetate buffer (pH 5.0). The experiments were performed with or without ABA (ABA/phospholipid, 1:2) at 30 °C.

permeabilities. However, leakage of CF from 80 mol % DOPC/20 mol % DMPC vesicles containing coexisting phases (10 °C) was shown to be enhanced by ABA (Figure 4). The results in Figures 2 and 4 thus identify an additional condition of membrane composition for which the hormone increases permeability, namely, the coexistence of gel and liquid-crystalline states.

Magnetic resonance experiments were performed to elucidate the molecular mechanism by which ABA enhances permeability of model membranes. ESR spectra were recorded for 5- and 12-doxylstearic acids intercalated into aqueous multilamellar dispersions of 80 mol % egg PC/20 mol % egg PE in the absence and presence of 50 mol % (with respect to phospholipid) ABA. The data are given in Table I. The hormone has only slight effect (<2%) on either the order parameter S at the 5-position or the correlation time τ_c at the 12-position. The former quantity is a measure of acyl chain ordering and can take values in the range of $0 \leq S \leq 1$, the respective upper and lower limits representing axial rotation with no off-axis flexing and isotropic reorientation, while the latter quantity is related to acyl chain fluidity (microviscosity). A lack of appreciable ABA-associated change in S or τ_c was similarly seen with egg PC multilamellar liposomes (Table I) and was also previously reported for sonicated unilamellar vesicles of egg PC and egg PC (80 mol %)/egg PE (20 mol %) (Stillwell et al., 1987). Thus, as monitored by spin-label ESR, it is apparent that ABA has negligible influence on phospholipid acyl chain ordering and dynamics.

Spin–lattice relaxation times T_1 measured for C1, $(CH_2)_n$, and CH_3 resonances in ^{1}H-decoupled ^{13}C NMR spectra of egg PC and egg PC (80 mol %)/*Escherichia coli* PE (20 mol %) vesicles with and without 10 mol % hormone (pH 5.0, 37 °C) support the finding that ABA has little effect on phospholipid acyl chain motion (Table II). Although the precise interpretation of the motions, and their time scales, responsible for spin–lattice relaxation in phospholipid membranes remains contentious (Brown, 1984), clearly the acyl chain T_1 values listed in Table II are essentially unchanged upon the addition of hormone. There is also no change in chemical shift. No ^{13}C NMR resonances were observable for ABA at the concentrations of hormone employed. ^{1}H NMR spectra recorded for soy PC vesicles in the absence and presence of 50 mol % ABA (pH 5.0, 37 °C) similarly confirm the lack of influence the hormone has on molecular motions within the bilayer interior (Wassall et al., 1985b). In these experiments showing ABA-induced enhancement of Pr^{3+} permeability, no change due to ABA occurred in the line widths of the $(CH_2)_n$ and CH_3 resonances over the several hours duration of experimentation.

No substantial ABA-associated modification of phospholipid molecular motion in the glycerol backbone or head-group region is further implied by Table II. Inspection reveals that the ^{13}C NMR T_1 values for the CHO and $N(CH_3)_3^+$ signals are unchanged by the addition of 10 mol % ABA to egg PC or egg PC (80 mol %)/*E. coli* PE (20 mol %) vesicles. The possibility that the rate of head-group motion is slightly reduced by the hormone may be suggested by ^{31}P NMR. ^{1}H-decoupled ^{31}P NMR spectra, together with line widths, are presented in Figure 5 for egg PC and egg PC (80 mol %)/*E. coli* PE (20 mol %) vesicles with or without ABA (10 mol %). As can be seen, separate chemically shifted resonances are resolved for PC and PE in the mixed bilayer system. Their integrated intensities are in the ratio 3.9 ± 0.5:1, respectively, which is consistent within experimental uncertainty with the membrane composition at preparation. Presence of the hormone causes no change in the rate of spin–lattice relaxation $1/T_1$ but an increase in line width $\Delta\nu_{1/2}$ for egg PC vesicles, while for egg PC/*E. coli* PE vesicles both $1/T_1$ and $\Delta\nu_{1/2}$ are increased for the PC and PE resonances (Table I and Figure 5). Although such behavior may be reconciled in terms of slower motion of the phospholipid head groups, the changes are on the order of 10% and would reflect only a small effect. Moreover, selectivity between PC and PE is not indicated. Head-group conformation appears independent of ABA, since the chemical shifts (^{13}C and ^{31}P NMR) remain constant within experimental uncertainty (Table II).

Discussion

In earlier studies we reported that ABA dramatically enhances permeability of PC/PE bilayers but not PC bilayers to erythritol (Stillwell & Hester, 1984ab), Cl^- (Stillwell et al., 1987), and Pr^{3+} (Wassall et al., 1985). This led us to propose that ABA can selectively interact with PE in PC/PE mixed bilayers (Stillwell & Hester, 1984b). The results presented

Table II: ^{13}C and ^{31}P NMR Spin–Lattice Relaxation Times (T_1) and Chemical Shifts for Egg PC and Egg PC (80 mol %)/*E. coli* PE (20 mol %) Vesicles in the Absence and Presence of 10 mol % ABA at pH 5.0 and 37 °C.

		acyl chain			glycerol backbone	head group	
		C1[a]	$(CH_2)_n$[a]	CH_3[a]	CHO[a]	$N^+(CH_3)_3$[a]	PO_4^-[b]
egg PC	T_1 (s)	2.17, 2.11[c]	0.59	3.45	0.22	0.62	1.05
	chemical shift (ppm)	174.3, 174.0	30.6	14.6	71.4	54.9	−0.2
egg PC + ABA	T_1 (s)	2.39, 2.23	0.60	3.37	0.21	0.65	1.04
	chemical shift (ppm)	174.3, 174.0	30.6	14.6	71.3	54.8	−0.3
egg PC/*E. coli* PE	T_1 (s)	2.11	0.61	3.50	0.21	0.59	0.88, 0.87[d]
	chemical shift (ppm)	174.2	30.6	14.6	71.4	54.9	−0.2, 0.4
egg PC/*E. coli* PE + ABA	T_1 (s)	2.17	0.61	3.45	0.20	0.59	0.78, 0.77
	chemical shift (ppm)	174.2	30.5	14.6	71.3	54.8	−0.2, 0.4

[a] ^{13}C NMR. [b] ^{31}P NMR. [c] Two carbonyl resonances resolved (outside and inside vesicle layer, respectively). [d] PC and PE resonances, respectively, resolved.

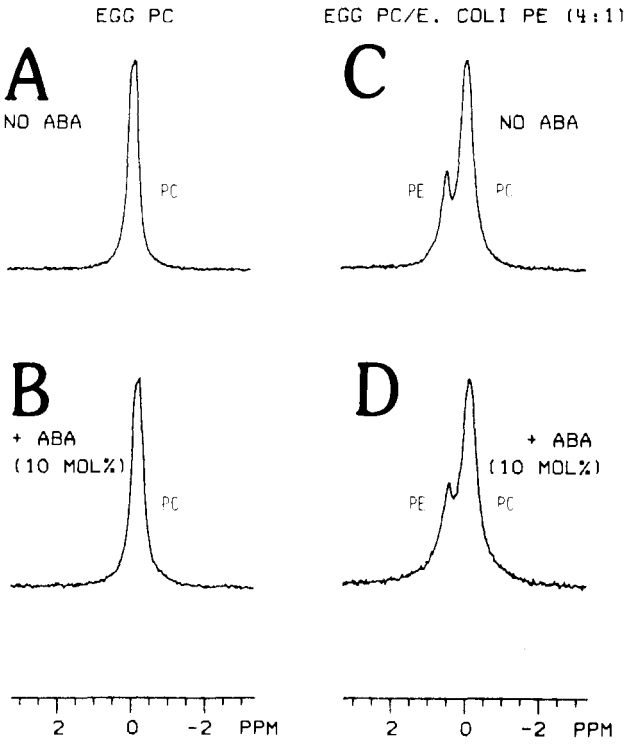

FIGURE 5: Effect of abscisic acid on ^{1}H-decoupled ^{31}P NMR spectra for egg PC and egg PC (80 mol %)/*E. coli* PE (20 mol %) vesicles [10% (w/v) phospholipid, pH 5.0, 37 °C]: (A) egg PC, no ABA, $\Delta\nu_{1/2}$ = 39 Hz (PC); (B) egg PC, 10 mol % ABA, $\Delta\nu_{1/2}$ = 44 Hz (PC); (C) egg PC/*E. coli* PE, no ABA, $\Delta\nu_{1/2}$ = 37 Hz (PE) and 42 Hz (PC); (D) egg PC/*E. coli* PE, 10 mol % ABA, $\Delta\nu_{1/2}$ = 41 Hz (PE) and 47 Hz (PC).

in Figure 1, however, establish that PE can be replaced by several types of lipids with different head groups, and these mixed bilayers are still responsive to ABA. Since ABA permeability was not enhanced when either cholesterol or a second liquid-crystalline-state PC was incorporated, we conclude that ABA-induced permeability is not a general feature of all mixed membranes but depends on the nature of the constituents. In addition, we show in Figures 3 and 4 that the hormone enhances permeability to a neutral solute (erythritol) as well as an anion (Cl^-) with mixed acyl chain PC membranes at temperatures where liquid-crystalline and gel states coexist. This suggests that ABA–membrane interactions are not with a specific phospholipid component as we previously proposed (Stillwell & Hester, 1984b) but instead are with membrane regions containing different types of head groups or lipid phases.

A large enhancement in membrane permeability often accompanies an increase in acyl chain disorder and/or fluidity. The NMR studies described here, which do not detect an appreciable change due to 10 mol % ABA in spin–lattice relaxation rate or line width throughout the model membranes studied (Table II and Figure 5), thus appear to contradict the permeability measurements. This conclusion is confirmed by ESR of 5- and 12-doxylstearic acid spin labels intercalated into multilamellar liposomes of egg PC and 80 mol % egg PC/20 mol % egg PE which show that ABA at up to 50 mol % has no appreciable effect on order parameters at the 5-position or correlation times at the 12-position in the bilayer interior (Table I). Furthermore, ESR spectra for membranes containing head-group spin-labeled PC were unchanged by the presence of the hormone (Cheng, Wassall, and Stillwell, unpublished results). Fluorescence polarization similarly demonstrates that ABA does not affect interior bilayer fluidity, as monitored with 1,6-diphenyl-1,3,5-hexatriene or 2-, 6-, 9-, or 12-(9-anthroyloxy)stearic acids, nor did it affect polarization values for the membrane surface probes 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene or octadecylanthracene-9-carboxylate (Stillwell and Wassall, unpublished results). The explanation for the apparent discrepancy may be in part the very different time scales of the two types of experiments. Permeability, as well as vesicle aggregation and fusion which are also promoted by ABA (Brengle et al., 1988; Stillwell et al., 1988), is measured over a period of seconds to hours and represents the time accumulation of an event. In contrast, the spectroscopic techniques (NMR, ESR, and fluorescence polarization) are monitoring motions on a much faster time scale ($<10^{-6}$ s) and, by comparison, look at the influence of ABA on the bilayer at essentially a single instant. In light of these considerations, it is suggested that ABA–phospholipid interactions at any one instant involve only a small proportion of the membrane so that the population weighted average effect detected by ESR, NMR, or fluorescence polarization is small.

A possible mechanism we propose is that the site of hormone action is localized to regions of membrane defect in mixed-component bilayers where a mismatch in molecular packing occurs at the interface between different polar head groups or between gel and liquid-crystalline acyl chains. Perhaps it is here that the hormone promotes bilayer instability with consequent leakiness. Support for this proposal is derived from previous studies ascribing enhanced vesicle permeability to membrane defects (Marsh et al., 1976). Moreover, the inducement of vesicle aggregation and fusion has been linked to the presence of bilayer defects (Hui et al., 1981). Another proposal by Lea and Collins (1979), who saw that ABA increases the electrical conductivity of egg PC planar bimolecular lipid membranes, has the hormone forming multimeric transient channels. It may be speculated that membrane defects would provide a preferential location for such channels. Other possibilities such as ABA-induced flip-flop or nonbilayer phases exist as well.

The ABA-induced changes in model membrane permeability observed here and the hypothesis that defects are responsible may explain many of the permeability changes associated with the hormone in natural membranes. One theory for stomatal closure proposes that ABA causes a general membrane permeability increase resulting in the energy-independent efflux of K^+ and other guard-cell-sequestered ions (MacRobbie, 1980). This theory is supported by the observation that the ionophore benzo-18-crown-6 can also close stomates (Richardson, 1979). Our results could also explain ion uptake in various storage disks, hydrolytic conductivity in roots (Glinka & Reinhold, 1971), and the report that ABA can serve as an uncoupler of oxidative phosphorylation (Hemberg, 1978) (presumably by discharging the transmembrane pH gradient).

To date, potential interaction of plant hormones with the predominant molecular component of membranes, lipids, has been generally ignored. Perhaps the major reason for the lack of interest in hormone–lipid interactions stems from the assumption that no specificity could possibly exist between a hormone and phospholipid membrane devoid of proteins. The failure after many years to detect any ABA-binding proteins or proteinaceous receptors that have a direct association with a subsequent physiological function makes the lipid component of membranes an attractive candidate for the initial site of the hormone's action. The results of this communication and others from our laboratory clearly exhibit lipid composition

dependent selectivity in ABA enhancement of permeability. In addition, we have recently reported large differences in permeability enhancement of bilayers exposed to *cis-trans*-ABA (active) vs *trans-trans*-ABA (inactive) (Stillwell & Hester, 1984b) and *cis*-zeatin (active) vs *trans*-zeatin (inactive) (Stillwell et al., 1985b). Harkers et al. (1986) similarly have seen isomer dependency for hormone-induced enhancement of K^+ permeability with bilayers composed of soybean PC. They also found *cis-trans*-ABA to be effective, while *trans-trans*-ABA was ineffective. These results on lipid bilayer permeability parallel physiological effects on biological systems. Jones and Paleg (1984) have reported binding selectivities among a series of auxins where the binding sequence is not just a reflection of the hormone lipid solubilities. From this wide variety of experiments, it may be proposed that at least some degree of selectivity may exist between plant hormones and the lipid component of membranes.

Another criticism aimed at experiments with lipid bilayers has been the use of "unnaturally" high amounts of plant hormones. However, ABA levels in plants are not certain and have been the subject of much debate (Zeevaart & Creelman, 1988). The recent paper by Behl and Hartung (1986) that ABA levels may even rise into the millimolar range in the apoplast region of guard cells furthermore renders the issue less contentious. In biophysical studies high hormone levels are required by the nature of the techniques employed, which measure a population weighted average effect over the entire membrane. Low hormone concentrations may indeed have a dramatic local effect (such as that at membrane defects), but will appear to have small effect when so observed. Interestingly, experiments with IAA by Misso et al. (1986) have shown a substantial reduction in the level of hormone required to exert a measurable influence on lipid bilayers. These workers reported a factor of up to 100-fold increase in the rates of IAA-facilitated movement of Pr^{3+} across PC membranes as a result of slight lipid oxidation. Thus, it seems reasonable to propose that a proper combination of phospholipids, salts, and pH may result in very tight binding of hormone and enhanced activity to protein-free membranes.

In conclusion, the present study establishes that ABA enhancement of phospholipid membrane permeability requires the presence of different polar head groups or the coexistence of gel-state and liquid-crystalline state acyl chains. Magnetic resonance experiments indicate that the permeability increase does not accompany significant changes in membrane molecular dynamics or ordering, and it is suggested that bilayer defects at the interface between phospholipids possessing different head groups or acyl chain states are the site of action for the hormone. Further experiments probing the nature of ABA-induced changes in bilayer properties are currently in progress. From these experiments, we eventually hope to define the molecular role of ABA in controlling physiological processes.

ACKNOWLEDGMENTS

It is a pleasure to thank Professors B. D. Nageswara Rao and M. D. Kemple for, respectively, NMR and ESR spectrometer time.

Registry No. ABA, 21293-29-8.

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^{31}P NMR Spectra of Ethidium, Quinacrine, and Daunomycin Complexes with Poly(adenylic acid)·Poly(uridylic acid) RNA Duplex and Calf Thymus DNA†

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Received October 6, 1988; Revised Manuscript Received December 14, 1988

ABSTRACT: ^{31}P NMR provides a convenient monitor of the phosphate ester backbone conformational changes upon binding of the intercalating drugs ethidium, quinacrine, and daunomycin to sonicated poly(A)·poly(U) and calf thymus DNA. ^{31}P chemical shifts can also be used to assess differences in the duplex unwinding angles in the presence of the drug. Thus a new ^{31}P signal, 1.8–2.2 ppm downfield from the double-stranded helix signals, is observed in the ethidium ion–poly(A)·poly(U) complex. This signal arises from phosphates which are in perturbed environments due to intercalation of the drug. This is in keeping with the hypothesis that the P–O ester torsional angle in phosphates linking the intercalated base pairs is more trans-like. Similar though smaller deshielding of the ^{31}P signals is observed in sonicated poly(A)·poly(U)–quinacrine complexes as well as in the daunomycin complexes. The effect of added ethidium ion, quinacrine, and daunomycin on the ^{31}P spectra of sonicated calf thymus DNA is consistent with Wilson and Jones' (1982) earlier study. In these drug–DNA complexes the drug produces a gradual downfield shift in the DNA ^{31}P signal without the appearance of a separate downfield peak. These differences are attributed to differences in the rate of chemical exchange of the drug between free and bound duplex states. The previous correlation of ^{31}P chemical shift with drug duplex unwinding angle (Wilson & Jones, 1982) is confirmed for both the RNA and DNA duplexes.

It is now widely appreciated that duplex DNA and RNA can exist in a number of different conformations (Saenger, 1984). Significant conformational differences can exist globally along the entire double helix, as in the A-, B-, C-, and Z-forms of DNA. In addition, local conformational heterogeneity in the sugar–phosphate backbone has been most recently noted in the form of sequence-specific variations (Calladine, 1982; Dickerson, 1983) or as the result of drug (Saenger, 1984) or protein binding (Anderson et al., 1987) to local regions of the poly(nucleic acids).

While ^{1}H NMR can provide detailed information on the overall conformation of the sugar rings and bases of oligonucleotides, it generally is unable to provide very much information on the conformation of the phosphate ester backbone. Of the six torsional angles that largely define the backbone structure, only the four involving the sugar ring are amenable to analysis by ^{1}H NMR techniques (via coupling constant or NOESY distance measurements). It has been suggested that the sugar ring and base form a rather rigid unit with the main conformational flexibility of the nucleic acid backbone being limited to the two P–O phosphate ester torsional angles (Sundaralingam, 1969). Thus, one of these, the C3′–O3′–P–O5′ torsional angle, ζ, is found to be the most variable one in the B-form of the DNA double helix, and the other, the O3′–P–O5′–C5′ torsional angle, α, is one of the most variable in the A-form of the RNA or DNA duplex (Saenger, 1984). Indeed, following the original suggestion of Sundaralingam (1969) and on the basis of recent X-ray crystallographic studies of oligonucleotides, Saenger (1984) has noted that the P–O bonds may be considered the "major pivots affecting polynucleotide structure".

We have proposed that ^{31}P NMR spectroscopy is potentially capable of providing information on the most important remaining two torsional angles involving the phosphate ester bonds that define the nucleic acid backbone. Our studies (Gorenstein & Kar, 1975; Gorenstein, 1978, 1981, 1983a,b, 1984) indicated that a phosphate diester monoanion in a gauche,gauche (g,g) conformation (referring to the α and ζ torsional angles) should have a ^{31}P chemical shift 1.5–2.5 ppm upfield from an ester in a non-g,g conformation. Our earlier ^{31}P NMR studies on poly- and oligo(nucleic acids) described in Gorenstein et al. (1976, 1982, 1988), Gorenstein (1978, 1981, 1983a,b, 1984), and Gorenstein and Luxon (1979)

†Supported by NIH (Grants GM36281 and AI27744), the Purdue University Biochemical Magnetic Resonance Laboratory which is supported by NIH (Grant RR01077 from the Biotechnology Resources Program of the Division of Research Resources), and the NSF National Biological Facilities Center on Biomolecular NMR, Structure and Design at Purdue (Grants BBS 8614177 and 8714258 from the Division of Biological Instrumentation).
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0006-2960/89/0428-2804$01.50/0