Phytochemistry, Vol. 26, No. 12, pp. 3145–3150, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

COMPARISON OF EFFECTS OF ABA AND IAA ON PHOSPHOLIPID BILAYERS

WILLIAM STILLWELL, BLAIR BRENGLE, DANIEL BELCHER and STEPHEN R. WASSALL*

Departments of Biology and *Physics, Indiana University-Purdue University at Indianapolis, P.O. Box 647, Indianapolis, IN 46223, U.S.A.

(*Received* 10 *February* 1987)

Key Word Index—Indole-3-acetic acid; abscisic acid; lipid vesicles; membrane permeability; vesicle aggregation; acyl chain mobility; ESR.

Abstract—The plant hormones indole-3-acetic acid (IAA) and abscisic acid (ABA) affect the properties of phospholipid bilayers differently. IAA enhances permeability of bilayers composed of phosphatidylcholine to the non-electrolyte erythritol while ABA requires an additional phospholipid in the membrane to produce substantial enhancement. Similar conclusions are obtained by measuring hormone-induced permeability to chloride ions; IAA is effective with single component phosphatidylcholine membranes while ABA requires a second phospholipid. Erythritol permeability is shown to be pH dependent for both hormones. Although IAA is more effective at increasing erythritol permeability at pH 4 than at pH 7, both dissociated and undissociated IAA affect the process. In comparison ABA is almost totally ineffective in the dissociated form (at pH 7). Spin label electron spin resonance measurements demonstrated that neither hormone substantially disrupts acyl chain mobility within the membrane, indicating that the mechanism of permeability enhancement is not a general non-specific pertubation of membrane ordering and fluidity. Both hormones can also effect the stability of small unilamellar (sonicated) vesicles. Phosphatidylcholine vesicles are relatively stable and do not rapidly aggregate with either ABA or IAA. However, when phosphatidylethanolamine is incorporated as a minor component (10 mol %) into phosphatidylcholine vesicles ABA causes rapid aggregation while IAA has no effect. These experiments indicate that the two hormones may exhibit completely different behaviour on membranes without the requirement for specific proteinaceous receptors.

INTRODUCTION

The plant hormones indole-3-acetic acid (IAA) and abscisic acid (ABA) are small (IAA, M_r 175.2; ABA, M_r 264.3), monocarboxylic acids (IAA, pK_a 4.7; [1] ABA, pK_a 4.85) [2] of similar organic/water solubilities known to cross phospholipid bilayer membranes only in the undissociated form [1, 3]. Despite these common properties each hormone affects different physiological functions. In fact, one of the first properties to be associated with ABA was that of an IAA antagonist inhibiting coleoptile growth [4]. Both hormones are believed to function initially by interacting with some as yet unknown component of the plasma (or tonoplast) membrane. This component is generally assumed to be a proteinaceous receptor [5]. Binding proteins (possibly receptors) have been detected for both hormones (reviewed in [6] for IAA and [7–9] for ABA) although direct links between binding and membrane function have not been established. Also, elucidation of the effects of these hormones on the lipid component of membranes has not been widely pursued.

Plant membrane–hormone interactions have been difficult to study at the molecular level due to the complex, heterogenous nature of membranes and the extremely low levels of hormone involved. For this reason, protein-free phospholipid bilayers have been employed to model natural membranes [10]. Bilayers are of well-defined composition, lend themselves to a variety of biophysical measurements, and have been successfully used to mimic numerous biological membrane functions. Since IAA and ABA have similar chemical properties and specific protein receptors are believed to distinguish the two hormones, it may be anticipated that IAA and ABA would affect protein-free bilayer membrane properties in a similar general manner. Here we report that these two hormones can exhibit completely different behavior on phospholipid bilayers. We propose that some of the specificity for hormone–membrane interaction may reside with the lipids.

RESULTS

We have extensively used the technique of measuring non-electrolyte permeability followed by light scattering of multilamellar vesicles (MLV) to demonstrate the effect of plant hormones on bilayer permeability [11–15]. Several years ago, Bangham showed that MLV's behave as almost perfect osmometers [16] and so permeability into or out of vesicles can be followed with a spectrophotometer as the vesicles swell or shrink. Here we compare the effects of IAA and ABA on the permeability of lipid vesicles composed of either 100 mol % phosphatidylcholine (PC) or 80 mol % PC/20 mol % phosphatidylethanolamine (PE) to the non-electrolyte erythritol.

The auxins IAA, IPA (indole-3-propionic acid) and IBA (indole-3-butyric acid) are shown to enhance the permeability of erythritol to bilayers composed of dioleoyl-

phosphatidylcholine (DOPC) (Fig. 1). Previousy we reported that model membrane permeability to the NMR shift reagent Pr^{3+} is enhanced by ABA. The enhancement, which is only slight for egg PC vesicles, is increased sixfold by incorporation of 20 mol% *E. coli* PE into PC vesicles [17]. Paleg, on the other hand, reported IAA substantially increased the permeability of Pr^{3+} to 100 mol% PC vesicles [18]. We tested this membrane composition requirement difference between the hormones with the erythritol experiment. IAA enhanced the permeability of 100 mol% DOPC (curve C) and 100 mol% egg PC (curve D) bilayers (Fig. 2). Upon addition of 20 mol% dioleylphosphatidylethanolamine (DOPE) to DOPC (curve A) and 20 mol% egg PE to egg PC (curve B) a slight enhancement in erythritol permeability is measured. However, when similar experiments were performed with ABA, a dramatic difference in behaviour is observed (Fig. 3). For several 80 mol% /20 mol% mixed PC vesicles (curves A, B, C and D) ABA did not significantly enhance bilayer permeability to erythritol. When the 20 mol% PC component was replaced with either soy PE (curve E) or *E. coli* PE (curve F) a dramatic increase in permeability to erythritol was produced by ABA.

Gutknecht and Walter [1] showed that only undissociated IAA is permeable to PC vesicles. They implied a similar fate for ABA. Here we report the effect of pH on the IAA-induced and ABA-induced permeability to erythritol. IAA substantially increased the permeability of several types of 100 mol% PC bilayers to erythritol (Table 1, Fig. 4). Although protonated IAA (pH 4) is more effective than dissociated IAA (pH 7), both forms of the hormone enhance permeability. By comparison dissociated ABA (pH 7) is almost totally ineffective at

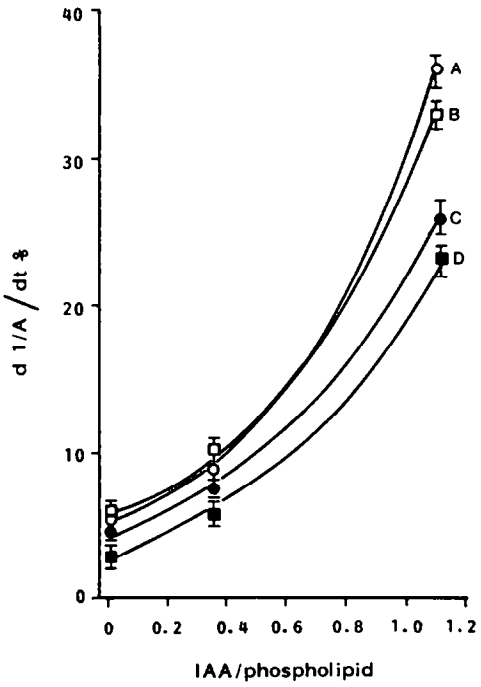

Fig. 2. Effect of IAA on erythritol permeability expressed as liposome swelling (dl/A/dt %) for vesicles composed of: Curve A, 80 mol% egg PC/20 mol% egg PE; curve B, 80 mol% DOPC/20 mol% DOPE; curve C, 100 mol% egg PC, curve D, 100 mol% DOPC.

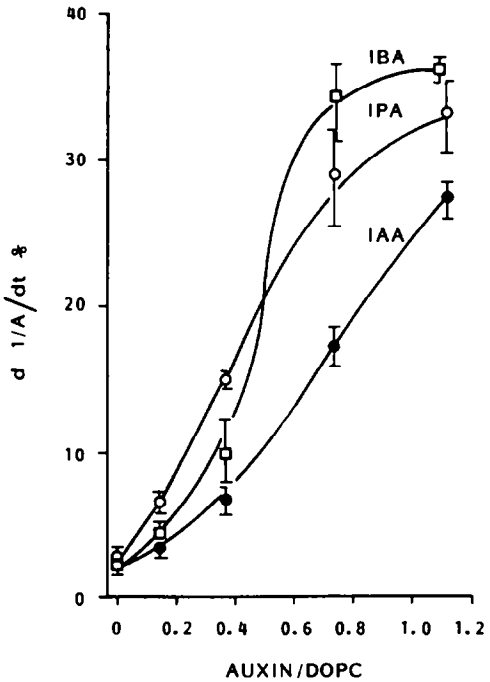

Fig. 1. Auxin-induced enhancement of erythritol permeability expressed as rate of liposome swelling (dl/A/dt %) with vesicles composed of 100 mol% DOPC.

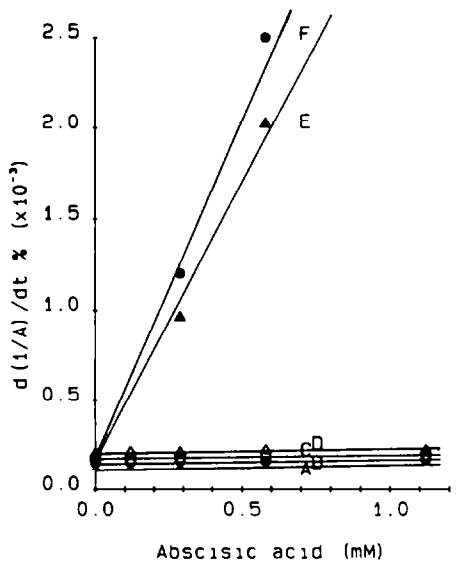

Fig. 3. Effect of ABA on the permeability rates of erythritol expressed as dl/A/dt % to liposomes composed of: A. 80 mol% egg PC/20 mol% DOPC; B. 80 mol% egg PC/20 mol% DLPC; C. 100 mol% egg PC; D. 80 mol% egg PC/20 mol% DMPC; E. 80 mol% egg PC/20 mol% soy PE; and F. 80 mol% egg PC/20 mol% *E. coli* PE.

Table 1. Effect of pH on the IAA-induced enhancement of lipid bilayer permeability to erythritol

	Erythritol permeability rate (6.5 mM IAA/O mM IAA) pH	
Lipid	4	7
Egg PC	15.4	5.1
Soy PC	10.1	2.7
DMPC	17.2	10.3
DOPC	15.7	8.1
Crude egg lecithin	6.7	6.3

Results are expressed as the swelling rate of vesicles with 6.5 mM IAA divided by the rate for hormone free vesicles.

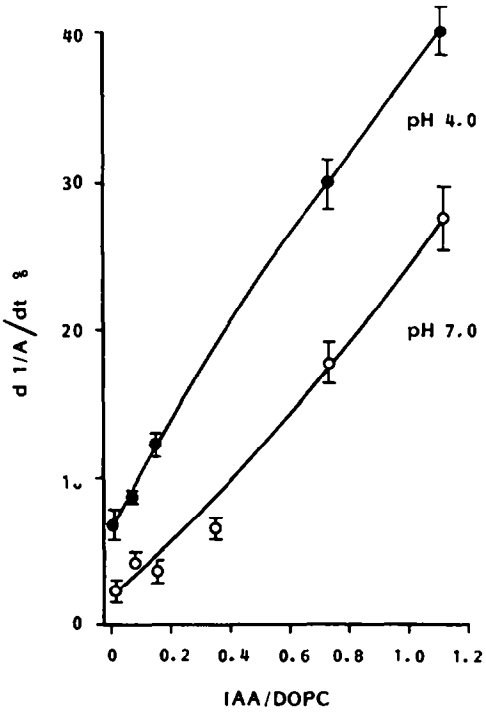

Fig. 4. Effect of pH on the IAA-induced permeability of erythritol expressed as rate of liposome swelling (dl/A/dt %) for vesicles composed of 100 mol % DOPC.

enhancing erythritol permeability of lipid bilayers composed of crude (mixed phospholipid) egg lecithin (Table 2). Undissociated ABA does not enhance erythritol permeability of 100% PC bilayers (Fig. 3).

Hormone enhancement of Cl^- permeability is followed by the use of a chloride specific electrode (Figs 5 and 6). IAA enhances permeability of 100 mol % DOPC bilayers (Fig. 5). Incorporation of 20 mol % DOPE into these membranes does not further increase IAA enhancement of permeability but appears to slightly decrease the

Table 2. Effect of pH on the ABA-induced enhancement in erythritol permeability with mixed phosphatide egg lecithin MLV's

pH	Enhancement in erythritol permeability rate (3 mM ABA/O mM ABA)
3	2.43
4	1.76
5	1.68
6	1.61
7	1.13

Results are expressed as the swelling rate of vesicles with 3 mM ABA divided by the rate for hormone-free vesicles.

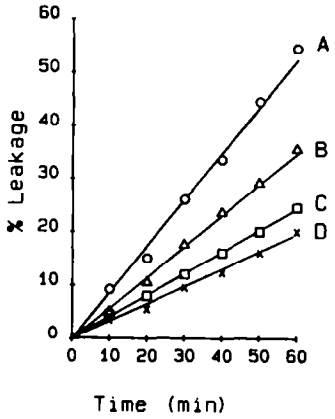

Fig. 5. Effect of IAA on Cl^- permeability expressed as the percentage of initially sequestered Cl^- leaking out over time. Curve A, 100 mol % DOPC; IAA/lipid 1:1; curve B, 80 mol % DOPC/20 mol % DOPE, IAA/lipid 1:1; curve C, 80 mol % DOPC/20 mol % DOPE, no IAA; curve D, 100 mol % DOPC, no IAA.

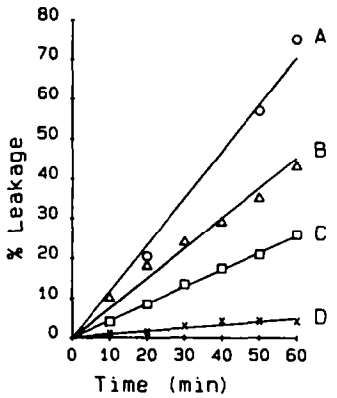

Fig. 6. Effect of ABA on Cl^- permeability expressed as the percentage of initially sequestered Cl^- leaking out over time. Curve A, 80 mol % DOPC/20 mol % DOPE, ABA/lipid 1:1; curve B, 100 mol % DOPC, ABA/lipid 1:1; curve C, 80 mol % DOPC/20 mol % DOPE, no ABA; curve D, 100 mol % DOPC, no ABA.

enhancement in relation to the pure PC membrane. ABA also enhances Cl^- permeability of 100 mol% DOPC bilayers. However, in comparison to IAA, ABA induced Cl^- permeability is greatly enhanced by incorporation of DOPE into the membranes (Fig. 6). These experiments on permeability of an anion followed by ion specific electrodes support observations made by very different techniques with a cation (Pr^{3+} by NMR [17]) and a non-electrolyte (erythritol by light scattering).

Electron spin resonance (ESR) of spin labelled stearic acids intercalated at low concentration (1 mol%) into phospholipid membranes was employed to investigate the molecular mechanism of permeability enhancement for both hormones. Table 3 presents order parameters S and correlation times τ_c measured with 5- and 12-doxyl stearic acids, respectively, for sonicated unilamellar vesicles (SUV's) of 100 mol% egg PC and 80 mol% egg PC/20 mol% egg PE in the absence and presence of 50 mol% (with respect to phospholipid) ABA or IAA. The order parameter is a measure of acyl chain ordering within the membrane and can take values in the range $0 \leqslant S \leqslant 1$, the respective limits representing isotropic motion and axial motion with no off-axis flexing. The correlation time is related to acyl chain fluidity (microviscosity). There is no appreciable effect ($\leqslant 5\%$) on these quantities by either ABA or IAA.

Although SUV obtained from sonication of MLV normally remain stable for extended periods of time, we noted previously that below the phase transition temperature of the lipids, PC/PE mixed bilayers aggregate (and/or perhaps fuse) rapidly in the presence of ABA [unpublished]. We have used vesicle aggregation as another assay of gross changes in bilayer properties caused by plant hormones. The results of Fig. 7 compares IAA with ABA on their ability to aggregate 100 mol% DMPC (dimyristoylphosphatidylcholine) or 90 mol% DMPE (dimyristoylphosphatidylethanolamine) bilayers.

IAA has little effect on aggregation with either membrane system while ABA does not affect DMPC bilayers but does rapidly aggregate DMPC/DMPE mixed bilayers. Furthermore, 20 mol% DMPE vesicles aggregated instantly upon addition of ABA so that no kinetic data could be obtained.

DISCUSSION

In a series of papers Paleg and coworkers [18, 19–24] demonstrated by high resolution 1H, ^{13}C and ^{31}P NMR techniques that IAA can interact with the phosphorylcholine head group component of PC in an organic solvent as well as in an aqueous dispersion. Their results in $CDCl_3$ showed that head group resonances are shifted by IAA in proportion to hormone concentration; formation of phospholipid-hormone complexes was discussed [19–22]. Ligand specific upfield shifts for head group resonances, especially the $N^+(Me)_3$ group, were subsequently seen in ^1H NMR spectra for soybean PC vesicles in the presence of IAA and other auxins [23]. These experiments essentially confirmed and considerably extended the earlier reports of Weigl [25] and Veen [26] that IAA does associate with PC. In contrast, using similar NMR techniques with PC and PC/PE vesicles we were not able to detect any significant ABA dependent change in chemical shift or relaxation time for any part of the PC molecule [unpublished, 27], nor was any effect seen for the phosphate group in PE. Furthermore, while both hormones have been reported to affect membrane permeability, the nature of the effects differ. Paleg's group reported a substantial IAA induced enhancement in

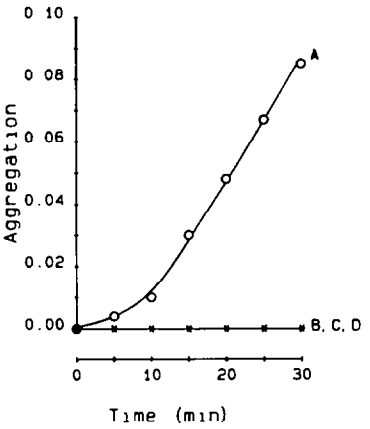

Fig. 7. Effect of IAA and ABA on aggregation rates of SUV's expressed as the increase in absorbance change induced by the hormone compared to hormone-free vesicles. Hormone/lipid 0.054, pH 5. Curve A, 90 mol% DMPE, 10 mol% DMPE with ABA; curve B, 100 mol% DMPC with IAA; curve C, 90 mol% DMPC, 10 mol% DMPE with IAA; curve D, 100 mol% DMPC with ABA.

Table 3. Effect of ABA and IAA on acyl chain motion in phospholipid bilayers as monitored by spin label ESR

Membrane composition	Spin label position	Order parameter, S		
		No hormone	+ ABA	+ IAA
100 mol% egg PC	5	0.393	0.412	0.416
80 mol% egg PC/20 mol% egg PE	5	0.423	0.418	0.426
		Correlation time, $\tau_c \times 10^{10}$ sec		
		No hormone	+ ABA	+ IAA
100 mol% egg PC	12	17.78	18.42	18.76
80 mol% egg PC/20 mol% egg PE	12	18.73	18.57	18.51

The samples were SUV's of 10 mM phospholipid/0.1 mM spin labelled stearic acid in 20 mM acetate buffer (pH 5). The experiments were performed at 30° in the absence the presence of 5 mM hormone.

permeability to the NMR shift reagent Pr^{3+} for single component PC membranes [18], whereas we measured a very small ABA enhancement in PC bilayers which was substantially increased (six-fold) by incorporating PE (20 mol%) into the bilayers [17]. Permeability to Cl^- exhibited a similar trend. Thus, IAA is able to enhance permeability by interacting with the PC head group, but ABA seems to require at least two different phospholipids for substantial enhancement and may be interacting at the region of defects in the bilayer due to disruption of head group packing at the interface between different phospholipids [unpublished, 27].

Vesicle aggregation can be greatly altered by both IAA and ABA. As with membrane permeability, the circumstances under which it occurs, however, are considerably different. Measurement of permeability to a cation (Pr^{3+}), an anion (Cl^-) and a non-electrolyte (erythritol) all demonstrate a similar trend. IAA is effective with pure PC vesicles while ABA requires the presence of at least two phospholipid components for substantial enhancement. Vesicle stability is also affected by the hormones in a parallel way. Paleg reported that his pure PC vesicles could become unstable and aggregate with IAA [22]. While we measured only small aggregation with IAA to PC vesicles, we demonstrated considerable instability in SUVs with mixed component vesicles and ABA. Hormone-induced changes in permeability and aggregation were pH dependent. IAA was somewhat effective at promoting permeability as either an anion (pH 7) or as a neutral solute (pH 4), but ABA was almost totally ineffective at pH 7.

Recently, Paleg demonstrated that by slightly oxidizing soy PC, IAA-induced bilayer permeability to Pr^{3+} was increased 100-fold [20] with enhanced binding of the hormone to the membranes. We previously reported that the two geometric isomers of ABA possessing identical chemical properties (biologically active *cis-trans* ABA and inactive *trans-trans* ABA) behave differently in bilayers[11]. *Cis-trans* ABA enhances permeability of erythritol in bilayers while the *trans-trans* isomer is ineffective. These surprising results, which demonstrate some selectivity with protein free bilayers, have been confirmed in ref. [28] measuring K^+ permeability. Here we report several differences in behavior between IAA and ABA in phospholipid bilayers. Thus, it is clear from the selectivity in effects observed that the hormone-phospholipid interactions being monitored are not merely general properties of small, monocarboxylic acids.

The ESR work adds further support to the above conclusion. The most likely general mechanism of enhanced permeability would be expected to be increased acyl chain disorder and/or fluidity within the membrane. Our data (Table 3) show that at the high concentration of 50 mol% (with respect to phospholipid) neither hormone has substantial effect on order at the 5-position or fluidity at the 12-position in either 100 mol% egg PC or 80 mol% egg PC/20 mol% egg PE membranes. Indeed, while with 80 mol% egg PC/20 mol% egg PE vesicles the effect is negligible, with pure egg PC vesicles the trend is towards higher order parameters and correlation times for both ABA and IAA.

As the experiments with bilayer membranes are just beginning and the possible combination of lipid types, fatty acid chain lengths and unsaturations, pH's, salt concentrations and temperatures is enormous, it may be possible that the lipid component of membranes plays a significant role in controlling plant hormone activity. Many additional experiments must be performed, however, to define the effect of IAA and ABA on bilayers of different compositions before the possible significance of plant hormone–phospholipid interactions can be ascertained.

EXPERIMENTAL

Plant hormones were purchased from ICN Pharmaceuticals (IAA, IPA and IBA) and Sigma Chemical Co. (*cis-trans* ABA). The natural phospholipids, egg PC (Type XI-E), crude egg lecithin (Type IX-E), egg PE (Type III), soy PC (Type III-S), soy (Type IV) and *E. coli* PE (Type V) were from Sigma. The synthetic phospholipids DLPC, DMPC, DOPC, DMPE and DOPE were from Avanti Polar Lipids, Birmingham.

Erythritol permeability was followed by light scattering in a temp controlled computing spectrophotometer as previously described [11]. MLV's were prepd from appropriate phospholipids at 10 mg/mol by the method of ref. [29]. Initial swelling velocities were determined as described in ref. [30] and are expressed as $dl/A/dt\%$, where A is absorbance at 350 nm. Each point is the mean of at least 5 determinations.

Chloride permeability was followed using a specific electrode linear through 5×10^{-5} M Cl^-. MLV's were made above the phase transition of the lipids in 200 mM KCl/10 mM NaOAC as described in ref. [11], and were then put through a 'Liposor' (Lidex Technologies, Bedford) 500 times. The resulting large unilamellar vesicles (LUV) were sized through 1.0 and 0.2 m Nucleopore filters. Non-sequestered Cl^- was removed on a Sephadex G-50 column pre-equilibrated with 400 mM glucose/10mM NaOAc. LUV's were mixed with appropriate amounts of IAA or ABA and Cl^- leakage determined for one hr. After this time, total sequestered Cl^- was determined by release with 2% Triton X100 [21].

Sonicated unilamellar vesicles of 100 mol% egg PC (SUV's) and 80 mol% egg PC/20 mol% egg PE were prepared from MLV's for ESR expts. The MLV's were 20 mM in phospholipid, 0.2 mM in 5- or 12-doxyl stearic acid (Molecular Probes, Inc., Eugene) and hydrated with 20 mM acetate buffer (pH 5). Sonication for *ca* 5 min under N_2 with a Tekmar Model VC 250 Ultrasonic Cell Disruptor or Heat Systems Model W-220F Cell Disruptor produced SUV's. A brief period of centrifugation of *ca* 5 min, ensured removal of titanium fragments. The vesicles were then mixed with additional buffer, ABA or IAA solns (both hormones 10 mM in 20 mM acetate buffer, pH 5) to result in a final phospholipid concn of 10 mM with or without 50 mol% (with respect to phospholipid) plant hormone.

ESR experiments were performed at 30° on an IBM/Bruker ER 200D x-band ESR spectrometer, interfaced and controlled by a computer system. Order parameters S (5-position) and correlation times τ_c (12-position) were obtained by direct computer calculation from the spectra according to

$$S = \frac{A_{\parallel} - A_{\perp} - C}{A_{\parallel} + 2A_{\perp} + 2C}(1.66) \qquad (1)$$

where $A_{\parallel}$ and $A_{\perp}$ are the apparent parallel and perpendicular hyperfine splitting parameters, the constant $C = 1.4 - 0.053\ (A_{\parallel} - A_{\beta})$ is an empirical correction for the difference between the true and apparent values of A, and the factor 1.66 is a solvent polarity correction factor [32]; and

$$\tau_c = 6.5 \times 10^{-10} W_o \left[\left(\frac{h_0}{h_{-1}}\right)^{1/2} - 1\right]$$

where W_0 is the peak to peak width of the central line, and h_0/h_{-1} is the ratio of the heights of the central and high field lines, respectively [33]. Spectral parameters are: centre field, 3294 G; sweep width, 100 G (5-position) or 80 G (12-position); sweep time, 200 sec (5-position) or 160 sec (12-position); modulation amplitude, 2.0 G (5-position) or 1.0 G (12-position); time constant, 500 msec; microwave power, 12 dB; and dataset, 1000 points (5-doxyl) or 2000 points (12-position).

SUV's for aggregation expts were prepd as for the ESR work except that the initial MLV's were made up pH-adjusted in 10 mM potassium phosphate. The SUV's obtained were mixed with IAA or ABA, as appropriate, and the aggregation then followed at 350 nm [11]. Results are expressed as the difference in A with and without hormone for each of the four lipid populations.

Acknowledgement—It is a pleasure to thank Professor M. D. Kemple for ESR spectrometer time.

REFERENCES

1. Gutknecht, J. and Walter (1980) *J. Memb. Biol.* **56**, 65.
2. Hipkins, M. F. and Hillman, J. R. (1981) *Z. Pflanzenphysiol.* **104s**, 217.
3. Rubery, P. H. and Sheldrake, A. R. (1973) *Nature New Biol.* **244**, 286.
4. Milborrow, B. V. (1967) *Planta* **76**, 93.
5. Venis, M. (1985) *Hormone Binding Sites in Plants.* Longman, New York.
6. Venis, M. (1985) in *Hormone Binding Sites in Plants* p. 69. Longman, New York.
7. Hornberg, C. and Weiler, E. W. (1984) *Nature* **310**, 321.
8. Hocking, T. J., Clapham, J. and Catell, K. J. (1978) *Planta* **138**, 303.
9. Curvetto, N., Delmastro, S. and Brevedan, R. (1982) *Plant Physiol.* **69**, suppl., 40.
10. Papahadjopoulos, D., ed. (1978) *Ann. N. Y. Acad. Sci.* **308**, 462.
11. Stillwell, W. and Hester, P. (1984) *Phytochemistry* **23**, 2187.
12. Stillwell, W. and Hester, P. (1983) *Plant Physiol.* **71**, 524.
13. Stillwell, W. and Hester, P. (1984) *Z. Pflanzenphysiol.* **114**, 65.
14. Hester, P. and Stillwell, W. (1984) *Biochim. Biophys. Acta* **770**, 105.
15. Stillwell, W., Hester, P. and Brengle, B. (1985) *J. Plant Physiol.* **118**, 105.
16. Bangham, A. D., de Gier, J. and Greville, G. D. (1967) *Chem. Phys. Lipids* **1**, 225.
17. Wassall, S. R., Hester, P. and Stillwell, W. (1985) *Biochim. Biophys. Acta* **815**, 519.
18. Misso, N. L. A., Jones, G. P. and Paleg, L. G. (1986) *Biochim. Biophys. Acta* 861, 1.
19. Paleg, L. G., Wood, A. and Spotswood, T. M. (1975) in *Plant Growth Substances* (Y. Sumiki, ed.) p. 732. Hirokawa, Tokyo.
20. Marker, A., Paleg, L. G. and Spotswood, T. M. (1977) in *Plant Growth Substances* (P. E. Pilet, ed.) p. 44. Springer, Berlin.
21. Marker, A., Paleg, L. G. and Spotswood, T. M. (1978) *Chem. Phys. Lipids* **22**, 39.
22. Jones, G. P., Marker, A. and Paleg, L. G. (1984) *Biochemistry* **23**, 1514.
23. Jones, G. P. and Paleg, L. G. (1984) *Biochemistry* **23**, 1521.
24. Jones, G. P. and Paleg, L. G. (1984) *Biochemistry* **23**, 1525.
25. Weigl, J. (1969) *Z. Naturforsch.* **24**, 1046.
26. Veen, H. (1974) *Z. Naturforsch.* **29**, 39.
27. Stillwell, W., Brengle, B. and Wassall, S. (1986) *Plant Physiol.* **80**, 93.
28. Harkers, C., Hartung, W. and Gimmler, H. (1986) *J. Plant Physiol.* **122**, 385.
29. Bangham, A. D. (1968) *Progr. Biophys. Mol. Biol.* **18**, 29.
30. Block, M. C., Van Deenen, L. L. M. and de Gier, J. (1976) *Biochem. Biophys. Acta* **433**, 1.
31. Bondeson, J., Wijkander, J. and Sundler, R. (1984) *Biochim. Biophys. Acta* 777, 21.
32. Gaffney, B. J. (1976) in *Spin Labelling—Theory and Applications* (L. J. Berliner, ed.) p. 567. Academic Press, New York.
33. Keith, A., Bulfield, G. and Snipes, W. (1970) *Biophys. J.* **10**, 618.