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Abscisic acid increases lipid bilayer permeability to cations as studied by phosphorus-31 NMR

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Using a ^{31}P-NMR lanthanide shift technique, abscisic acid is shown to enhance the permeability to praeseodymium of lipid bilayers composed of 80 mol% phosphatidylcholine and 20 mol% phosphatidylethanolamine. Praeseodymium permeability is immeasurably slow in the absence of the hormone whether or not phosphatidylethanolamine is present in the bilayers. Only in the presence of abscisic acid is praeseodymium permeability observed, the effect being significantly greater when phosphatidylethanolamine is present. These results substitate prior reports from nonelectrolyte permeability studies that abscisic acid interacts with phosphatidylethanolamine in lipid bilayers.

Although the molecular mechanism for any plant hormone is not understood [1], it has been suggested that the initial site of action may be at the membrane [2] where interaction with specific, but unknown components affects movement of solutes and water across the membranes. Numerous reports demonstrating the effect of the plant hormone abscisic acid on membrane transport processes have appeared [3]. The preponderance of these reports have involved studies with highly complex biological membrane systems and as a result it is not even clear if abscisic acid enhances [4,5] or decrease [6,7] general membrane permeability. Recently, using simple, protein-free liposomal systems, we have shown that undissociated abscisic acid markedly increases the permeability of phosphatidylcholine (PC) bilayers to urea and erythritol only if phosphatidylethanolamine (PE) is incorporated into the membranes [8,9]. We have proposed a general model for abscisic acid action involving abscisic acid-phosphatidylethanolamine interactions on membranes [8]. Clearly confirmation of these results by another method would be an essential step in establishing the mode of action for this significant plant hormone.

The present communication describes a study in which a ^{31}P-NMR lanthanide-induced shift technique is employed to determine the effect of abscisic acid on membrane permeability to ions. The method consists of introducing Pr^{3+} into the external aqueous solution of a sonicated phospholipid dispersion. The phosphorus nuclei of phospholipid molecules in the outer layer of vesicles interact with Pr^{3+} and their ^{31}P-NMR signal is consequently shifted downfield from the signal from the essentially unaffected inner layer [10]. Observation of the ^{31}P-NMR spectrum as a function of time then allows Pr^{3+} permeation into the unilamellar vesicles to be monitored from the resultant modification of the signal due to the inner layer [11].

Multilamellar liposomes were made from 8%

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

(w/v) aqueous dispersions of PC (Type XI-E from egg yolk, Sigma) or 4:1, PC/PE (molar ratio) mixtures (Type V from *Escherichia coli,* Sigma) [12]. The 2.6 ml aqueous solutions were adjusted to pH 5.0 with or without abscisic acid ((±)*cis-trans* isomer, Sigma) at a 1:5 hormone to lipid molar ratio. The mixtures were sonicated with cooling under N_2 for 10 min on a Heat Systems Model W-220F Cell Disrupter and were then centrifuged on a bench top centrifuge to remove titanium fragments and non-dispersed lipids. The samples were transferred to 12 mm o.d. NMR tubes and maintained at 37°C.

^{31}P-NMR spectra at 37°C were recorded at 121.5 MHz on a Nicolet NTC-300 NMR spectrometer interfaced to a 1280 computer. ^{1}H decoupling was not employed and the spectra were run unlocked. Spectral parameters are: pulse width, 24 μs (90° flip angle); sweep width, ±2000 Hz; datatest, 2K zero-filled to 4K; and line broadening, 2 Hz. Number of acquisitions were 512 and the repetition time (2.756 s) ensures that the effect of spin-lattice relaxation on signal intensity may effectively be ignored. Chemical shifts are with respect to external H_3PO_4 (85%).

Data were collected at various times over 72 h following the addition of 30 μl 0.5 M Pr^{3+} to the sonicated dispersions. Finally the non-sequestered Pr^{3+} was chelated with EDTA and the spectra redetermined.

Fig. 1 shows ^{31}P-NMR spectra recorded at 37°C as a function of time following the addition of Pr^{3+} to PC/PE vesicles prepared in the presence and absence of abscisic acid. The initial spectra for both systems are quite similar, consisting of a pair of peaks from the outer vesicle layer that are shifted downfield and broadened by Pr^{3+} and another pair of essentially unshifted peaks due to the inner vesicle layer. The extent of the shift is greater in the presence of abscisic acid, 10–11 ppm, than in its absence, 5–6 ppm. In both cases the ratio of outside:inside intensity of the NMR signals, which is a direct measure of the ratio of phospholipid in the outer and inner vesicle layers, is (1.9 ± 0.1):1. This ratio as well as the Pr^{3+} induced shifts are comparable to those reported for pure PC vesicles [13,14] of average diameter 220 Å [15]. For a given pair of peaks the more and less intense signals are assigned to PC and PE,

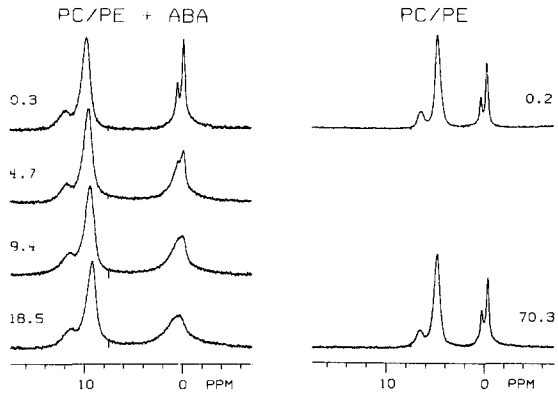

Fig. 1. ^{31}P-NMR spectra at 37°C for PC/PE (4:1) vesicles in the presence and absence of abscisic acid. The spectra are labeled in number of hours following the addition of Pr^{3+}, the time referring to the start of data acquisition for a given spectrum.

respectively, on the basis of relative intensity and chemical shift [16].

Inspection of the two sets of spectra in Fig. 1 clearly reveals a difference in variation with time. Whereas in the absence of abscisic acid the spectra are essentially unchanged after 70.3 h, in the presence of abscisic acid the pair of upfield peaks from the inner vesicle layer progressively become broadened and shifted downfield slightly over the period of 18.5 h depicted. The degree of broadening, indeed, is so great that separate signals produced by inner layer PC and PE are eventually unresolvable. The ratio of signals from outer and inner vesicle layers remains the same at (1.9 ± 0.1):1 for all spectra.

^{31}P-NMR spectra at 37°C as a function of time after the addition of Pr^{3+} are presented in Fig. 2 for PC vesicles with and without abscisic acid. The initial spectra are again similar for the two systems, consisting of a downfield signal due to PC in the outer layer of vesicles and an essentially unshifted signal from the inner layer. As before, the shift produced by Pr^{3+} is greater in the presence of abscisic acid, 9 ppm, than in its absence, 6 ppm. This indicates that interaction of Pr^{3+} with the head groups is modified in some way by the plant hormone.

The dependence on time of the two sets of spectra in Fig. 2 also differs. Essentially no change is apparent in 70.6 h when abscisic acid is absent.

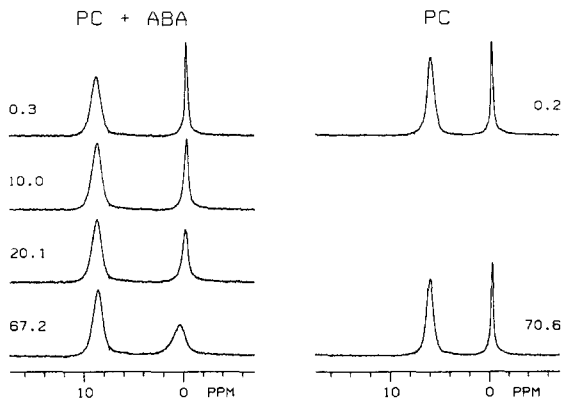

Fig. 2. ^{31}P-NMR spectra at 37°C for PC vesicles in the presence and absence of abscisic acid. The spectra are labeled in number of hours following the addition of Pr^{3+}, the time referring to the start of data acquisition for a given spectrum.

This contrasts with the gradual broadening and very slight downfield shift with time observed for the ^{31}P-NMR signal from the inner layer of PC vesicles when abscisic acid is present. It should be noted, however, that the amount of broadening after comparable intervals is substantially greater in PC/PE than in PC vesicles. The ratio of outside vesicle layer to inside vesicle layer signal intensities for all spectra in Fig. 2 is $(1.8 \pm 0.1) : 1$.

The addition of excess EDTA (EDTA/Pr^{3+}, 4 : 1) upon completion of the experiment complexes the accessible Pr^{3+} and so eliminates its spectral shift. In all samples the downfield portion of the spectrum, assigned to the outer layer of the vesicles, was shifted upfield and narrowed while the upfield portion, assigned to the inner layer, was essentially unaffected.

The ^{31}P-NMR spectra in Figs. 1 and 2 demonstrate a clear enhancement of membrane permeability to ions by abscisic acid. In the absence of abscisic acid the spectra for both PC/PE and PC vesicles are independent of time following the addition of Pr^{3+}, which shows that a negligible movement of the ion across the membrane has occurred in approximately three days of experimentation. This contrasts with the behavior in the presence of abscisic acid, when the upfield portion of the ^{31}P-NMR spectrum due to the inner layer of vesicles progressively becomes broadened and shifted slightly downfield with increasing time for PC/PE and PC vesicles. Such a variation with time of the spectra is interpreted in terms of enhanced ion permeation through the membrane into the aqueous interior of the vesicles, where interaction of Pr^{3+} with the phosphorus nuclei of phospholipid molecules in the inner vesicle layer occurs [11]. To support this interpretation, experiments were run in which low concentrations of Pr^{3+} were introduced into the aqueous interior of the phospholipid vesicles, while a fixed higher concentration was present in the exterior aqueous solution. This allowed the ^{31}P-NMR signals from the inner and outer layers to be distinguished. ^{31}P-NMR spectra were obtained that, for the inner layer signal, exhibit broadening with only slight shifts at low interior Pr^{3+} concentrations, followed by larger shifts at higher concentrations (Wassall, S.R. and Stillwell, W., unpublished results). By comparison with these spectra, moreover, rate constants for abscisic acid-induced Pr^{3+} permeation on the order of 3 and 16 μM/h could be estimated for PC and PC/PE vesicles, respectively.

The possibility that a time-dependent process involving vesicle rupture (e.g. fusion) is responsible for Pr^{3+} coming into contact with the phospholipid molecules producing the upfield portion of the ^{31}P-NMR spectra is discounted. Throughout the experiments in all samples the total signal intensities remained constant and the ratio of signal intensities for the downfield portion of the spectra with respect to the upfield portion were also constant. This implies that the ratio of the number of phospholipid molecules in the outside and inside vesicle layers is a constant, and hence that the size and integrity of the vesicles is maintained. Furthermore, upon completion of the experiment the introduction of EDTA to complex all accessible Pr^{3+} was seen to only affect the downfield portion of the spectra ascribed to the outer vesicle layer. This again indicates the maintenance of vesicle integrity.

Thus, it is concluded that membrane permeability to Pr^{3+} is enhanced by abscisic acid. This is consistent with the increased membrane permeability to non-electrolytes in the presence of ABA that we have previously reported [8,9]. Other plant hormones have also been found to increase non-electrolyte permeability through model membranes [17]. Using a similar ^{1}H-NMR technique to the one reported here, Paleg and co-workers [18,19]

have very recently found that the permeability to Pr^{3+} and Mn^{2+} of soybean phosphatidylcholine vesicles is increased by indole-3-acetic acid and related auxins.

Significantly, the spectra in Figs. 1 and 2 furthermore indicate that the abscisic acid-induced increase in ion permeability is greater for PC/PE than for PC membranes. This conclusion follows from the observation that the time scale as Pr^{3+} enters the vesicles of the broadening and shifting of the ^{31}P-NMR resonances from the inner vesicle layer is much shorter for PC/PE vesicles. The difference in rate of signal broadening is estimated to correspond to a factor of approximately six in enhancement of permeability rate. These results extend to cations our prior observations, based on measurement of urea and erythritol permeability, that abscisic acid interacts with PE in lipid bilayers increasing permeability [8,9]. We believe this is a head group interaction rather than a function of acyl chain composition since ESR and NMR data indicate that abscisic acid has no effect on the acyl chains (Wassall, S.R., Stillwell, W. and Kemple, M.D., unpublished results). The previous osmotic swelling experiments, which employed synthetic phospholipids of well defined structure, also support the conclusion by indicating that abscisic acid enhancement of permeability to nonelectrolytes is dependent upon the presence of PE rather than acyl chain composition [8,9].

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