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The effect of plant hormone abscisic acid on model membranes – differential scanning calorimetry and planar bilayer membranes investigations

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The effect of abscisic acid on the thermotropic properties of dipalmitoylphosphatidylcholine (DPPC) and on phosphatidylethanolamines (natural (PE) and dipalmitoylphosphatidylethanolamine (DPPE)) bilayers was investigated by differential scanning calorimetry (DSC). Abscisic acid eliminates the pretransition of DPPC, causes a downward shift of its temperature of melting (T_m) and broadens the melting peak without changing the enthalpy of melting. In natural PE bilayers interacting with abscisic acid a small decrease in the enthalpy of melting almost without change of T_m was detected, whereas in synthetic DPPE abscisic acid caused a small shift of T_m and small broadening of the melting peak without changing the enthalpy of melting. Abscisic acid increases the conductance to Na^+ or K^+ by three orders of magnitude in planar lipid membranes formed from PE monolayers and by less than two orders of magnitude in membranes formed from PC monolayers.

The plant hormones can be divided into four classes of compounds: abscisic acid, auxins, cytokinins and gibberillins. In spite of the vast amount of research published, the molecular mechanism of the action of the plant hormones is not clear. It was suggested that the cell membranes might be their site of action, as the hormones influence the permeability to ions and water [1,2]. Lipid bilayers are the simplest model system for investigation of the effect of hormones, drugs etc. on the cell membrane lipids. By employing DSC it was shown that auxins and gibberillins cause a perturbation of synthetic phospholipid membranes [3,4]. Lea and Collins [5] found that in black lipid membranes formed from phosphatidylcholine + cholesterol in *n*-decane abscisic acid causes appearance of very quick conductance channels. Recently it was reported that abscisic acid increases the permeability of model membranes to non electrolyte-erythriol and to praeseodymium ion [6,7]. The increase of permeability is obtained only in the presence of phosphatidylethanolamine in the lipid mixture. The aim of the current work was to study the effect of abscisic acid on the thermotropic properties of synthetic and natural phospholipid membranes and to investigate the change in permeability to Na^+ or K^+ ions induced by abscisic acid in solvent-free planar bilayer membranes (PLM) formed either from phosphatidylethanolamine or phosphatidylcholine monolayers.

Dipalmitoylphosphatidylcholine was purchased from Dr. Berchtold Lab, Bern, Switzerland. Phosphatidylethanolamine grade I from egg yolk (in chloroform/methanol) was from Lipid Products South Nutfield, U.K. Diphytanoylphosphatidylcholine was from Avanti-Birmingham, AL, U.S.A. Dipalmitoylphosphatidylethanolamine and abscisic acid ((+)*cis-trans* synthetic) were bought

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from Sigma, St. Louis, MO, U.S.A. Abscisic acid was dissolved in Spectra Grade methanol. For differential scanning calorimetry experiments abscisic acid was added at appropriate concentrations to the solutions of the phospholipids. After mixing, the solvents were driven off by a stream of nitrogen, the samples were kept under high vacuum (0.1 Torr) for 3 h. The dry lipids were weighted directly into the aluminum pans of the instrument and an excess of salt solution (0.15 M NaCl at pH 5.3) was added. In some experiments with PE the salt solution contained also 25% (v/v) ethylene glycol. The samples were incubated for 1 h at 60°C (PE), 80°C (DPPE) and 70°C (DPPC) followed by DSC measurements. The DSC experiments were performed on a DuPont 990 Thermal Analyser equipped with cell base II.

Planar lipid membranes, solvent free were formed from monolayers by the method of Montal and Mueller [8]. Planar bilayer membranes were formed from PE in symmetrical solutions of 1 M KCl or 1 M NaCl and from diphytanoylPC in 1 M NaCl. Silver/silver chloride electrodes were used and the electrical response (current) was fed into a 427 Keithley current amplifier, the voltage was recorded on a BD8 Kipp and Zonen recorder.

(1). DSC. Fig. 1 presents the thermograms of DPPC alone and with increasing concentrations of abscisic acid . As seen from Fig. 1B at very low concentrations of abscisic acid (5 mol%) the pretransition characteristic for DPPC disappears and the melting peak becomes broader. With increase of abscisic acid concentration further broadening of the peak and a decrease of T_m (temperature of the middle of the peak) is seen. Upon addition of 18 mol% abscisic acid T_m is shifted down by about 5 K and the transition half-width is 3.5 K as compared to 1.5 K for DPPC itself. However, no change in the enthalpy of melting due to interaction with abscisic acid was detected. The increase of the width of transition indicates that abscisic acid interacting with the phospholipid decreases the size of the cooperative unit undergoing melting and the decrease of temperature of melting without change of the enthalpy of melting indicates that the perturbation induced by abscisic acid is mainly in the head group region. The effect of abscisic acid on DPPC is similar to the one reported for gibberilins and auxins [3,4].

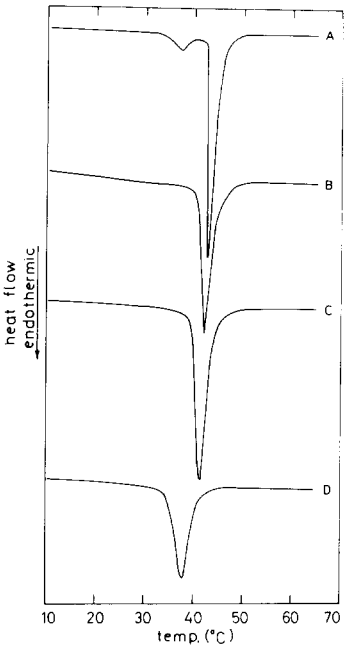

Fig. 1. Thermograms of DPPC interacting with abscisic acid: A, DPPC only; B, DPPC + 5 mol% abscisic acid; C, DPPC + 9 mol% abscisic acid; D, DPPC + 18 mol% abscisic acid. The mole percentages of abscisic acid indicate the initial concentrations. Total lipid concentration 5.5–7.5%, dispersing medium 0.15 M NaCl; scan rate 5 K/min.

In an attempt to evaluate the effect of the structure of the head group and of the degree of unsaturation of the hydrocarbon chains on the interaction of abscisic acid with phospholipids the influence of abscisic acid on the thermotropic properties of phosphatidylethanolamines: natural PE (from egg yolk) and on dipalmitoylphosphatidylethanolamine was investigated. Abscisic acid interacting with PE bilayers influences also their thermotropic properties, but the effect is different from this exerted on DPPC bilayers (Fig. 2). In the case of egg yolk PE interacting with abscisic acid (Fig. 2a) almost no decrease of T_m is seen (about 1.5 K), however, a decrease of the enthalpy of melting is detected amounting to about 25% at the highest concentration of abscisic acid investigated (19 mol%, Fig. 2aD). As the melting temperature of PE is low (approx. 11°C) data presented in Fig. 2a were obtained for the lipid dispersed in 25% (v/v) ethylene glycol/salt to prevent the in-

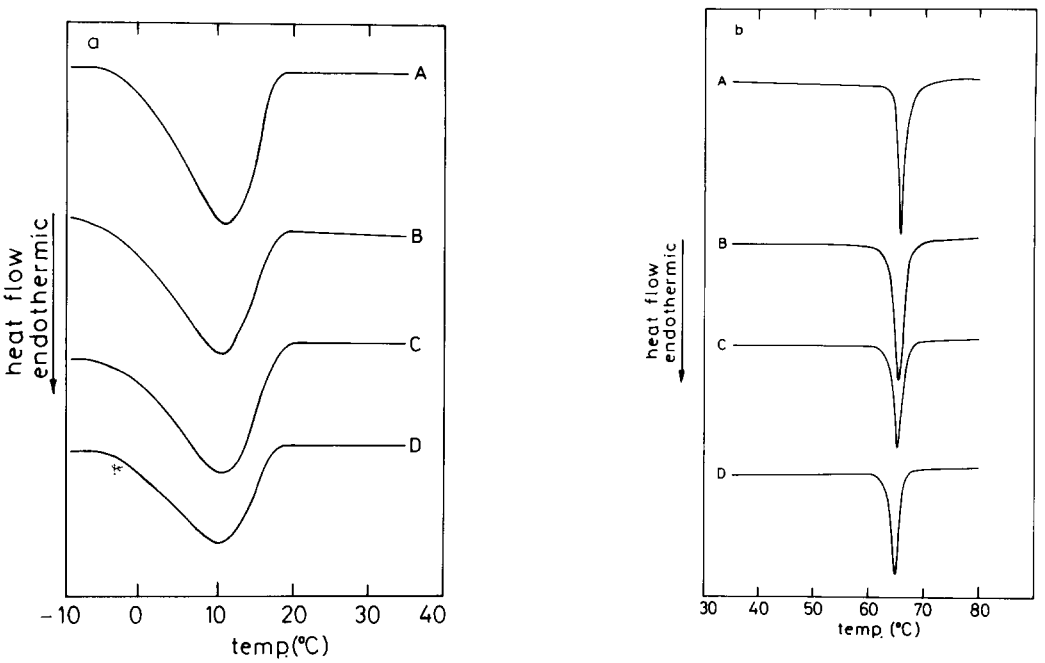

Fig. 2. Thermograms of phosphatidylethanolamines interacting with abscisic acid. (a) Natural PE: A, PE only; B, PE + 5 mol% abscisic acid; C, PE + 10 mol% abscisic acid; D, PE + 19 mol% abscisic acid. Dispersing medium 25% (v/v) ethylene glycol/0.15 M NaCl (b) DPPE: A, DPPE only; B, DPPE + 6 mol% abscisic acid; C, DPPE + 11 mol% abscisic acid; D, DPPE + 20 mol% abscisic acid. Lipid concentration 5–6%. Scan rate 5 K/min.

terference from water melting. The presence of ethylene glycol did not influence the results as similar results were obtained when the lipid was dispersed in salt solution only.

There are two main differences between DPPC and PE: DPPC is a synthetic disaturated lipid with two equal acyl residues, high melting temperature and it is devoid of hydrogen bonding. PE from egg yolk is a mixture of acyl chains with various lengths and degrees of saturation resulting in its lower melting temperature and higher fluidity and its head group lattice is stabilized by hydrogen bonding as in all phosphatidylethanolamines. The synthetic phosphatidylethanolamine investigated in the present work (dipalmitoylphosphatidylethanolamine (DPPE)) is a disaturated lipid and as in DPPC the acyl residues are palmitic chains. By comparing the effect of abscisic acid on the thermotropic properties of DPPC and DPPE the influence of the head group on the interaction can be evaluated. As seen from Fig. 2b the effect of abscisic acid on the thermotropic properties of DPPE is very small. Shift in T_m of about 1.5 K and broadening of the peak are seen. Within experimental error on change in ΔH is found. By comparing Fig. 1 with Fig. 2b it is possible to see that the head group of the phospholipid has very strong influence on the interaction with abscisic acid. The presence of hydrogen bonding in DPPE decreases strongly the modifying effect of abscisic acid on the phospholipid. By comparing the effect of abscisic acid on DPPE with this on natural PE the effect of acyl chains can be evaluated. In the case of PE the effect on T_m is similar to this on DPPE as it is predominantly determined by the head group, but in PE a small decrease of ΔH was detected probably due to higher fluidity and higher degree of unsaturation of PE enabling deeper penetration of the hormone with concomitant withdrawing of some molecules of PE from melting.

(2) PLM. The electrical conductivity of planar lipid membranes found from PE monolayers in 1 M NaCl or 1 M KCl at pH 5.3–5.5 is very low

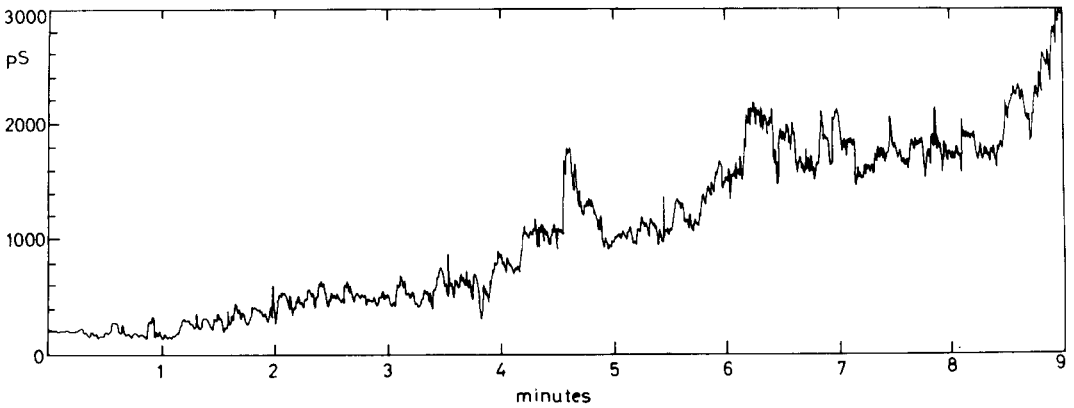

Fig. 3. Conductance of planar lipid membranes formed from PE. One monolayer PE + 7 mol% abscisic acid, the second one PE only. Applied potential −50 mV, 1 M NaCl on both sides of the bilayer.

(lower than 4 pS). Addition of abscisic acid in methanol to one side of the bilayer (to the side of the virtual ground) at a concentration of $3 \cdot 10^{-4}$ M has a very small effect on the electrical conductance and only after very long time, about 3 h. This lack of effect is probably due to aggregation of abscisic acid in aqueous solution and formation of aggregates with very low diffusion coefficient. When abscisic acid is incorporated directly into the PE monolayer an immediate effect on the conductance is obtained as seen from Fig. 3. In these experiments abscisic acid was dissolved in methanol, added at a concentration of 7 mol% to PE, the solvents were evaporated and the PE/abscisic acid mixture dissolved in hexane at a concentration of 2%. After determination of the base line conductance for pure PE bilayer (< 4 pS) the solution on one side (virtual ground) was withdrawn and replaced by a new solution on which a monolayer of PE containing abscisic acid was spread and a mixed bilayer was formed. The initial conductance of this bilayer was much higher than that of the pure PE bilayer (Fig. 3). The conductance increased further with time and the final values were over three orders of magnitude higher than those of pure PE bilayers. As seen from Fig. 3 the conductance rises in 'jumps' probably due to conducting structures formed by abscisic acid. Planar bilayer membranes formed from monolayers containing abscisic acid on both sides

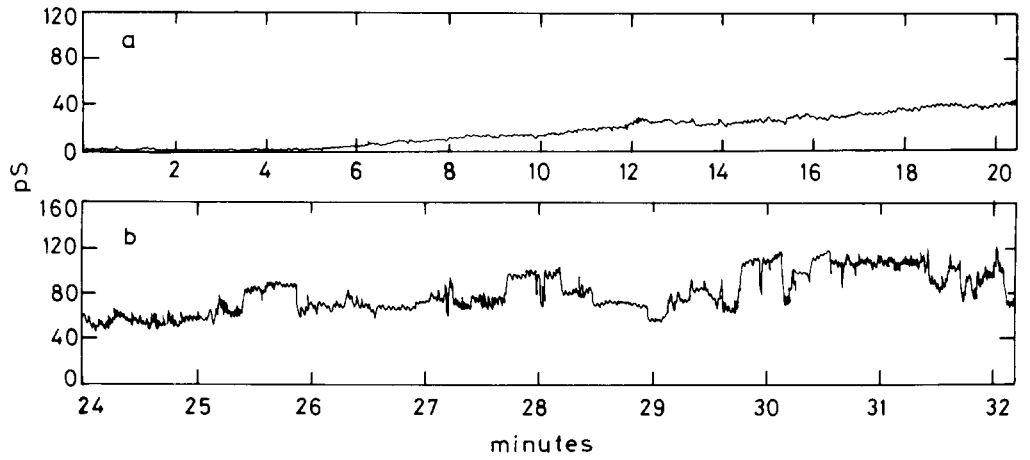

Fig. 4. Conductance of planar lipid membranes formed from diphytanoylPC. One monolayer diphytanoylPC + 7 mol% abscisic acid, the second one diphytanoylPC only. Applied potential −50 mV, 1 M NaCl on both sides of the bilayer. Trace b is the continuation of trace a.

were very unstable, possibly due to the introduction of too much perturbation. The conductance as shown on Fig. 3 is independent of the cation used (Na^+ vs. K^+) and of the sign or magnitude of the applied voltage (up to 100 mV). Modification of the permeability of diphytanoylPC bilayers to Na^+ by abscisic acid was also investigated. Fig. 4 presents the conductance of diphytanoylPC, planar bilayer membranes (formed from one monolayer of diphytanoylPC + 7 mol% abscisic acid and the second one pure diphytanoylPC) as a function of time. By comparing Figs. 3 and 4 it is possible to see that the effect of abscisic acid on the permeability of PC bilayers is much smaller than on PE bilayers. After formation of the mixed bilayer a small and gradual increase of the conductance is seen (trace a) and the total increase of G is up to two orders of magnitude (trace b) being over three orders of magnitude for PE bilayers (Fig. 3). In case of PE bilayers the raise of conductance is also much quicker. These findings are in agreement with the data of Wassall et al. [7] who claim that the effect of abscisic acid on the permeability of membranes containing PE is bigger than on pure PC membranes. The data presented in this communication show that the perturbations induced by abscisic acid are dependent on the type of the lipid employed. As in plants several glycolipids and phospholipids are present, future experiments will show more clearly the possible involvement of abscisic acid in the various membranal processes.

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