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Abscisic acid–lipid interactions: a phospholipid monolayer study

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Lipid monolayer studies were performed on a Langmuir trough in the absence and in the presence of the plant hormone abscisic acid (ABA). The ABA-induced effects on the lipid monolayers can be summarized as follows: (i) ABA as the free acid (pH below 5.3) increased the molecular area and slightly decreased the surface pressure in the collapse points of monolayers made of saturated, unsaturated and of mixed lipids; ABA as the anion showed only minor effects. (ii) The ABA-induced area increase of the lipid monolayers decreased when the surface pressure increased, but some ABA remained in the monolayers made of unsaturated phospholipids even at collapse pressure. (iii) The incorporation of ABA into the monolayers could be inhibited by adding the plant sterol β-sitosterol to the monolayer forming phospholipids. (iv) There was no substantial difference of ABA action on plant phospholipids as compared with other phospholipids. (v) ABA had a much stronger influence on unsaturated phospholipids than on saturated ones. (vi) ABA decreased the phase-transition temperature of saturated phospholipids. These results, which agree with those obtained from phospholipid vesicle studies, indicate that the physical state of the lipid is important for the ability of ABA penetrating into the lipid monolayer. Finally, a possible relevance of these results is discussed in terms of the action of ABA on guard cell membranes of plants.

Introduction

It is well-known that abscisic acid (ABA) acts on the plasma membrane of guard cells, rapidly altering the transmembrane distribution of solutes, primarily K^+ [1]. Attempts to isolate the reputed membrane-bound proteinaceous receptor, however, have continuously met with failure. The early report of an ABA receptor by Hocking et al. (1978) [2] was later shown to be an artefact resulting from the low specific activity of the [^{3}H]ABA used, while the subsequent elegant report of Hornberg and Weiler [3] identifying three binding sites for ABA has never been extended or confirmed. Furthermore, Smart et al. [4], using a variety of binding assays, were not able to detect any ABA-receptors. If ABA affects the plasma membrane of guard cells, as it most certainly does, but does not bind to a receptor protein, it is possible that the plant hormone is primarily interacting instead with the other major component of the membrane, the lipid bilayer. On the other hand, it cannot be ruled out completely that ABA is interacting with a combination of both lipid and a possibly existing receptor.

Recently several laboratories have confirmed that indeed ABA can alter a variety of properties of protein-free phospholipid bilayers. By an assortment of techniques ABA has been shown to enhance membrane permeability to cations [5,6], anions [7–9] and neutral solutes [7–13]. ABA has been reported to increase electrical conductivity of planar bimolecular lipid membranes [14,15] and to induce lipid vesicle aggregation [16] and fusion [17]. Furthermore, ABA has also been implicated in altering microheterogeneity of lipid bilayers [18]. From these experiments a new proposal is emerging: ABA might act on guard cells through interaction with the membrane lipids [8].

Recent studies by Leshem et al. [19,20] have extended the ABA-lipid studies to lipid monolayers and have suggested that ABA may interact with a very minor component of the guard cell plasma membrane,

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Abbreviations: ABA, abscisic acid; PL, phospholipid; PC, L-α-phosphatidylcholine, PE, L-α-phosphatidylethanolamine; DPPC, dipalmitoyl-L-α-phosphatidylcholine; DSPC, distearoyl-L-α-phosphatidylcholine; DOPC, dioleoyl-L-α-phosphatidylcholine; DLPC, dilinoleoyl-L-α-phosphatidylcholine; DLinPC, dilinolenoyl-L-α-phosphatidylcholine.

dipalmitoylphosphatidylcholine (DPPC). Here, we report firstly on the adsorption of ABA onto monolayers composed of unsaturated phosphatidylcholine (PC) and phosphatidylethanolamine (PE) extracted from biological sources. Secondly, we present the action of ABA onto monolayers of pure unsaturated lipids (DOPC, DLPC and DLinPC); all these lipids are in the liquid-expanded state. Thirdly, we employed pure saturated lipids (DPPC and DSPC) with DPPC exhibiting a surface pressure-induced phase transition and DSPC being in the liquid-condensed state throughout the surface pressure–area isotherm. In contrast to Leshem et al. [20] our results suggest, regardless of the lipid used, that ABA enlarges the area per lipid molecule in a pressure-dependent way, but never reduces the molecular area of the lipids. Furthermore, monolayer studies with DPPC suggest that 1 mM ABA influences the phase transition of the lipid and has a strong effect on the measured area per lipid molecule only in the liquid-expanded state.

Materials and Methods

Materials. Natural phospholipids (egg and soy bean phosphatidylcholine (PC) and phosphatidylethanolamine from *Escherichia coli* and soy bean (PE)) and dilinolenoyl-L-α-PC (DLinPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). (±)-*cis-trans*-ABA was obtained from Fluka (Buchs, Switzerland). The mixed isomers of ABA (24% (±)-*cis-trans*-ABA, 76% (±)-*trans-trans*-ABA), citric acid, β-sitosterol and the synthetic phospholipids dipalmitoyl-L-α-phosphatidylcholine (DPPC), distearoyl-L-α-PC (DSPC), dioleoyl-L-α-PC (DOPC) and dilinoleoyl-L-α-PC (DLPC)) were supplied by Sigma (St. Louis, MO, USA). Benzene was spectroscopically pure and the ethanol was analytical grade (Merck, Darmstadt, Germany). Ultrapure water was obtained by passing deionized water through a Milli-Q equipment (Millipore, Bedford, MA, USA).

The purity of the lipids was checked by thin layer chromatography. All lipids with the exception of egg PC (purity about 95%) did not contain any contaminant (purity > 99%) and were used without further purification.

Buffers and solutions. The different lipids were dissolved in benzene or a mixture of benzene and ethanol (1 : 1 (v/v)) in a final concentration between 0.6 and 2.3 mM. The lipid solutions were mixed according to the requested molar ratios. In all monolayer experiments the buffer in the subphase consisted of 0.1 M NaCl and 10 mM citric acid buffer dissolved in ultrapure water, adjusted to the desired pH by using either concentrated solutions of NaOH or HCl, respectively. ABA was insoluble at low pH in high concentrations. Thus ABA was dissolved near pH 10 to reach concentrations of up to 1 mM. Subsequently, the appropriate pH of the ABA-containing solutions was adjusted.

Monolayer experiments. Monolayer experiments were performed with a commercial Lauda FW 1 monolayer trough (MGW Lauda, Lauda, Germany). The Teflon coated metal trough was cleaned with acetone and rinsed with ultrapure water at the beginning of each set of experimental conditions. The trough was filled with the buffer solution with or without ABA. Then, the buffer's surface was cleaned by moving a mobile barrier over the surface towards a measuring barrier and by subsequently removing all surface-active material that could create a surface pressure between the two barriers. The balance was calibrated mechanically with a well-defined weight. Afterwards the mobile barrier was moved away from the measuring barrier to provide a 562-cm^2 large surface. The different lipids were spread upon from the organic solutions using a Hamilton microsyringe (Hamilton, Bonaduz, Switzerland). After evaporation of the solvent the lipid monolayer was compressed. The speed of the movable barrier was 1.7 cm/min, which corresponds to a decrease of the surface area of 25.5 cm^2/min or about 0.07 nm^2/lipid molecule per min, respectively. This speed was chosen to minimize hysteresis effects [21], which comprised about 0.5 to 2.5 mN/m depending on magnitude of surface pressure. Hysteresis was similar in experiments with and without ABA. The normalized film pressure and the area per lipid molecule were recorded with an X-Y-recorder (ABB-Goerz, Vienna, Austria).

Results

Surface-active properties of ABA

In control experiments performed with a Teflon trough equipped with a Wilhelmy balance we found out that 1 mM ABA in solution diminished the buffer's surface tension by about 3 mN/m in the first 10 min and by about 6 mN/m within 1 h. After this time ABA's surface adsorption had gained a state of equilibrium. This phenomenon was not pH dependent within the range chosen (pH 4 to pH 7).

When a phospholipid monolayer was spread upon the buffer's surface in the film balance (subphase: buffer (pH 4.8)), an enhanced surface activity of dissolved ABA could be detected: Using this measuring method the molecular area vs. surface pressure isotherms are metered directly by comparing the same buffer's surface with and without the amphiphilic molecule spread on the surface. When ABA alone was placed upon the film balance from concentrated benzene/ethanol solutions no monolayer could be detected. Also when ABA was spread from benzene/ethanol solutions together with dilinoleoyl-PC no enhanced area per lipid molecule was observed.

These results suggested that ABA did not adsorb to the surface until a minimum concentration of ABA was present in the subphase. Performing experiments with decreasing ABA concentrations the minimum concentration for a detectable interaction with the monolayers was determined to be about 0.1 mM.

Furthermore, we did not observe a more pronounced hysteresis in the presence of ABA. This means that the effects of ABA on the lipid monolayer isotherms were reversible and reproducible.

Influence of ABA on monolayers made of natural unsaturated lipids

Lipid monolayers were made from PC/PE mixtures over a subphase containing various concentrations of ABA in 0.1 M NaCl/10 mM citric acid buffer. The lipids were in the liquid-expanded state at 22°C. The surface pressure–molecular area isotherms were measured on a Lauda FW 1 Film Balance as described in Materials and Methods. Fig. 1 depicts typical surface pressure isotherms for egg PC/*E. coli* PE (molar ratio 4:1) monolayers with and without 1 mM ABA. Interestingly, the presence of ABA lead to a strong pressure-dependent increase of the area per lipid molecule. At small surface pressure, just at the end of the gas phase, the increment of the area per molecule was as large as 0.45 nm^2. With rising surface pressure, the area increase per lipid molecule descended and at collapse pressure the accretion was only 0.03 nm^2.

It is interesting to note that using films made of natural, unsaturated lipids, ABA in the subphase always enlarged the surface area per molecule and slightly decreased the surface pressure at the collapse point. From these isotherms it is clear that ABA is integrated into lipid monolayers. Furthermore, this process is surface-pressure dependent. As surface pressure intensified, ABA was gradually squeezed out of the monolayer, but even at the collapse point of the monolayer some ABA remained in the monolayer.

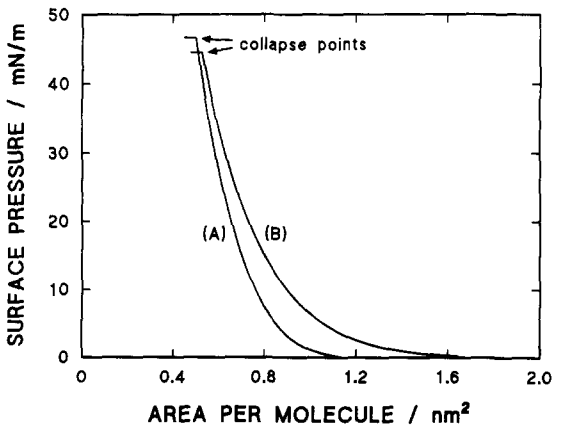

Fig. 1. Surface pressure-molecular area isotherms of monolayers composed of PC/PE (molar ratio 4:1) in the absence (A) and in the presence (B) of 1 mM ABA. The subphase contained 0.1 M NaCl/10 mM citrate buffer (pH 5); the temperature was 22°C.

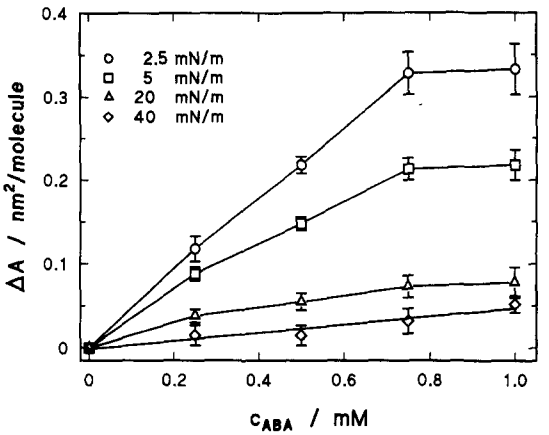

Fig. 2. ABA-induced surface area increase per lipid molecule of mixed PC/PE monolayers (molar ratio 4:1), given as a function of the ABA-concentration in the subphase. The data points correspond to the average of five measurements taken at different surface pressures of the monolayers. The subphase contained 0.1 M NaCl/10 mM citrate buffer (pH 5); the temperature was 22°C.

Experiments with lipids extracted from different biological sources showed that the uptake of ABA into monolayers is dependent on the type of lipid used for monolayer formation. At the collapse pressure, 1.0 mM ABA in the subphase resulted in an area increase per molecule of 0.02 nm^2 for monolayers composed entirely of egg PC. An increase of nearly 0.03 nm^2 was observed for a mixed component egg PC/*E. coli* PE (4:1) monolayer. We gained similar results with soy bean PC and soy bean PE. However, we could not observe an increased ABA incorporation with rising PE mol fractions. Additional experiments with mono- and digalactosyldiglycerides extracted from wheat (data not shown) resulted in quite similar area increases per lipid molecule. This indicates that the head groups of the phospholipids do not determine the rate of ABA adsorption and that the phase of the lipid is more important. Employing any lipid, the ABA-induced extension in surface area was hormone concentration dependent and was much larger at lower surface pressures than it was at higher pressures (Fig. 2).

Effect of β-sitosterol on ABA-phospholipid interaction

By permeability and membrane fusion studies Stillwell et al. [9] have proposed that plant sterols occupy the same locations in phospholipid bilayers as does ABA. They have demonstrated that ABA-enhancement of membrane permeability and fusion can be reversed by the incorporation of plant sterols. When β-sitosterol, which is one of the major plant sterols [22], is incorporated in increasing concentrations into the mixed PC/PE (4:1) monolayers, the enhancement

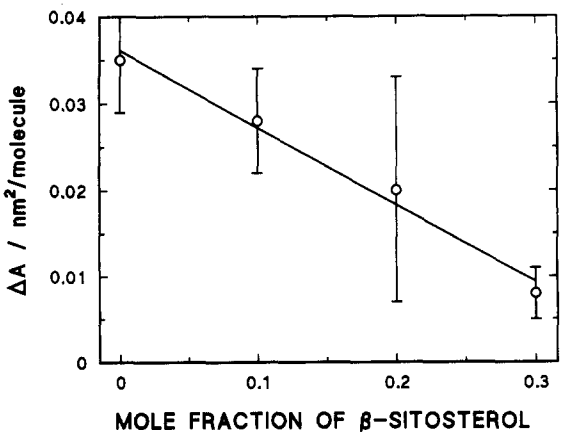

Fig. 3. ABA-induced surface area increase per lipid molecule of mixed PC/PE monolayers (molar ratio 4:1), given as a function of mol fraction of β-sitosterol in total lipid. The data points correspond to the average of five measurements taken at collapse pressures of the monolayers. The subphase contained 0.1 M NaCl/10 mM citrate buffer (pH 5) and either no ABA (control) or 1 mM ABA; the temperature was 22°C.

in molecular area caused by ABA is substantially diminished (Fig. 3).

Effect of pH

ABA is a monocarboxylic acid with a pK_a of 4.8 in aqueous solutions [23]. At pH values above this, the plant hormone dissociates increasingly. The insertion into the lipid phase should be more difficult for the charged form of ABA than for the free acid. Both the area per molecule (Fig. 4A) and the collapse pressure measured for monolayers in the presence of ABA (Fig. 4B) confirm this assumption. As the pH of the subphase containing ABA was increased from 4.0 to 7.0, the area per molecule decreased while the collapse pressure became higher. Both measurements indicated that less ABA was incorporated into the monolayers when the anion was present in the subphase. This effect was used for the determination of the pK of ABA adsorbed to the water-lipid interface. From the data given in Figs. 4A and 4B a pK of about 5.3 was determined for ABA adsorbed to the monolayer. It has to be noted that a pK increase for carboxylic acids adsorbed to a membrane-water interface is not unusual. It may be caused by a larger partition coefficient of the free acid to a membrane [24].

Effects of ABA on monolayers made of synthetic saturated and unsaturated lipids

In another set of experimental conditions with pure lipids we investigated the influence of saturated side chains on the ABA-lipid interaction. DPPC has a transition temperature (T_m) of 42.3°C and exhibits phase transitions on the Langmuir trough in the temperature range between 15°C and 40°C [21]. Fig. 5 demonstrates the isotherms for DPPC recorded at a temperature of 22°C. ABA affected the area per molecule as described above, but only in the range from 0 to approx. 12 mN/m (i.e., in the liquid-expanded state of the lipid). Interestingly, ABA had also an effect on the phase transition: The surface pressure at which the phase transition occurred increased from approx. 9 mN/m to around 12 mN/m. Another interesting result was that ABA was squeezed out completely from the monolayers above a surface pressure of approx. 35 mN/m (i.e., in the liquid-condensed state of the lipid, see Fig. 5).

Another saturated phospholipid, DSPC, has a transition temperature of 58°C and shows no phase transition in monolayers at 22°C but is in the liquid-condensed state throughout the measured isotherm. In experiments with this lipid, only a few ABA molecules were incorporated into the monolayers at low surface

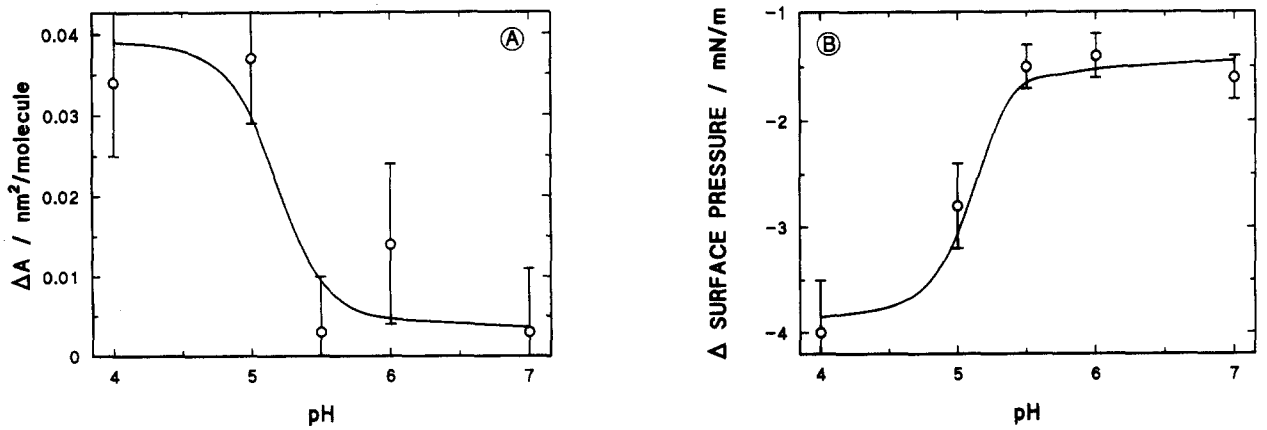

Fig. 4. (A) ABA-induced surface area increase per lipid molecule of mixed PC/PE monolayers (molar ratio 4:1), given as a function of the pH of the subphase. The data points correspond to the average of five measurements taken at collapse pressures. The subphase contained 0.1 M NaCl/10 mM citrate buffer and ABA in a concentration of 0 mM (control) or 1 mM; the temperature was 22°C. (B) ABA-induced decrease of collapse pressure of mixed PC/PE monolayers (molar ratio 4:1), given as a function of the pH of the subphase. The data points correspond to the average of five measurements. The subphase contained 0.1 M NaCl/10 mM citrate buffer and ABA in a concentration of either 0 mM (control) or 1 mM; the temperature was 22°C.

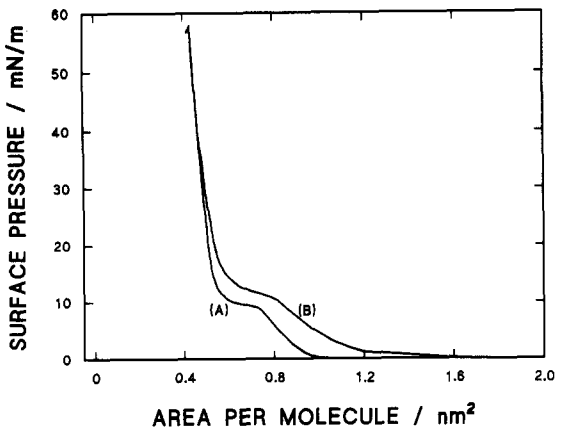

Fig. 5. Surface pressure–molecular area isotherms of monolayers made of pure DPPC in the absence (A) and in the presence (B) of 1 mM ABA. The subphase contained 0.1 M NaCl/10 mM citrate buffer (pH 5); the temperature was 22°C.

pressure (Fig. 6). Furthermore, these ABA molecules were completely squeezed out as the surface pressure exceeded about 20 mN/m (see Table I). Interestingly, β-sitosterol, which also exists in the condensed state under our experimental conditions (T_m = 140°C), showed a similar behaviour in monolayers as DSPC. Again, the small amount of ABA present in the monolayers at low surface pressure was completely squeezed out in the surface pressure range up to 20 mN/m.

A comparison between the ABA effects on natural lipids and on the defined DPPC and DSPC suggested that ABA showed only strong interaction with monolayers when the lipids were in the liquid-expanded state. To test this we performed some monolayer experiments with defined unsaturated lipids (DOPC, one double bond in each fatty-acid chain; DLPC, two double bonds, DLinPC, three double bonds, see Table I) being in the liquid-expanded state. The interaction between ABA and the monolayers was similar to that

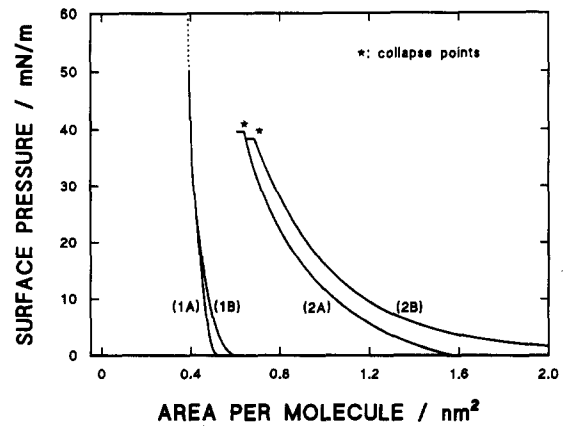

Fig. 6. Surface pressure–molecular area isotherms of monolayers made of pure DSPC (1) and pure DLinPC (2) in the absence (A) and in the presence (B) of 1 mM ABA. The subphase contained 0.1 M NaCl/10 mM citrate buffer (pH 5); the temperature was 22°C.

TABLE I

ABA effects on the surface pressure–molecular area isotherms of the phospholipids DSPC, DOPC, DLPC and DLinPC

The effects are expressed as enhancement in area per phospholipid molecule and the values were taken at different surface pressures. The temperature of the subphase (10 mM citrate, 0.1 M NaCl (pH 5), with or without 1 mM ABA) was thermostatically held at 22°C. The values represent the data derived from at least five measurements with and without ABA, respectively. The relative standard deviations (not noted) were below 1% for DSPC and below 3% for DOPC, DLPC and DLinPC.

Lipid	Side chains		Surface area increase (mN/m) at (nm²/PL molecule)			
	C1	C2	2.5	10	20	40
Distearoyl-PC	18:0	18:0	0.04	0.02	0.01	0
Dioleoyl-PC	18:1	18:1	0.19	0.07	0.03	0.02
Dilinoleoyl-PC	18:2	18:2	0.26	0.11	0.06	0.04
Dilinolenoyl-PC	18:3	18:3	0.36	0.11	0.06	– [a]

[a] Not detectable because the collapse point is below 40 mN/m when ABA is present in the subphase.

described above for PC/PE mixtures. This means that ABA led to an increase of the area per molecule and to a small decrease of the collapse pressure, too. The effects were obviously stronger for two and three double bonds. These results suggested that ABA effects are correlated with the number of double bonds, i.e., they are stronger when the lipid has a higher degree of fluidity (see also Fig. 6).

Incorporation of the ABA isomers and other organic molecules

ABA exists in two geometric and two optical isomers. The *cis-trans* geometric isomer is biologically active while the *trans-trans* isomer lacks activity [25]. Experiments with pure (+)-*cis-trans*-ABA (biologically active) could not be done for cost reasons. Two forms of the geometric isomers ((±) racemic mixtures) are commercially available. One is the 100% *cis-trans* isomer (which is used in all experiments presented in this paper except this one) and the mixed isomers containing 24% *cis-trans* and 76% *trans-trans*-ABA [12]. The 100% *cis-trans* isomer of ABA is found to decrease the collapse pressure of mixed PC/PE monolayers, compared to the hormone-free control, by about 1 mN/m more than the 24% *cis-trans* isomer (data not shown). From this we conclude that the biologically-active geometric *cis-trans* isomer is also the one which is incorporated into lipid monolayers in a more effective way. In additional experiments with 1 mM benzoic acid (which has a similar pK as ABA) we demonstrated that there was no difference of the isotherms compared to those recorded on pure buffer. Benzoic acid did not influence the surface pressure and the area per molecule of the monolayers (data not shown).

Discussion

Extensive works exist about the different functions ABA covers in plants, but the mode of action in each case is unclear. Apart from developmental responsibilities ABA affects the guard cell membranes so that the guard cell internal osmoticum, particularly the concentration of potassium ions, decreases, and the stomates close. The mechanism for this process and the type of the target molecules are still unknown [26]. One possibility involves the direct action of ABA on the lipid bilayer component of the guard cell plasma membrane resulting in enhanced leakage. In fact, more than 10 years ago Lea and Collins [14], using planar bimolecular lipid membranes, proposed that ABA may be inducing channels in membranes. Previous works with lipid vesicles have demonstrated a phospholipid dependence on the ABA-induced increase in permeability [8,12], lipid vesicle aggregation and fusion [16,17] and changes in membrane microheterogeneity [18]. While ABA has a small effect on single component PC bilayers, the effect is greatly magnified by incorporation of a second lipid into the bilayers [8,12]. All attempts to monitor the nature of the molecular interaction between ABA and the phospholipids that comprise the altered membranes with ^{1}H-, ^{31}P- and ^{13}C-NMR, electron paramagnetic resonance and fluorescence polarization have met with failure. This has been attributed to the assumption that ABA does not act uniformly over the bilayer surface but instead primarily does at selected regions of membrane defects between different phospholipid types or between different lipid phases [8]. The ABA-lipid signal would, therefore, be lost in the population-weighted average signal of the entire membrane. Supporting this hypothesis, which is based on phospholipid compositional analysis, is the reversal of ABA-induced permeability and lipid vesicle fusion by plant sterols [9]. Plant sterols will compete with and perhaps replace ABA at the membrane defect sites. It is through the defects that the membranes become leaky and lipid bilayer integrity is decreased enough to induce vesicle aggregation and fusion. This may be of physiological importance because plant sterols were shown to be affected under stress conditions [27].

In the experiments presented here using a Langmuir trough, ABA effects on monolayer surface area and pressure support those previously reported for phospholipid vesicles. In particular ABA caused a strong increase of the surface area per lipid molecule, implicating incorporation of ABA into the lipid phase. The cross section of ABA integrated into the membrane is about 0.4 nm^2 according to space-filling models. This means that the PC/PE monolayers contain approx. 1 ABA molecule per lipid at the end of the gas phase when the ABA concentration in the subphase is 1 mM. At collapse pressure, the monolayer contains still 1 ABA per approx. 20 lipids. This enhanced surface activity of ABA only occurred when a lipid film was present on the surface of an ABA solution; ABA neither formed a monolayer when spread alone nor when spread together with phospholipids on pure buffer. Furthermore, in all experiments using different lipids being in the liquid-expanded state, the collapse pressure was decreased by ABA in the subphase. This indicates that ABA influences also the Van der Waals forces between the fatty-acid chains.

The additional area per molecule and diminution in surface pressure at the collapse point is caused primarily by non-dissociated *cis-trans* ABA. This is derived from the fact that we observed a strong effect of ABA on the monolayer isotherms at low pH. A sharp decrease of area increase per lipid molecule occurred between pH 5 and 6 (see Fig. 4). ABA has an aqueous pK of 4.8 [23]. The surface pK of ABA is (according to our data) approx. 5.3. The partition coefficient, β_{HA}, of the free acid between membrane and aqueous phase is given in Ref. 24:

$$\beta_{HA} = N_{HA}/c_{HA} \quad (1)$$

and that of the charged form, β_A is also given in Ref. 24:

$$\beta_A = N_A/c_A \quad (2)$$

where N_{HA} and N_A are the total numbers of free acids and anions (expressed in mol/cm^2) adsorbed to the monolayer, respectively. Their aqueous concentrations are c_{HA} and c_A, respectively (given in mol/cm^3). The ratio β_{HA}/β_A is given in Ref. 28:

$$\beta_{HA}/\beta_A = K_m/K_a \quad (3)$$

where $K_m = 10^{pKm}$ and $K_a = 10^{pKa}$ are the stability constants for the free acid at the membrane-water interface and the bulk aqueous phase, respectively. When we introduce the pK values into Eqn. 3, the ratio of the partition coefficients is approx. 3. Interestingly, ABA shows also in biological systems a similar shift of pK as found here for monolayers [29]. This supports our assumption that ABA interacts with lipids in the cytoplasmic membrane of guard cells.

Another important observation is made from these monolayers. ABA has a much higher potential to penetrate PL-monolayers at low surface pressures. For example in Fig. 2, 1 mM ABA increases the area per molecule about 0.03 nm^2 at 40 mN/m but increases the area per molecule 0.33 nm^2 at 2.5 mN/m. This indicates an inverse relationship between the amount of ABA incorporated into membranes and the surface pressure. The proposed site of ABA action on lipid bilayers, regions of membrane defects, would also likely

be places of low surface pressure and so would accumulate the hormone.

Recently, Leshem et al. [19,20] have reported a study measuring interactions of ABA with lipid monolayers composed of dipalmitoylphosphatidylcholine (DPPC). Their results are completely opposite to ours. They measured an ABA-induced reduction in molecular area, no phase transition and a large increase in the surface pressure at the collapse point (compare Figs. 1 and 5 to Leshem et al. [19] Fig. 1). DPPC would exist in the gel state under physiological conditions (T_m 42.3°C), as well as under the conditions in Leshem's experiments (20°C [19,20]). Guard cell plasmalemma lipids are highly unsaturated and so exist in the liquid-crystalline state. Two results were obvious from Fig. 5:

The first one was that ABA penetrates DPPC only if it is in the liquid-expanded state, which corresponds to the normal condition in biological membranes. Accordingly, we observed a strong effect of ABA on monolayers from unsaturated lipids (see also Fig. 6).

The second observation was that the surface pressure at phase transition increased in the presence of ABA by approx. 3 mN/m, which corresponds to a decrease of the transition temperature T_m by about 1.3°C [21]. Using differential scanning calorimetry Bach [15] has shown that 18 mol% ABA in DPPC caused a decrease of T_m by 5°C. This would mean that in our experiments about 5 mol% ABA was incorporated from the ABA solution into the DPPC monolayer in the region of phase transition.

We made another important observation during the experiments with DPPC monolayers: when the lipid was spread from an unsuitable solvent it showed the tendency to form lipid aggregates within the monolayer, i.e., the area per molecule was underestimated. However, when ABA was present in the subphase, DPPC formed a perfect monolayer even when the same solvent was used. This represents another indication for the influence of ABA on the phase behaviour of lipids. ABA prefers to interact with lipids being in the liquid-crystalline state and it slightly decreases the transition temperature of phospholipids thus favouring its interaction with lipids. With ABA greatly magnifying aggregation and fusion processes in PL-vesicles only when unsaturated PE and PC are part of the membranes [8,12] it is obvious that the fatty-acid chains are responsible for ABA adsorption and the headgroups for the ABA functions [8,12] in protein-free systems.

Incorporation of ABA into the monolayers is also inhibited by inclusion of β-sitosterol into the monolayers (see Fig. 3). β-sitosterol (T_m = 140°C) alone is in the condensed state during the whole surface pressure/molecular area isotherm and a monolayer made of this sterol was not significantly affected by ABA. There was a slight effect (an area increase per sterol molecule of about 0.05 nm^2/molecule) at a surface pressure of 1 mN/m, but no increase could be detected when the surface pressure exceeded 20 mN/m. De Kruyff et al. showed that sterols preferentially interact with lipids being in the liquid-crystalline state when there are lipids in both the crystalline and in the liquid-crystalline state [30]. Only lipids of the latter type being at low surface pressures contain considerable amounts of ABA. So one can argue that when β-sitosterol is placed between those lipids the ABA molecules are squeezed out by both the higher surface pressure and the growing incompetence of the sterol containing monolayer to accumulate ABA. Ghosh and Tinoco [31] described the effects of β-sitosterol and compared them to those of cholesterol. They showed that β-sitosterol tends to decrease the molecular area of some expanded lecithins more than cholesterol. Our results coming from the raw data of Fig. 3 show that the condensing effect of 30% β-sitosterol in the PC/PE monolayers decreases the area per molecule by 0.034 nm^2 at 5 mN/m. When 1 mM ABA is dissolved in the subphase the deviation from the simple additive rule is 0.057 nm^2. Since ABA incorporation is strongly dependent on the area per molecule, the amount of ABA in the monolayer would decrease with increasing β-sitosterol concentration.

We do not exclude the possibility of the existence of a potential receptor which binds ABA as the second step, but there is clear evidence of the ability of ABA to interact with protein-free lipid systems. In addition, the concentration of ABA used to interact with the lipid monolayers (0–1.0 mM) may be of the correct magnitude. Behl and Hartung [32] and later Lahr and Raschke [33] using completely different techniques have reported surprisingly high levels of ABA associated with guard cells. They found ABA in guard cells to be in the same range as used in the lipid monolayer experiments reported here.

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