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Abscisic Acid Enhances Aggregation and Fusion of Phospholipid Vesicles

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SUMMARY: The plant hormone abscisic acid (ABA) is shown to enhance the aggregation and fusion of small unilamellar lipid vesicles composed of 80 mol% dimyristoylphosphatidylcholine (DMPC) and 20 mol% dimyristoylphosphatidylcholine (DMPE). Aggregation and fusion did not occur with single component (100 mol%) DMPC vesicles. Fusion was followed by two fundamentally different techniques, fluorescence resonance energy transfer which monitors intermixing of bilayers and ANTS-DPX which monitors intermixing of the sequestered aqueous interiors. It is suggested that a previously unreported role of ABA may be as a membrane fusagen.

The plant hormone abscisic acid (ABA) participates through poorly understood mechanisms in a large variety of physiological processes (1). Among the rapid time responses affected by ABA are events clearly associated with membranes (2). The best defined of these are the ABA-induced closure of stomates (3) and ABA alteration in membrane permeability of various root tissues (4,5). Recently we have been studying the effect of ABA on phospholipid bilayer model membranes to elucidate the mode of action of ABA on natural membranes (6-11). Using a variety of biophysical techniques we have demonstrated that ABA significantly enhances membrane permeability to anions, cations and neutral solutes only if the hormone is in its undissociated state (pK_a 4.85) and the membranes are composed of at least two different types of lipids. Despite the large effects of ABA on permeability, we could not demonstrate by ESR, NMR and fluorescence polarization significant binding of ABA to any bilayer membrane. From these observations we proposed that ABA is not affecting the entire membrane but instead is acting at regions of bilayer defects found between either two

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DLPE, dilauroylphosphatidylethanolamine; RET, fluorescence resonance energy transfer; NBP-PE, N-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine; Rho-PE, lissamine rhodamine-B-Sulfonyl dioleoylphosphatidylethanolamine; ANTS, 8-aminonaphthalene-1,3,6-trisulfonic acid; DPX, p-xylene-bis-pyridinium bromide, ABA, abscisic acid.

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different phospholipid head group types or between gel and liquid crystalline states with mixed acyl chain PC's. We also reported that ABA enhances membrane aggregation with bilayers of similar composition to those displaying ABA induced permeability (10). Since membrane aggregation is a necessary prerequisite to membrane fusion (12), and the process of fusion has been proposed to occur at regions of membrane defects (13), it is reasonable to propose that ABA may cause the fusion of membranes composed of the appropriate types of phospholipids. Experiments confirming this potential, unique role for ABA are presented here.

MATERIALS AND METHODS

Materials: The phospholipids DMPC, DMPE, DSPC and DLPE, and the fluorescent labeled lipids NBD-PE and Rho-PE were purchased from Avanti Polar Lipids, Birmingham, AL. The aqueous fluorescent molecule ANTS and its quencher DPX were bought from Molecular Probes, Eugene, OR. ±, *cis-trans* abscisic acid was from Sigma Chemical Co., St. Louis, MO.

Vesicle Preparation and Aggregation: Multilamellar vesicles (MLV's) were made as described by Bangham (14) from 90 mol% DMPC/10 mol% DMPE (10.3 mM) in 10 mM sodium acetate, pH 5.0. Small unilamellar vesicles (SUV's) were made by sonicating the MLV's for 6 min on ice at level 7 of a Heat Systems 220-F Cell Disruptor. After a brief centrifugation to remove titanium particles, the SUV's were rapidly mixed with appropriate amounts of ABA, also in 10 mM sodium acetate, pH 5.0. Vesicle aggregation was followed by an increase in turbidity at 350 nm (19°C) on a Beckman DU-8 Computing Spectrophotometer. Results are expressed as the change in absorbance as a function of time.

Fluorescence Resonance Energy Transfer: Fusion of vescicular membranes is followed by fluorescence resonance energy transfer (RET) which monitors the exchange of energy from a light absorbing membrane bound "donor" (NBD-PE) to an acceptor (Rho-PE)(15). Tow populations of SUV's containing 0.3 mol% NBD-PE and Rho-PE, respectively, were made as described above and were mixed at time zero. Lipid content was 2.5 mM in 40 mM sodium acetate, pH 5.0. RET was followed by exciting the mixed vesicles at 464.2 nm and measuring emission at 593.9 nm on a Perkin-Elmer MPF-66 Fluorescence Spectrophotometer. Results, expressed as the percentage of membranes fused over time, are calculated as described by Wang and Huang (16).

Aqueous Compartment Mixing: Fusion of vesicles was also followed with an ANTS-DPX assay that measures the intermixing of the aqueous sequestered compartments of vesicles (17). The highly fluorescent ANTS, initially sequestered in one population of vesicles, is quenched upon fusion by DPX initially trapped in a second vesicle population. SUV's were made as described above in buffers containing either 25 mM ANTS, 40 mM NaCl, 10 mM sodium acetate, pH 5.0 or 90 mM DPX, 10 mM sodium acetate, pH 5.0. Lipid concentration was 10 mM. Excitation was at 420 nm and emission at 513.6 nm. Results are expressed as the percentage of fusion as a function of time. A control experiment measuring fluorescence quenching due to vesicle leakage showed that less than 2.7% DPX diffused during the length of the presented experiments.

RESULTS

The results presented here demonstrate that ABA can induce aggregation and fusion of a mixed component bilayer membrane. The experiments are all

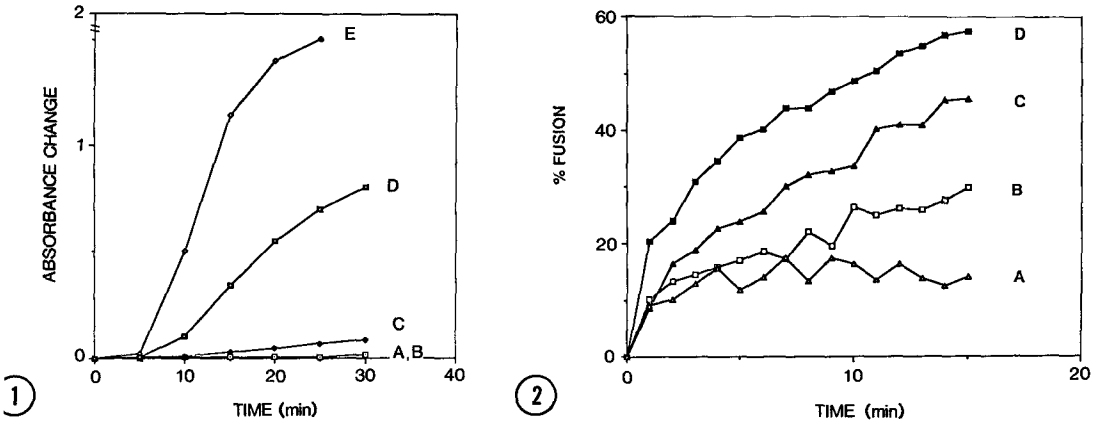

Figure 1. Effect of ABA on inducing aggregation of 90 mol% DMPC/10 mol% DMPE unilamellar vesicles at 19°C. Results are expressed as the increase in absorbance (turbidity) at 350mm. ABA:lipid molar ratios: A. 0.0; B. 0.0054; C. 0.054; D. 0.27; and E. 0.54.

Figure 2. Effect of ABA on inducing fusion of 90 mol% DMPC/10 mol% DMPE vesicles at 10°C as followed by the fluorescence resonance energy transfer method. ABA:lipid molar ratios: A. 0.0; B. 0.125; C. 0.25; and D. 0.50.

run at pH 5.0 which is close to the pK_a of ABA, 4.85 (18). This pH is chosen because at lower pH's the solubility of ABA becomes limiting and our previous work measuring bilayer permeability showed that at higher pH's dissociated ABA was not very membrane active.

The ability of ABA to enhance aggregation of lipid vesicles composed of 90 mol% DMPC/10 mol% DMPE is presented in Figure 1. Clearly aggregation is ABA dependent as no increase in turbidity could be detected over 30 min without ABA. Experiments were also run with 20 mol% DMPE but, in the presence of ABA, they became turbid too rapidly for meaningful data to be obtained. Also 100 mol% DMPC bilayers were stable in the presence of ABA and did not aggregate (results not shown).

Fluorescence resonance energy transfer was used to follow the fusion of vesicles by observation of the intermixing of bilayer membranes. ABA did not enhance fusion at all with 100 mol% DMPC bilayers (results not shown). However, when a second component (DMPE) was included at 10 mol%, substantial ABA enhanced fusion was measured (Figure 2). A second mixed component membrane consisting of 90 mol% DSPC/10 mol% DLPE was also tested and, as shown in Figure 3, this membrane also demonstrated considerable ABA dependent fusion.

Finally, fusion was confirmed by the ANTS-DPX method which measures mixing of the vesicles aqueous cores. ABA did not enhance fusion for 100 mol% DMPC vesicles (results not shown) but demonstrated considerable fusion with the mixed component (90 mol% DMPC/10 mol% DMPE) vesicles (Figure 4).

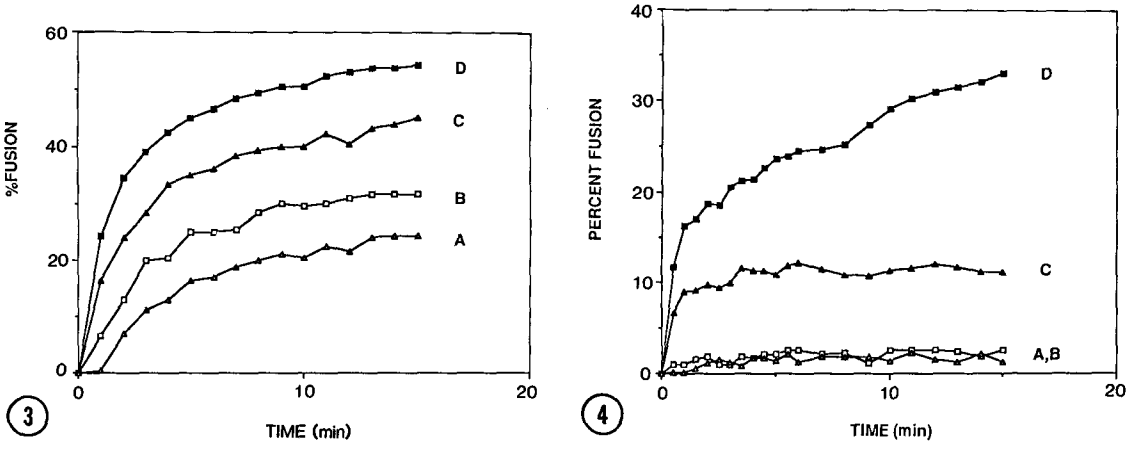

Figure 3. Effect of ABA on inducing fusion of 90 mol% DSPC/10 mol% DLPE vesicles at 15°C as followed by the fluorescence resonance energy transfer method. ABA:lipid molar ratios: A. 0.0; B. 0.125; C. 0.25; and D. 0.50.

Figure 4. Effect of ABA on inducing fusion of 90 mol% DMPC/10 mol% DMPE vesicles at 10°C as followed by the ANTS-DPX method. ABA:lipid molar ratios: A. 0.0; B. 0.125; C. 0.25; D. 0.50.

DISCUSSION

Fusion of various cellular membranes is essential for many physiological processes including membrane biogenesis, endocytosis, secretion and fertilization (19). This important mechanism has been studied for years without producing a clear picture of the sequence of molecular events. By use of protein free lipid bilayer model membranes some basic properties common to all membrane fusions have emerged. The process of fusion must: a. kinetically follow that of membrane aggregation (12); b. occur only over a very small portion of the coalescing membranes (20,21); and c. occur in regions of defects associated with fluctuations in lipid packing (22).

From our previous work with ABA and various phospholipid membranes we demonstrated that the hormone can greatly alter basic membrane properties such as permeability (6-9) and vesicle aggregation (10). Without significantly binding to any portion of the tested membranes from the aqueous interface all the way through to the membrane interior (11). From these experiments we proposed that ABA must be acting at regions of membrane defects where either differing lipid head groups do not pack properly or two different physical states coexist. Therefore conditions necessary for ABA-induced permeability and aggregation clearly parallel the conditions required for general membrane fusion.

In the experiment presented here for the first time we have investigated the possible role of a plant hormone in the process of membrane fusion. In Figure 1 we report the effect of ABA on promoting aggregation of a mixed

component DMPC/DMPE phospholipid membrane. More detailed results comparing ABA-induced aggregation for a variety of mixed phospholipid membranes have been published elsewhere (10). The DMPC/DMPE membranes that undergo aggregation also fuse as followed by two techniques, one monitoring the intermixing of the membranes themselves (Figures 2 and 3) and the other following the intermixing of the aqueous interiors of the vesicles (Figure 4). Since mixing of membrane components does not necessarily imply core mixing and *vica versa* (23), both methods were required.

Our experiments establish that for DMPC/DMPE mixed membranes, ABA induces vesicle aggregation and fusion. Although they by no means prove ABA is inducing these processes *in vivo*, it is emphasized that model membranes have produced most of the basic understanding of how fusion may occur (24). Since ABA meets at least some of the criteria of a membrane fusagen and is shown here to fuse phospholipid vesicles, its potential role in inducing fusion in plant cells should be given serious consideration.

In related experiments Hartung (personal communication) has recently employed a laser light scattering technique to demonstrate that non-dissociated ABA increases the size of particles formed from large unilamellar phospholipid vesicles. ABA-induced particle size changes were attributed to vesicle aggregation and (or) fusion. In addition Behl and Hartung (25) have shown, when stressed, ABA concentrations may rise to the mM range in the acidic apoplast surrounding guard cells. Therefore, the fusion conditions reported here (pH 5.0, mM ABA) are not reasonable and may be of physiological significance.

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