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Effect of Ethylene on Plasma Membrane Density in Kidney Bean Abscission Zones[1]

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ABSTRACT

Plasma membranes from abscission zones of kidney bean seedlings were isolated on sucrose density gradients using cellulase and KCl-stimulated ATPase as marker enzymes. Following treatment of the seedling explants with 50 microliters per liter ethylene, an increase in the buoyant density of the plasma membrane fraction from 1.165 to 1.175 grams per cubic centimeter was observed. Such a change is consistent with the loss of lipids from the senescent cells of the abscission zone.

In examining the role of cellulase during leaf abscission of kidney bean, it has been shown that one form of cellulase appears to be bound to the plasma membrane (5).

Although the physiological function of this cellulase form is not known, it does appear to be a reliable marker for plasma membranes in this system. Previous studies have shown the bean abscission zone to contain buffer-soluble and high salt-soluble forms of cellulase, the latter being correlated with the progress of abscission (6, 9).

Further characterization of the membrane-bound form of cellulase has been carried out during the course of ethylene treatment of bean seedlings in an effort to learn about the mechanisms of cellulase synthesis and secretion and the steps involved in ethylene-induced abscission.

In this paper we describe an increase in the buoyant density of the plasma membrane due to ethylene treatment of bean abscission zones. This increase in density was observed by following the shift of cellulase and KCl-stimulated ATPase activities in isopycnic sucrose density gradients of membrane preparations.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L., cv. Red Kidney, were grown for 10 to 12 days at 24 C and a light intensity of 2,000 ft-c. Control tissue was harvested directly from such seedlings by excising 1-cm sections of petiole which included the laminar abscission zone. For ethylene-treated tissue, seedlings were first debladed, then cut off at the base of the stem and placed in water in cups. The cups were incubated in a closed chamber in the presence of 50 μl/l ethylene for 24 h at 20 C.

Incubation of seedling explants with ethylene for 24 h at this suboptimal temperature results in no increase in cellulase activity in the abscission zone during this first 24 h and no observable induction of abscission during this time period. Both of these responses are observed during the subsequent 24-h incubation.

The preparation of the membrane fraction was described previously (5). Tissue was homogenized in a polytron (Brinkmann Instruments) in a buffer containing 20 mM Tris-Mes (pH 7.2), 0.3 mM sucrose, 3 mM EDTA, and 0.1% (w/v) BSA. The homogenate was squeezed through nylon cloth and centrifuged for 15 min at 13,000g. The membrane fraction was collected on a cushion of 55% (w/w) sucrose by centrifugation at 80,000g for 45 min. This fraction was washed by suspending in homogenization buffer without sucrose and pelleting again onto a sucrose cushion. The membrane fraction was analyzed in a linear 20 to 50% (w/w) sucrose density gradient prepared with the homogenization buffer. Centrifugation of the gradient was for 15 h at 2 C at 25,000 rpm in a Spinco model SW 25.2 rotor.

Cellulase was determined viscometrically by the method of Lewis and Varner (6) and expressed as relative units. All cellulase assays were run in the presence of 0.17% (w/v) Triton X-100. ATPase was measured by the method of Hodges and Leonard (2) in the presence of 3 mM Mg^{2+} and 50 mM KCl. Isoelectric focusing of solubilized enzyme activities was carried out on a membrane fraction collected at 80,000g and resuspended in a buffer containing 1% (w/v) glycine and 1% (w/v) Triton X-100. This solubilized membrane preparation was either applied directly to an isoelectric focusing column (LKB Instruments) or was centrifuged at 80,000g to remove nonsolubilized components and the supernatant subjected to isoelectric focusing for 40 h at 4 C. The ATPase assay on fractions from the isoelectric focusing column was terminated by the addition of 20% (w/v) SDS to the reaction to prevent precipitation of the Triton X-100 from the column fractions.

RESULTS

Plasma membranes isolated from ethylene-treated bean abscission zones had a greater buoyant density than similarly isolated membranes from untreated zones. A treatment with 50 μl/l ethylene for 24 h produced plasma membranes with a buoyant density of 1.175 g/cm^3 (Fig. 1) compared with 1.165 g/cm^3 for the plasma membrane from freshly harvested control tissue.

The determination of the buoyant density of the plasma membrane was based on the position of two membrane-bound enzyme markers, Triton X-100-solubilized cellulase and KCl-stimulated ATPase, in a sucrose density gradient. A 12-h exposure to ethylene caused about the same shift in buoyant density as the 24-h treatment.

A statistical analysis of the equilibrium density of the peak cellulase activity from a total of 12 gradients (five untreated, seven ethylene-treated) showed that the cellulase activity from untreated zones peaked at an average of 37.5 ± 0.577% (w/w) sucrose compared with 39.02 ± 0.866 for treated zones. This difference corresponds to a density shift of 0.0078 g/cm^3 (Iscotables) and was significant at the 1% level by Student's t criterion.

The ethylene effect on membrane density appears to be restricted to the plasma membrane fraction. A change in density

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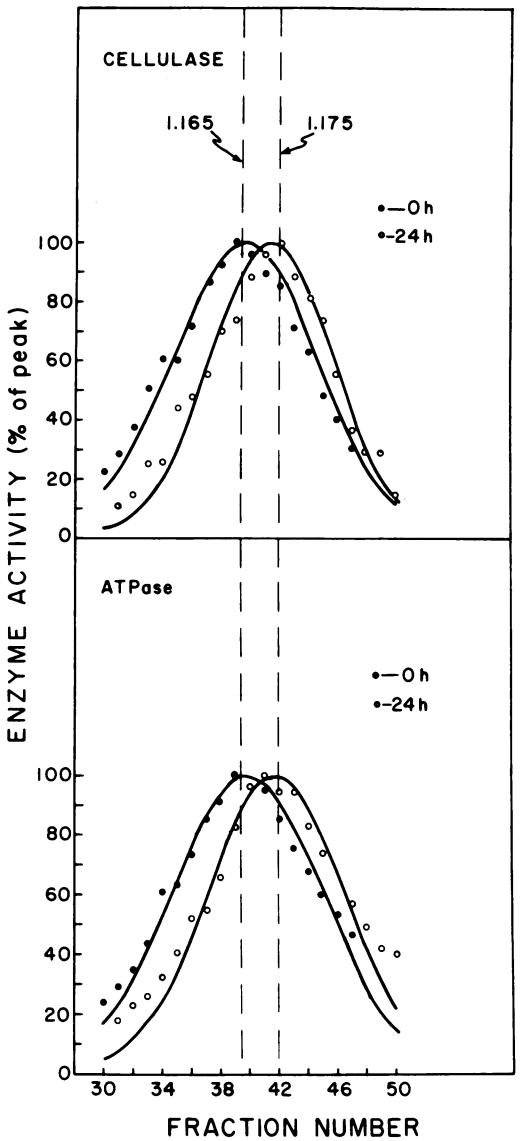

FIG. 1. Effect of ethylene treatment on buoyant density of plasma membranes from kidney bean abscission zones. Membrane fraction sedimenting between 13,000*g* and 80,000*g* was prepared from untreated abscission zones (●——●) and from abscission zones prepared from seedling explants which had been exposed to ethylene for 24 h (○——○). Data points represent raw data plotted as a percentage of maximum peak height. Curves are the best fitting lines from a Gaussian plot of the raw data.

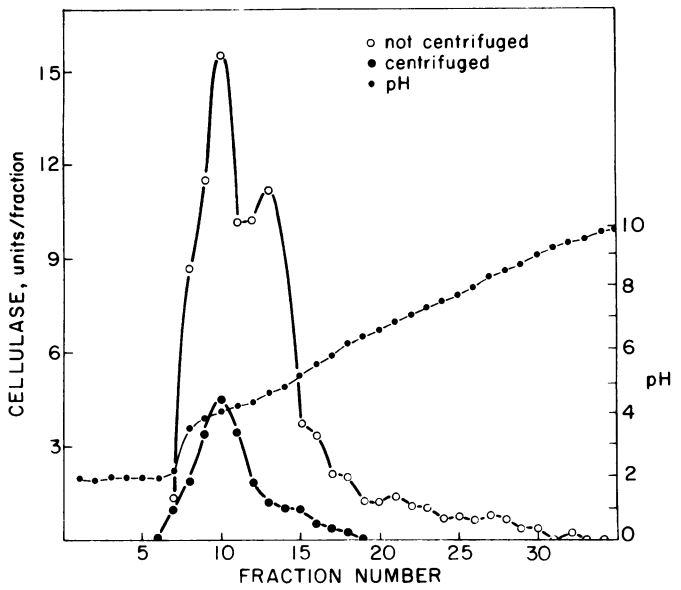

FIG. 2. Isoelectric focusing profiles of cellulase solubilized from the 13,000*g* to 80,000*g* membrane fraction from untreated abscission zones by 1% Triton X-100. In one experiment, the membrane pellet was treated with Triton X-100 and focused directly. The second profile shows a membrane fraction which was centrifuged after Triton X-100 treatment to remove undissolved membrane components.

was not observed for NADH-Cyt *c* reductase, a marker for ER. Chl, marking the equilibrium density of broken chloroplasts, was consistently found at a density of 1.195 g/cm^3 whether or not the abscission zone tissue had been exposed to ethylene.

Cellulase solubilized by Triton X-100 from plasma membranes of untreated tissue had a pI[3] of 4.0 to 4.3 (Fig. 2). Presumably the second peak of cellulase activity was due to nonsolubilized membranes, since this peak was removed by a subsequent centrifugation of the Triton X-100-treated membrane fraction (Fig. 2). Triton X-100 solubilization of this membrane fraction was previously shown to be incomplete (5). The membrane-associated cellulase activity from ethylene-treated tissue had the same pI as that from untreated tissue.

DISCUSSION

Treatment with ethylene causes an increase in the buoyant density of the plasma membrane of abscission zone cells. Such an effect is significant in that a basic physical parameter of the membrane has been altered by treatment with the plant hormone, ethylene. An increase in buoyant density of a membrane would normally be ascribed to an increase in the protein to lipid ratio of the membrane. This could be due either to a loss of lipids or an increase in protein in the membrane. The former seems to be the most likely alternative in view of observations on the loss of phospholipids from membranes of senescent cotyledons (1). Certainly the cells distal to the separation layer in the petiole are senescent (4, 8). Ethylene treatment inhibited glycerol incorporation into phospholipids of pea stems, bringing about a decrease in phospholipid levels in that tissue (3). The shift in plasma membrane density may be a reflection of the senescent character of at least a portion of the cells in the abscission zone.

The increase in membrane density cannot be attributed to an increase in cellulase bound to the membrane *per se*, since plasma membrane-bound cellulase activity did not increase after 24 h of ethylene treatment (data not given).

Changes in membrane density in plant systems have been described by Moore and Beevers (7) working with soybean suspension cultures. In this system the maturation of the microbody was accompanied by an increase in the density of the organelle.

The pI of the membrane-associated cellulase resembles that of the cellulase found inside abscission zone cells rather than the wall-associated activity (9). This may be an intracellular enzyme that has become fixed in the plasma membrane. If it is destined for secretion from the cell, further processing or alteration of the protein must take place to raise the pI to 9.5, characteristic of the extracellular enzyme (9).

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[3] Abbreviation: pI: isoelectric point.

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