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Transport of Gibberellin A_1 in Cowpea Membrane Vesicles[1]

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ABSTRACT

The permeability properties of gibberellin A_1 (GA_1) were examined in membrane vesicles isolated from cowpea hypocotyls. The rate of GA_1 uptake was progressively greater as pH decreased, indicating that the neutral molecule is more permeable than anionic GA_1. Membrane vesicles used in this study possessed a tonoplast-type H^+-translocating ATPase as assayed by MgATP-dependent quenching of acridine orange fluorescence and methylamine uptake. However, GA_1 uptake was not stimulated by MgATP. At concentrations in excess of 1 micromolar, GA_1, GA_5, and GA_9 collapsed both MgATP-generated and artifically imposed pH gradients, apparently by shuttling H^+ across the membrane as neutral GA. The relatively high permeability of neutral GA and the potentially detrimental effects of GA in uncoupling pH gradients across intracellular membranes supports the view that GA_1 accumulation and compartmentation must occur by conversion of GA_1 to more polar metabolites.

Membrane transport of phytohormones plays a fundamental role in translocation to sites of action and in subcellular compartmentation of these growth regulating compounds. The relatively hydrophobic and weakly acidic nature of the three major phytohormones (auxin, ABA, and gibberellins) (12) is a major factor in determining their passive membrane transport properties. Carrier-mediated mechanisms of membrane transport of IAA have been described and proposed to account for polar auxin transport (13, 14, 28), yet evidence for carrier-mediated transport of ABA (2, *cf.* 6) and gibberellins (20, 23) has been scarce.

Recent experiments utilizing isolated membrane vesicles have substantially overcome technical difficulties associated with hormone metabolism (19) by physically separating the site of membrane transport from sites of metabolic conversion and by providing greater experimental control over factors likely to influence membrane transport of hormones (*i.e.* pH, pH gradients, and inhibitors). The results of these experiments have conclusively demonstrated the role of membrane carriers in IAA transport (15). In this report, we have used isolated cowpea membrane vesicles to assess membrane transport properties of gibberellin A_1.

MATERIALS AND METHODS

Plant Material. Etiolated hypocotyl segments of cowpea (*Vigna sinensis* cv Blackeye pea No. 5; Lagomarsino Seeds, Inc., Sacramento, CA) were used for all membrane isolations. Seedlings were grown in moist vermiculite in the dark at 24°C and 40 to 50% RH for approximately 5 d.

Membrane Isolation. Microsomal membranes were prepared from etiolated cowpea seedlings in the following manner. Approximately 10 g fresh weight of etiolated hypocotyl segments were harvested into aerated 0.1 mM $CaCl_2$ at room temperature. Tissue was homogenized with an ice cold mortar and pestle in 180 ml of 250 mM sucrose, 25 mM Tris/Mes (pH 7.4), 2 mM EDTA, 2 mM DTT, and 0.1% BSA. The homogenate was filtered through six layers of cheesecloth, and centrifuged for 10 min at 13,000*g* (Sorvall SS34 rotor). The supernatant was centrifuged for 30 min at 100,000*g* (Beckman SW 27 rotor) and the resulting microsomal pellets resuspended in either 250 mM sucrose, 10 mM Tris/Mes (pH 7.0 or as indicated), 2 mM DTT or in 250 mM sucrose, 50 mM citrate/phosphate (pH 5.0–7.0) for the pH series experiments. Membrane vesicles were either used fresh or frozen in liquid N_2 and stored at −70°C until use. Continuous sucrose gradients (15–45%, w/w) were prepared as previously described (3), using freshly prepared cowpea microsomes.

ATPase Assay. ATPase activity was determined by measuring the release of Pi from ATP (Tris salt) according to the method of Ames (1). ATPase activity was assayed for 30 to 60 min at 38°C. The basic reaction mix contained 5 mM ATP/Tris (pH 7.0), 5 mM $MgSO_4$, 30 mM Tris/Mes (pH 7.0), and appropriate salts and/or ionophores and inhibitors in a final volume of 0.5 ml. Nitrate-sensitive and gramicidin-stimulated ATPase activities were assayed as described previously (25).

Transport Assays. Formation of acid-interior pH gradients across cowpea vesicle membranes due to the activity of a H^+-translocating ATPase were monitored continuously as quenching of fluorescence of the permeant amine dye, acridine orange. Cowpea vesicles were added to a reaction mix containing 250 mM sucrose, 10 mM Tris/Mes (pH 7.0), appropriate salts (50 mM KCl, 25 mM K_2SO_4) and/or inhibitors (50 mM NO_3^-), and 5 μM acridine orange. Fluorescence quenching was initiated by the addition of ATP:Mg to a final concentration of 5 mM. Approximately 300 to 400 μg of membrane protein were added per transport assay. Nigericin (2 μM) was added where indicated to relieve the pH gradient. For determining the effect of the gibberellins on ΔpH, the hormone in a small volume of buffer was added prior to the initiation of fluorescence quenching. Fluorescence was measured using a Perkin-Elmer 650-40 spectrofluorimeter equipped with a temperature-controlled cuvette. The temperature of all transport assays was 25°C.

Filtration assays of uptake of [^{14}C]methylamine or [^{3}H]GA_1 were performed by incubating cowpea membrane vesicles at 25°C in a reaction medium consisting of 10 mM Tris/Mes (pH 7.0) or 50 mM citrate/phosphate (pH 5.0–7.0), 250 mM sucrose, additions as indicated in figure legends, and 1×10^6 cpm/ml [^{14}C]methylamine or [^{3}H]GA_1. The concentration of GA_1 was maintained at 25 nM. Aliquots of membranes (100 μl) were withdrawn at the indicated time intervals and diluted into 5 ml of reaction medium without radioactive substrate. This was

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rapidly filtered onto a pre-wet 25 mm Millipore type HA, 0.45 μm filter disk. Filter disks were then transferred to scintillation vials and radioactivity counted using Aquasol (New England Nuclear) scintillation fluid. Blank values were obtained by pipetting an equivalent amount of either [^{14}C]methylamine or [^{3}H] GA_1 onto filter disks and rinsing with 5 ml of reaction medium. Binding of both [^{3}H]GA_1 or [^{14}C]methylamine was less than 0.2% of the radioactivity applied.

Octanol/Water Partitioning. Partitioning of GA_1 into octanol was measured by adding [^{3}H]GA_1 to vials containing equal volumes of 2-octanol and 50 mM citrate/phosphate buffer ranging from pH 3.0 to pH 9.0. The phases were well mixed by vigorously inverting the vials 100 times. Vials were then centrifuged at 1000*g* for 2 min and aliquots of each phase were removed and radioactivity determined by scintillation counting. After correcting for counting efficiency, the partition coefficient was calculated by dividing the amount of radioactivity in the octanol phase by the amount in the aqueous phase.

Protein Determination. Proteins were determined by a modified Lowry procedure (18).

Gibberellins. (1,2-$^3H_2$)GA_1 (9.25×10^{11} Bq mmol^{-1}) was synthesized and purified according to the procedure of Nadeau and Rappaport (20). Immediately prior to use [^{3}H]GA_1 was subjected to reverse phase HPLC on a preparative ODS-5μm C18 column (Jones Chromatography, Inc., Columbus, OH 43214) with a gradient of 25 to 35% methanol in 0.05 M phosphoric acid. After reducing the eluate to the aqueous phase, the latter was partitioned four times against ethyl acetate. The ethyl acetate phase was reduced to dryness and redissolved in aqueous buffer. The radiochemical purity of [^{3}H]GA_1 was greater than 99%.

The purity of unlabeled GAs used in this study was confirmed, using GLC, by injecting the MeTMS[3] or Me (for GA_9) derivatives at 220°C onto wall-coated open tubular (WCOT) fused silica columns (25 m × 0.2 mm i.d.) coated with DB-1 (J. and W. Scientific, Rancho Cordova, CA). Me derivatives were prepared by successive treatment with ethereal diazomethane and (for MeTMS derivatives) Sil-Prep (hexamethyldisilizane:trimethylchlorosilane:pyridine, 3:1:9, v/v/v; Applied Science Labs.). Percentage purity of GAs generally exceeded 97% and in some cases, no detectable impurities were present.

Source of Chemicals. ATP was obtained from Boehringer Mannheim as the disodium salt and converted to the Tris salt by ion exchange chromatography through Dowex 50W. Nigericin was obtained from Calbiochem-Behring Corp. The 2-octanol was obtained from Fisher Scientific Co. All other chemicals were obtained from Sigma. [^{14}C]methylamine hydrochloride (43.3 mCi mmol^{-1}) was obtained from New England Nuclear.

RESULTS

Previous research on membrane transport of GA_1 in cowpea leaves suggested the existence of GA_1 carriers in both the plasma membrane (24) and tonoplast (16, 17). Attempts to isolate intact membrane vesicles from cowpea leaves were unsuccessful due to heavy contamination of all fractions by chloroplast membranes. Membranes isolated from cowpea hypocotyls, however, were largely chloroplast free and subcellular membrane fractions could be resolved by density gradient centrifugation on sucrose gradients (Fig. 1). The presence of a peak of NO_3^--inhibited and gramicidin-stimulated ATPase (Fig. 1, C) at a density of 1.12 g/cc indicated the presence of sealed tonoplast membrane vesicles (3) in the cowpea microsomal membranes. The competence of these membranes to generate and maintain a pH gradient is shown in Figure 2, where ATP-dependent H^+ transport was measured using the fluorescent amine pH gradient indicator, acridine orange. The initial rate of ATP-dependent H^+ transport

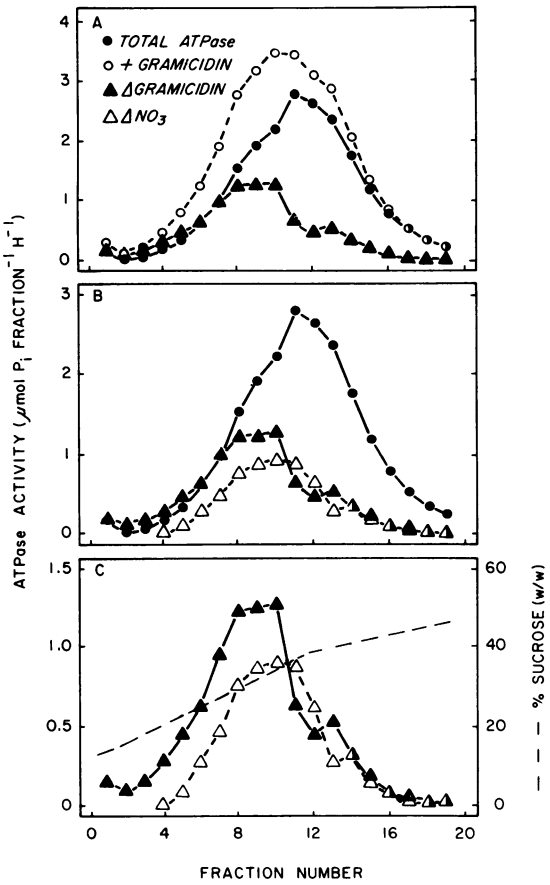

FIG. 1. Continuous sucrose gradient distribution of ATPase activity isolated from dark-grown cowpea hypocotyl tissue. A, Total ATPase (●), total ATPase in the presence of 2 μM gramicidin (○), and the difference between the two as the gramicidin-stimulated activity (▲). Nitrate-sensitive ATPase activity (△), calculated as the difference in enzyme activity in the absence or presence of 50 mM NO_3^- (B), along with total ATPase (●) and gramicidin-stimulated ATPase (▲) activity. Panel C overlays the NO_3^--sensitive ATPase (△) with the gramicidin-stimulated ATPase (▲) activity.

was inhibited 75% by NO_3^- (Fig. 2) but was unaffected by vanadate (not shown). This result suggests that for cowpea hypocotyl microsomal membranes, only tonoplast-derived vesicles are sealed. Active transport of GA_1 had been previously reported in isolated vacuoles (16, 17), and we felt, therefore, that the isolated vesicles would be a useful system to assess membrane transport properties of GA_1.

Partition coefficients of various gibberellins in ethyl acetate/water and of GA_3 in octanol/water have been reported (8, 12). Because a great deal of information is available regarding the relationship between octanol/water partition coefficients and membrane permeability, we measured the octanol/water partitioning of GA_1 as a function of pH (Fig. 3). The high octanol/water partition coefficient at low pH is indicative of the high hydrophobicity of the neutral (undissociated) species of GA_1. If we assume that all of the dissociated species remains in the aqueous phase, we can calculate an octanol/water partition coefficient for neutral GA_1 of 5.95. From the data of Collander (4), a permeability coefficient of approximately 1×10^{-7} m s^{-1} can be estimated for neutral GA_1. This value is similar to that measured for neutral GA_3 with regard to spinach protoplast plasma membrane (12).

If only passive processes are involved in GA_1 membrane transport, one would expect the rate of GA_1 uptake into mem-

[3] Abbreviation: MeTMS, methyl ester trimethylsilyl ether.

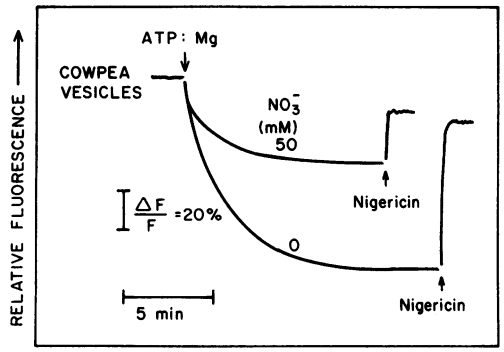

FIG. 2. H^+ transport activity of cowpea membrane vesicles. The formation of acid-interior pH gradients across vesicle membranes due to the activity of an H^+-translocating ATPase was monitored as quenching (Q) of fluorescence (F) of the permeant amine dye, acridine orange. Vesicles were equilibrated in reaction media consisting of 10 mM Tris/Mes (pH 7.0), appropriate salts (KCl, K_2SO_4) and/or inhibitor (NO_3^-), 250 mM sucrose, and 5 μM acridine orange. Fluorescence quenching was initiated by the addition of ATP:Mg to a final concentration of 5 mM. Nigericin (2 μM) was added where indicated to abolish the pH gradient. Initial rates of quenching were 13.3% Q/min and 3.5% Q/min in the absence and presence of NO_3^-, respectively.

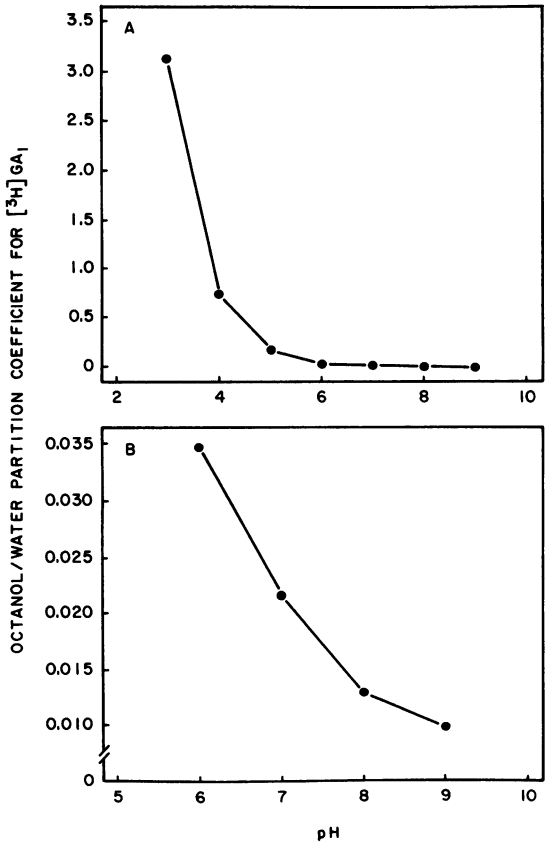

FIG. 3. Octanol/water partitioning of [^{3}H]GA$_1$ as a function of pH. Citrate/phosphate buffer was used to generate a 50 mM buffer series ranging from pH 3.0 to 9.0.

brane vesicles to be greatest at low pH. The time course of GA$_1$ uptake into cowpea membrane vesicles at various pH is shown in Figure 4. After 20 min, uptake of GA$_1$ was greatest at pH 5.0 and progressively less at pH 5.5 and 6.0. At later time points, GA$_1$ accumulation declined at pH 5.0 and 5.5, indicating instability of membrane vesicles at low pH. The time course of GA$_1$ uptake demonstrates the importance of determining the pH-

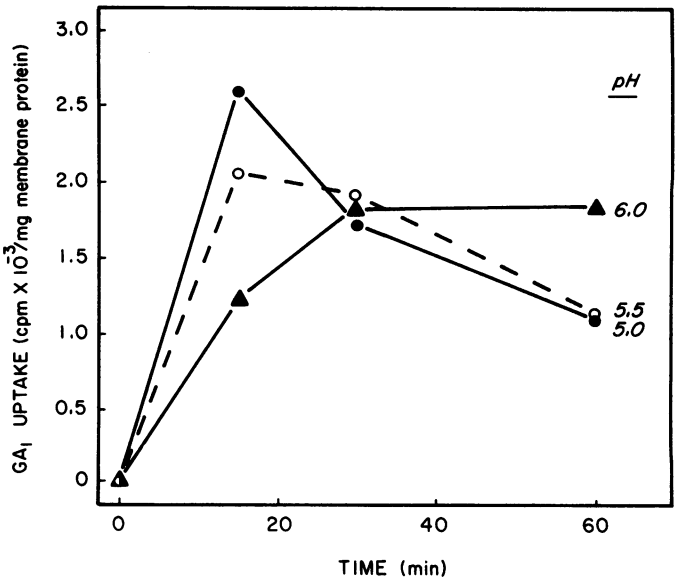

FIG. 4. Time course of GA$_1$ uptake into sealed cowpea vesicles as a function of pH. The incorporation of [^{3}H]GA$_1$ was determined at 25°C using the filtration method described in "Materials and Methods." Assay pH was adjusted using 50 mM citrate/phosphate buffer. Approximately 100 μg membrane protein were filtered per treatment. Maximal uptake of [^{3}H]GA$_1$ at pH 5.0 after 15 min amounted to about 0.35 pmol GA$_1$/mg membrane protein filtered.

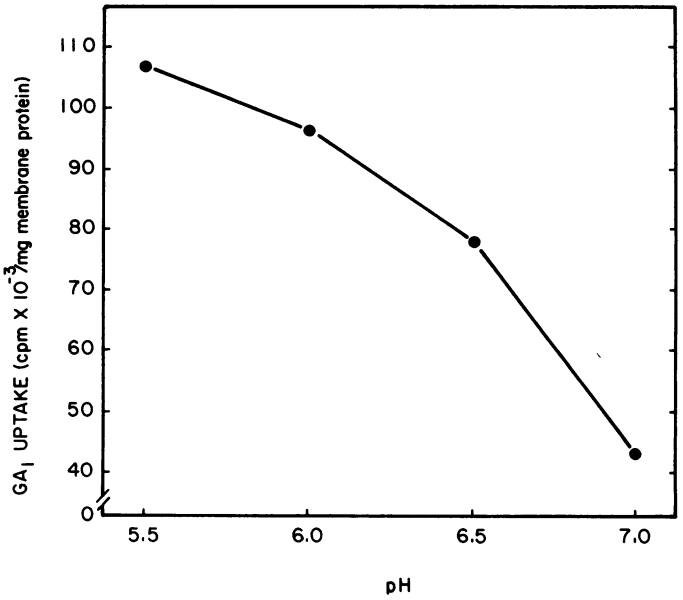

FIG. 5. Uptake of GA$_1$ into cowpea vesicles as a function of pH. The incorporation of [^{3}H]GA$_1$ was determined after 2.5 min at 25°C using the filtration method described in "Materials and Methods." Approximately 100 μg membrane protein were filtered per treatment.

dependence of uptake rates over short time periods since membrane instability at low pH could result in an apparent pH optimum for GA$_1$ uptake that merely reflects a pH optimum for general membrane stability. Consequently, GA$_1$ uptake as a function of pH was measured after a 2.5 min uptake period (Fig. 5). The pH dependence of GA$_1$ uptake is similar to the pH dependence of GA$_1$ octanol/water partitioning (Fig. 3), suggesting that the major mechanism of membrane transport of GA$_1$ is by permeation of neutral GA$_1$. This result is similar to that described for GA$_1$ by Drake and Carr (7) using oat coleoptiles.

To examine the possible existence of energy-dependent mech-

anisms of GA_1 transport we measured ATP-dependent uptake of [^{14}C]methylamine into cowpea membrane vesicles (Fig. 6). Methylamine uptake occurs in response to acid-interior pH gradients (27) and, in these cowpea membrane vesicles, is stimulated by MgATP and inhibited by NO_3^-. These results confirm the fluorescence transport result shown in Figure 2 and establish the feasibility of using filtration assays to measure active membrane transport processes in isolated cowpea vesicles. When the effects of Mg^{2+}, MgATP, and inhibitors on [^{14}C]methylamine and [^{3}H]GA_1 uptake were compared, quite different results were observed (Table I). Methylamine uptake was stimulated by MgATP and was inhibited by NO_3^-. GA_1 uptake was slightly stimulated by Mg^{2+} alone, but was neither stimulated further by ATP added in the presence of Mg^{2+}, nor inhibited by NO_3^-. The effect of Mg^{2+} on GA_1 uptake is similar to that observed in cowpea vacuoles (16, 17). The differences in Mg^{2+} and ATP stimulation of methylamine and GA_1 uptake indicate that only methylamine and not GA_1 uptake is driven by the activity of a primary transport MgATPase. The effect of Mg^{2+} alone in stimulating GA_1 uptake is not understood but may result from a general effect of Mg^{2+} in stabilizing membrane structures.

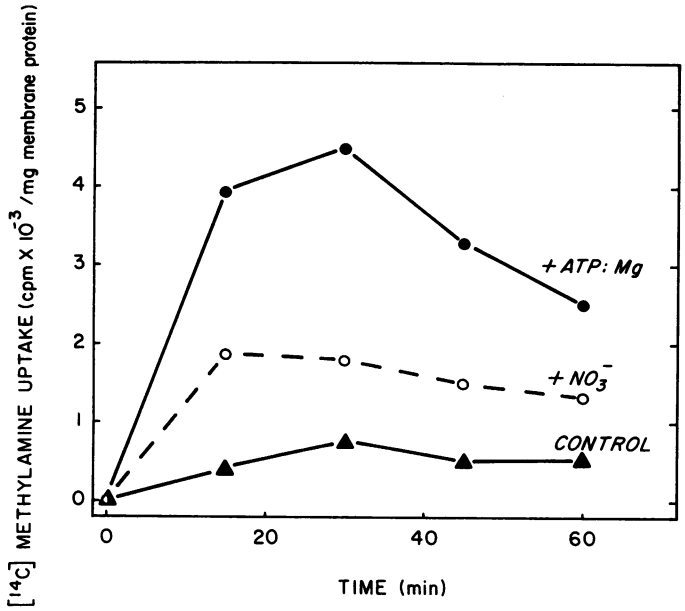

FIG. 6. Time course of [^{14}C]methylamine uptake into sealed cowpea vesicles in the absence or presence of ATP:Mg and ±50 mM NO_3^-. Reaction media contained 50 mM Tris/Mes (pH 7.0), 50 mM KCl, 25 mM K_2SO_4, or 50 mM KNO_3, and 5 mM $MgSO_4$ plus 5 mM ATP/Tris (pH 7.0) where indicated. Vesicles were added to initiate the uptake. Transport of [^{14}C]methylamine was assayed at pH 7.0 and 25°C using the filtration method. Approximately 110 μg membrane protein were filtered per treatment.

Table I. *Effect of Mg^{2+}, MgATP, and Inhibitors on [^{14}C]Methylamine and [^{3}H]GA_1 Uptake into Cowpea Vesicles*

Uptake was measured after 30 min at pH 7 and 25°C in a reaction medium consisting of 50 mM Tris/Mes (pH 7.0), 50 mM KCl, 25 mM K_2SO_4, or 50 mM KNO_3, ±50 μM vanadate, ±5 mM $MgSO_4$, and/or ATP/Tris (pH 7.0).

	[^{14}C]-Methylamine[a]	[^{3}H]-GA_1
	% of control	
Control	100	100
+ $MgSO_4$ (5 mM)	194	141
+ MgATP (5 mM)	3,582	111
+ NO_3^- (50 mM)	1,041	107
+ Vanadate (50 μM)	3,571	

[a] Values are based on cpm incorporated/mg membrane protein.

To further investigate possible interactions between GA_1 and H^+-ATPase activity in tonoplast vesicles, the effect of GA_1 on ATP-dependent H^+ transport was determined (Fig. 7). It was observed that GA_1 inhibited the formation of a pH gradient at concentrations as low as 1 μM (Fig. 7, A). A direct effect of GA_1 on ATPase activity was ruled out (not shown). To test whether GA_1 acted by shuttling H^+ across the membrane and thereby dissipating the pH gradient, the effect of GA_1 on the maintenance of artificially imposed pH gradients was examined (Fig. 7, B). In this experiment, NaOH was added to the vesicle exterior to create an acid-interior pH gradient of approximately 1 unit. In the presence of GA_1, the extent of fluorescence quenching was decreased and the rate of fluorescence recovery increased. These results indicate that GA_1 acts to increase the permeability of the membrane vesicles to H^+ (26).

The question may arise whether GA at μM concentrations could collapse a pH gradient in the presence of millimolar buffer concentration. An alternative explanation for how GA dissipates the pH gradient could be that GA acts by inserting into the membrane structure, making the membrane more permeable to H^+. We feel that a mechanism of H^+ shuttling is far more likely a mechanism than one whereby GA forms channels in the membrane. Many proton ionophores (such as FCCP and CCCP)

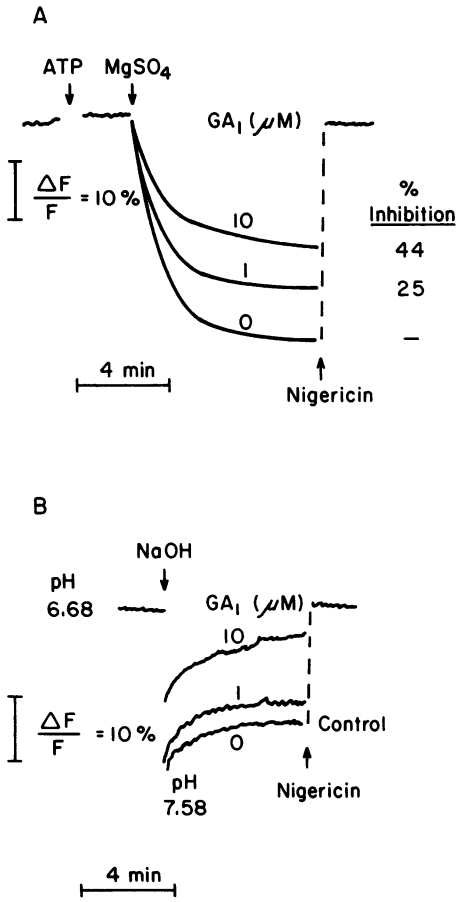

FIG. 7. Inhibition by GA_1 of H^+ transport into cowpea vesicles. A, Progressive inhibition by GA_1 when vesicles were preincubated with increasing concentration from 0 to 10 μM. Fluorescence quenching was not initiated until both ATP and Mg^{2+} were present, demonstrating the substrate specificity of the H^+-ATPase. B, A parallel experiment investigating the effect of GA_1 (0–10 μM) on the maintenance of an acid-interior pH gradient of approximately 1.0 pH unit created artificially by adding a base pulse of NaOH. Additional details are in "Materials and Methods."

act at nanomolar concentrations by shuttling H^+ across membranes (22) in a fashion similar to that which we have proposed for GA.

The effect of another biologically active gibberellin, GA_9, and a less biologically active gibberellin, GA_5 (11), were tested for their ability to dissipate H^+ gradients (Fig. 8). Both GA_9 and GA_5 were nearly as effective as GA_1 in abolishing pH gradient formation, indicating that this ability to dissipate H^+ gradients is probably not related to the biological activity of gibberellins (30).

The ability of gibberellins to dissipate pH gradients across tonoplast vesicle membranes could arise from two possible mechanisms of membrane transport of gibberellins (Fig. 9). Mechanism I would account for an increased H^+ leak by permeation of the neutral form of GA (GAH) (and to a limited extent of GA^-) serving to shuttle H^+ across the membrane. Alternatively, Mechanism II accounts for an increased H^+ leak by a specific, carrier-mediated H^+/GA^- antiport. These two mechanisms give rise to two distinct and testable predictions regarding gibberellin accumulation in response to a pH gradient. If Mechanism I is operative, GA will accumulate inside the vesicle in response to an alkaline-interior pH gradient. Conversely, if Mechanism II is operative, GA will accumulate inside the vesicle in response to an acid-interior pH gradient. These predictions were tested by monitoring the accumulation of GA_1 in membrane vesicles equilibrated at either pH 6.0, 6.75, or 7.5 and then shifted to a medium of pH 6.75 (Fig. 10). GA_1 was transiently accumulated in response to an alkaline-interior pH gradient. As the pH gradient decayed, GA_1 accumulation also decreased to a level observed in the absence of a pH gradient. These results are consistent with the operation of Mechanism I of gibberellin membrane transport (Fig. 9) in cowpea tonoplast vesicles. This mechanism simply involves passive partitioning of GAH and anion trapping of GA^- in alkaline compartments.

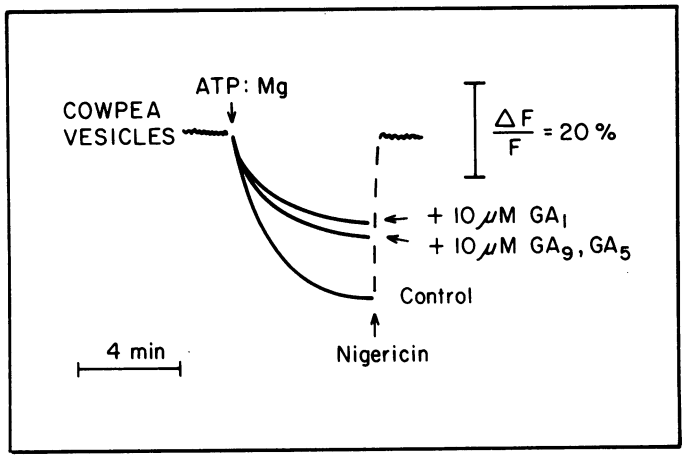

FIG. 8. Dissipation of H^+ gradients in cowpea vesicles by several gibberellins: GA_1, GA_5, and GA_9.

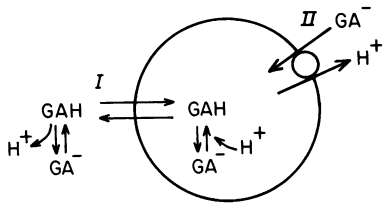

FIG. 9. Two possible mechanisms of membrane transport of gibberellins. Mechanism I involves passive partitioning of GAH and anion trapping of GA^- in alkaline compartments. Mechanism II involves a specific carrier-mediated H^+/GA^- antiport.

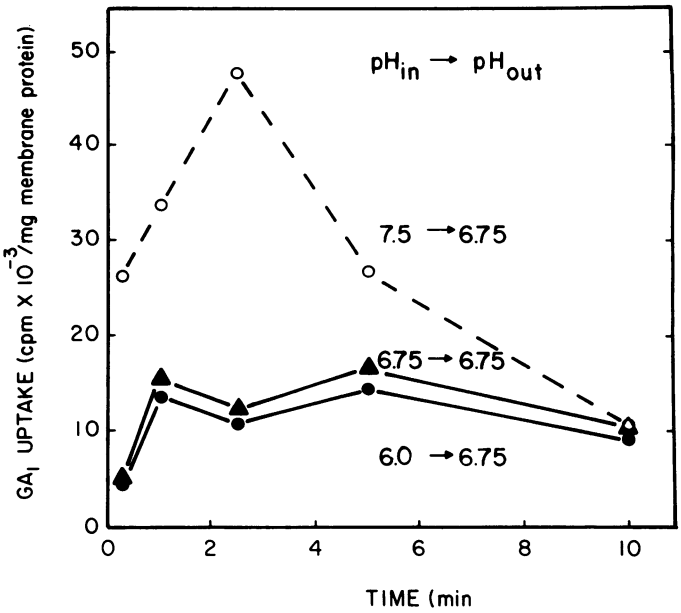

FIG. 10. Effect of a pH gradient on [^{3}H]GA_1 uptake into cowpea vesicles over time. Membrane vesicles were equilibrated at 25°C in sucrose resuspension buffer at either pH 6.0 (●), 6.75 (▲), or 7.5 (○). Filtration of aliquots began immediately following a shift to medium at pH 6.75.

DISCUSSION

In the present study, we have examined the permeability properties of GA_1 in membrane vesicles isolated from dark-grown cowpea hypocotyls. Our results, based on effects of pH on uptake by vesicles and the effect of imposition of artificially created pH gradients across vesicles, support the view that GA_1 is transported passively and predominantly as the neutral form of GA_1 (Fig. 9). We have not observed any response suggestive of carrier-mediated transport of GA_1. However, additional studies of the effect of GA_1 concentration on uptake into vesicles are needed before conclusions about the role of carriers in GA_1 uptake can be made. It is clear though that GA_1 uptake into cowpea vesicles does not show any MgATP-stimulated component as previously reported (*cf.* 16, 17) even though a tonoplast-type H^+-translocating ATPase was demonstrated to be present in these vesicles. Since the ATPase acidifies the vesicle interior, we would not expect MgATP to stimulate GA_1 uptake unless an antiport transport system was involved as previously discussed.

In studies on the effect of pH on GA_1 transport by cowpea vesicles, we did find that long-term measurements of GA fluxes could lead to an erroneous pH optimum for GA transport since the membranes we tested were unstable at low pH (*cf.* 16, 17, 24). Short-term uptake measurements indicated that only neutral GA_1 permeated membranes as predicted from measurements of octanol/water partition coefficients. Due to the high permeability of neutral GA_1, concentration gradients of GA_1 established by active transport mechanisms would rapidly decay. This suggests that GA_1 accumulation above that predicted for pH partitioning of a weak acid into alkaline compartments cannot occur. Some of the discrepancies concerning the effects of pH and ATP on GA_1 transport by vacuoles (16, 17) compared to results obtained here using vesicles may be explained by the differences in methodology and in the tissue (leaf *versus* hypocotyl) and transport system (vacuole *versus* vesicle) studied.

Our observation that GA_1 at concentrations of 1 μM or above uncoupled pH gradients across cellular membranes is unlikely to be related to the primary mechanism of action of this GA (*cf.* 30) since GA_5, a weakly active GA (11), was just as effective in

uncoupling pH gradients. GA_9, which may be active only through its ability to be metabolized to an active form (21), was also as effective an uncoupler of pH gradients as GA_1 and GA_5. This result indicates that substantial accumulation of GA_1 in a cell could be detrimental. These considerations suggest that accumulation and compartmentation of gibberellins can only occur through metabolic conversion to more polar forms. This is consistent with the observations of Garcia-Martinez *et al.* (9) who reported that the major form of a vacuolar pool of gibberellin is GA_8-glucoside. A similar conclusion was also drawn by Musgrave *et al.* (19) who observed that accumulated radioactivity in barley aleurone cells was associated with polar metabolites of the gibberellins applied.

The high permeability coefficient (1×10^{-7} m s^{-1}) for neutral GA_1 estimated from the octanol/water partition coefficient has implications for long distance transport of GA_1. With respect to phloem mobility of GA_1, this permeability coefficient is far too high for GA_1 to be effectively translocated according to the 'intermediate permeability' theory of phloem mobility (29). Weak acids, however, are generally thought to be phloem mobile because of their ability to partition into the relatively alkaline compartment of the phloem sieve tube (5). When both factors related to phloem mobility of organic solutes are considered, it is predicted that GA_1 will be translocated well in the phloem (D. Kleier, personal communication). This prediction arises only from consideration of the passive membrane transport properties of GA_1 and is consistent with the observation that gibberellins synthesized in the leaves are translocated to the epicotyl where elongation is promoted (10).

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