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Specific photoaffinity labeling of two plasma membrane polypeptides with an azido auxin

(auxin receptors/aqueous phase partitioning/3-indoleacetic acid/*Cucurbita pepo*)

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ABSTRACT Plasma membrane vesicles were isolated from zucchini (*Cucurbita pepo*) hypocotyl tissue by aqueous phase partitioning and assessed for homogeneity by the use of membrane-specific enzyme assays. The highly pure (*ca.* 95%) plasma membrane vesicles maintained a pH differential across the membrane and accumulated a tritiated azido analogue of 3-indoleacetic acid (IAA), 5-azido-[7-^{3}H]IAA ([^{3}H]N_3IAA), in a manner similar to the accumulation of [^{3}H]IAA. The association of the [^{3}H]N_3IAA with membrane vesicles was saturable and subject to competition by IAA and auxin analogues. Auxin-binding proteins were photoaffinity labeled by addition of [^{3}H]N_3IAA to plasma membrane vesicles prior to exposure to UV light (15 sec; 300 nm) and detected by subsequent $NaDodSO_4$/PAGE and fluorography. When the reaction temperature was lowered to −196°C, high-specific-activity labeling of a 40-kDa and a 42-kDa polypeptide was observed. Triton X-100 (0.1%) increased the specific activity of labeling and reduced the background, which suggests that the labeled polypeptides are intrinsic membrane proteins. The labeled polypeptides are of low abundance, as expected for auxin receptors. Further, the addition of IAA and auxin analogues to the photoaffinity reaction mixture resulted in reduced labeling that was qualitatively similar to their effects on the accumulation of radiolabeled IAA in membrane vesicles. Collectively, these results suggest that the radiolabeled polypeptides are auxin receptors. The covalent nature of the label should facilitate purification and further characterization of the receptors.

The plant hormone auxin (3-indoleacetic acid, IAA) plays an important role in a variety of developmental responses such as cell division, growth, and differentiation. While the molecular mechanism of auxin action is unknown, it is likely that auxin activates cellular responses by binding to specific receptor proteins (see refs. 1–3 for reviews). We are particularly interested in the role of auxin-specific plasma membrane (PM) receptors in plant growth and development. At least three auxin-specific binding proteins are thought to be present in the PM: (*i*) an auxin uptake carrier (4–10), (*ii*) an auxin efflux carrier (11–13), and (*iii*) an auxin receptor that is associated with an outwardly directed proton pump thought to be involved in elongation growth (see refs. 1 and 3 and *Discussion*). The approach we are using to isolate and identify putative auxin receptors involves the preparation of highly enriched PM vesicles by aqueous phase partitioning and photoaffinity labeling of polypeptides in those vesicles with the auxin analogue 5-azido-[7-^{3}H]IAA ([^{3}H]N_3IAA). N_3IAA has auxin activity in several bioassays (14) and exhibits a rate of polar transport similar to that of IAA (D.L.R. and T.L.L., unpublished data). The photoaffinity technique has advantages over radiolabeled auxins for the study and isolation of ligand–receptor complexes (15). Foremost among these advantages is the ability of [^{3}H]N_3IAA to covalently link to its binding protein by photolysis, providing a stable chemical marker for the putative receptor proteins.

Exposure of N_3IAA to UV light (300 nm) results in photolysis of the aryl azide (azido group). The products of this reaction are N_2 gas and a highly reactive nitrene, which, via a nucleophilic reaction, would be expected to covalently label specific binding proteins. Using this compound, Jones *et al.* (16, 17) were able to demonstrate quantitative differences in labeling of maize microsomal proteins in the presence of competing auxin or auxin analogues, although much background polypeptide labeling was evident (17). Ripp *et al.* (18) recently reported that a photoaffinity analogue of sucrose labeled a 62-kDa membrane protein associated with sucrose transport in soybean. They found that lowering the temperature of the reaction mixture to −196°C (liquid N_2) resulted in reduced background labeling. Here we report that nonspecific [^{3}H]N_3IAA labeling of membrane proteins can also be largely eliminated by conducting the photolysis at −196°C. Under these conditions, photolysis of zucchini (*Cucurbita pepo* L.) PM proteins with [^{3}H]N_3IAA results in the high-specific-activity labeling of a low-abundance PM polypeptide doublet that displays properties consistent with those expected for a PM auxin receptor.

MATERIALS AND METHODS

Radiochemicals and Chemicals. [5-^{3}H]IAA, 22 Ci/mmol (1 Ci = 37 GBq), was purchased from Amersham; [^{3}H]N_3IAA, 16 Ci/mmol, was synthesized as described (17). All other chemicals were purchased from Sigma. The ionophore mixture (ION3), containing valinomycin, nigericin, and carbonyl cyanide *m*-chlorophenylhydrazone, was prepared as a stock in 100% ethanol with each compound at 4 mM (9).

Plant Material and Preparation of PM Vesicles. Seeds of zucchini squash (*Cucurbita pepo* L., cv. Dark Green, Ferry Morse Seed, Mountain View, CA) were sown in moist vermiculite and grown for 4 days in the dark at 28°C. Hypocotyl sections (2 cm) were excised 5 mm below the apical hook and homogenized for 15 sec with a Polytron (Brinkmann) (at setting 7–8) in 1 volume (1 ml/g of fresh weight) of ice-cold buffer 1 (0.25 M sucrose/10 mM Tris/HCl, pH 7.5/1 mM Na_2EDTA/1 mM dithiothreitol/0.1 mM $MgSO_4$/0.2 mM phenylmethylsulfonyl fluoride with pepstatin and leupeptin at 1 μg/ml). This slurry was filtered through four layers of cheesecloth. The particulate material collected in the cheesecloth was homogenized again with an additional

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Abbreviations: IAA, 3-indoleacetic acid; N_3IAA, 5-azido-IAA; ION3, mixture of ionophores (valinomycin, nigericin, and carbonyl cyanide *m*-chlorophenylhydrazone); PM, plasma membrane; TIBA, 2,3,5-triiodobenzoic acid.

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volume of buffer 1 for 15 sec. The combined filtrates were pooled and centrifuged at 4°C for 20 min at 3000 × *g* (GPR H3.7 rotor, Beckman) and the pellets were discarded. The supernatant was centrifuged at 100,000 × *g* for 40 min at 4°C (33,000 rpm, Ti50.2 rotor, Beckman), and the resulting microsomal pellet was suspended in 2 ml of Buffer 2 (5 mM potassium phosphate, pH 7.8/0.25 M sucrose/4 mM KCl). Purified PM vesicles were then prepared by a version of the aqueous phase partitioning procedure recently reviewed by Larsson (19). The resuspended microsomal vesicles were layered onto a phase-separation medium [6.5% (wt/vol) dextran/6.5% (wt/vol) PEG 4000/0.25 M sucrose/4 mM KCl/5 mM potassium phosphate, pH 7.8]. The mixture was degassed by vacuum for 20 min and centrifuged at 1000 × *g* for 10 min at 4°C. The upper phase, containing PM vesicles, was extracted twice more, then removed, diluted with 4 volumes of buffer 1, and centrifuged at 100,000 × *g* for 30 min. The resulting PM pellet was resuspended in ≈0.5 ml of Buffer 1, aliquoted, frozen in liquid N_2, and stored at −80°C.

Marker Enzyme Analysis. Cytochrome-*c* oxidase, a mitochondrial inner membrane marker (20), was assayed using freshly prepared PM vesicles. The membrane sample (20 μl) was mixed with 10 μl of 3% (wt/vol) digitonin in a cuvette and incubated 60 sec before addition of 1 ml of assay solution (0.2% reduced cytochrome *c*/0.02% EDTA/40 mM potassium phosphate, pH 7.4). Cytochrome *c* was reduced with sodium dithionite until A_{550}/A_{565} was 9–10; excess reducing agent was removed by bubbling air through the solution. NADH-dependent cytochrome-*c* reductase, an endoplasmic reticulum marker (21), was assayed in the same manner using unreduced cytochrome *c* (0.27 mg/ml in 50 mM Tris/Mes, pH 7.5/ 1 mM KCN/0.1 mM NADH). Detergent-activated inosine diphosphatase (IDPase, Golgi marker) was assayed according to Nagahashi and Kane (22) in the presence and absence of 0.03% digitonin. The nitrate-sensitive ATPase (a tonoplast marker), azide-sensitive ATPase (mitochondrial marker), and vanadate-sensitive ATPase (PM marker) were assayed as described by Sandstrom *et al.* (23) except that 0.1 mM $(NH_4)_6Mo_7O_{24}$ was included in the assay buffer.

[^{3}H]IAA and [^{3}H]N_3IAA Association Curves. Association of labeled IAA with plasma membrane vesicles was assayed according to Benning (7). Radiolabeled auxins were diluted to 4 nM in 10 mM disodium citrate/citric acid, pH 5.5/0.25 M sucrose/5 mM $MgSO_4$. Tubes (1.5 ml) were prefilled with 0.9 ml of radiolabeled auxin solution (with or without ION3 or unlabeled competitors) to which 1 g (fresh-weight equivalent) of PM vesicles was added in 100 μl of buffer 1. After 5 min, samples were centrifuged at 200,000 × *g* for 5 min at 4°C (Beckman TLA 100.2 rotor). The supernatants were discarded, and the radioactivity was extracted from the pelleted membranes with 0.5 ml of methanol. The methanol extract was then added to Beckman Ready-Safe scintillant for measurement of radioactivity.

Photoaffinity Labeling. All manipulations were performed under red light. A quantity of PM vesicles equivalent to 100 μg of protein, as assayed by Coomassie brilliant blue G250 binding (24), was diluted to 50 μl with binding buffer (10 mM Mes/1,3-bis[tris(hydroxymethyl)methylamino]propane, pH 6.5/0.25 M sucrose/0.5 μM [^{3}H]N_3IAA. For competition experiments, all competitors were added at 1 mM except 2,3,5-triiodobenzoic acid (TIBA) which was added at 0.1 mM. Triton X-100, when present, was at 0.1%. Thirty seconds after addition of PM vesicles to the [^{3}H]N_3IAA mixture, samples were transferred to glass coverslips, which were placed on an aluminum block surrounded by liquid N_2 (−196°C samples). After the samples were solidly frozen (1 min) they were irradiated for 15 sec with 300-nm UV light from a Fotodyne model 3-3000 transilluminator at a distance of 2.5 cm. Following irradiation, −196°C samples were stored overnight in the dark at −20°C. The vesicles were then washed by dilution into 1 ml of binding buffer and pelleted at 200,000 × *g* for 5 min at 4°C. The pelleted PM vesicles were suspended in 20 μl of $NaDodSO_4$ loading buffer and the PM proteins were separated by electrophoresis through 7.5–15% acrylamide gradient gels (25) and stained with Coomassie brilliant blue R250 (26) or silver (27). Gels were soaked in a fluorographic enhancer (Fluoro-hance, Research Products International) prior to drying and exposure to Kodak XAR-5 x-ray film for 5–10 days. For concentration of the proteins, the regions corresponding to the labeled bands were excised and proteins were electroeluted according to Lomax *et al.* (25).

Table 1. Marker enzyme analysis of microsome (M) and PM preparations

Marker enzyme (organelle)*	Spec. act.† M	Spec. act.† PM	PM/M ratio
VO_4^--sensitive ATPase (PM)	21.0	105.0	5.0
pH-dependent IAA transport (PM)	19.3	205.0	10.6
NO_3^--sensitive ATPase (Tp)	7.5	0.0	0
N_3^--sensitive ATPase (Mt)	6.8	0.0	0
Cytochrome-*c* oxidase (Mt)	34.7	0.5	0.01
Detergent-sensitive IDPase (Golgi)	360.0	60.0	0.17
Cytochrome-*c* reductase (ER)	156.6	30.2	0.19

*Tp, tonoplast; Mt, mitochondria; ER, endoplasmic reticulum.
†Specific activity, nmol per mg of protein per min, except for pH-dependent IAA transport, pmol of IAA per mg of protein per min.

RESULTS

Phase-Separated Zucchini Membrane Vesicles. Aqueous phase partitioning produces PM preparations from zucchini hypocotyl tissue that are substantially free of contaminating vacuolar, mitochondrial, endoplasmic reticulum, and Golgi membranes (Table 1). PM enrichment is indicated by the increase in vanadate-sensitive ATPase activity with an ≈5-fold reduction of the endoplasmic reticulum marker (cytochrome-*c* oxidase) and Golgi apparatus marker (IDPase) and >100-fold reduction in marker enzyme activities for the tonoplast and mitochondria (nitrate-sensitive ATPase and azide-sensitive ATPase, respectively). The purified vesicles are also enriched for pH-dependent IAA accumulation when compared to microsomes, indicating that the resulting PM preparations contain vesicles that are tightly sealed and able to maintain a pH gradient.

Uptake of [^{3}H]N_3IAA into Membrane Vesicles. Previous data (14) have shown that N_3IAA is an active auxin in several bioassays, including polar transport in tobacco pith explants. However, before initiating attempts to photoaffinity label membrane proteins, we felt it important to verify that N_3IAA is accumulated in PM vesicles in a manner analogous to IAA and that it exhibits similar competition kinetics when compared with auxin analogues.

As shown in Table 2, zucchini PM vesicles were able to accumulate [^{3}H]IAA and [^{3}H]N_3IAA in similar quantities in the presence of a pH gradient. Accumulation was reduced by

Table 2. Ionophore-sensitive association of [^{3}H]IAA or [^{3}H]N_3IAA with zucchini PM vesicles

Labeled compound	Radiolabel associated, cpm − ION3	Radiolabel associated, cpm + ION3
[^{3}H]N_3IAA	4840	867
[^{3}H]IAA	6668	815

The association of [^{3}H]N_3IAA or [^{3}H]IAA (4 nM) with zucchini PM vesicles was measured by centrifugation assay in the presence and absence of ION3 (4 μM final concentration).

Table 3. Effect of auxin analogues on the inhibition of accumulation of radiolabeled IAA or N_3IAA

Labeled compound	−log IC_{50} (M)				
	IAA	2-NAA	2.4-D	1-NAA	BA
$[^3H]N_3IAA$	7.3	7.3	6.2	5.7	4.7
$[^3H]IAA$	6.7	7.4	6.2	5.5	4.4
$[^{14}C]IAA$*	7.1	6.4	5.4	4.7	4.5

IC_{50} values were calculated from experiments measuring the pH-gradient-dependent (ION3-sensitive) accumulation of $[^3H]N_3$-IAA, $[^3H]IAA$, or $[^{14}C]IAA$ in the presence of seven concentrations of auxin analogue ranging from 10^{-8} to 10^{-4} M. Nonspecific radioligand association in the presence of 10^{-4} M unlabeled IAA was 25% of the control value. NAA, naphthaleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; BA, benzoic acid.
*Taken for comparison from ref. 8, data generated by using microsomal membrane preparations rather than PM.

the addition of ionophores (ION3), which dissipate both the pH gradient and any membrane potential that is present. Competition curves for both $[^3H]IAA$ and $[^3H]N_3IAA$ were obtained by using various synthetic auxins and auxin analogues, and the concentrations giving 50% inhibition of association (IC_{50}) were calculated (Table 3). For comparison, we have included values obtained for $[^{14}C]IAA$ uptake into zucchini microsomal membrane vesicles (8). In each case, nonradioactive IAA and 2-naphthaleneacetic acid competed well for association, whereas 1-naphthaleneacetic acid and benzoic acid were much less effective and 2,4-dichlorophenoxyacetic acid was intermediate in ability to compete. Collectively, these data indicate that N_3IAA and IAA are taken up into PM vesicles in a similar manner and that competitors of IAA association also alter N_3IAA association in a qualitatively similar fashion.

A Polypeptide Doublet Is Labeled to High Specific Activity at −196°C. Initial attempts to photoaffinity label PM auxin receptors were performed at 0–4°C. After 300-nm UV irradiation followed by denaturing gel electrophoresis and fluorography, $[^3H]N_3IAA$ was found to be associated with more than 10 polypeptides (Fig. 1). The intensity of polypeptide labeling under these conditions appeared to be roughly proportional to the stained quantity of protein (compare Fig. 1 to Coomassie-stained gel in Fig. 2*b*). These findings suggested that since $[^3H]N_3IAA$ photoaffinity labeling at 0–4°C was relatively nonspecific, the identification of putative auxin receptors would be problematical.

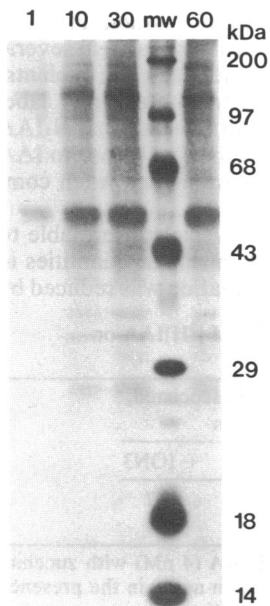

FIG. 1. Fluorogram showing nonspecific photoaffinity labeling at 0–4°C. Zucchini PM vesicles were irradiated with 300-nm UV light in the presence of 0.5 μM $[^3H]N_3IAA$ for 1, 10, 30, or 60 sec. Each lane contained 100 μg of protein. Sizes of molecular weight marker proteins (lane mw) are given at right.

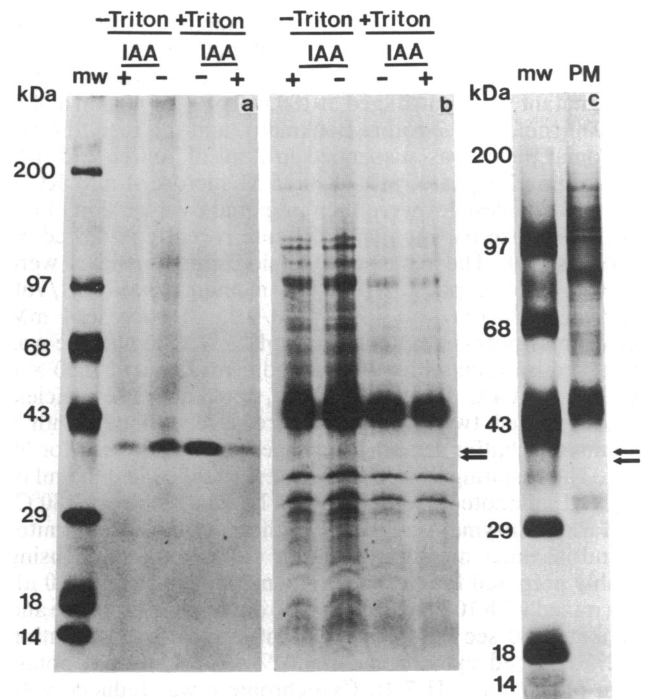

FIG. 2. High-specific-activity photoaffinity labeling of zucchini PM proteins at −196°C. Fluorogram (*a*) shows labeling with $[^3H]N_3IAA$ (0.5 μM) in the absence (−) or presence (+) of 1 mM unlabeled IAA. The labeling was performed with and without the addition of 0.1% Triton X-100. The corresponding Coomassie-stained gel (*b*; 100 μg of protein per lane) and a silver-stained gel of 15 μg of PM protein (*c*) are shown for comparison. Arrows indicate positions of the 40-kDa and 42-kDa polypeptides estimated from alignment with the fluorogram. Molecular masses of molecular weight marker proteins (lanes mw) are given.

A different result was obtained when the temperature of the PM/$[^3H]N_3IAA$ mixture was lowered to −196°C prior to a 15-sec UV exposure. Under this condition fluorography revealed that labeling was limited to a polypeptide doublet of 40 kDa and 42 kDa (Figs. 2*a* and 4). Since we could not visualize the doublet after either Coomassie or silver staining (Fig. 2 *b* and *c*), high-specific-activity labeling was indicated. After a 9-fold concentration of the protein from the gel region containing the doublet, polypeptide bands became visible by silver staining (Fig. 3). Addition of unlabeled IAA prior to irradiation reduced photoaffinity labeling by $[^3H]N_3IAA$ (Fig. 2*a*). Competition was more pronounced in the presence of Triton X-100, which both increased the intensity of labeling in the absence of unlabeled IAA and reduced the amount of

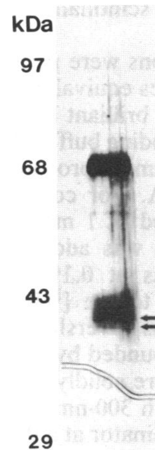

FIG. 3. Silver-stained gel demonstrating visualization of polypeptides following 9-fold enrichment by preparative electrophoresis and electroelution of the $[^3H]$-N_3IAA-labeled region. Each band (arrows) was estimated to contain about 50 ng of protein. The 68-kDa protein band is bovine serum albumin used to enhance protein precipitation.

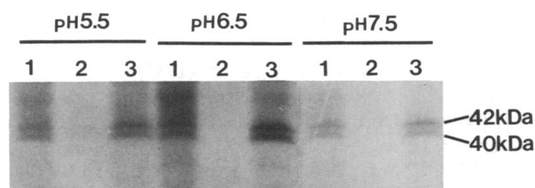

FIG. 4. Fluorogram showing the effect of pH {10 mM Mes/1,3-bis[tris(hydroxymethyl)methylamino]propane buffer} on [^{3}H]N_3-IAA labeling of the 40/42-kDa doublet (compare lanes 1 at pH 5.5, 6.5, and 7.5). Also shown is the effect of 1 mM IAA (lanes 2) and 0.1 mM TIBA (lane 3) at each pH. Photoaffinity labeling was performed at −196°C with 100 μg of PM protein, 0.5 μM [^{3}H]N_3IAA, and 0.1% Triton X-100.

background per lane. The enhancement effect of Triton X-100 is likely to be due to solubilization of extrinsic PM proteins, accounting for the reduced background in these lanes; this explanation would also account for the reduced quantity of visible protein in the matching Coomassie-stained gel lanes (Fig. 2*b*) and suggests that the labeled 40/42-kDa doublet is intrinsic in the PM.

The effect of varying the pH of the medium in which the PM vesicles were suspended on labeling of the polypeptide doublet was also investigated. The greatest amount of photoaffinity labeling by [^{3}H]N_3IAA was observed at pH 6.5 (Fig. 4). Significant fluorographic signals were also produced at pH 5.5, but medium with a pH of 7.5 resulted in a greatly diminished signal.

Auxins that Compete for Uptake *in Vitro* Reduce [^{3}H]N_3IAA Labeling. When competitors of IAA and N_3IAA uptake were added to the photoaffinity reaction prior to UV irradiation, a pattern of inhibition emerged that was qualitatively similar to the effects of the analogues on uptake (compare Fig. 5 with Table 3). IAA and 2-naphthaleneacetic acid largely prevented labeling, whereas 2,4-dichlorophenoxyacetic acid and 1-naphthaleneacetic acid had lesser effects. Addition of tryptophan, which is a precursor of IAA and which does not compete for uptake *in vitro,* had no effect on labeling (Fig. 5). Though the overall intensity of signal could be reduced by the addition of auxin analogues, the polypeptides of the doublet appeared to be labeled with equal intensity relative to each other regardless of the competitor present during the photoaffinity reaction. Addition of the auxin transport inhibitor TIBA to the photoaffinity reaction mixture produced no significant reduction in labeling (Fig. 4). This result suggests that the auxin efflux carrier was not being labeled, since TIBA has been shown to specifically inhibit auxin association with this site *in vivo* (4) and *in vitro* (5, 28).

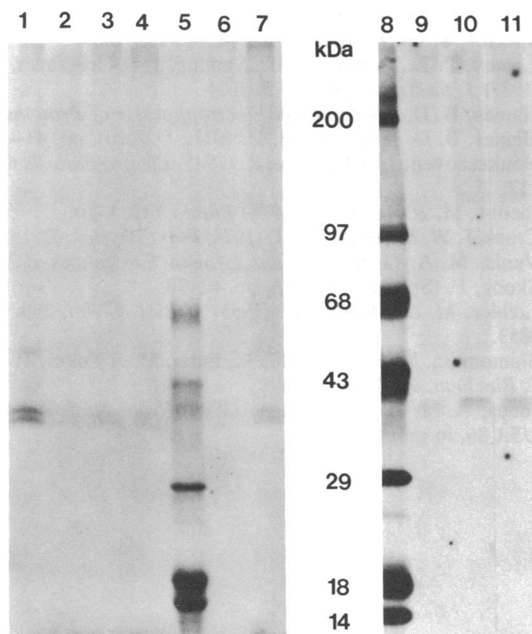

FIG. 5. Intensity of photoaffinity labeling by [^{3}H]N_3IAA in the presence of unlabeled auxin or auxin analogues (1 mM): IAA (lanes 2 and 9), 1-naphthaleneacetic acid (lane 3), 1-naphthaleneacetic acid plus IAA (lane 4), 2-naphthaleneacetic acid (lane 6), 2,4-dichlorophenoxyacetic acid (lane 7), and tryptophan (lane 11). Controls (no added competitor) are shown in lanes 1 and 10. The fluorogram on the left (lanes 1–7) and the fluorogram on the right (lanes 8–11) are from two experiments performed on different days. Lanes 5 and 8 contained molecular weight marker proteins; sizes (molecular mass in kilodaltons) are given between the fluorograms. Conditions were as for Fig. 4 at pH 6.5.

DISCUSSION

PM vesicles from zucchini have been demonstrated to accumulate [^{3}H]IAA *in vitro*. An integral component of this accumulation is believed to be an auxin-specific uptake symport (5, 6). Here, we have shown that [^{3}H]N_3IAA is accumulated by PM vesicles with competition characteristics similar to those of IAA. Thus, in theory, the use of [^{3}H]N_3IAA should result in specific labeling of at least the auxin-uptake carrier protein.

Our initial attempts to label auxin receptors in the PM with [^{3}H]N_3IAA were carried out at 0–4°C and resulted in the labeling of a variety of polypeptides. Since the intensity of labeling was proportional to the abundance of PM proteins, it seems likely that these conditions produce a high background of nonspecific labeling. Previous attempts to label auxin-binding proteins in microsomal membrane preparations from maize by photoaffinity labeling (17) have also met with limited success as a result of nonspecific labeling of membrane proteins. We have overcome this problem by lowering the temperature of the [^{3}H]N_3IAA/membrane mixture to −196°C prior to photolysis. At this temperature, we observed high-specific-activity labeling of a low-abundance polypeptide doublet of 40 kDa and 42 kDa with little labeling of other polypeptides. One possible reason that low temperatures increase the specificity of photolabeling is that the residence time of the photogenerated nitrene at −196°C is greater than the half-life of this intermediate; thus diffusion of the reactive nitrene from the binding site to nonspecific sites would be greatly reduced. Another possibility may be the effect low temperatures have on the lipid phase present in these samples. N_3IAA, being a lipophilic molecule, may partition less into the lipid phase below the phase-transition temperature of the bilayer. This in turn would reduce nonspecific N_3IAA associations with the hydrophobic domains of membrane proteins.

The qualitative pattern of competition by auxin analogues for [^{3}H]N_3IAA labeling (Fig. 5) is similar to that observed for accumulation of [^{14}C]IAA into microsomal vesicles (8) and [^{3}H]IAA and [^{3}H]N_3IAA into PM vesicles (Table 3). In particular, 2-naphthaleneacetic acid, which strongly competes for uptake and presumably the uptake carrier *in vitro,* prevents virtually all photoaffinity labeling, whereas 1-naphthaleneacetic acid, which competes poorly for uptake, has less effect. These data, along with the fact that the labeled polypeptides may be integral membrane proteins, suggest that the uptake carrier is being labeled. However, the putative PM auxin receptor site thought to be involved with proton secretion and thus with growth may have a similar specificity pattern and could not be distinguished from the uptake carrier by these experiments. The lack of interaction of TIBA with [^{3}H]N_3IAA labeling indicates that the efflux carrier responsible for the polar transport of IAA is not likely to be related to the 40-kDa and 42-kDa polypeptides labeled

under these conditions. At this time we cannot fully explain why competition by auxin analogues requires higher-than-expected concentrations (1 mM) to substantially reduce labeling. However, this does not appear to be a unique problem, since Ripp *et al.* (18) encountered a similar difficulty when they used sucrose or other sugar analogues to compete with azidosucrose in photolabeling of a sucrose-transport protein in soybean.

That the two polypeptides are labeled indicates either (*i*) a multimeric protein, (*ii*) different processing states of the same protein (e.g., preprotein, glycosylation, or proteolytic cleavage), or (*iii*) two different auxin-binding proteins. We do not know whether the $[^3H]N_3IAA$-labeled polypeptides reported here are related to auxin-binding proteins that have been isolated and solubilized by other means. In solubilization studies of naphthaleneacetate-binding proteins in maize, polypeptides of approximately 80 kDa (29), 40 kDa (30), and 20 kDa (31–33) have been isolated, some of which (31, 32) appear to be of PM origin. It is possible that these polypeptides are related to one another as monomers of a multimeric protein, and the sizes might suggest that they are related to our polypeptides as well. However, this may not be the case, since the solubilized maize polypeptides exhibit different binding specificities and pH optima than those described here.

The density of PM auxin binding sites has been calculated by Jacobs and Hertel (28) to be ≈20 pmol/g of fresh weight for zucchini hypocotyls. Given the molecular weights of our polypeptides ($M_r \approx 40,000$), the hypothetical yield of our presumptive receptor would be 80 ng/100 g of fresh weight. We found that preparative electrophoresis and electroelution (Fig. 3) yielded ≈100 ng of each protein per 100 g of fresh weight of zucchini hypocotyls. The yield was determined by comparing the intensity of doublet staining to known quantities of control protein (data not shown). This result confirms that the concentrations of the labeled doublet proteins are near that predicted for a PM receptor. The yield is also sufficient for the production of monoclonal antibodies and, perhaps, for direct polypeptide microsequencing. Specific antibodies would allow both the immunological localization and characterization of the auxin-binding protein and the eventual reconstitution of the protein in an active form, which is a prerequisite for the ultimate identification of its role in auxin action.

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