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Azido Auxins[1]

PHOTOAFFINITY LABELING OF AUXIN-BINDING PROTEINS IN MAIZE COLEOPTILE WITH TRITIATED 5-AZIDOINDOLE-3-ACETIC ACID

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ABSTRACT

Tritiated 5-azidoindole-3-acetic acid (5-N_3-[7-^{3}H]IAA), a photoaffinity labeling agent, was used to photolabel proteins of a crude microsomal preparation from maize (*Zea mays* L., Bear Hybrid, WF9 × BR38) coleoptile. Approximately 50% of the bound radioactivity was solubilized in 5 molar urea containing Triton X-100, and the extract was fractionated using a variety of techniques. High performance liquid chromatography demonstrated that, although many membrane proteins incorporated tritiated label, only a few showed reduced incorporation in the presence of excess indole-3-acetic acid. By contrast, no detectable reduction in incorporation was observed in the presence of excess naphthalene-1-acetic acid. Results from isoelectric focusing gel electrophoresis indicate that the proteins that showed reduced incorporation of photolyzed 5-N_3-[7-^{3}H]IAA in the presence of IAA fell into two main groups: one which focuses between pH 5.2 and 5.7 (pI 4.8–5.3) and another around pH 6.2 (pI 5.8). In sodium dodecylsulfate polyacrylamide gel electrophoresis, the proteins migrated as four bands with apparent molecular weights of 60, 49, 45, and 37 kilodaltons. The auxin-transport inhibitor, 2,3,5-triiodobenzoic acid, competes for the labeling by 5-N_3-[7-^{3}H]IAA, suggesting that some of these proteins may be involved in auxin transport.

The technique of photoaffinity labeling allows covalent attachment of analogs to biologically important sites (3). If an analog is appropriately labeled (*i.e.* if it is fluorescent or radioactive), attachment to these sites can be visualized, which makes the sites potentially isolable. We have previously shown that in the dark three photoaffinity labeling agents, 4-, 5-, and 6-N_3IAA,[5] cause elongation in a variety of plant tissues, including maize mesocotyl (16). We have determined the affinities of these compounds for auxin-binding sites in maize coleoptile in the dark, and we have calculated the efficiency of attachment of the auxin analogs to these sites on photolysis (11). We now report the labeling of potential IAA-binding proteins with 5-azido-[7-^{3}H]indole-3-acetic acid (5-N_3-[7-^{3}H]IAA) and their partial characterization.

MATERIALS AND METHODS

Plant Material. Etiolated maize seedlings (*Zea mays* L., Bear Hybrid, WF9 × BR38; Custom Farm Seeds, Decatur, IL) were grown and coleoptiles were harvested as described in Jones *et al.* (11).

Chemicals. Tritiated 5-azidoindole-3-acetic acid (5-N_3-[7-^{3}H] IAA) was prepared according to the method of Melhado *et al.* (17) except that the tritium (20 Ci) in the reduction step was not diluted with hydrogen and the reaction pressure was one atmosphere. Tritiated 5-N_3IAA, 29 Ci/mmol, was purified on a silica-18 column (IBM) using an IBM HPLC apparatus and a Romac radioactivity detector. The solvent was 50% methanol. The purified product (>99% radiochemical purity by radiochromatography [17]) was stored in 50% aqueous methanol in the dark at −5°C at a concentration of 405 μCi/ml. High quality, white, crystalline IAA, purchased from Sigma Chemical Company, was used without further purification. NAA of high specified purity (>98%) from Sigma was further purified by recrystallization from water/ethanol. TIBA, purchased from Eastman Organic Chemicals, and NPA were purified by water/ethanol recrystallization to obtain white grains of TIBA (mp 226–228°C) and pinkish-white needles of NPA (mp 195–196°C), respectively. Urea, SDS, and acrylamide were analytical grade.

Preparation of Sample. A washed microsomal fraction was prepared as described by Jones *et al.* (11), except that the membrane pellet represented the 5,000 to 133,000*g* fraction. The pellet was resuspended in 10 ml of assay buffer (10 mM Na-citrate, 250 mM sucrose, 0.5 mM $MgCl_2$ (pH 5.5) with citric acid (from 21) containing 2 μM carrier-free 5-N_3-[7-^{3}H]IAA. FCCP and PMSF were omitted. The resuspension, containing approximately 2 mg protein/ml, was distributed to the wells of a photolysis chamber (see Fig. 1b of Jones *et al.* [11]), which contained 0.1 mM IAA, NAA, or an auxin-transport antagonist such as TIBA or NPA or simply buffer alone. Wells containing the membrane resuspension were irradiated (365 nm peak emission, 2 mw cm^{-2}) for 1 h at 4°C. After photolysis, the membrane suspension was extensively washed by pelleting (133,000*g*) and resuspending in buffer (25 ml) until unbound photoproduct and unused starting material were removed, usually with two centrifugation/resuspension cycles. Approximately 1% of the added radioactivity was bound irreversibly to the final pellet, which was

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[5] Abbreviations N_3IAA, azidoindole-3-acetic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; IEF, isoelectric focusing; NAA, naphthalene-1-acetic acid; NPA, naphthylphthalamic acid; PMSF, phenylmethylsulfonyl fluoride; TIBA, 2,3,5-triiodobenzoic acid.

resuspended in assay buffer (pH 6) and stored frozen.

Extraction. Proteins were extracted from labeled pellets by several methods: (a) 5 M urea; (b) incubation for several days in 5 M urea; (c) sonication in 5 M urea; (d) 5 M urea containing 1% Triton X-100. Extractable protein contained approximately 600 cpm/μg.

HPLC. Labeled membrane proteins were separated by gel permeation chromatography using an Altex (Berkeley, CA) TSK-3000 column on a Beckman HPLC apparatus. Absorbance, at 280 nm when Triton X-100 was absent and at 290 nm when present in the solvent, was measured with an Hitachi spectrophotometer (model 100-10) and recorded and integrated with an Altex integrator (model C-R1A). Samples were chromatographed using 5 M urea, 10 mM Tris-HCl (pH 7.0), and 0.1% Triton X-100 at a flow rate of 0.5 ml/min. Fractions were collected directly in scintillation vials, the solvent was evaporated, a toluene-base scintillation fluid was added, then vials were counted for 10 min each in a Beckman scintillation counter (model LS7500).

IEF Gel Electrophoresis. IEF gel electrophoresis was performed by the method of O'Farrell (19). The pH gradient was pH 4 to 8, determined directly by placing a flat-end electrode on the gels. TCA precipitation technique (14) was used to determine incorporation of label into macromolecules. The same amount of radioactivity was applied to each tube. Gels stained with Coomassie blue were first rinsed with distilled H_2O, laid side by side on a light box, and the gels were carefully matched. After being frozen in this position with powdered dry ice, they were sliced with a Mickle gel slicer (Brinkmann) at 1-mm intervals. Each slice was placed in a scintillation vial and incubated with 0.4 ml of 90% NCS (Amersham) at 50°C for 3 h to digest the sample. After the samples were cooled, ACS (Amersham) scintillation fluid was added and the vials were counted for 10 min.

SDS-PAGE. The apparent mol wts of potential auxin-binding proteins were determined by SDS-PAGE. Labeled protein was extracted with urea, diluted with SDS-PAGE sample buffer (8% SDS, 125 mM Tris, pH 6.8, 14 mM 2-mercaptoethanol, 50% glycerol, 0.01% bromophenol blue), loaded onto 10% gels, electrophoresed, stained with Coomassie blue or silver (23), sliced, and counted as described above.

Chromatofocusing. A 16 × 1.0 cm glass column (13-ml bed volume) was packed with Polybuffer exchanger (PBE 94, Pharmacia Fine Chemicals, AB, Uppsala, Sweden). The column was equilibrated with 5 M urea, 25 mM bis-Tris-HCL (pH 7). Before sample was injected, 5 ml of Polybuffer (Polybuffer 74, Pharmacia) was added to the column. The proteins in the sample were eluted at a rate of 1 ml/min and collected with a Gilson microfractionator. Fractions of the indicated pH were pooled, dialyzed against water, and lyophilized.

Sample Nomenclature. Samples of proteins photolyzed in the presence of 5-N_3-[7-^{3}H]IAA alone are called control samples. When agonists such as IAA or NAA or antagonists such as TIBA or NPA are included with 5-N_3-[7-^{3}H]IAA during photolysis, the sample is called 'plus IAA', 'plus NAA', etc.

RESULTS

It was apparent that certain membrane proteins isolated from maize coleoptile were labeled with 5-N_3-[7-^{3}H]IAA after photolysis. Urea plus Triton X-100 solubilized about 50% of the total radioactivity in the pellet, most of which was associated with proteins, *i.e.* it was precipitable by TCA. Incorporation of radioactivity was approximately 0.5 μCi/mg of extractable protein. Several techniques, including HPLC, IEF, SDS-PAGE, and chromatofocusing, were used to fractionate the membrane proteins and to determine which ones were labeled in a specific manner.

HPLC. Proteins of a control sample were extracted with 5 M urea and 1% Triton X-100 and were then chromatographed. Figure 1 shows the chromatogram of a typical experiment. The profile consists of three major peaks. Peak A, a minor peak, runs close to the void volume. If samples are extracted with urea alone and run under identical conditions, then a single peak corresponding to peak A, although 50% smaller, is observed. Therefore, enrichment of peak A proteins can be achieved at the first extraction step by omitting Triton X-100. Peak B is seen when a nonionic detergent, such as Triton X-100, is included in the sample extraction buffer. Sonication of samples extracted with urea alone and/or incubation of these samples for several days with urea alone are two additional methods of obtaining peak B. Accordingly, proteins associated with peak B are tenaciously associated with the lipid bilayer, since they are released with urea

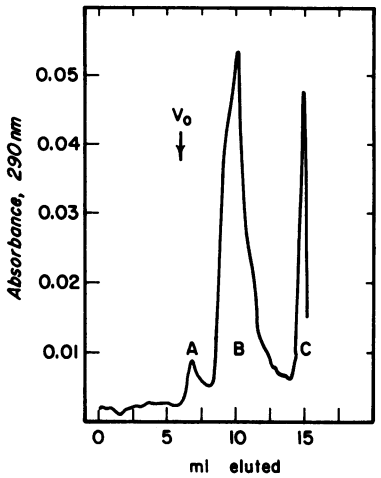

FIG. 1. Chromatograph of maize microsomal protein extracted with urea and Triton X-100. Approximately 100 μg of protein was injected. The solvent was 5 M urea, 10 mM Tris (pH 7), and 0.1% Triton X-100. The column was an Altex TSK-3000 and the flow rate was 0.5 ml/min. Similar results were obtained in four other experiments.

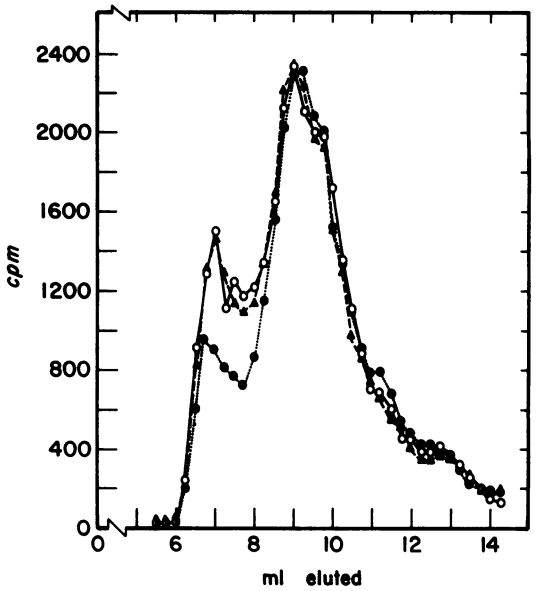

FIG. 2. Radioactivity profiles of photolabeled proteins extracted and chromatographed as described in Figure 1. Extracted proteins are from samples photolyzed with 5-N_3-[7-^{3}H]IAA alone (2 μM) (○), photolyzed with 5-N_3-[7-^{3}H]IAA (2 μM) plus IAA (100 μM) (●), and photolyzed with 5-N_3-[7-^{3}H]IAA (2 μM) plus NAA (100 μM) (▲). Each sample contained the same amount of radioactivity (30,000 cpm). IAA, but not NAA, competes for labeling of a protein or proteins migrating in the first peak. Similar results were obtained in four other experiments.

only when disruptions such as sonication or detergents are used. Nonionic detergents also facilitate the release of proteins in peak A, but extraction of these proteins is not dependent upon such a treatment. The detergent, either in the extraction buffer or the eluent, does not affect the mobility of these proteins. If the sample is extracted first with urea and then chromatographed with detergent, peak A proteins elute at the same position as the same sample chromatographed without detergent (data not shown). Peak C in Figure 1 appears to be free detergent.

The radioactivity associated with proteins of the control sample (Fig. 2, open circles) has a profile similar to the chromatograph of Figure 1 except that the first peak is relatively larger, indicating that the specific activity of peak A proteins is greater than that for peak B proteins. Figure 2 shows that numerous proteins incorporate the label, probably as a function of both specific and nonspecific interactions. We have found that under these denaturing conditions mol wt standards do not migrate as predicted strictly on the basis of size. In addition, the poor resolution this column offers in the presence of urea precludes its use as the sole method of isolating specifically labeled proteins. Nevertheless, the speed and reproducibility of this method have allowed us to use it as a quick assay to detect gross differences between samples of proteins labeled with 5-N_3-[7-^{3}H]IAA in the presence or absence of auxin agonists. Binding of 5-N_3-[7-^{3}H]IAA in the presence of agonists is mainly nonspecific. The reasoning is that an agonist such as IAA, by binding to the active site or sites, can block subsequent labeling by 5-N_3-[7-^{3}H]IAA (11). The agonist, in theory, would not affect nonspecifc labeling since the number of available nonspecific sites is not limiting. This reasoning suggests a method to discriminate between specifically and nonspecifically labeled proteins. In all the experiments separating labeled proteins, two samples are processed in parallel: one sample contains proteins labeled with 5-N_3-[7-^{3}H]IAA when an agonist is included as well. Control samples represent total labeling and the 'plus agonist' samples represent nonspecific binding. The difference between these represents specifically labeled fractions.

Figure 2 shows that a protein or proteins migrating in the shoulder of peak A fail to incorporate, or incorporate less, label when IAA, but not NAA, is included during photolysis. These fractions are interpreted as being IAA-binding proteins with a low affinity toward NAA.

IEF Gel Electrophoresis. Labeled proteins of the control samples were compared with those of the plus IAA sample using IEF gel electrophoresis. Tube gels, stained with Coomassie blue, are shown in the top of Figure 3, and the radioactivity profile corresponding to migrating distance along the gel is shown in the bottom. Many, but not all, of the stained bands correspond to peaks of radioactivity. Incorporation of the label by the proteins falls into two main categories: a high pI group focusing around pH 6.2 and a low pI group focusing between pH 5.2 and 5.7. The high group always showed less radioactivity than the low pI group, but the amount relative to the low pI group varied. The more interesting group, at this point, was the low pI group, since there was always a large incorporation of the label and there was a consistent decrease of label in the plus IAA samples (compare control to plus IAA, Fig. 3). The low pI group has five peaks of radioactivity corresponding to five stained bands located at approximately pH 5.2, 5.4, 5.5, 5.6, and 5.7. Because of the effect of urea on the charged sidechains of proteins, 0.42 pH units should be subtracted from the above values to calculate actual pI values (29). Thus, the pI values of the low pI group are: 4.8, 5.0, 5.1, 5.2, and 5.3; and approximately 5.8 for the high pI group.

Figure 4 compares labeled proteins of the control sample to those of the plus NAA sample. No large differences of incorporation of label were observed, indicating that proteins separated

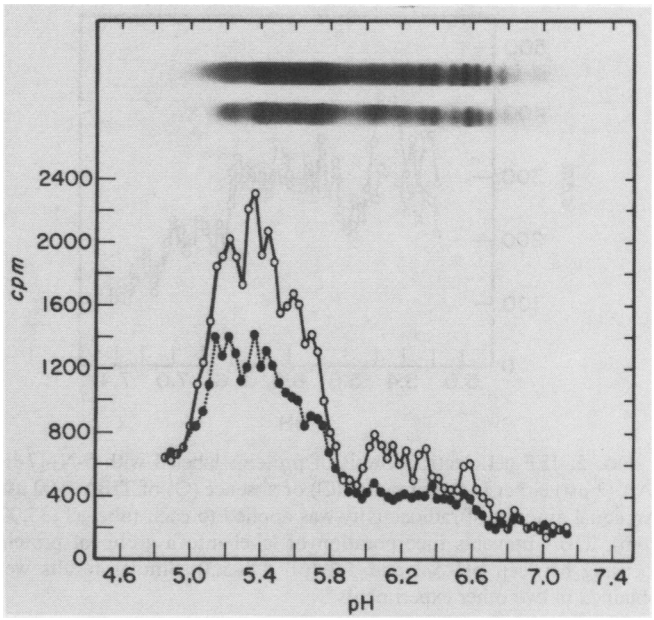

FIG. 3. IEF gel electrophoresis of proteins labeled with 5-N_3-[7-^{3}H]IAA (2 μM) either in the presence (plus 100 μM IAA) or absence (control) of IAA. An equal amount of radioactivity (122,000 cpm) was added to each tube gel. The gels, shown at the top of the figure, were stained with Coomassie blue, sliced, and the radioactivity of each slice was determined. The radioactivity profile for the control sample (○) is compared to the plus IAA sample (●). Differences between the two profiles indicate the positions of proteins that incorporate less label when IAA is present (specific labeling). Similar results were obtained in two other experiments.

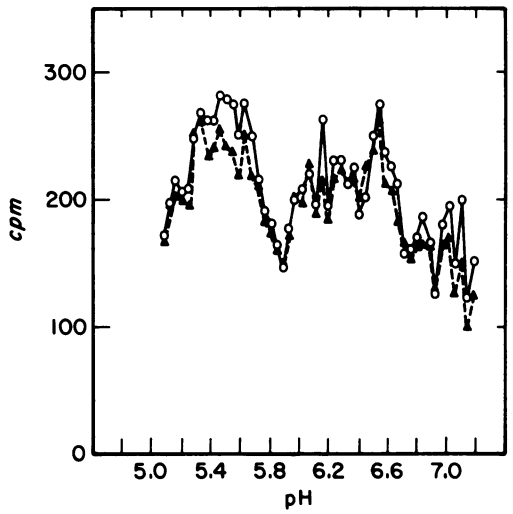

FIG. 4. IEF gel electrophoresis of proteins labeled with 5-N_3-[7-^{3}H]IAA (2 μM) either in the presence (▲) or absence (○) of NAA (100 μM). An equal amount of radioactivity was applied to each tube gel (30,000 cpm). NAA has no apparent effect on the incorporation of label. Similar results were obtained in two other experiments.

under these conditions are apparently not involved in NAA binding or else that they have low affinities toward NAA. This observation agrees with the results obtained with HPLC.

TIBA, an auxin-transport antagonist (12, 13), was also included during photolysis to determine its effect on labeling of these proteins. The radioactivity profile of control samples is compared to 'plus TIBA' samples in Figure 5. When TIBA was present during photolysis, less radioactivity was incorporated into proteins of the low pI group, but the amount of radioactivity

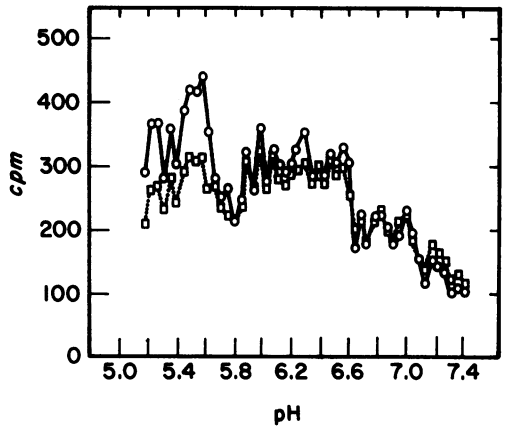

FIG. 5. IEF gel electrophoresis of proteins labeled with 5-N_3-[7-^{3}H] IAA (2 μM) either in the presence (□) or absence (○) of TIBA (100 μM). An equal amount of radioactivity was applied to each tube gel (35,000 cpm). TIBA prevents incorporation of label into a group of proteins focusing between pH 5.2 and 5.7 (pI, 4.8–5.3). Similar results were obtained in two other experiments.

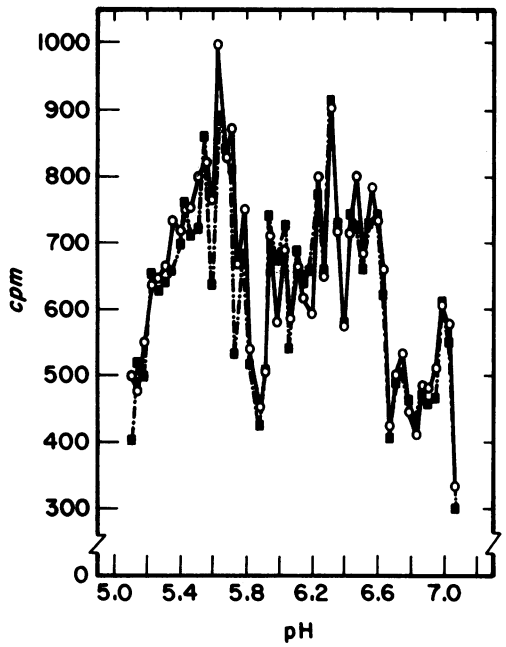

FIG. 6. IEF gel electrophoresis of proteins labeled wtih 5-N_3-[7-^{3}H] IAA (2 μM) either in the presence (■) or absence (○) of NPA (100 μM). An equal amount of radioactivity was applied to each tube gel (84,000 cpm). NPA had no apparent effect on the incorporation of label. Similar results were obtained in another experinent.

incorporated into proteins of the high pI group remained unchanged. NPA, a noncompetitive inhibitor of auxin transport, had no apparent effect on the photolabeling of proteins in either the high or the low pI group (Fig. 6).

SDS-PAGE. We preferred IEF gel electrophoresis over SDS-PAGE because the maize proteins were resolved much better with the former; nevertheless, it was possible to use SDS-PAGE to obtain limited information on the apparent mol wts of these potential auxin-binding proteins. We had a problem with protein aggregation, as have other workers (4), but it could be partially overcome by using a higher SDS concentration (4–6%) in the sample buffer. Figure 7 compares radioactivity profiles of labeled proteins in the control sample and in the plus IAA sample. Differences between the profiles were seen at four bands corresponding to 60, 49, 45, and 37 kD. The largest difference was consistently observed at 37 kD. If the sample was first treated with proteinase K, then electrophoresed, there was no radioactivity significantly above background in the gel, thereby confirming the protein nature of the fractions. When the same samples were chromatofocused and the proteins of pH 5.7 to 5.45 were pooled and subjected to electrophoresis as before, discrete bands at 49, 45, and 37 kD were observed. There was a group of darkly stained bands around 60 kD that precluded visualization of a discrete band corresponding to a 60-kD IAA-binding protein.

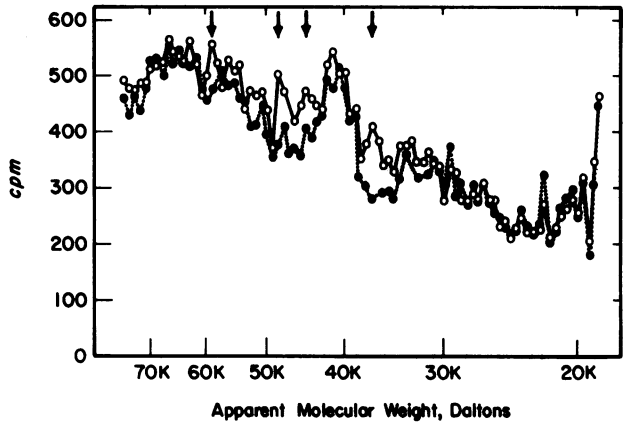

FIG. 7. SDS-PAGE of proteins labeled with 5-N_3-[7-^{3}H]IAA (2 μM) either in the presence (●) or absence (○) of IAA (100 μM). An equal amount of radioactivity was applied to each lane (50,000 cpm). Four bands (arrows), which migrated at 60, 49, 45, and 37 kD, failed to incorporate label when IAA was present during the photolysis reaction. Similar results were obtained in another experiment.

DISCUSSION

Although many proteins are labeled with 5-N_3-[7-^{3}H]IAA on photolysis, only a few of these proteins incorporate less label when IAA is included in the photolysis reaction. IEF gel analysis reveals that five bands between pH 5.2 and 5.7 (pI 4.8–5.3) and several bands around pH 6.2 (pI 5.8) are potential IAA-binding proteins. Also, we conclude that four groups of proteins migrating in SDS-PAGE with apparent mol wt of 60, 49, 45, and 37 kD are to be considered as candidate IAA-binding proteins. We are not certain of the relationship between the proteins observed in IEF gel analysis and those observed in SDS-PAGE. It is possible that the charge variation is due to the isozyme pattern of a single mol wt class. Another possibility is that each different mol wt class may have a different net charge. We have been unable to eliminate the latter possibility with two-dimensional gel analysis or chromatofocusing in combination with SDS-PAGE. Also, because we did not attempt to retard proteolysis by the inclusion of PMSF or other protease inhibitors (4), yet another possibility cannot be excluded, namely, that the many candidate IAA-binding polypeptides may be degradative products of a single protein. The observation that a number of proteins specifically bind IAA is perhaps not surprising considering that the auxin economy of the cell is managed by many proteins (*e.g.* for auxin metabolism, transport, catabolism).

Results of experiments described here provide partial characterization of potential auxin-binding proteins and also provide hints as to function. TIBA, an auxin-transport antagonist, competes for labeling of the low pI group. Also, we have found that NAA included with 5-N_3-[7-^{3}H]IAA in the photolysis reaction has no effect on the labeling of proteins analyzed by all the methods described. An affinity for TIBA (efflux site) and a low affinity for NAA (influx site) are both characteristics of the auxin transport system (6, 8, 25–27), suggesting that proteins described

here are involved in auxin transport. Additionally, we have tested for an effect of NPA, an auxin-transport inhibitor, on labeling and found none, supporting the current interpretation by Goldsmith (7) that NPA and IAA bind at different sites and that the data of Jacobs and Hertel (10) may need to be reinterpreted to mean that NPA causes increased accumulation of IAA into sealed vesicles rather than increased binding of IAA to the IAA-transport site (8). Recently, Jacobs and Gilbert (9) made an interesting discovery. They have used monoclonal antibodies against the NPA site to locate the auxin-transport carrier in pea. Immunofluorescence staining of cortical pea cells shows that certain vertical files of cells contain the antigen only at the morphological base. If these antibodies to pea protein cross-react with a maize protein, they may be useful to confirm the hypothesized transport function of the specifically labeled proteins described here.

The so-called auxin-binding site I, characteristic of a high affinity for NAA, is in low abundance in the cell (1, 21, 22). We have calculated that NAA included in the photolysis reaction at 0.1 mM would decrease the label incorporated by approximately 200 cpm. This value, which takes into account the observed efficiencies of labeling, protection by NAA (11), and counting, is at the limit of detection by methods used in the current study. If this difference were divided among many peptides, as might be the case if the NAA receptor were composed of heterogeneous subunits, then it would be undetectable above the current level of nonspecific binding. Also, we have been able to extract and fractionate only about half of the radioactivity in the pellet, so that site I, the NAA-binding site, may reside in the part of the membrane that is not easily solubilized.

We have concentrated our efforts on characterizing the proteins of the low pI group, since it is reproducible and the difference of incorporated label between control and plus IAA in this pH region is convincingly large. We may find, after we learn how to reduce the background and to make the control *versus* plus auxin differences reproducible in the high pI region, that a protein with characteristics of site I binding focuses in the high pI group.

Several groups of researchers have experimented with the solubilization of auxin-binding proteins of maize (2, 4, 5, 18, 30, 31). Batt *et al.* (2) first demonstrated that NAA-binding activity could be retained in a Triton X-100 extraction of a membrane preparation from maize. Subsequently, Cross and Briggs (4, 5) found that auxin-binding activity, solubilized by Triton X-100 in the presence of PMSF, a serine protease inhibitor, migrated in gel filtration chromatography with an apparent mol wt of 80 kD. Also, they showed that the affinity for NAA increased approximately 10-fold after solubilization without a significant change in the total binding sites, suggesting solubilization-induced conformational change of the active site as observed with solubilized prolactin receptor (24). This observation may be useful later to understand the mechanism whereby auxin receptors are regulated. Venis (30, 31), using the Triton X-100 solubilization as well as other extraction methods in combination with various types of column chromatography, determined the apparent mol wt of the solubilized binding fraction to be between 40 and 45 kD. This fraction resolved into two broad peaks in IEF at pH 5.2 and 4.5 when urea was absent. The discrepancy between the findings quoted has not yet been resolved and is currently assumed to be due to cultivar or batch differences (Cross: Bear Hybrid, WF9 × BR38; Venis: Kelvedon 33). We have used the Bear Hybrid cultivar as did Cross and Briggs, but by our method we did not observe auxin-binding proteins at 80 kD as they report. However, we find potential auxin-binding proteins in a 37 to 45 kD fraction with apparent pI values between 5.3 and 4.8, characteristics which are in rough agreement with results by Venis. Therefore, differences may not be cultivar specific as was suggested (22). In addition, Murphy (18) demonstrated that a fraction from maize cytosol (cultivar Golden Bantam) containing an NAA-binding site migrated through Sephadex G-100 with an apparent mol wt of 39 kD. There is an important difference between our results and those of Cross and Briggs, Venis, and Murphy. These groups used NAA as the probe in the assay for auxin-binding proteins, indicating that these clearly bind NAA, while we report IAA-binding proteins that have little or no affinity for NAA, suggesting that they are a different class or related in a complex manner not yet understood.

The question remains as to the purity of the solubilized fractions that we and others have observed. At best, the solubilized auxin-binding protein is probably only 1% pure since Venis (31) claims that a 200-fold increase in specific activity is possible with his purification protocol after extraction (also by estimate of J. Cross, personal communication). Here, photoaffinity labeling has an important advantage: labeled proteins can be partially characterized while still impure due to their radioactive tag.

Proteins labeled wtih 5-N_3-[7-^{3}H]IAA are associated with the total membrane fraction isolated from maize coleoptile. We chose this fraction in a first attempt to isolate auxin-binding proteins, since extensive work has already shown that within this fraction exist auxin-binding sites probably associated with cell growth (32) and transport of auxin (6; and from another system, 8, 10). By using a crude membrane fraction, we maximized the possibility of finding potential auxin-binding proteins, but we also decreased the resolution because of nonspecific labeling. We are convinced that specific labeling does occur; the next task is to find ways to reduce nonspecific labeling. One possibility is that the enrichment for certain membrane fractions before photolysis would select for auxin-binding sites obligatively associated with the membranes. Enriching for the ER (20) and for the plasmalemma (10) may provide the needed discrimination. It may also be possible to link N_3IAA covalently onto sites before new nonspecific sites are generated, for example, with intact tissue, such as was done with 4-N_3IAA in soybean hypocotyl (15) or with cell cultures.

The problem of assigning physiological function has not been circumvented with this novel technique. We are still dependent upon the use of agonists to auxin binding (12, 13) and to correlations between plant development and auxin binding (32). The exacting criterion for assigning a function is reconstitution of candidate auxin-binding proteins into membranes of specific auxin mutants or mutants of auxin transport, metabolism, etc., or an artificial system (28). Researchers in this area are still in need of all of the components of this approach, namely, pure auxin-binding protein, a method to reconstitute these proteins, and auxin mutants. We have reported here the partial characterization of a group of potential IAA-binding proteins, and this information may be useful in designing methods to achieve the goal of obtaining purified auxin-binding proteins.

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