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Preparation and characterisation of monoclonal and polyclonal antibodies to maize membrane auxin-binding protein

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Abstract. Binding proteins, thought to be auxin receptors, can be solubilised from maize (*Zea mays* L.) membranes after acetone treatment. From these crude extracts, receptor preparations of over 50% purity can be obtained by a reliable, straightforward procedure involving three chromatographic steps – anion exchange, gel filtration and high-resolution anion exchange. Such preparations have been used to immunise rats for subsequent production of monoclonal antibodies. By the further step of native polyacrylamide gel electrophoresis the semi-purified preparations yield homogeneous, dimeric (22-kilodalton, kDa) auxin-binding protein, which has been used to produce a polyclonal rabbit antiserum. The preliminary characterisation of this antiserum and of the five monoclonal antibodies is presented. Two of the monoclonal antibodies specifically recognise the major 22-kDa-binding protein polypeptide whilst the other three recognise, in addition, a minor 21-kDa species. All the monoclonal antibodies recognise the polypeptide rather than the glycan side chain and the polyclonal antiserum also recognises deglycosylated binding protein. The antibodies have been used to quantify the abundance of auxin-binding protein in a number of tissues of etiolated maize seedlings. Root membranes contain 20-fold less binding protein than coleoptile membranes.

Key words: Antibody (auxin) – Auxin (receptor, antibody) – *Zea* (auxin-binding protein)

Abbreviations: ABP = auxin-binding protein; DEAE = diethylaminoethyl; Ig = immunoglobulin; kDa = kilodalton; NAA = naphthalene-1-acetic acid; M_r = relative molecular mass; PAGE = polyacrylamide gel electrophoresis; SDS = sodium dodecyl sulfate

Introduction

Auxin-binding sites in maize coleoptile membranes, first studied by Hertel et al. (1972), have been extensively investigated in several laboratories and appear to fulfil many of the criteria expected of genuine receptors (see Venis 1985). The binding sites can be readily solubilised from the membranes and have been partially purified, revealing a native molecular mass (M_r) of 40–45 kDa (Murphy 1980; Venis 1980; Tappeser et al. 1981).

Two groups have applied auxin affinity chromatography to the isolation of the maize auxin-binding protein (ABP). Löbler and Klämbt (1985) obtained partial purification on a column of 3,5-diiodosalicylic acid coupled via the hydroxyl group to epoxy-Sepharose, produced a heterogeneous antibody and then, by a sequence of immunoaffinity steps, derived small amounts of both ABP (contaminated with fragments of immunoglobulin G, IgG) and monospecific antiserum. Subsequently Shimomura et al. (1986) described purification of the same ABP by a procedure incorporating chromatography on a matrix of NAA (naphthalene-1-acetic acid) linked through the carboxyl group to aminohexyl-Sepharose.

Ingenious though the Löbler-Klämbt purification protocol is, it is a laborious procedure and not one that can be readily reproduced. We have obtained indifferent results from diiodosalicylate and related columns (Venis 1987) and also from the matrix of Shimomura et al. (1986). It would be advantageous to have reliable and reproducible procedures for preparing extensively purified or homogeneous ABP for the generation of monoclonal and polyclonal antibodies respectively, and for further biochemical characterization. We de-

scribe here protocols based on commercially available chromatographic materials that yield preparations of suitable antigenicity, and report on the initial characterization of the resulting antibodies.

Materials and methods

Chemicals. Naphthalene-[1-^{14}C]acetic acid, 2.26 GBq·mmol^{-1} was from Amersham International, Amersham, Bucks., UK. Laboratory chemicals were normally of analytical reagent grade. Peroxidase-labelled second antibodies were obtained from Sigma (Poole, Dorset, UK) and Serotec (Kidlington, Oxford, UK) and 0.2 µm nitrocellulose membranes from Schleicher and Schuell (via Anderman, Kingston-upon-Thames, UK).

Membrane preparation and solubilisation. Microsomal membranes were prepared from 5-d-old etiolated coleoptiles and enclosed leaf rolls of *Zea mays* L. (cv. Beaupré or Cobra) as previously described (Batt et al. 1976). The resuspended membrane pellet was injected into acetone at −15° C, yielding a preparation from which the auxin-binding activity was solubilised with buffer (Venis 1977) consisting of 0.25 M sucrose, 5 mM $MgSO_4$, 10 mM 2-(N-morpholino)ethanesulfonic acid (Mes)-NaOH, pH 5.5 with 0.25 mM phenylmethylsulfonyl fluoride (PMSF) added just before use (Mes buffer, 1 ml per 6 g tissue fresh weight). After standing for at least 2 h with occasional homogenization (Teflon-glass), the extract was centrifuged for 30 min at 50000·*g* and the pellet discarded.

Purification procedures. These were carried out at 4° C, apart from Mono Q chromatography, which was at room temperature.

Anion-exchange chromatography. The above supernatant (from 140 g tissue) was loaded on a column (5 cm long, 1.5 cm i.d.) of diethylaminoethyl (DEAE) Bio-Gel A (Bio-Rad, Watford, UK) previously equilibrated in Mes buffer, and washed with the same buffer until the A_{280} returned to near baseline. Auxin-binding activity was then eluted with 0.1 M NaCl in Mes buffer. The salt eluate was precipitated with ammonium sulphate (75% saturation), taken up in 1 ml of gel-filtration buffer and clarified by centrifugation.

Gel filtration. Post-DEAE fractions derived from approx. 300 g tissue were applied (in a volume of 2 ml) to a column (81 cm long, 1.5 cm i.d.) of Sephacryl S-200 (Pharmacia, Milton Keynes, UK) equilibrated and eluted in 0.25 M sucrose-0.1 M NaCl-20 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl pH 7.3 with 0.25 mM PMSF freshly added. Fractions of 2.6 ml were collected, the A_{280} and-or protein content determined (Bradford 1976) and aliquots were assayed for auxin-binding activity.

High-resolution anion exchange. The most active fractions from the Sephacryl column were pooled, diluted with an equal volume of the same Tris buffer minus NaCl and PMSF and applied to a Mono Q HR 5/5 column (Pharmacia) equilibrated in this buffer, using a 10 ml Superloop. The column was eluted at 1.5 ml·min^{-1} with a 20 ml gradient of 50–350 mM NaCl in 0.25 M sucrose-20 mM Tris-HCl pH 7.3, and 1-ml fractions were collected. Elution was controlled with the Pharmacia GP-250 gradient programmer and dual P-500 pumps. Aliquots were assayed for auxin binding, and the most active fractions (usually two) were pooled, desalted to 0.25 mM PMSF on a short Sephadex G25 (Pharmacia) column and lyophilized.

Native polyacrylamide gel electrophoresis (PAGE). The lyophilized fractions were taken up in 5% (w/v) sucrose (400 µl per 300 g preparation), applied to two 2.5-cm wells of a 1.5-mm-thick 10% non-dissociating polyacrylamide gel and electrophoresis carried out at 4° C in a neutral-pH discontinuous system (Hames 1981). Electrophoresis was continued at 35 mA for 45 min after the bromophenol blue applied in adjacent wells reached the bottom of the gel. The gel was then briefly electroblotted (10 mA, 5 min in a Bio-Rad Trans-Blot cell) to a 0.2-µm nitrocellulose membrane using 25 mM Tris-192 mM glycine pH 8.3 and the small fraction of transferred proteins visualised by the rapid and sensitive staining method of Kumar et al. (1985). This blot was then used to locate precisely the bulk-protein bands remaining in the gel. Auxin-binding assays (see below) had identified activity with the protein band of highest mobility and this was excised and used for polyclonal antiserum production. The ABP content was estimated from scanning densitometry of the blot (transmitted light, Vitatron densitometer type MPS940.800) and the total protein content of the sample applied to the gel.

Antibody production. Rabbit polyclonal antiserum. Receptor-containing gel slices from native PAGE were homogenized in 1 ml of distilled water, emulsified with 1 ml of Freund's complete adjuvant and used to immunise a single female rabbit by multi-site subcutaneous and intradermal plus a single intramuscular injection. Two boosts were given similarly, but with Freund's incomplete adjuvant, at 17-d intervals. The first bleed was taken from the marginal ear vein 10 d later and further bleeds at 14-d intervals. An additional boost was given after the second bleed. Each immunisation used from 200–270 µg of ABP, derived from approx. 600 g of tissue. A pre-immune bleed was collected 6 d prior to the primary immunisation.

Monoclonal antibodies. Each of four female F344 rats was injected intramuscularly with 200 µl (approx. 100 µg) of post-Mono Q auxin-binding fraction in Freund's complete adjuvant. Similar boosts were given subcutaneously 40 d and 74 d later using Freund's incomplete adjuvant and an intravenous boost of 50 µg protein in saline was given 3 d prior to fusion. Sera were collected 10 d after each boost and screened for reaction against the auxin receptor by immunoblotting.

Fusions and subsequent hybrid-myeloma selection were carried out as described by Galfrè and Milstein (1981) except that azaserine was used in place of thymidine in the selection medium (Foung et al. 1982). Half the spleen cells were fused with cells of the rat myeloma line IR983F (Bazin 1982) and half with the mouse myeloma line NSO/unc (Galfrè and Milstein 1981). Initial screening of the culture supernatants was done by enzyme-linked immunosorbent assay (ELISA). Electroeluted pure ABP was diluted to 3 µg·ml^{-1} with 0.1 M $NaHCO_3$, pH 8.3 and 50 µl used to coat wells of 96-well, flat-bottomed ELISA plates (Nunc, from Gibco, Uxbridge, UK) at 4° C overnight. The coating solution was collected and could be reused. The plates were blocked with 10% foetal calf serum in 0.1 M $NaHCO_3$ pH 8.3 and 50 µl of culture supernatant tested for reaction to the auxin receptor following the method of Notton et al. (1985). Strong positives were further screened by immunoblotting of post-DEAE ABP preparations subjected to sodium dodecyl sulfate (SDS)-PAGE. Selected lines were cloned on soft agar. Antibody isotyping was done by Ouchterlony double diffusion with rat isotype-specific antisera.

Endoglycosidase-H digestion. An ABP preparation purified on Mono Q was separated by SDS-PAGE (see below), electroeluted and desalted (Sephadex G.25, Pharmacia) into citrate digestion buffer (20 mM trisodium citrate-acetic acid pH 5.5, 5 mM

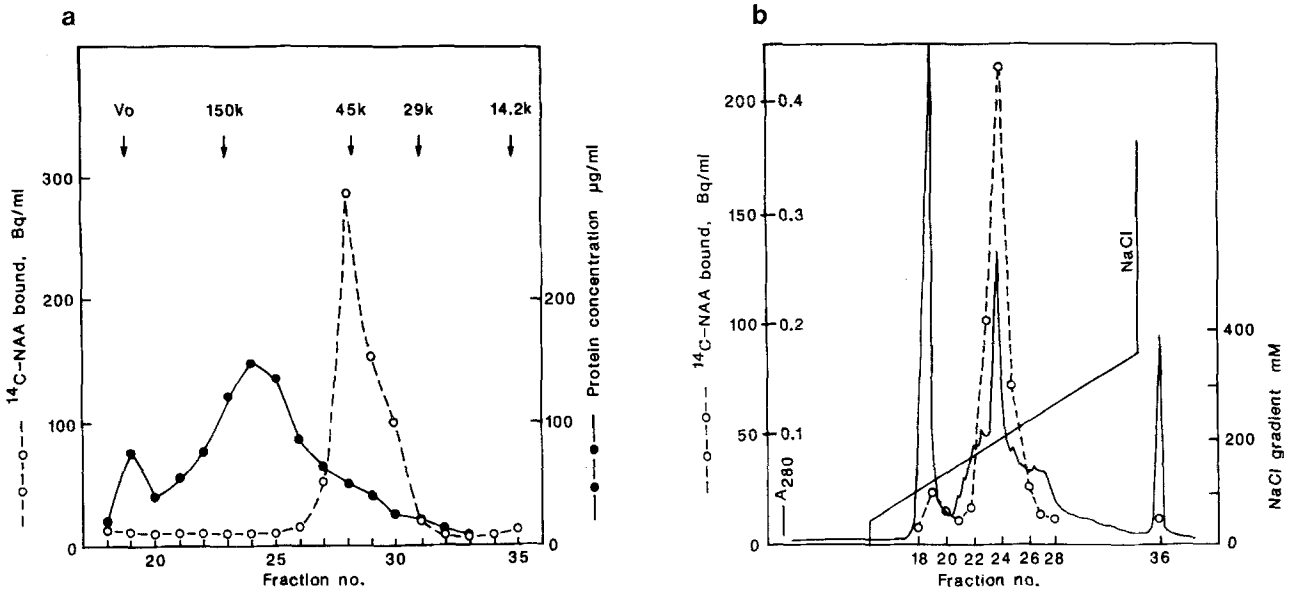

Fig. 1a, b. Purification of auxin-binding proteins from maize coleoptile microsomal membranes. Fractionation of 0.1 M NaCl eluate from DEAE Bio-Gel on Sephacryl S-200 (**a**), followed by Mono Q (**b**). Auxin-binding assays were done by ammonium-sulphate precipitation, and total NAA binding (including nonsaturable) is shown, *Arrows* in **a** indicate M_r markers (kDa). V_o = void volume

$MgSO_4$, 0.5 mM dithiothreitol, 0.5 mM PMSF). Endoglycosidase H (EC 3.2.1.96; Boehringer Mannheim, Lewes, UK) 0.1 mU, was added to approx. 20 µg of ABP and incubated at 23° C for 18 h. The reaction was stopped by adding an equal volume of 20% (w/v) trichloroacetic acid, the precipitate solubilised in SDS sample buffer and subjected to SDS-PAGE.

Tissue titrations. Microsomal membranes were prepared from various tissues of 5-d-old etiolated maize seedlings as described by Batt et al. (1976). Resuspended membranes, 5 g fresh weight equivalent·ml^{-1} were added to an equal volume of Laemmli (1970) sample buffer, 5 mM 6-amino-n-hexanoic acid and 1 mM 4-aminobenzamidine, and boiled for 3 min. Sequential threefold dilutions of these solutions were made in the same sample buffer. After SDS-PAGE and immunoblotting (see below), ABP content was quantitated by scanning densitometry.

Analysis by SDS-PAGE and immunoblotting. The SDS-PAGE was carried out in 12% gels of 1.5 mm thickness using the buffer system of Laemmli (1970). Molecular-weight markers were the Dalton Mark VII range of Sigma. Electrophoretic blotting to nitrocellulose was performed at 4° C, 100 mA overnight, in the pH 9.9 buffer system of Dunn (1986) after first incubating the gel in 50 mM Tris-HCl pH 7.4 for 30 min. Blots were stained either for total protein (Kumar et al. 1985), for glycoprotein (Faye and Chrispeels 1985) or immunochemically as follows. The nitrocellulose was washed 2 × 5 min in Tris-buffered saline (TBS)-Tween (10 mM Tris-HCl pH 7.4, 140 mM NaCl, 0.1% v/v Tween 20), then incubated for 45 min at room temperature with gentle agitation in primary antibody diluted in TBS-Tween (rabbit serum 1:1000; culture supernatant 1:10). The blot was washed 5 × 5 min in TBS-Tween before incubation for 45 min in peroxidase-labelled second antibody (anti-rabbit IgG or anti-rat IgG+IgM) diluted 1:1000 or 1:2000, respectively, in TBS-Tween. After washing 7 × 5 min in TBS-Tween and 1 × 5 min in TBS, colour was developed by incubation for 5–15 min in freshly prepared substrate (53 mg 4-chloro-1-naphthol in 5 ml methanol plus 95 ml water and 60 µl of 100 volume hydrogen peroxide).

Auxin-binding assays. Binding of [1-^{14}C]NAA by solubilised fractions was carried out in citrate-acetate buffer pH 5.5 (Batt et al. 1976) by one of three methods (described in Venis 1984) depending on the speed required: equilibrium dialysis (slowest), ammonium-sulphate precipitation, or centrifugal ultrafiltration (fastest).

Binding activity was located on native polyacrylamide gels as follows. After electrophoresis and partial blotting, gel slices were excised and each was finely crushed in 1.5 ml of citrate-acetate buffer pH 5.5. After standing for 1 h in ice with occasional mixing, the liquid was removed carefully from the crushed gel and filtered through a small cotton-wool plug in a Pasteur pipette, followed by a 200-µl wash. The extract was cleared of remaining fine gel fragments by centrifugation (12000·*g*, 5 min) and desalted to citrate-acetate buffer pH 5.5 on a Sephadex G25 column. Duplicate aliquots were assayed for auxin binding by equilibrium dialysis.

Results

Purification of ABP. The auxin-binding peak from solubilised maize microsome membranes elutes from Sephacryl S-200 close to the position of ovalbumin (45 kDa Fig. 1a), as expected from earlier gel-filtration data, with a purification of six- to eightfold. Fractionation of the active Sephacryl eluate on Mono Q (Fig. 1b) generally provides a further eight- to tenfold enrichment, though recovery of activity in this particular run was unusually low. Monitoring of the Mono Q elution profile by SDS-PAGE (Fig. 2) reveals a predominant 22-kDa

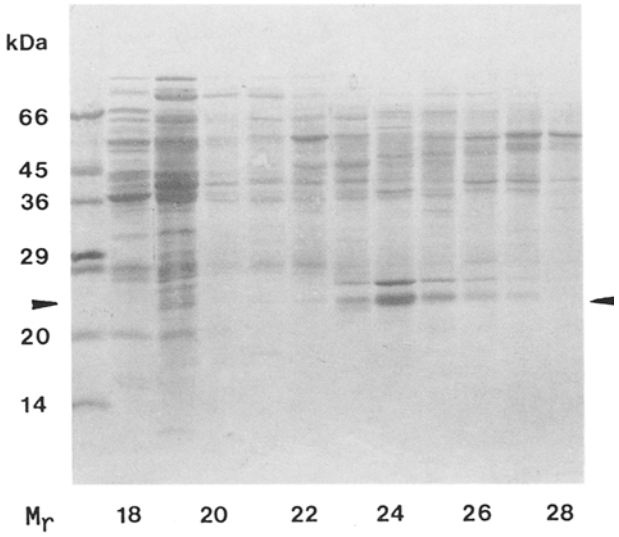

Fig. 2. Analysis by SDS-PAGE of the maize microsomal membrane proteins separated by Mono Q. From each of the fractions collected in Fig. 1b, 70 µl were run out on SDS-PAGE, transferred to nitrocellulose and stained for total protein. *Arrowhead* indicates the 22-kDa band

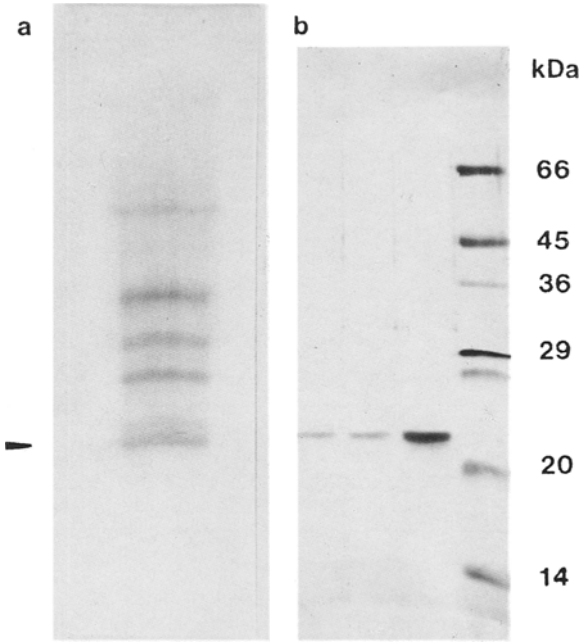

Fig. 3. a Non-dissociating PAGE blots of pooled auxin-binding fractions from Mono Q chromatography. *Arrowhead* indicates the native band excised for immunisation. **b** SDS-PAGE blots of the excised native gel bands from three different preparations. M_r markers at *right*

polypeptide at the binding peak (fraction 24, 195 mM NaCl). A minor band at 21-kDa elutes slightly earlier (fraction 23, 190 mM NaCl) and is consistently associated with the major 22-kDa species. Fractionation of mesocotyl extracts (not shown) yields a similar pattern. An example of ABP purification is shown in Table 1.

When the active post-Mono Q eluate is resolved by native PAGE, auxin-binding assays (data not shown) show that activity is associated only with the protein region of highest mobility (arrowed in Fig. 3a). On dissociation by SDS-PAGE this band runs as a single polypeptide at 22 kDa (Fig. 3b).

Antibody production. Gel slices from native PAGE containing pure ABP were used to generate a polyclonal antiserum. When this antiserum was used to probe nitrocellulose blots of a post-DEAE ABP fraction after SDS-PAGE, a band at 22 kDa was selected (Fig. 4). On the original blot, reaction was still readily detectable at a loading equivalent to 45 mg fresh weight of coleoptile tissue and, by direct dissociation of membrane pellets in SDS sample buffer prior to electrophoresis, ABP equivalent to 3 mg of tissue can be detected (data not shown). A distinct reaction with immune serum is still obtained at a dilution of 1:15000 on blots, whereas pre-immune serum is completely unreactive at dilutions of 1:25 and greater (data not shown). Although not resolved in Fig. 4, other blots show that the 21-kDa minor band is also recognised by the serum. In addition, the antiserum reacts specifically with the blotted ABP band after native PAGE (not shown).

Table 1. Example of purification of ABPs from maize coleoptile microsomal membranes. Auxin binding was determined at 0.125 µM [^{14}C]NAA by ammonium-sulphate precipitation (Venis 1984). Non-saturable binding (determined in the presence of 25 µM unlabelled NAA) was subtracted from total [^{14}C]NAA binding to give saturable binding

Sample	Total protein (mg)	Saturable binding (pmol [^{14}C]NAA)	Yield (%)	Specific activity (pmol·mg^{-1})	Purification (-fold)
Solubilised membrane extract	29.7	2186	100	73.6	
Post-DEAE Bio-Gel	12.4	2171	99	175.1	2.4
Post-Sephacryl	0.499	571	26	1144	15.5
Post-Mono Q	0.041	374	17	9122	124

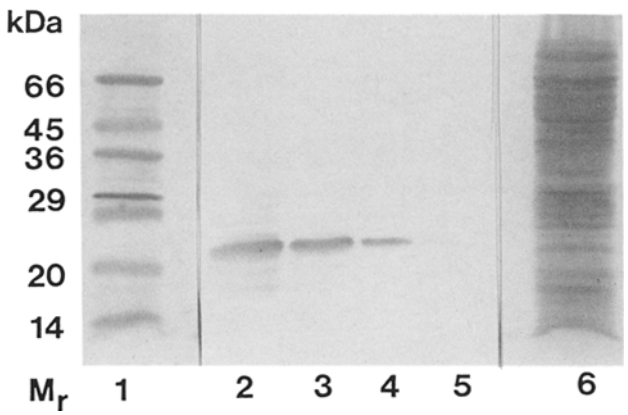

Fig. 4. Blots obtained by SDS-PAGE of post-DEAE fraction (*lanes 2–6*) or M_r markers (*lane 1*). Blot stained for total protein (*lanes 1, 6*) or probed with rabbit anti-ABP polyclonal antiserum (*lanes 2–5*). Loadings equivalent to 1500 mg (*lanes 2, 6*), 450 mg (*lane 3*), 150 mg (*lane 4*) or 45 mg (*lane 5*) of maize coleoptile tissue

Post-Mono Q fractions (e.g. fraction 24 in Fig. 1b) were used to immunise rats to raise monoclonal antibodies (mAbs). Selection of hybridomas and clones was done by enzyme-linked immunosorbent assay (ELISA) using pure ABP preparations electroeluted after native PAGE. Figure 5 shows an immunoblot of a post-DEAE eluate resolved on SDS-PAGE and probed with the five mAbs raised. Two of the mAbs, MAC 256 and MAC 259 recognise only the 22-kDa polypeptide, while the other three recognise in addition the minor 21-kDa polypeptide. The mAb isotypes are shown on the figure.

Digestion with endoglycosidase H. In order to determine whether the mAbs recognise the polypeptide and not the glycan of this glycoprotein (Löbler et al. 1987) the ABP was digested with endoglycosidase H (Fig. 6a) which cleaves N-linked oligosaccharide side chains from glycopeptides between the two β-1,4-linked N-acetylglucosamine residues of the chitobiose core (Tarentino and Maley, 1974). As the digestion progresses the 22-kDa receptor band disappears to yield a single product, running at 20-kDa, consistent with the removal of one glycan unit of M_r approx. 2 kDa. This is confirmed in complementary tracks probed with concanavalin A which binds only to the 22-kDa band. In Figure 6b the endoglycosidase digestion has been allowed to go to completion and both glycosylated and deglycosylated ABP probed with mAbs and polyclonal serum after SDS-PAGE. More ABP was loaded onto the deglycosylated lanes than onto control lanes. Densitometer traces (not shown) of lanes 1 and 2, (control and deglycosylated, respectively, stained for total protein), show that after digestion there is approximately twice as much protein in the deglycosylated bands as in the control bands. The staining intensity of the deglycosylated ABP is at least twice the intensity of the control for the mAbs and the polyclonal serum, and considerably more for MAC 256 and MAC 258. MAC 257, which reacts with both the 22- and 21-kDa polypeptides (Fig. 5), also detects a minor band about 1 kDa smaller than the major 20-kDa deglycosylated ABP polypeptide, indicating that the 21-kDa polypeptide has also been deglycosylated with a reduction in M_r of approx. 2 kDa.

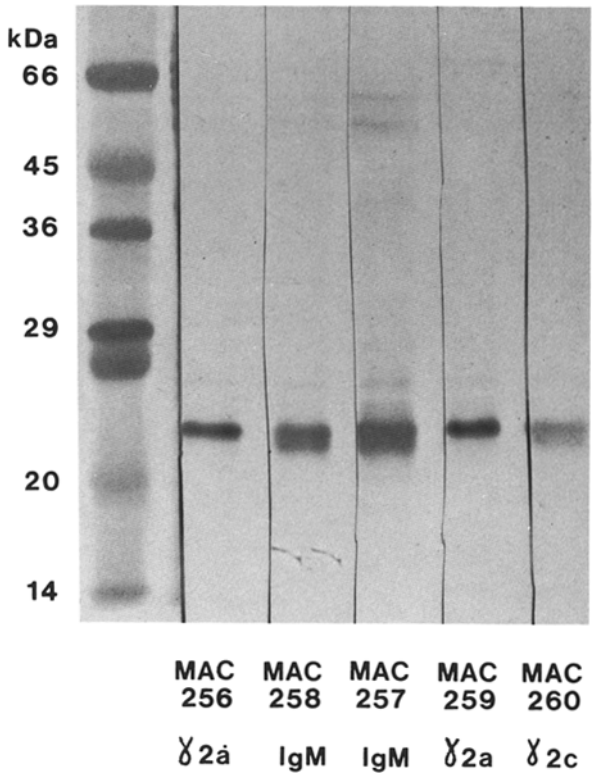

Fig. 5. Blot obtained by SDS-PAGE of post-DEAE fraction probed with mAbs. Protein equivalent to 420 mg tissue was loaded per track. M_r markers at *left*. The mAb isotype is shown below each track

Tissue receptor titration. The mAbs have been used to quantify the ABP present in various tissues of etiolated maize seedlings. Membranes from the coleoptile, enclosed leaf, mesocotyl and roots were prepared and a number of threefold dilutions subjected to SDS-PAGE and immunoblotted using MAC 257 (Fig. 7). Although MAC 257 will recognise both 22- and 21-kDa ABP polypeptides (Fig. 5) only a single band of M_r 22 kDa was detected in all four tissues. The ABP is most abun-

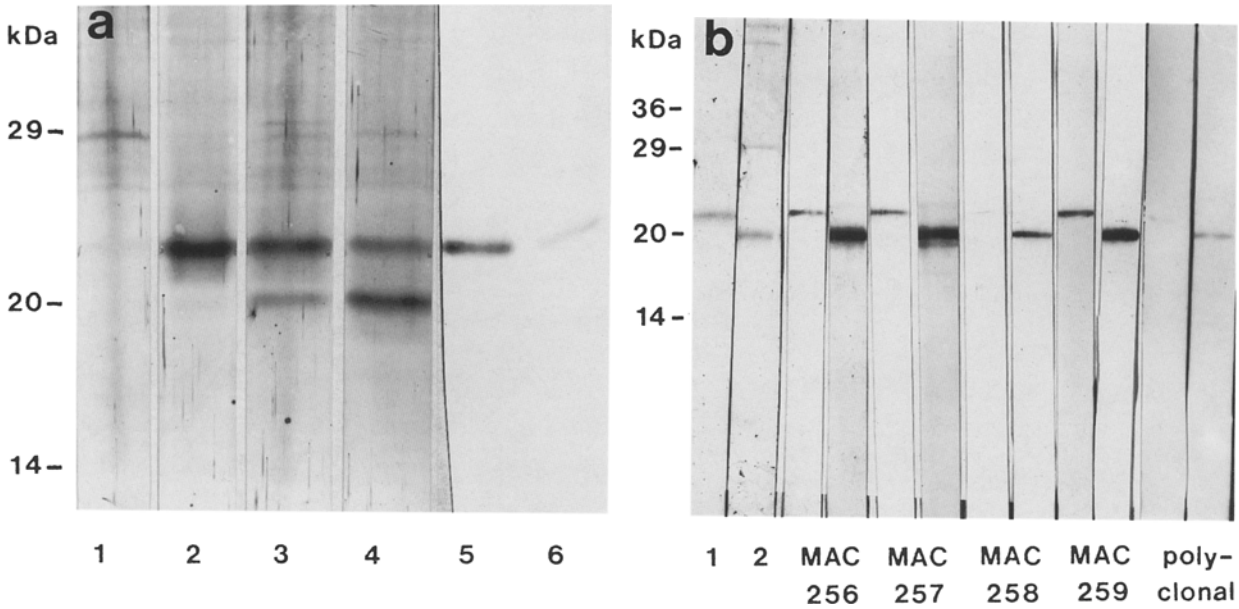

Fig. 6a, b. Endoglycosidase-H digestion of the ABP from maize coleoptile microsomal membranes visualised by SDS-PAGE and protein blotting. **a** Approximately 2 μg of purified ABP (*track 2*) and 0.3 mU of endoglycosidase H (*track 1*) were loaded for each sample. Digestion was at 15° C for 3 h (*tracks 3, 5*) or 20 h (*tracks 4, 6*). *Tracks 1–4* have been stained for total protein, *tracks 5, 6* for glycoprotein. (**b**) Digestion at 23° C for 18 h. Undigested ABP (approx. 0.7 μg) in *track 1* and *alternate tracks*, digested ABP (approx. 2 μg) in the *remaining tracks*. *Tracks 1* and *2* stained for total protein with colloidal gold (Aurodye; Janssen, Maidenhead, UK), the *remaining tracks* with antibody as indicated

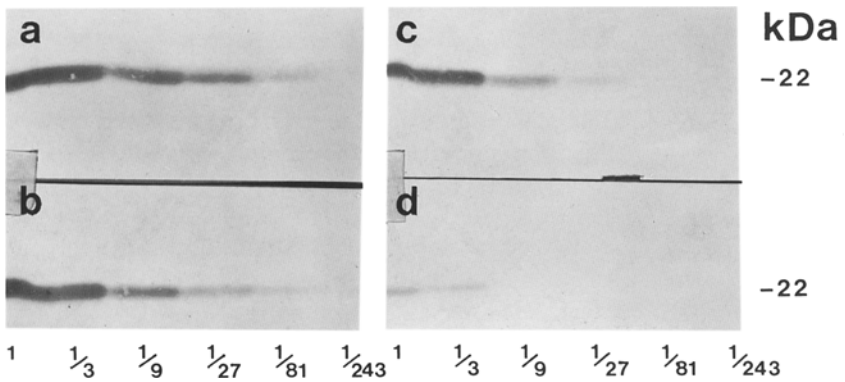

Fig. 7a–d. Blot obtained by SDS-PAGE of serial threefold dilutions of membrane proteins from maize coleoptiles (**a**), leaves (**b**), mesocotyls (**c**) and roots (**d**). The most concentrated *track 1* of each titration is loaded with protein from 320 mg tissue except for leaves in which 195 mg was loaded. Protein loading in these tracks was **a** 332 μg, **b** 299 μg, **c** 280 μg and **d** 179 μg

dant in coleoptile membranes, leaf membranes containing 65%, mesocotyls 25% and roots 2.5% of the ABP concentration of coleoptiles, on a fresh-weight basis. If calculated per milligram of membrane protein these differences remain broadly unchanged except that in roots the relative concentration of ABP rises to 4.5% of that of the coleoptile. Similar relative concentrations are obtained if MAC 256 or the polyclonal serum are used. These data are in broad agreement with the auxin-binding data presented by Ray et al. (1977) for these tissues.

Discussion

The column purification procedure described provides a reproducible and straightforward procedure for obtaining highly enriched ABP preparations from maize membranes. After the Mono Q step, ABP purity ranges from 40–80%, depending largely on how selectively the chromatographic peaks are cut. Such preparations were used to immunise rats in order to produce monoclonal antibodies. This high degree of enrichment was dictated by the earlier failure to generate a suitable anti-

genic response using preparations of lower (approx. 10%) purity. Native PAGE provided pure ABP (Fig. 3) and an excellent rabbit polyclonal antiserum was produced.

Löbler and Klämbt (1985) reported a single 20-kDa polypeptide by SDS-PAGE of their ABP preparations. Our findings are more in accord with those of Shimomura et al. (1986), who found major 21-kDa and minor 20-kDa subunits. In our hands, both major and minor bands migrate more slowly than the 20-kDa standard (soybean trypsin inhibitor) and correspond to M_rs of approx. 22 and 21 kDa, respectively. We agree with Shimomura et al. (1986) that the minor species elutes fractionally ahead of the major protein on gradient anion-exchange chromatography (Fig. 2). Since the peak of auxin-binding activity corresponds to a native molecular mass close to 45 kDa (Fig. 1a), we conclude that the major ABP species is a dimer of 22-kDa subunits. Rapid ABP separation by SDS-PAGE in conditions designed to inhibit proteolysis yields only the 22-kDa polypeptide (Fig. 7), but the two polypeptides show strong similarities in terms of chromatographic properties (Fig. 2), glycosylation (Fig. 6b) and immunoreactivity (Fig. 5). It is likely, therefore, that the 21-kDa species is a breakdown product of the 22-kDa subunit. We assume that MAC 256 and MAC 259, which recognise only the 22-kDa polypeptide, can be mapped to a terminal epitope within or substantially within the 1-kDa peptide that is lost from the 22-kDa species. The two sets of mAbs will allow us to examine the relationship of these two ABP polypeptides more closely.

Löbler et al. (1987) showed that the maize ABP is glycosylated, as are many membrane-bound proteins. By removal of the oligosaccharide side-chain with endoglycosidase H, followed by immunoblotting, we have shown that all five monoclonal antibodies recognise the deglycosylated polypeptide at least as effectively as they recognise the glycosylated form. Two monoclonals (MAC) show apparently higher affinity for deglycosylated ABP as determined by the intensity of staining on the blot (Fig. 6b). The polyclonal serum also exhibits approximately equal affinity for glycosylated and deglycosylated forms.

Auxin-binding sites, possibly with differing kinetic properties, appear to be present in more than one class of cellular membrane in maize (see Venis, 1985 for a full discussion), but it is not known whether more than one ABP species is involved. We now aim to use the polyclonal and monoclonal antibodies to address this question as well as for immunopurification and as probes of putative receptor structure and function in maize and in other species.

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