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Enzymic synthesis of 1-*O*-(indol-3-ylacetyl)-β-D-glucose

Purification of the enzyme from *Zea mays*, and preparation of antibodies to the enzyme

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The enzyme indol-3-ylacetylglucose synthase (UDP-glucose:indol-3-ylacetate β-D-glucosyltransferase) catalyses the reaction:

Indol-3-ylacetic acid + UDP-glucose ⇌ 1-*O*-(indol-3-ylacetyl)-β-D-glucose + UDP

This is the first step in the series of reactions leading to the indol-3-ylacetic acid conjugates found in maize. Previous attempts to purify this enzyme from the liquid endosperm of kernels of *Zea mays* (sweet corn) were not entirely successful owing to the lability of partially purified preparations during column chromatography. Thus this enzyme has not previously been purified to homogeneity. During the present study it was found that retention of enzyme activity required the combined presence of glycerol and dithiothreitol. Adding these requirements permitted purification of the enzyme to homogeneity with retention of catalytic activity. These purified preparations were used for preparation of rabbit polyclonal antibodies to the enzyme. Antibodies to the *Zea mays* endosperm enzyme cross-react with the enzyme from *Zea mays* vegetative tissues and with an enzyme from the liquid endosperm of oak acorns (*Quercus* sp). In this paper we report a simplified purification procedure adaptable to the preparation of milligram amounts of the enzyme.

INTRODUCTION

Kernels of *Zea mays* (sweet corn) contain 99% of the growth hormone indol-3-ylacetic acid (IAA) as ester conjugates (Cohen & Bandurski, 1982) and less than 1% as the free acid, which is presumed to be the physiologically active form (Bandurski *et al.*, 1990). The first step in the synthesis of the ester conjugates is the formation of the acyl alkyl acetal 1-*O*-(indol-3-ylacetyl)-β-D-glucose (IA-glucose) by means of the reaction:

$$\text{IAA} + \text{UDP-glucose} \rightleftharpoons \text{IA-glucose} + \text{UDP} \quad (1)$$

The equilibrium of reaction (1) is unfavourable (Leznicki & Bandurski, 1988*a*,*b*; Kowalczyk & Bandurski, 1990) and is followed by the energetically favourable transacylation of IAA to *myo*-inositol (Michalczuk & Bandurski, 1982):

$$\text{IA-glucose} + \textit{myo}\text{-inositol} \rightleftharpoons \text{IA-}\textit{myo}\text{-inositol} + \text{glucose} \quad (2)$$

Reaction (2) is followed by a second energetically favourable reaction, the glycosylation of IA-*myo*-inositol to form the IA-*myo*-inositol glycosides characteristic of maize (Corcuera *et al.*, 1982; Corcuera & Bandurski, 1982).

The unusual transacylation reaction from IA-glucose to form IA-*myo*-inositol was first observed by Michalczuk & Bandurski (1980), and slightly later the same mechanism was observed by Tkotz & Strack (1980) for the synthesis of sinapoyl-L-malate by transfer of the sinapoyl group from 1-sinapoylglucose to L-malate. Strack & Gross (1990) have summarized two mechanisms for the synthesis of glucose esters by plants. They are transacylation from an acyl-glucose, as described above, or via an acyl-CoA (Kopcewicz *et al.*, 1974).

Growing vegetative tissues of maize, although limited in growth rate by unesterified IAA, contain 90% of their IAA as ester conjugates (Bandurski *et al.*, 1990). Thus understanding the mechanism of IAA conjugation and the regulation of the relative amounts of free and conjugated IAA is important in understanding the hormonal control of plant growth.

In the present paper we describe a five-step procedure for the extraction and purification of IA-glucose synthase adaptable to the processing of large amounts of maize liquid endosperm, and yielding a homogeneous product, 580-fold purified, and in 22% yield from the crude endosperm homogenate.

The involvement of IAA ester conjugates in transport of the hormone from kernel to shoot and in regulating hormone concentrations has been studied (Bandurski *et al.*, 1977, 1990; Nowacki & Bandurski, 1980). The availability of an antibody to the enzyme catalysing the first step in the synthesis of the conjugates may contribute to localizing the enzyme in plant tissues and thus to understanding how plant hormone concentrations are controlled and how the tip of the plant regulates plant growth (Ciesielski, 1872; Darwin, 1880).

EXPERIMENTAL

Plant material

Ears of sweet corn (var. Seneca) were purchased from the Gascon Farms (Fowlerville, MI, U.S.A.) at the table-ready stage and chilled to 4 °C, and the liquid endosperm was extracted from the kernels. This was accomplished by cutting the kernels with a razor blade and extruding the liquid against the lip of a beaker (Leznicki & Bandurski, 1988*b*). After filtering through cheesecloth, the liquid endosperm was stored in freezer bags in 300 g lots at −20 °C (with 50% loss of activity after 1 year), or at −85 to −195 °C for periods of greater than 1 year (without loss of activity).

Reagents

Alkaline-phosphatase-conjugated anti-(rabbit IgG) antibody, 5-bromo-4-chloroindol-3-yl phosphate, EDTA, Freund's

Abbreviations used: IAA, indol-3-ylacetic acid; IA-glucose synthase, 1-*O*-(indol-3-ylacetyl)-β-D-glucose synthase (UDP-glucose:indol-3-ylacetate β-D-glucosyltransferase, EC 2.4.1.121).

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adjuvants (complete and incomplete), γ-globulins, glycine, IAA, UDP-glucose, molecular-mass standards (MW-SDS-70L), nitrocellulose membrane (0.2 μm pore size), phenylmethanesulphonyl fluoride, polyvinylpyrrolidone, Protein A–Sepharose CL-4B and Sepharose 4B were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); dithiothreitol, Hepes, Tris and glycerol were from Boehringer Mannheim Corp. (Indianapolis, IN, U.S.A.); Coomassie Brilliant Blue G-250, all chemicals for PAGE and a silver stain kit were from Bio-Rad Laboratories (Richmond, CA, U.S.A.); DEAE-Sephadex A-25 and DEAE-Sephacel were from Pharmacia (Gaithersburg, MD, U.S.A.); Pansorbin cells and Miracloth were from Calbiochem (San Diego, CA, U.S.A.); indol-3-yl[2-^{14}C]acetic acid (50 mCi/mmol) was from Amersham (Arlington Heights, IL, U.S.A.); TSK-Gel Toyopearl DEAE-650M was from Supelco (Bellefonte, PA, U.S.A.); octyl-agarose was from P–L Biochemicals (Milwaukee, WI, U.S.A.); glycerol was from Baker Scientific Co. (Phillipsburg, PA, U.S.A.); Safety Solve scintillant was from Research Products International (Mount Prospect, IL, U.S.A.). Blue Sepharose with a low degree of substitution was synthesized according to the method of Bohme *et al.* (1972).

Analytical methods

Protein was determined by the Bradford (1976) method, with globulins as a standard. Radioactivity was determined with a Beckman LS-7000 scintillation counter. Mass spectrometry was performed with a Hewlett–Packard 5970 instrument coupled to a 5890 g.l.c. apparatus.

Enzyme homogeneity and determination of molecular mass

Native gel electrophoresis was used to verify the effectiveness of the purification procedures. PAGE in an 8% slab gel was with a vertical slab electrophoresis apparatus (BRL model V-16) by the method of Ogita & Markert (1979) and SDS/PAGE was in the same apparatus. Protein bands were detected by using the Bio-Rad Silver Stain Kit. Molecular mass was determined from a graph of distance migrated versus the logarithm of the molecular masses of standard proteins (Sigma-MW-SDS-70L) and by column chromatography according to the procedure of Andrews (1965).

Assay of enzyme activity

The standard reaction mixture (0.5 ml final volume) contained 4 mM-IAA and 0.05 μCi of indol-3-yl[2-^{14}C]acetic acid (50 mCi/mmol), 5 mM-UDP-glucose, 0.1 mM-dithiothreitol and 50 mM-Hepes/NaOH buffer, pH 7.4. The reaction was started by the addition of 50 μl of enzyme solution. After a 30 min incubation at 30 °C the reaction was stopped by the addition of 1 ml of propan-2-ol. If necessary, proteins were removed by heating samples at 80 °C for 1 min and centrifugation after cooling. Then 1 ml of supernatant solution was transferred to a 1.5–2.0 ml-bed-volume DEAE-Sephacel (acetate) column in 50% (v/v) propan-2-ol. The uncharged reaction product was eluted with 5 ml of aq. 50% (v/v) propan-2-ol, and 1 ml of that eluate was used for determination of radioactivity in a Beckman LS-7000 scintillation counter. The non-ionic product was previously shown to be pure IA-glucose by g.l.c.–m.s. (Michalczuk & Bandurski, 1982; Leznicki & Bandurski, 1982*b*) by comparison with a synthetic standard (Keglevic & Pokorny, 1969; Ehmann, 1974).

Production of antiserum

Antiserum against the purified IA-glucose synthase was prepared by injecting 177 μg of purified protein in 200 μl of 0.9% NaCl emulsified with 0.8 ml of Freund's complete adjuvant subcutaneously into two rabbits. After 9 weeks a first booster injection of 174 μg and after 11 weeks a second booster injection of 186 μg of purified protein in 1 ml of incomplete Freund's adjuvant were given. Then 12 days after the last injection blood was taken from the ear and tested for the presence of anti-(IA-glucose synthase) antibodies by using an immuno-inactivation method (see below).

Purification of IgG

After collection the blood was clotted by incubation at 37 °C for 45 min. Serum was removed from the clot and centrifuged at 10000 *g* for 10 min and stored in 5 ml portions at −20 °C. A Protein A–Sepharose column was used for antibody purification essentially as described by Harlow & Lane (1988). Serum collected from the same animals before immunization was purified as above and used as a control.

Immuno-inactivation of IA-glucose synthase

The effect of purified IgG on IA-glucose synthase activity was determined in a reaction mixture containing 0.1% BSA, 50 mM-Tris/HCl buffer, pH 7.6, and 6.1 μg of IA-glucose synthase (50 μl volume). Into each sample 50 μl of IgG (from 3.9 to 254 μg of protein) was added and, after incubation at room temperature for 45 min, 100 μl of 10% (w/v) Pansorbin cells was added. The samples were kept at 4 °C for 30 min with gentle shaking and then centrifuged at 10000 *g* for 5 min. Residual IA-glucose synthase activity in the supernatant solution was determined as described above. In control samples pre-immune IgG (from 7 to 56 μg per sample) was added.

Protein blotting

Crude extracts of different tissues were prepared by homogenizing 2–5 g portions of tissue in twice their weight of extraction buffer [25 mM-Tris/HCl buffer, pH 7.8, containing 1 mM-EDTA, 2 mM-dithiothreitol, 0.5 mM-phenylmethanesulphonyl fluoride and 2% (w/v) polyvinylpyrrolidone]. The homogenates were centrifuged at 13000 *g* for 30 min and the supernatant solutions were precipitated with sufficient $(NH_4)_2SO_4$ to adjust the concentration to 85% saturation. The precipitated proteins were centrifuged (13000 *g* for 20 min) and, after suspension of the pellet in 0.5 ml of 25 mM-Tris/HCl buffer, pH 7.3, containing 1 mM-dithiothreitol and 20% (v/v) glycerol, were dialysed against the same buffer.

Proteins were resolved on 8% polyacrylamide gel with a 4% stacking gel according to the procedure of Ogita & Markert (1979) in a Mini-Protean II cell (Bio-Rad Laboratories). Partially purified IA-glucose synthase from corn endosperm and from 2-day-old corn seedlings [after 15%-poly(ethylene glycol) and DEAE-Sephacel chromatography] were used for SDS/PAGE in a 12% polyacrylamide gel with a 4% stacking gel. Separated proteins or peptides were transferred overnight to nitrocellulose membranes in a Bio-Rad Mini Trans-Blot cell by using wet electrophoretic transfer. Transfer, blotting and detection with alkaline phosphatase were as described by Harlow & Lane (1988).

RESULTS

Liquid endosperm preparation and fractionation with poly(ethylene glycol) 6000

For most preparations a 300 g portion of frozen endosperm was homogenized with 450 ml of 25 mM-Tris/HCl buffer, pH 7.6, containing 2 mM-EDTA, 2 mM-dithiothreitol, 0.5 mM-phenylmethanesulphonyl fluoride and 2% (w/v) polyvinylpyrrolidone.

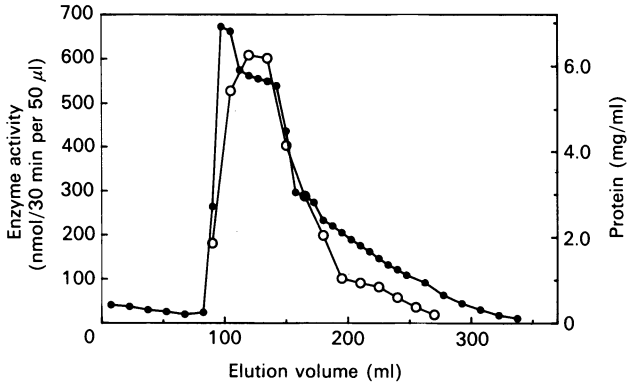

Fig. 1. Elution profile of proteins not precipitated by 15% poly(ethylene glycol) from a DEAE-Sephacel column

The proteins were first eluted with 82 ml of equilibrating buffer and then with equilibrating buffer containing 0.2 M-NaCl. IA-glucose synthase activity (○) (Kowalczyk & Bandurski, 1990) was determined as described in the Experimental section and protein concentration (●) was determined by Coomassie Blue binding.

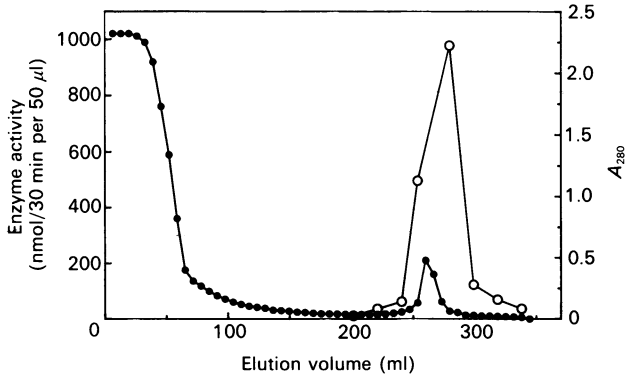

Fig. 2. Elution profile of IA-glucose synthase from a Blue Sepharose column

After dilution, the active fractions from the DEAE-Sephacel column were applied to a Blue Sepharose column and eluted with 150 ml of equilibrating buffer. Remaining proteins were then eluted with that buffer but also containing 0.5 M-NaCl. IA-glucose synthase activity (○) was determined as described in the Experimental section and protein (●) was determined spectrophotometrically at 280 nm.

The homogenate was filtered through Miracloth and the filtrate, after pH adjustment to 7.6, was centrifuged at 13000 *g* for 40 min (step I). To 2 vol. of the supernatant solution, 1 vol. of 45% (w/v) poly(ethylene glycol) 6000 in 25 mM-Tris/HCl buffer, pH 7.6, containing 2 mM-dithiothreitol, 1 mM-EDTA and 0.2 mM-phenylmethanesulphonyl fluoride was added slowly with stirring to obtain a 15% (w/v) final concentration of poly(ethylene glycol). The mixture was left to stand for 2 h in a cold-room, and after centrifugation 760 ml of clear supernatant fluid was obtained (step II). The increase in specific activity (Table 1) is partially due to an increase in enzyme units after poly(ethylene glycol) fractionation.

DEAE-Sephacel chromatography

The supernatant fluid was applied to a 5 cm × 12 cm DEAE-Sephacel column equilibrated with 25 mM-Tris/HCl buffer, pH 7.6, containing 0.1 mM-EDTA and 1 mM-dithiothreitol. The column was washed with the same buffer but also containing

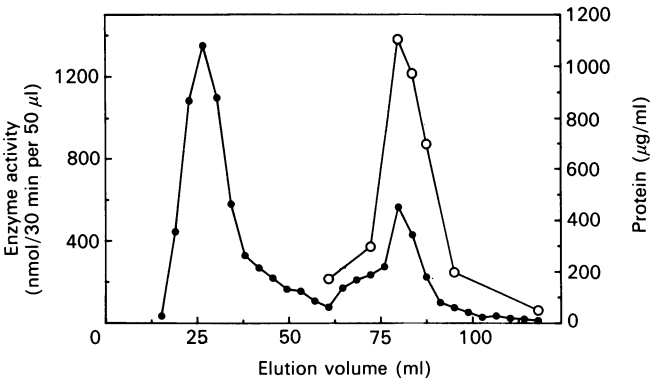

Fig. 3. Hydrophobic-interaction chromatography of IA-glucose synthase on an octyl-agarose column

The concentrated fractions from Blue Sepharose chromatography were applied to an octyl-agarose column in 0.8 M-NaCl. Weakly retained proteins were eluted with the equilibrating buffer containing 0.8 M-NaCl. Proteins retained by the column were eluted with 25 mM-Tris/HCl buffer, pH 7.6, containing 1 mM-dithiothreitol and 20% (v/v) glycerol. IA-glucose synthase activity (○) was eluted at 68–114 ml and was determined as described in the Experimental section after a 10-fold dilution to lower the NaCl concentration. Protein concentration (●) was determined by Coomassie Blue binding.

20% (v/v) glycerol, and finally the adsorbed proteins were eluted with 200 mM-NaCl in the glycerol-containing buffer. The flow rate was 0.8 ml/min, and 7.5 ml fractions were collected (Fig. 1). Fractions containing enzyme activity (nos. 11–36) were combined (step III).

Blue Sepharose chromatography

The combined fractions were diluted with 25 mM-Tris/HCl buffer, pH 7.6, containing 1 mM-dithiothreitol and 20% (v/v) glycerol and applied to a 2.5 cm × 38 cm Blue Sepharose column with a low dye loading and at a flow rate of 50 ml/h. The column was washed with equilibrating buffer [25 mM-Tris/HCl buffer, pH 7.6, containing 1 mM-dithiothreitol, 20% (v/v) glycerol and 0.1 M-NaCl], and the adsorbed proteins were eluted with 0.5 M-NaCl in equilibrating buffer as shown in Fig. 2. Active fractions (228–325 ml) were combined and concentrated to 3–4 ml by ultrafiltration with an Amicon Diaflo YM-10 filter (step IV).

Octyl-agarose chromatography

The enzyme concentrate was adjusted to 0.8 M-NaCl by adding 1.5 M-NaCl and applied to an octyl-agarose column (1.5 cm × 7 cm). The column was equilibrated with 25 mM-Tris/HCl buffer, pH 7.3, containing 1 mM-dithiothreitol, 20% (v/v) glycerol and 0.8 M-NaCl and washed with 50 ml of the equilibrating solution. The protein was then eluted with equilibrating solution without NaCl (Fig. 3). Fractions collected at 68–114 ml were combined and diluted so as to lower the NaCl concentration to 0.1 M (step V).

Toyopearl DEAE-650M chromatography

The eluate, after dilution with 25 mM-Tris/HCl buffer, pH 7.3, containing 1 mM-dithiothreitol and 20% (v/v) glycerol to an NaCl concentration below 0.1 M, was applied to a column of Toyopearl DEAE-650M equilibrated with 25 mM-Tris/HCl buffer, pH 7.3, containing 1 mM-dithiothreitol and 20% (v/v) glycerol. The column was washed with buffer, and the enzyme was eluted with a linear gradient of 100 ml of equilibrating buffer in the mixing chamber and 100 ml of the same buffer containing 0.15 M-NaCl in the reservoir. The flow rate was 0.6 ml/min, and

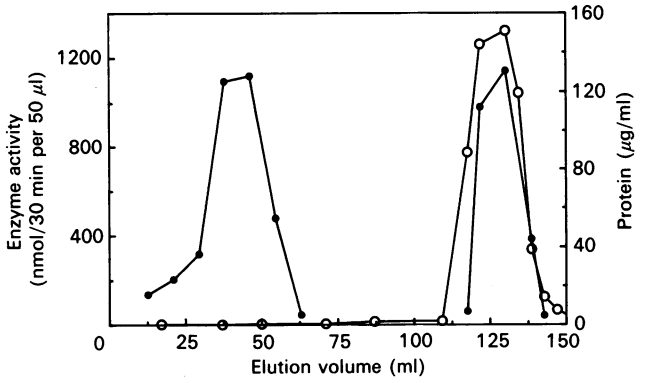

Fig. 4. Ion-exchange chromatography of IA-glucose synthase on Toyopearl DEAE-650M

The active fractions after octyl-agarose chromatography were combined and diluted to lower the NaCl concentration below 0.1 M and applied to a Toyopearl DEAE-650M column (1.5 cm × 12 cm). IA-glucose synthase activity (○) was eluted with a linear gradient of 0–0.15 M-NaCl with the peak activity emerging at 0.12 M-NaCl. No activity was detected until 110 ml of elution volume. Protein concentration (●) was determined by the Coomassie Blue method.

4.2 ml fractions were collected (Fig. 4). The active fractions (113–155 ml), after concentration to about 0.5 ml by Amicon YM-10 ultrafiltration, were mixed with glycerol to 70% (v/v) and stored at −20 °C (step VI).

A typical purification procedure is shown in Table 1. The specific activity of the final preparation increased 580-fold with 21% recovery of enzyme units. Native gel electrophoresis of a step VI preparation showed two diffuse bands when stained with silver reagent. A final preparation showing but a single band was obtained by gel filtration on a Zorbax GF-250 h.p.l.c. column (duPont). The column was equilibrated with 100 mM-Tris/HCl buffer, pH 8.0, containing 1 mM-dithiothreitol and eluted at a flow rate of 1 ml/min under 3 MPa (30 atm) pressure. A 280 nm-absorbing band possessing IA-glucose synthase activity was observed at 13.7 min. Since Zorbax chromatography was performed in the absence of glycerol some protein aggregation occurred, resulting in a very small peak at 8 min. Upon storage, further aggregation occurred, and the broad band at 8 min intensified and a second sharp peak appeared that was eluted at 10.3 min. The 10.3 min peak may be formed by disulphide bond formation since chromatography on Zorbax in the absence of dithiothreitol yielded the 10.3 min peak in 80–90% abundance with total disappearance of the 13.7 min peak. The peak at 10.3 min corresponds to a dimer with molecular mass equal to 100 kDa.

The enzyme, together with four marker proteins (BSA, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase and carbonic anhydrase), was chromatographed on the Zorbax column. The estimated molecular mass for the enzyme was 52 kDa, somewhat higher than the 46.5 kDa previously estimated (Leznicki & Bandurski, 1988*b*).

Fig. 5(*a*) demonstrates the purity of the preparation and shows an estimation of the molecular mass as determined by SDS/PAGE. The SDS/12%-polyacrylamide gel was first stained with Coomassie Blue, destained and then stained with silver. The IA-glucose synthase enzyme in lane A shows only one band, at 51 kDa. Fig. 5(*b*) is a graphical determination of molecular mass from the data of Fig. 5(*a*).

Immuno-inactivation of IA-glucose synthase

Fig. 6 shows an antibody titration curve for IA-glucose synthase after Toyopearl DEAE-650M chromatography (step VI). Titrations were performed with IgG purified on Protein A–Sepharose. The results show that the immune serum is effective in precipitation of IA-glucose synthase. No loss of activity occurs with control sera.

Immunological cross-reactivity

Extracts from several different tissues and from different plants were precipitated with $(NH_4)_2SO_4$ and analysed by 8%-PAGE. The separated proteins, after being blotted on to nitrocellulose membranes, were tested for immunoreactivity with anti-(IA-glucose synthase) antibody. Single immunoreactive bands were observed at a locus corresponding to 50 kDa from a partially purified corn endosperm extract, from 2-day-old germinated corn vegetative tissue and from extracts of 6-day-old corn shoots. An immunoreactive band was also observed with extracts of the acorns of oak (*Quercus* sp.) collected in late August. The cross-reactive protein from *Quercus* had a somewhat lower R_F than that from corn endosperm. Extracts of the leaves of horse chestnut (*Aesculus* sp.), liquid endosperm of the horse chestnut and extracts of *Arabidopsis* leaves did not yield proteins showing visible cross-reactivity. The data are not shown owing to poor photographic quality.

Table 1. Purification scheme for IA-glucose synthase

For experimental details see the text.

Step	Volume (ml)	Total protein (mg)	Enzyme activity (nmol/min per 50 μl)	Total activity (μmol/min)	Specific activity (nmol/min per mg of protein)
I Crude homogenate	710	6450	3.17	45	6.9
II 15%-poly(ethylene glycol) supernatant fluid	950	846	2.49	47	60
III DEAE-Sephacel (Fig. 1)	140	434	11.1	31	71
IV Blue Sepharose (Fig. 2)	74	21	18.1	27	1231
V Octyl-agarose (Fig. 3)	74	5.8	9.8	14.5	2512
VI Toyopearl DEAE-650M (Fig. 4)	39	2.4	12.4	9.7 (26%)	4029 (580×)

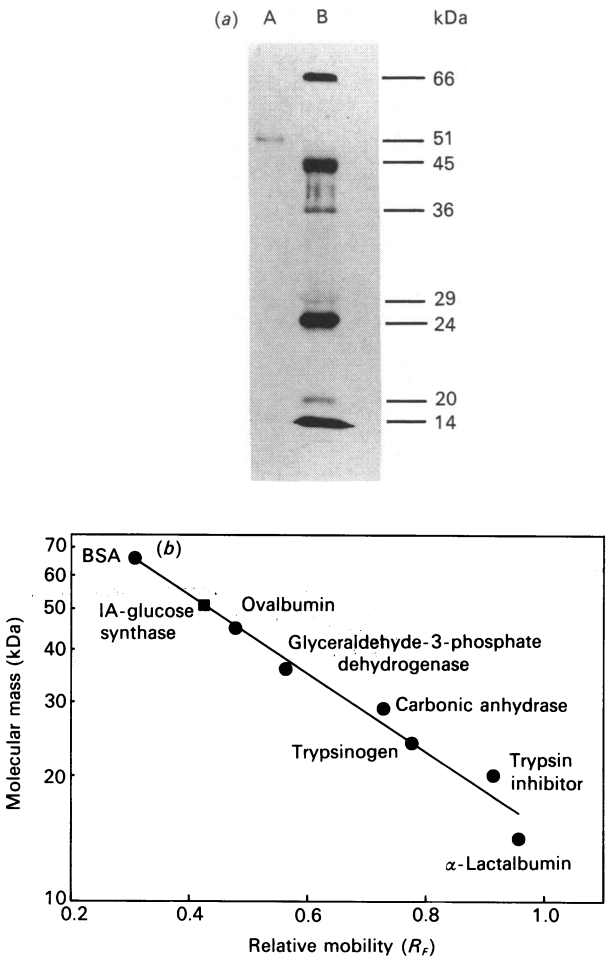

Fig. 5. Determination of enzyme homogeneity and molecular mass by SDS/PAGE

(*a*) The resolving gel (15 cm × 17 cm) was 12% polyacrylamide and was prestained with Coomassie Blue, destained and then restained with the Bio-Rad silver reagent (Harlow & Lane, 1988). IA-glucose synthase (6–7 μg) is in lane A. BSA, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor and α-lactalbumin in lane B were used as markers having molecular masses of 66, 45, 36, 29, 24, 20 and 14 kDa respectively. (*b*) Plot of the data of (*a*) showing mobility as a function of molecular mass, indicating that of IA-glucose synthase to be 51 kDa.

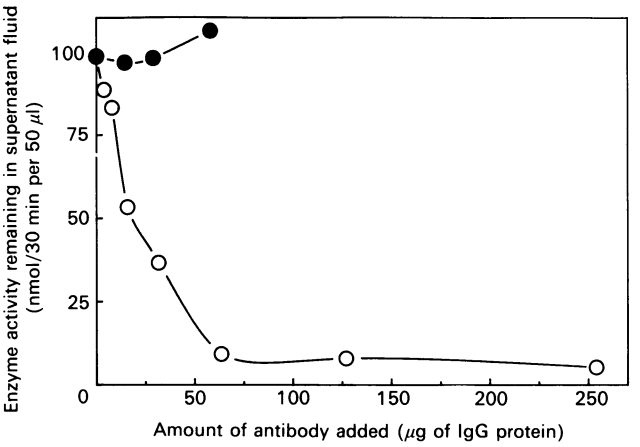

Fig. 6. Immuno-inactivation of the IA-glucose synthase from sweet-corn endosperm

Titrations were done with IgG purified on a Protein A–Sepharose column as described in the text. Results obtained with serum from a control animal are indicated by ● symbols and those obtained with serum from an enzyme-immunized animal by ○ symbols.

DISCUSSION

Conjugate synthesis and hydrolysis has been observed *in vivo* and *in vitro* (Nowacki & Bandurski, 1980; Hall & Bandurski, 1986; Chisnell & Bandurski, 1988). Since the synthesis of IA-glucose, followed by transacylation to *myo*-inositol, represents the first steps in the IAA-conjugation process, the enzymes catalysing these steps are candidates for the regulation of IAA concentration by converting hormonally active free IAA into growth-inactive IAA ester (Bandurski *et al.*, 1977, 1990), as occurs, for example, in the tropic responses to gravity and light. Conjugate hydrolysis and synthesis would represent a reversible regulatory process following the irreversible process of release of hormone from the vascular tissue into cortical target cells.

The physical properties of this enzyme are noteworthy. There is the failure of this enzyme to be precipitated by concentrations of poly(ethylene glycol) as high as 30%. The mechanism by means of which poly(ethylene glycol) precipitates, or in this case fails to precipitate, a protein is not understood, except that it appears not to involve protein–poly(ethylene glycol) interaction but rather may involve an excluded solution volume into which the protein cannot penetrate (Atha & Ingham, 1981; Suelter, 1985). Most of the protein of a corn endosperm preparation is precipitated by poly(ethylene glycol) concentrations slightly above 5%, such as, for example, the enzyme catalysing transfer of IAA from IA-glucose to *myo*-inositol (Michalczuk & Bandurski, 1982; J. Kesy, unpublished work). The amino acid composition of this protein, as well as the hydrophobic nature of the *N*-terminal sequence, may explain the failure of the enzyme to be precipitated by poly(ethylene glycol) (S. Kowalczyk, unpublished work).

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REFERENCES

Andrews, P. (1965) Biochem. J. **96**, 595–605
Atha, D. H. & Ingham, K. C. (1981) J. Biol. Chem. **256**, 12108–12117
Bandurski, R. S., Schulze, A. & Cohen, J. D. (1977) Biochem. Biophys. Res. Commun. **79**, 1219–1223
Bandurski, R. S., Schulze, A., Desrosiers, M. F., Jensen, P. J., Epel, B. & Reinecke, D. M. (1990) in Inositol Metabolism in Plants (Morre, J. & Boss, W., eds.), pp. 289–300, Wiley–Liss, New York
Bohme, H. J., Kopperschlager, G. & Schultz, J. (1972) J. Chromatogr. **69**, 209–214
Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
Chisnell, J. R. & Bandurski, R. S. (1988) Plant Physiol. **86**, 79–84
Ciesielski, T. (1872) Beitr. Biol. Pflanz. **1**, 1–82
Cohen, J. D. & Bandurski, R. S. (1982) Annu. Rev. Plant Physiol. **33**, 403–430
Corcuera, L. J. & Bandurski, R. S. (1982) Plant Physiol. **70**, 1664–1666
Corcuera, L. J., Michalczuk, L. & Bandurski, R. S. (1982) Biochem. J. **207**, 283–290
Darwin, C. (1880) The Power of Movement in Plants, 1st edn., John Murray, London
Ehmann, A. (1974) Carbohydr. Res. **34**, 99–114
Hall, P. J. & Bandurski, R. S. (1986) Plant Physiol. **80**, 374–377
Harlow, E. & Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor
Keglevic, A. & Pokorny, M. (1969) Biochem. J. **114**, 827–832

Kopcewicz, J., Ehmann, A. & Bandurski, R. S. (1974) Plant Physiol. **54**, 846–851
Kowalczyk, S. & Bandurski, R. S. (1990) Plant Physiol. **94**, 4–9
Leznicki, A. J. & Bandurski, R. S. (1988*a*) Plant Physiol. **88**, 1474–1480
Leznicki, A. J. & Bandurski, R. S. (1988*b*) Plant Physiol. **88**, 1481–1485
Michalczuk, L. & Bandurski, R. S. (1980) Biochem. Biophys. Res. Commun. **93**, 582–592
Michalczuk, L. & Bandurski, R. S. (1982) Biochem. J. **207**, 273–281
Nowacki, J. & Bandurski, R. S. (1980) Plant Physiol. **65**, 422–427
Ogita, Z.-I. & Markert, C. L. (1979) Anal. Biochem. **99**, 233–241
Strack, D. & Gross, W. (1990) Plant Physiol. **92**, 41–47
Suelter, C. H. (1985) A Practical Guide to Enzymology, p. 85, John Wiley and Sons, New York
Tkotz, N. & Strack, D. (1980) Z. Naturforsch. C **35**, 835–837

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