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UDP-GLUCOSE: INDOLEACETIC ACID GLUCOSYL TRANSFERASE AND INDOLEACETYL-GLUCOSE: *MYO*-INOSITOL INDOLEACETYL TRANSFERASE

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SUMMARY: We have demonstrated the *in vitro* enzymatic synthesis of an ester of indole-3-acetic acid (IAA) and glucose and of IAA and *myo*-inositol by the following reaction sequence:

1) IAA + UDPG ↔ IAA-glucose + UDP
2) IAA-glucose + *myo*-inositol → IAA-*myo*-inositol + glucose

The enzymes were partially purified from extracts of immature kernels of *Zea mays* sweet corn and the two activities separated on a Sephadex G-150 column. Products were characterized, primarily, by comparison of their 70 eV mass spectra with those of authentic synthetic standards. To our knowledge this is the first example of enzymatically catalyzed acylation by a 1-*O*-acyl sugar.

We previously reported on the enzymatic synthesis of IAA-*myo*-inositol utilizing the thiol ester IAA-CoA as the acylation reagent (1). We now wish to report a second pathway to IAA-*myo*-inositol involving the enzymatic synthesis of IAA-β-1-D-glucose from IAA and UDPG followed by transacylation from IAA-β-1-D-glucose to *myo*-inositol to form IAA-*myo*-inositol. As trivial names for these enzymes we propose IAA glucose synthetase for the enzyme catalyzing reaction 1 and IAA-transferase for that catalyzing reaction 2. An abstract of these studies has appeared (2). IAA-β-1-D-glucose is formed by plants following exogenous application of IAA (3, 4) and a report has appeared, without data, that lentils can synthesize IAA-glucose from UDPG and IAA (5).

Studies from this laboratory demonstrated that seeds and vegetative tissues of all plants examined contain most of the growth hormone, indole-3-acetic acid (IAA), as ester or amide-linked IAA (6, 7, 8). We further demonstrated, for seedlings of corn *(Zea mays)*, that conjugation and hydrolysis of the esters, in particular that of the IAA ester of *myo*-inositol, is a reversible process (8, 9, 10). This provides the plant with a system for hormonal homeostasis involving formation and hydrolysis of a covalently bonded hormone conjugate permitting control of growth rate (8, 9). Thus, knowledge of how IAA esters are synthesized and hydrolyzed becomes important for control of plant growth.

MATERIALS AND METHODS

Enzyme and substrate preparations:

Sweet corn, at the table-ready stage, was purchased from local markets. The activity of the enzymes extracted varied greatly depending upon the freshness of the corn and the stage of maturity. Stage I enzyme was prepared by the method of Kopcewicz *et al.*, (1) and, essentially, was a 10,000 g supernatant of the crude homogenate. Stage II enzyme had been precipitated with 85% $(NH_4)_2SO_4$ and then dialyzed for 24 hours at pH 7.6 and 4°C. Stage III enzyme was prepared from the 40 to 85% $(NH_4)_2SO_4$ precipitable fraction followed by

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chromatography on Sephadex G-150 and elution with 50 mM TRIS-HCl, pH 7.6 buffer. The fraction eluted immediately after the void volume was the transacylase called III-B, whereas the UDPG linked enzyme emerged at 1.6 X the void volume and was called III-A.

Reagents:

UDPG, *myo*-inositol, glutathione, indole-3-acetic acid, TRIS and DEAE-Sephadex A-25, were from Sigma Chemical Co., St. Louis, MO; Sephadex G-150 was from Pharmacia Inc., Piscataway, NJ; $(NH_4)_2SO_4$-enzyme grade, Mann Research Lab., Inc., New York, NY; and β-[2-^{14}C]indole-3-acetic acid 50.1 mCi/mmole was from New England Nuclear, Boston, MA.

Assay and product characterization:

Enzyme from about 0.2 g of corn kernels in 0.3 ml of 0.05 M TRIS, pH 7.6 was incubated with 1 μmol of UDPG, 2×10^{-3} μmol of ^{14}C-IAA (45×10^3 dpm), 5 μmol of $MgCl_2$, 5 μmol of reduced glutathione (GSH), and, as indicated 5 μmol of *myo*-inositol in a total volume of 0.6 ml for 1 hr at 37°C. The reaction was terminated by the addition of 0.6 ml of 2-propanol and the resultant suspension plus 2 ml of 50% aqueous 2-propanol washings freed of anionic substances by passage through a 1 ml bed volume column of DEAE-Sephadex-acetate. The eluate was made to 5 ml and a 1 ml aliquot used for counting the amount of non-anionic (esterified) IAA. About 100 μg of synthetic, non-radioactive IAA-*myo*-inositol (11) was added, as carrier, to the remaining 4 ml; the mixture was then chromatographed on a PA-28 HPLC column (12) and eluted with 50% 2-propanol. UV absorbance of the eluate was determined at 282 nm on a Gilson 240 spectrophotometer and radioactivity was determined using a Packard TriCarb Liquid Scintillation Counter and 5 ml of ACS scintillation fluid (Amersham-Searle). As shown in Fig. 1, the total radioactivity eluting between 9 and 16 ml was a measure of IAA-glucose formation, whereas that eluted between 21 and 37 ml was a measure of IAA-*myo*-inositol synthesis. These elution volumes corresponded to those of the authentic compounds.

RESULTS

Product characterization:

IAA glucose: a) The putative ^{14}C-IAA glucose was ammonolyzed and the products thin layer chromatographed on Silica Gel *60* (E. Merck) with $CHCl_3$-CH_3OH-H_2O (85:14:1). Two radioactive spots were found identical in R_f to authentic IAA and IAA-amide (13,14) thus proving that an ester had been formed. b) Utilizing a 500 fold increased reaction scale mixture, products were obtained which migrated on Silica Gel [methyl ethyl ketone:ethyl acetate:ethanol:water, (3:5:1:1)] coincidentally with the 1-O, 2-O and 4-O esters of IAA-glucose (14,15). Examination by GC-MS of the trimethylsilyl (TMS) derivatives, under conditions previously described, yielded one pentatrimethyl silyl derivative with retention time identical to that of authentic pentakis TMS IAA-1-O-glucose and a molecular ion at 697 and major fragment ions at m/z 202, 217, and 361, all identical to those of the authentic compound (14,15). TMS derivatives corresponding to the 2-O and 4-O IAA esters of glucose were also observed, as expected for a compound exhibiting facile acyl migration (14).

IAA-*myo*-inositol: a) The enzymatically formed, putative, ^{14}C-IAA-*myo*-inositol esters were eluted from a PA-28 column in two peaks with elution volumes identical to those of synthetic IAA-*myo*-inositols (11). b) The radioactive hexa trimethylsilyl derivatives had GC retention times of 15.9, 17.4 and 18.2 minutes identical to those of the mixed isomeric synthetic IAA-*myo*-inositols, (11,16). c) Incubation of synthetic IAA-1-O-β-D-glucose (15) with the transacylase enzyme and *myo*-inositol yielded products with tlc migration identical to those of authentic synthetic IAA-*myo*-inositols using the methyl ethyl ketone solvent described above. d) Analysis of the TMS derivatives of the reaction products by GC-MS showed m+ = 769 with major fragment ions at m/z = 202, 229, 318, 433, 507 and 697, all identical to those of hexakis TMS-IAA *myo*-inositols (16).

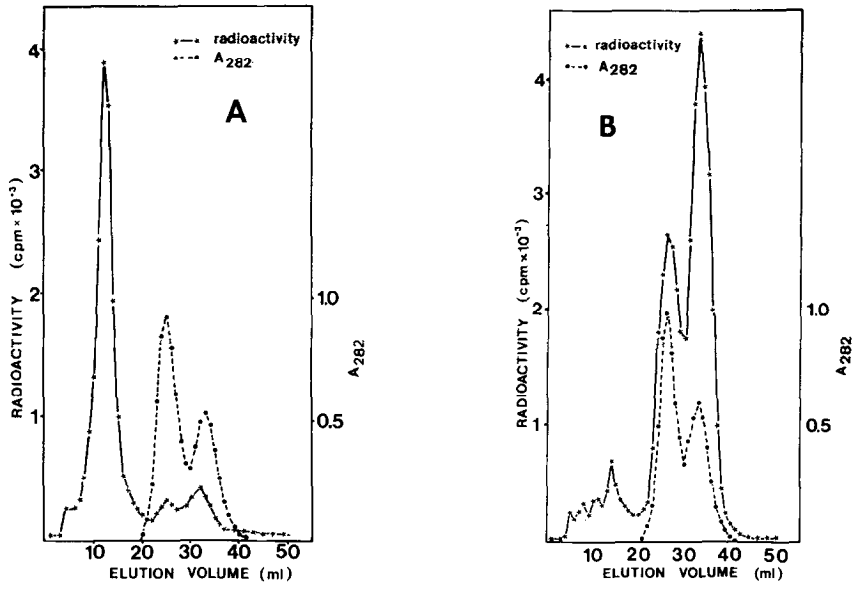

Fig. 1. Elution pattern of IAA-glucose (9 to 16 ml) and IAA-*myo*-inositol (21 to 37 ml) from a PA-28 column by 50% 2-propanol. Fig. 1 A shows the products formed by stage II enzyme from UDPG and ^{14}C-IAA in the absence of added *myo*-inositol. Fig. 1 B shows the products formed by an identical reaction mixture to which *myo*-inositol has been added. Radioactivity is shown as a solid line with a counting efficiency of 81%. Amounts of compound formed were determined by integrating the area under the solid line. The A_{282} observed is owing to the addition of synthetic IAA-*myo*-inositol which served as a carrier and marker for the elution volume of the IAA-*myo*-inositol. Other conditions are as described in the text.

Separation of the UDPG glucosyl transferase from the IAA-glucose acyl transferase:

We first noted that the relative amounts of IAA-glucose and IAA-*myo*-inositol formed upon incubation of the enzyme, UDPG and *myo*-inositol were inversely related as shown in Fig. 1, A and B. For this experiment Stage II enzyme, possessing both activities was used and, as can be seen, in the absence of added *myo*-inositol (Fig. 1 A) the product is almost totally IAA-glucose, whereas with added *myo*-inositol, IAA-*myo*-inositol becomes the predominant product (Fig. 1 B).

Separation of the two enzymes on Sephadex G-150 was possible as described above, and as illustrated by the data of Table I. Incubation of enzyme III-A (Table I, Exp. a) with IAA and UDPG leads to IAA-glucose formation. Enzyme III-B is totally without ability to form either IAA-glucose or IAA-*myo*-inositol (Table I, Exp. b). Addition of enzyme III-B to III-A causes conversion of IAA glucose to IAA-*myo*-inositol (Table I, Exp. c).

Lastly, incubation of 1 μmole of synthetic IAA-1-*O*-β-D-glucose (15) with 4 ml of enzyme III-B (IAA transferase) and 20 μmoles of *myo*-inositol results in the formation of 0.8 μmoles of IAA-*myo*-inositol having a mass spectral fragmentation pattern identical to that of authentic, synthetic IAA-*myo*-inositol as described above (11, 16). Enzyme III-A (IAA-glucose synthetase) was totally inactive in transferring IAA from IAA-glucose to *myo*-inositol.

TABLE I

SEPARATION OF IAA GLUCOSE SYNTHETASE AND INDOLEACETYL TRANSFERASE ACTIVITIES

	Incubation Mixture	IAA-glucose (dpm)	IAA-*myo*-inositol
a)	IAA, 5×10^3 dpm; UDPG, 1 μmol; *myo*-inositol, 5 μmol; enzyme III-A, 0.3 ml. Incubation, 1 hr at 37C.	12,700±40	not detectable
b)	As in "a" but 0.3 ml of enzyme III-B substituted for III-A.	not detectable	not detectable
c)	As in "a" but with 0.3 ml of enzyme III-B in addition to III-A.	2230±20	8100±21

DISCUSSION

The reactions described are an alternative to the synthesis of IAA-*myo*-inositol by the IAA-CoA requiring reaction previously described by Kopcewicz *et al.*, (1). Thus, kernels of corn can synthesize IAA-*myo*-inositol, a major IAA ester (17), using either an acyl-thiol ester or an acylglucoside as the acylation reagent. These multiple mechanisms could explain the occurrence of the more than 16 different IAA esters found in corn kernels including the various IAA-*myo*-inositol glycosides (8, 17). Owing to the ease of acyl migration (8, 16) we cannot determine which of the hydroxyls of *myo*-inositol is the initial acyl acceptor. Studies on the specificity of the transacylase should be of interest.

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