Plant Physiol. (1980) 65, 761-767
0032-0889/80/65-0761/07/$00.50/0

Biological Activities of Indoleacetylamino Acids and Their Use as Auxins in Tissue Culture[1]

Received for publication July 26, 1979 and in revised form November 1, 1979

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ABSTRACT

The auxin activities of a number of indoleacetylamino acid conjugates have been determined in three test systems: growth of tomato hypocotyl explants (*Lycopersicon esculentum* Mill. cv. Marglobe); growth of tobacco callus cultures (*Nicotiana tabacum* L. cv. Wisconsin 38); and ethylene production from pea stems (*Pisum sativum* L. cv. Alaska). The activities of the conjugates differ greatly depending on the amino acid moiety. Indoleacetyl-L-alanine supports rapid callus growth from the tomato hypocotyls while inhibiting growth of shoots and roots. Indoleacetylglycine behaves in a similar manner but is somewhat less effective in supporting callus growth and in inhibiting shoot formation. The other amino acid conjugates tested (valine, leucine, aspartic acid, threonine, methionine, phenylalanine, and proline) support shoot formation without supporting root formation or much callus growth. The tobacco callus system, which forms abundant shoots in the presence or absence of free indoleacetic acid, produces only rapid undifferentiated growth in the presence of indoleacetyl-L-alanine and indoleacetylglycine. The other conjugates inhibit shoot formation weakly if at all. Most of the conjugates induce sustained ethylene production from the pea stems but at rates well below the initial rates observed with free indoleacetic acid. Many, but not all of the effects of conjugates such as indoleacetyl-L-alanine can be mimicked by frequent renewals of the supply of free indoleacetic acid.

The components of plant tissue culture media which are most critical in determining the nature of the growth are the auxins and the cytokinins. Commercially available auxins differ greatly in their stability, in their effectiveness, and in their influence on organogenesis. IAA is destroyed rapidly by many tissues and is often not very effective in supporting the growth of cultured plant tissues. When it is effective, it is the least likely of the auxins to inhibit organogenesis. In contrast, the stable auxin-like growth regulator, 2,4-D is very active in stimulating the growth of cultures but it strongly represses organized growth and usually only callus is produced (20). Because 2,4-D is so effective in supporting the growth of many plant tissues it is probably the most widely used auxin in tissue culture studies but, unfortunately, its presence can cause problems if one desires to regenerate plants from the tissues.

In the 1940's, it was shown that IAA can exist in bound forms in plant tissues (6, 12, 13, 26, 28). Since these first observations a number of bound forms of IAA have been identified as metabolites of exogenous IAA and as natural products (1, 2, 14, 30). Careful measurements of IAA and its derivatives have shown that most of the IAA in a plant is bound, either through amide or through ester linkages, and that free IAA occurs in extremely low amounts (3, 4), perhaps because of its susceptibility to peroxidation *in vivo*. Conjugates of IAA are protected from peroxidative destruction (1, 7) and can act as reserve sources of IAA (2, 6) or perhaps as transport forms of IAA (2).

The widespread occurrence of stored, stable IAA conjugates in plant tissues suggested to us that conjugates of IAA might be better sources of auxin for plant tissue cultures than the too labile free IAA or the too persistent synthetic IAA analogs. IAA conjugates have been shown to support the growth of certain tissue cultures (9, 24). However, the use of IAA conjugates in tissue culture has not been systemically studied previously. Here, we have looked at the influence of several IAA conjugates on callus induction and organogenesis in tomato and tobacco tissue cultures. At the same time, we have studied both auxin-induced ethylene synthesis and auxin transport in pea stems in an attempt to gain some insight into the mode of action of these IAA conjugates.

MATERIALS AND METHODS

Synthesis of IAA-amino Acids. Most of the amino acid conjugates were synthesized by the mixed anhydride method of Weiland and Horlein (29). The synthesis of IAA-L-alanine is described as an example. Ten mmol IAA were dissolved with stirring in 30 ml tetrahydrofuran plus 10 mmol triethylamine in a vessel cooled in an ice bath. Over a period of 30 s, 10 mmol ethyl chloroformate were added dropwise with rapid stirring. After 5 min of stirring, 10 mmol of the sodium salt of the L-alanine in 8 ml water were added. The mixture was allowed to warm to room temperature with continuous stirring. The reaction mixture was separated into two phases by the addition of water (50 ml) and chloroform (50 ml). The aqueous phase was adjusted to pH 4 with HCl and extracted three times with 50 ml chloroform. The aqueous phase was then further acidified to pH 3 with more HCl and extracted three times with 50 ml 1-butanol. The butanol phases were pooled and concentrated at reduced pressure until a viscous residue remained. This residue was dissolved in approximately 15 ml 2-propanol-water (1:1, v/v) then purified by chromatography on a Sephadex LH-20 column (4 × 30 cm) using 2-propanol-water (1:1, v/v) as the eluting solvent. The fractions containing most of the product were pooled and concentrated by reduced pressure. The product was then crystallized from ethyl acetate by the addition of hexane and recrystallized from the same solvent.

Isolation of the other IAA-amino acid conjugates differed somewhat depending on the amino acid used. The Gly, Thr, Val, and Leu conjugates precipitated directly from the aqueous phase on acidification. The Phe conjugate remained in the organic phase and was then extracted into bicarbonate solution. It crystallized on acidification of the bicarbonate solution. These conjugates

[1] Michigan Agricultural Experiment Station Journal Article No. 9094. This investigation was supported in part by National Science Foundation Grant PCM-76-07581-A01 to N. E. G. and Department of Energy Grant EY-76-S-02-2528.M005 to Peter S. Carlson.

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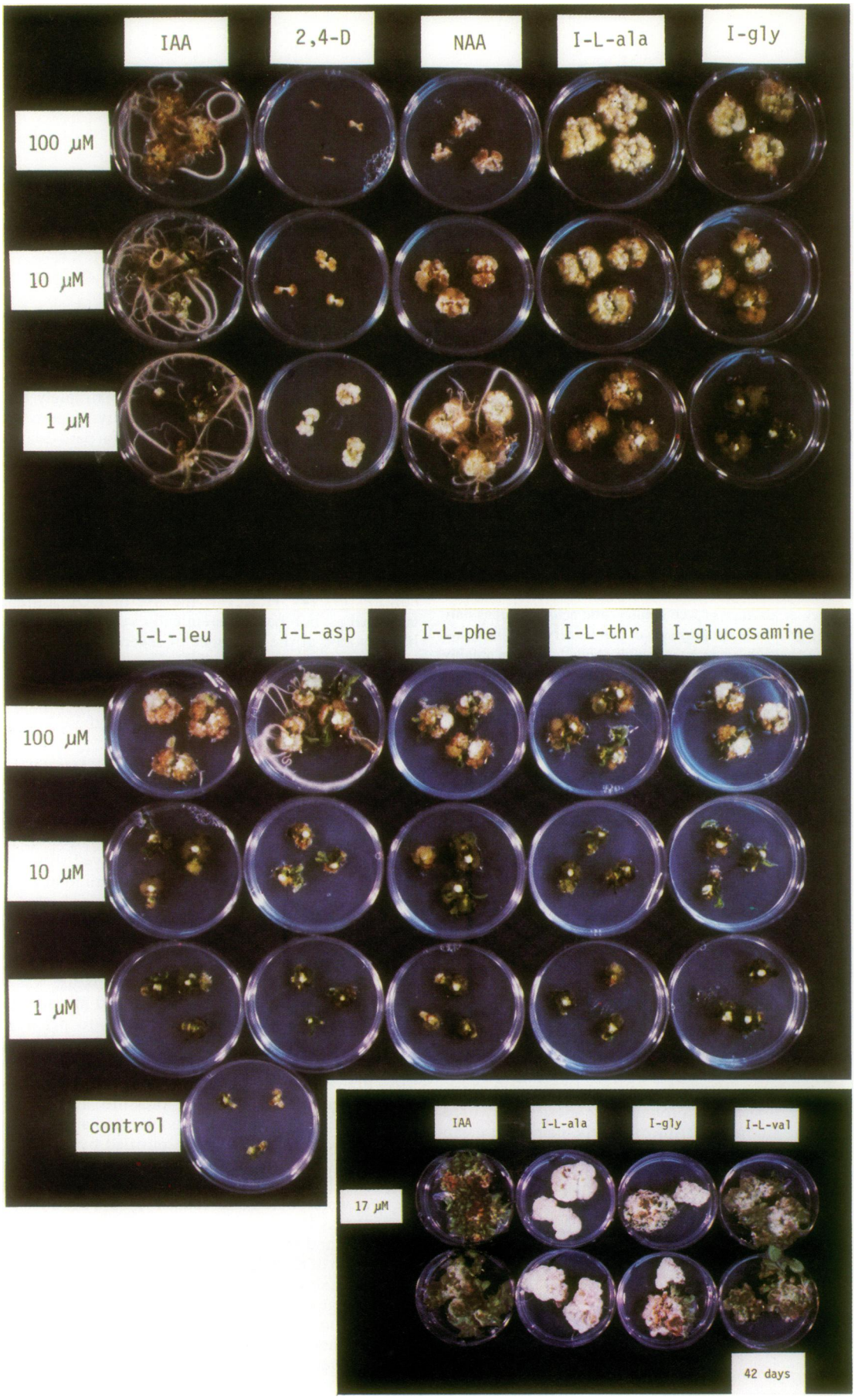
IAA
2,4-D
NAA
I-L-ala
I-gly
100 µM
10 µM
1 µM
I-L-leu
I-L-asp
I-L-phe
I-L-thr
I-glucosamine
100 µM
10 µM
1 µM
control
IAA
I-L-ala
I-gly
I-L-val
17 µM
42 days

were recrystallized from ethanol and water. The methionine and proline conjugates were prepared and isolated in the same way as the L-alanine conjugate. IAA-glucosamine was prepared and isolated by the same method as was IAA-L-alanine and identified as a single spot on TLC, yielding indole and reducing sugar color tests on chromatograms.

IAA-L-aspartate was synthesized by the method of Mollan *et al.* (18). The product was purified by the same method as was IAA-L-alanine and was stored in 2-propanol-water (1:1, v/v). Propanol was removed at reduced pressure prior to the use of the material in biological experiments. Yields for all conjugates were usually between 40 and 50%. TLC in chloroform-methanol-acetic acid (75:20:5) using Ehrlich's reagent for detection of indoles showed that our products were contaminated with less than 0.05% free IAA and with no other indole compound in detectable amounts.

Radioactive IAA-L-alanine and IAA-glycine were synthesized by the above described mixed anhydride procedure (29) with the following modifications. [2-^{14}C]IAA (49 mCi/mmol) was obtained from New England Nuclear, diluted to a specific radioactivity of 10 mCi/mmol by the addition of unlabeled IAA and stored in acetonitrile. One μmol of the [^{14}C]IAA was added to a 250-μl reaction vessel and the acetonitrile distilled off at reduced pressure until about 50 μl remained. To this was added 1 μmol triethylamine in 10 μl acetonitrile in an ice bath. One μmol ethyl chloroformate in 10 μl acetonitrile was added with stirring. After 5 min of stirring, 1 μmol of the sodium salt of the amino acid in 30 μl water was added. The mixture was allowed to warm to room temperature with continuous stirring. The acetonitrile was removed at reduced pressure and 150 μl of 2-propanol-water (1:1, v/v) was added. The reaction mixture was acidified with phosphoric acid and the [^{14}C]IAA-amino acid conjugates were purified by chromatography on a 1 × 30 cm column of Sephadex LH-20 with elution by 2-propanol-water (1:1, v/v). The [^{14}C]IAA-amino acid conjugates were stored in 80% ethanol. Yields were again around 50%. All products were stored in a freezer.

Tomato Tissue Culture. Tomato seeds (*Lycopersicon esculentum* Mill. cv. Marglobe) were sterilized by soaking them for 30 s in 95% ethanol followed by 30 min in 0.5% sodium hypochlorite solution containing 0.01% sodium laurylsulfate. The seeds were then rinsed several times in distilled H_2O and germinated on a solid agar medium in Petri dishes. Seven days after planting, the hypocotyls were excised aseptically, cutting them 1 cm below the cotyledons and 1 cm above the radical. The hypocotyls were then cut into 5-mm segments. The segments, randomly selected with respect to treatment, were placed on agar incubation media. Nine- (five segments) or 4.5-cm (three or four segments) disposable Petri dishes were used. All experiments contained four to six replicates and were repeated several times. Typical results are shown in the photographs. The results are very reproducible.

Tobacco Shoot Induction. Tobacco callus, *Nicotiana tabacum* L. cv. Wisconsin 38 was used. This callus had been established from pitch explants and maintained in culture for over 2 years. Two pieces of callus (approximately 300 mg each) were placed onto the appropriate experimental medium in 4.5 cm disposable Petri dishes 3 weeks after their last transfer onto the maintenance medium. Again all experiments contained four to six replicates and were repeated several times. Typical results are shown in the photographs. The results are very reproducible.

Growth Media. Slightly modified Murashige and Skoog culture media (MS) were used for all tissue culture experiments (21). For tomato germination the MS medium contained 20 mM sucrose, 3 μM thiamine, 4.9 μM pyridoxine, and 8.1 μM nicotinic acid. For tomato hypocotyl experiments the medium contained 88 mM sucrose, 3 μM thiamine, 4.9 μM pyridoxine, 8.1 μM nicotinic acid, 8.9 μM benzyladenine, and the particular auxin being tested. Tobacco shoot induction experiments were carried out on MS medium containing 88 mM sucrose, 3 μM thiamine, 49 μM 6-δ-δ-dimethylallylaminopurine, and the indicated auxin source. All media were solidified with 0.9% (w/v) Difco Bacto Agar. Prior to autoclaving all constituents including hormones were added and the pH was adjusted to 6.0 with KOH.

Conditions for Tissue Culture Experiments. Growth experiments were conducted at 22 ± 1 C on a 16-h light/8-h dark cycle under General Electric Delux cool-white fluorescent lights at a distance giving 40–54 microE/m^2·s as measured with a LiCor Radiometer LI-185A.

Auxin-Induced Ethylene Production. Pea seeds (*Pisum sativum* L. cv. Alaska) were soaked overnight in running tap water.They were sown in Vermiculite and grown in the dark at 25 C for 6–7 days. Stem sections from just below the plumular hook were cut under a green safelight and incubated for 16 h on filter paper soaked with distilled H_2O in 9-cm Petri dishes. The segments were transferred to 25-ml Erlenmeyer flasks containing a 3.5 cm filter paper disc and either 1 ml distilled H_2O or 1 ml 5 mM K-phosphate containing the indicated auxin adjusted to pH 6.5. Each flask was flushed with ethylene-free air, closed with a serum vial cap and incubated in darkness at 25 C. Ethylene was measured by GC as described by Kende and Hanson (16). All ethylene experiments were repeated several times.

Auxin Transport. Auxin transport was measured in pea stems by the agar block technique. Blocks of 1.5% agar (1 cm × 1 cm × 2 mm) were prepared with the appropriate ^{14}C-auxin (10 mCi/mmol) at 5 μM or without auxin (donor and receiver, respectively). Etiolated peas were grown as for the ethylene production experiments. Stem sections (3.5 mm) were cut with spaced razor blades 1 cm below the apical hook and placed on receiver blocks in the indicated orientation (12 segments/block). Donor blocks were positioned carefully so that the free stem ends came into uniform contact with the donor block. The transport was allowed to proceed in a humid chamber in darkness. After the appropriate time, the receiver blocks were placed into scintillation vials containing Tritosol scintillation fluid (10) and allowed to extract overnight. The radioactivity was determined on a Packard Tri-Carb scintillation counter with a counting efficiency of 83%.

RESULTS

Growth Responses of Tomato Hypocotyl Explants (Figs. 1A—4). In the absence of any exogenous auxin, the excised hypocotyl sections usually failed to grow and often died, although the medium contained an abundant carbon source, vitamins, and cytokinin. The addition of free IAA caused the growth of callus, roots, and shoots, but concentrations of IAA high enough to favor good callus growth always strongly inhibited shoot formation. Similar concentrations of 2,4-D allowed some growth of callus but totally inhibited organogenesis. However, all of the levels of 2,4-D employed in the experiment depicted in Figure 1A were much too high for good growth. The concentrations of 1-naphthaleneacetic acid were also somewhat excessive and totally inhibited shoot formation, but 1-naphthaleneacetic acid concentrations low

FIG. 1. A: growth response of tomato hypocotyls treated with various auxins. Seven-day-old hypocotyl segments were placed on nutrient agar containing 8.9 μM benzyladenine and the indicated auxin. The photograph was taken after 30 days. In this figure and all of the other photographs, I before the name of an amino acid or glucosamine means IAA. B: inhibition of tobacco shoot induction by IAA-amino acid conjugates. Friable tobacco callus was placed on nutrient agar containing 49 μM 6-δ,δ-dimethylallylaminopurine and the indicated auxin at 17 μM. The photograph was taken after 42 days. Duplicate plates are shown.

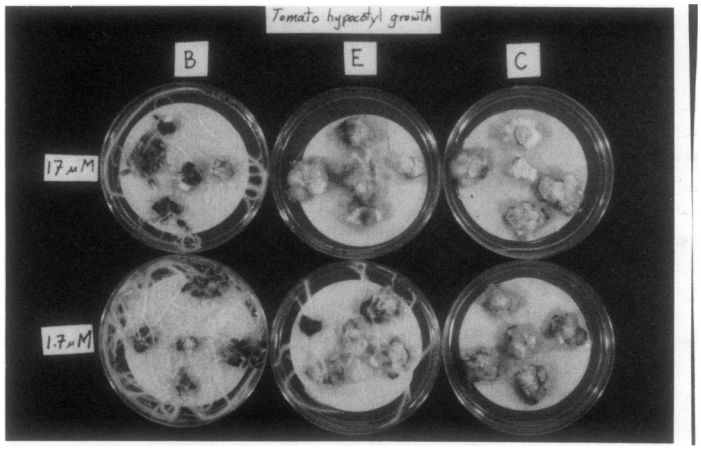

FIG. 2. Comparison of a "constant" supply of free IAA to a "slow release" source of IAA in the growth and differentiation of tomato hypocotyl sections. Seven-day-old hypocotyl segments were placed onto nutrient agar containing 8.9 μM benzyladenine and given the following treatments: (B) tissues were grown on the same plate containing 17 or 1.7 μM free IAA; (E) tissues were transferred to new plates containing fresh media with 17 or 1.7 μM free IAA every 4 days; (C) tissues were grown on the same plate containing 17 or 1.7 μM IAA-L-alanine. The photograph was taken after 30 days.

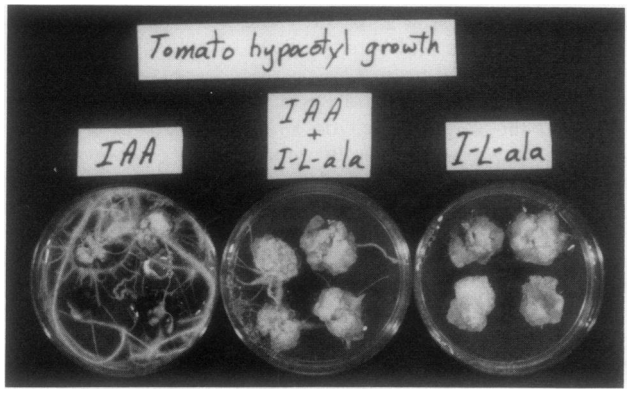

FIG. 3. Interaction of free IAA and IAA-L-alanine on the growth and differentiation of tomato hypocotyls. Seven-day-old hypocotyl segments were placed on nutrient agar containing 8.9 μM benzyladenine and the indicated auxins at 5.7 μM each. The photograph was taken after 32 days. Note that the addition of free IAA in the presence of IAA-L-alanine gave the root initiation effect of free IAA without affecting the callus formation characteristic of the alanine conjugate. See Figure 4 for a quantitative description of callus growth and root initiation in the same experiment.

enough to permit good callus growth also permitted considerable root development.

The responses to the IAA conjugates were quite different. None was inhibitory to growth at any of the concentrations used in the studies reported here. To the extent that they permitted growth, they encouraged callus or shoots and tended to inhibit roots. The responses to the different conjugates varied greatly depending on the conjugating moiety. (*a*) IAA-L-alanine supported vigorous callus growth, even at low concentrations. At very low concentrations (1 μM) the L-alanine conjugate sometimes permitted a little shoot formation. IAA-D-alanine was almost but not completely inactive; with the conjugate containing the unnatural amino acid there was no more growth than in the controls without auxin, but the explants often became green instead of dying (data not shown). (*b*) IAA-glycine was only slightly less active in supporting callus growth than was the L-alanine conjugate and it was not quite as effective in inhibiting organogenesis. At the lower concentrations

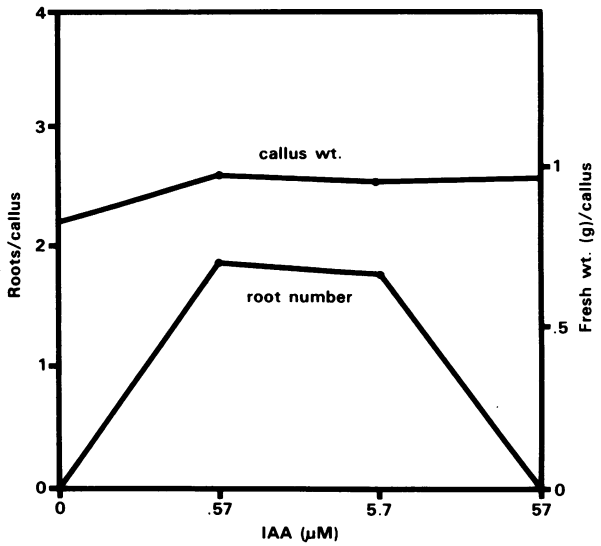

FIG. 4. Callus growth and root initiation from tomato hypocotyls as affected by combinations of free IAA and IAA-L-alanine. Seven-day-old hypocotyl segments were placed on nutrient agar containing 8.9 μM benzyladenine, 5.7 μM IAA-L-alanine and the indicated concentrations of free IAA. After 32 days, the number of roots initiated from the callus tissue was determined and the fresh weight of the callus was measured. Note again that root initiation was a function of the concentration of exogenous free IAA whereas callus growth depended only on the IAA-L-alanine.

(1 μM) the callus tended to become green, shoots were regularly formed and, in a few instances, there were some roots. (*c*) IAA-L-valine, although quite effective in supporting callus growth at concentrations above 50 μM, was appreciably less effective at lower concentrations (data not shown in these figures). It permitted shoot growth and sometimes root growth at concentrations which were suboptimal for callus formation (<50 μM). (*d*) IAA-L-aspartic acid (which is formed from exogenous IAA in most higher plants) was not very effective in supporting callus growth, even at 100 μM, and did not appreciably inhibit organogenesis. At this high concentration some callus formed, shoots grew readily and a few roots developed. (*e*) The other amino acid conjugates tested (those with L-Leu, L-Phe, L-Thr, L-Pro, and L-Met), although not eliciting identical responses, all supported growth of callus and shoots at high concentrations but did not often support root formation. None of the other amino acid conjugates of IAA was as effective as the L-alanine conjugate in supporting callus growth or in inhibiting organogenesis. In fact, IAA-L-alanine converts already established roots or shoots into rapidly growing callus.

The amino acid conjugates were not unique in supporting callus growth or in inhibiting organogenesis. IAA-glucosamine had effects which were indistinguishable from the effects of the less active amino acid conjugates. IAA-*myo*-inositol, which occurs naturally in considerable amounts in maize seeds (5), was similar to IAA-L-alanine, both in supporting rapid callus growth and in suppressing all organogenesis. However, it became inhibitory at 57 μM (data not shown).

Exogenous IAA is notoriously labile in the presence of plant tissue, sometimes disappearing completely from the medium within a few days (8, 25). It seemed possible that the action of the conjugates could be explained simply on the basis of the effects of a continuous supply of IAA made available by the slow enzymic hydrolysis of the L-isomers of the conjugates. In an attempt to maintain a constant supply of free IAA, the tomato hypocotyl explants were transferred every 4 days to fresh media containing free IAA. This treatment resulted in vigorous callus growth and in inhibition of organogenesis, which is a response similar to the

response observed with IAA-L-alanine (Fig. 2). However, it must be realized that a renewed supply of nutrients and cytokinin was provided and that metabolic products were also removed with each transfer.

It is not easy to explain all of the effects of IAA-L-alanine in terms of a persistent release of free IAA. If the IAA-L-alanine supports callus growth for no reason other than that it provides a continuous supply of free IAA, then the addition of free IAA to a medium containing callus-forming amounts of IAA-L-alanine should be without effect or should increase callus growth. The combination of IAA and IAA-L-alanine regularly produced roots in addition to vigorous callus growth (Fig. 3). Organogenesis, as measured by the number of roots initiated, depended on the concentration of the free IAA applied in the presence of callus-forming levels of IAA-L-alanine. The callus growth, on the other hand, seemed to depend only on the IAA-L-alanine (Fig. 4).

Several experiments were conducted in which the conjugating moiety in its free form (*i.e.* L-alanine, etc.) was added to medium along with an equal amount of free IAA. In all cases tested we found such treatments to be no different from treatments with free IAA itself. The unique properties of the IAA conjugates must therefore be entirely due to the covalent bonding of the complex and in no way represent a contribution by amino acids derived from the complex.

Shoot Induction and Callus Growth in Tobacco Cultures (Fig. 1B). The undifferentiated tobacco callus employed in these experiments produced abundant shoots when grown on the high cytokinin medium, whether or not free IAA was added. However, the callus grew very little if at all and became brown with or without IAA. Again the effects of the IAA-amino acid conjugates were quite different and depended very much on the amino acid moiety. (*a*) IAA-L-alanine supported vigorous callus growth which tended to be white and compact. This was true even when the conjugate was only 0.17 μM (data not shown). At the same time shoot induction was competely inhibited at concentrations of the conjugates down to 1.7 μM and was still greatly reduced at 0.17 μM. Very surprisingly 1.7 μM IAA-L-alanine, which inhibited shoot formation in the absence of exogenous free IAA, permitted shoot formation in the presence of free IAA (data not shown). A similar type of interaction of the two auxin forms has already been described in the tomato hypocotyl system (Figs. 3 and 4). (*b*) IAA-glycine also supported good callus growth but without inhibiting shoot formation quite as effectively as did the L-alanine conjugate. (*c*) IAA-L-valine was appreciably less effective in supporting callus growth and also less effective in inhibiting shoot formation than were the alanine and glycine conjugates. (*d*) The other IAA-amino acid conjugates tested (L-Met, L-Leu, L-Phe, and L-Pro) were almost without effect, although some of them supported a little callus growth or at least prevented the browning of the callus (data not shown).

Production of Ethylene by Pea stems (Fig. 5 and Table I). Ethylene production by plant tissues is stimulated by addition of auxins and, at least in some cases, the amount of ethylene produced is correlated with the amount of free auxins in the tissues (17). Since the various IAA conjugates tested in this study clearly are acting as auxins themselves or as sources of auxins, we investigated their abilities to induce ethylene formation in pea stem sections.

Free IAA and 2,4-D induced approximately the same rate of ethylene production in pea stems when they were both 100 μM. In less than 9 h the production of ethylene in the IAA-treated stems virtually ceased unless new IAA was added. In contrast ethylene formation by the 2,4-D-treated stems continued unabated for at least 15 h. None of the IAA-amino acid conjugates was as effective as either IAA or 2,4-D in inducing ethylene formation, although IAA-L-alanine at 1,000 μM was almost as effective as free IAA at 100 μM. However, the ethylene production with IAA-L-alanine did not fall off as with free IAA after 9 h, but rather continued as with 2,4-D. This persistence of ethylene production with IAA conjugates is consistent with their proposed stability *in vivo*.

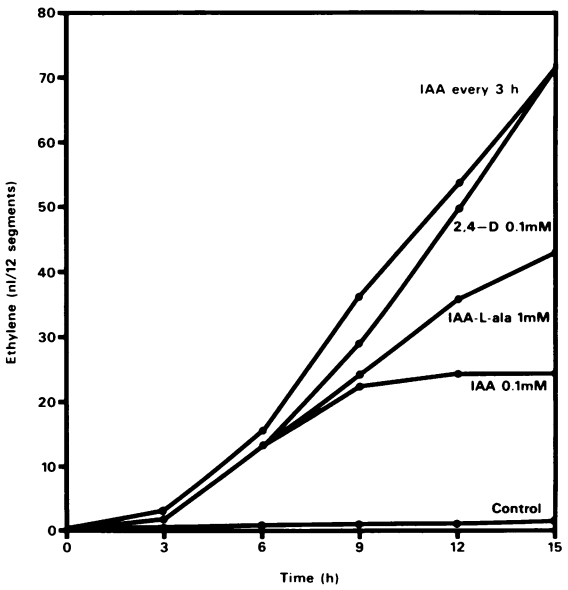

FIG. 5. Time course of ethylene production from pea stem segments treated with various auxins. Twelve segments were incubated in 1 ml of the auxin solutions at pH 6.5. Controls received distilled water only. The treatment labeled "IAA every 3 h" received additions of 17.5 μg of IAA in 20 μl aliquots every 3 h. At the time of the last addition (12 h) the final concentration of IAA would have been 0.45 mM if no IAA had been destroyed by the tissue.

Table I. *Ethylene Production from Etiolated Pea Stems Treated with IAA-Amino Acid Conjugates*

Twelve 1-cm segments were incubated in 1 ml of 5 mM K-phosphate buffer and 0.1 mM auxin at pH 6.5. Ethylene was measured after a 9-h incubation. IAA-treated segments produced 15.8 nl ethylene.

Compound Tested	Ethylene Production
	% of IAA Treatment
IAA	100
IAA-L-alanine	77
IAA-L-methionine	38
IAA-L-threonine	32
IAA-L-leucine	30
IAA-L-valine	23
IAA-L-aspartate	14
IAA-L-phenylalanine	9
IAA-glycine	8
IAA-L-proline	7
IAA-D-alanine	7
No auxin	7

Again, in this assay, the auxin activity of the conjugates depended very much on the amino acid moiety (Table I). As in the tissue culture experiments, the most active conjugate was the IAA-L-alanine. Not surprisingly, the D-alanine conjugate caused no measurable ethylene production. There was a good correlation between the ability of the conjugates to support callus growth in the tomato system and the ability of the conjugates to cause ethylene production in the pea system. The only discrepancy involved IAA-glycine which failed to stimulate ethylene production in peas. This apparent discrepancy can be explained by the difference between pea and tomato, since in subsequent experiments with tomato stem sections instead of pea stem sections the glycine conjugate was one of the most active in stimulating

ethylene production.

Transport of IAA and IAA-Amino Acid Conjugates (Fig. 6). Free IAA is transported by a very specific directed transport system (11) although there is some evidence that IAA-L-aspartic acid is an immobilized form of auxin (19). To determine whether the callus-forming IAA-amino acids are transported in the same way as free IAA, we synthesized [^{14}C]IAA-L-alanine and [^{14}C]-IAA-glycine and measured their basipetal and acropteal movements in pea stem segments. Only free IAA was transported by the characteristic basipetal transport system. The IAA-amino acids did not seem to have any specific transport, and they probably move only by diffusion.

DISCUSSION

Theoretical Considerations. The presence of IAA conjugates in plants has been known for years but the biological activities of such compounds, other than IAA-L-aspartic acid, have been studied in only a few instances (9, 15, 22). The data reported here clearly show that a number of IAA conjugates can serve as auxins in auxin-requiring systems and that various conjugates have widely different activities depending on the conjugating moiety (Fig. 1, A and B and Table I).

Many of the effects of the IAA conjugates can probably be explained in terms of a slow hydrolysis which gives a constant low steady-state concentration of free IAA. In long-term assays some of the conjugates seem to be more active than free IAA itself but in short term assays the free IAA always seems to be the more active (Fig. 1, A and B, Table I, and ref. 9). Constant renewals of free IAA gives results which mimic those obtained with the most active of the conjugates, IAA-L-alanine. The known lability of free IAA and the greater stability of the conjugates makes this interpretation reasonable; thus, the greater activities of the conjugates in the long-term experiments may be due to their persistence after the free IAA has been destroyed (Figs. 2 and 5).

Free IAA is transported by a directed transport system (*cf.* 11). The IAA conjugates on the other hand seem to move in tissues in a nonregulated manner, presumably by diffusion (Fig. 6). Free IAA is also destroyed very rapidly by peroxidative degradation whereas the IAA in the conjugates is probably not destroyed (7) except as the IAA is released. As a consequence of the nonregulated transport of the conjugates, the nondestruction of the conjugates, and the slow release of IAA from the conjugates, each cell in a tissue might well be exposed to the same steady supply of auxin as each other cell. If auxin gradients within tissues are required for the differentiation of various cell types and ultimately for organogenesis, then the controlled transport of auxin and its rapid destruction at its target may be necessary simply to create and maintain these gradients. If so, the marked dedifferentiating effects of IAA-L-alanine are easily explained in terms of the frustration of the tissues' attempts to create essential auxin gradients.

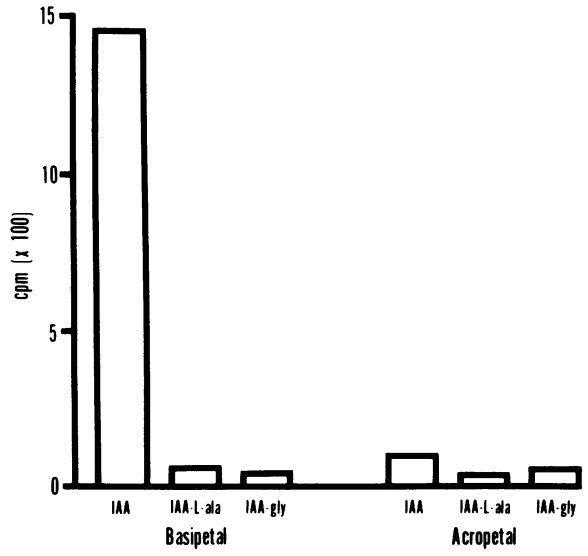

FIG. 6. Transport of [^{14}C]IAA, [^{14}C]IAA-L-alanine, and [^{14}C]IAA-glycine in pea stem segments. Auxin transport is shown as the amount of radioactivity moving from donor blocks through twelve 3.5-mm pea stem segments into the receiver blocks during a 3-h period.

It is possible, however, that the IAA conjugates are auxins in their own right and that they have effects that are qualitatively different from the effects of free IAA. Thus, if IAA-L-alanine acts as an auxin only be releasing free IAA, it is not clear why it prevents organogenesis when used alone and permits organogenesis when it is combined with free IAA in the medium (Figs. 3 and 4). This implies that the two auxins have different actions, but such a conclusion would be premature. It remains possible that the presence of the faster moving IAA with its controlled transport can create auxin gradients even in the presence of the slowly diffusing IAA-L-alanine, since the latter may be hydrolyzed so slowly that it contributes a very small part of the total IAA and therefore cannot wholly obliterate the gradient formed from the exogenous IAA.

Practical Applications. Free IAA is not very effective in supporting the growth of a number of plant tissue cultures, probably because it is rapidly destroyed (20). NAA and 2,4-D are much more stable and very active in stimulating the growth of cultures. However, 2,4-D has some serious disadvantages. Organized tissue growth is repressed (20), there is some indication that it causes chromosome damage (27), and the cytokinin requirement of some tissues can be replaced by it (23). Obviously, 2,4-D is not doing exactly the same things as the natural auxin.

IAA conjugates are very stable to peroxidative atack (7) and probably release the natural auxin IAA at a steady but slow rate. The IAA conjugates can supply a controlled level of auxin activity over a long period of time without the changes in activity expected with the labile, free IAA. In this sense, an IAA conjugate can be thought of as a sort of auxin buffer. In the case of the IAA-amino acid conjugates there is a very low level of toxicity so that large reservoirs of IAA can be provided.

IAA conjugates offer a great diversity of activities in the wide range of conjugates potentially available. Our results show that IAA-L-alanine and IAA-glycine are good auxin sources for the production of callus from tomato hypocotyl explants while the commercially available auxins are not nearly as good. Cultures have been maintained on these conjugates for several months. In the tomato hypocotyl system, where free IAA favors root development, we found a number of the IAA conjugates to favor shoot development instead (Fig. 1). The unique morphogenic responses caused by IAA conjugates deserves further study. Some conjugates may provide necessary stimuli for regeneration of organs from tissue cultures which have not responded successfully to other treatments. Combinations of IAA conjugates and free IAA may also prove generally useful in controlling morphogenesis as in the example shown in Figures 3 and 4. In preliminary experiments we have been able to regenerate shoots from tomato roots, even after the roots had been in culture for more than a year. There is some evidence that this phenomenon requires a combination of free IAA and one of the amino acid conjugates (B. Martin, personal communication). Friable callus and finely divided suspension cultures have been obtained from explants of field corn seedlings using IAA-L-alanine (S. McCormick, personal communication). The glycine conjugate may be useful in the multiplication of orchid protocorms (R. Griesbach, personal communication).

Acknowledgments—The authors wish to express their gratitude to P. S. Carlson

for use of facilities and for advice regarding tissue culture experiments, to H. Kende for use of facilities and for advice regarding ethylene experiments and to J. D. Cohen for the gift of IAA-*myo*-inositol.

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