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Interaction of Free Indole-3-Acetic Acid and Its Amino Acid Conjugates in Tomato Hypocotyl Cultures

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Abstract. The interaction of free IAA and its amino acid conjugates on growth and development of cultured tomato hypocotyl tissue (*Lycopersicon esculentum* Mill. cv. Marglobe) was studied. In a nutrient medium containing 10 μmol/L of benzyladenine, free IAA stimulated shoot and root development with little callus proliferation. In contrast, all IAA–amino acid conjugates tested supported mostly callus growth. Simultaneous application of free IAA and its conjugates resulted in the expression of mixed morphogenetic responses (i.e., both vigorous callus growth and organogenesis resulted). Growth kinetics and the effect of temporal exposure of the tissues to the bound and the free auxin suggest that some IAA–amino acid conjugates may specifically influence plant morphogenesis in ways that cannot be easily explained as simply a function of their slow hydrolysis to release free IAA.

Conjugates of IAA with several amino acids have been isolated from plants and plant pathogens (e.g., Cohen 1982, Epstein et al. 1986, Hutzinger and Kosuge 1968); others have been synthesized for comparative purposes (Feung et al. 1975, Hangarter et al. 1980, Good 1956, Magnus et al. 1992, Mollan et al. 1972, Wieland and Hörlein 1955). At least in some cases, evidence has been presented indicating that the biological activity of these compounds depends on their conversion to the free growth hormone (e.g., Bialek et al. 1983, Hangarter and Good 1981). However, Hangarter et al. (1980) have observed interactions between IAA and IAA-Ala[1] in plant tissue culture, and the responses noted have been difficult to explain solely on the basis of the hydrolysis of the supplied conjugate. We attempt herein to define the extent and nature of those interactions using IAA-Ala, as well as several previously untested IAA conjugates applied to *in vitro* cultures of tomato hypocotyl sections. Such explants formed predominantly unorganized callus in the presence of the conjugates, whereas shoot and root formation prevailed with free IAA. With mixtures of free and conjugated IAA, both morphogenetic responses were observed on the same explant.

Materials and Methods

Chemicals

Commercial, analytical grade chemicals were used except for the following: IAA-Ala and IAA-Asp were synthesized by dicyclohexylcarbodiimide-mediated condensation of IAA with the amino acid benzyl ester (Good 1956) followed by removal of the protective ester group by short alkaline hydrolysis. IAA-Lys was prepared according to Hutzinger and Kosuge (1968); essentially the same method was used for the synthesis of IAA-Orn. IAA-Thr was obtained as described by Hangarter et al. (1980). Except for DL-aspartic acid, L-amino acids were used in the syntheses and no major racemization was noted [see Magnus et al. (1992) for details]. The conjugates were purified on a column (4 × 30 cm) of Sephadex LH-20 eluted with 2-propanol:water (1:1, vol/vol) and/or recrystallization from water (IAA-Asp, IAA-Lys, IAA-Orn) or 30–50% (vol/vol) aqueous ethanol (IAA-Ala, IAA-Thr) until homogeneous and completely free of nonconjugated IAA [TLC, Ehmann's reagent (Ehmann 1977), detection limit ~0.1%]. IAA-Lys and IAA-Orn were subjected to Sephadex chromatography as the hydrochlorides. The fractions containing the conjugates were then neutralized for recrystallization.

[1] **Abbreviations:** IAA, indole-3-acetic acid; IAA-Ala, *N*-(indol-3-ylacetyl)-L-alanine; IAA-Asp, *N*-(indol-3-ylacetyl)-DL-aspartic acid; IAA-Lys, N_ϵ-(indol-3-ylacetyl)-L-lysine; IAA-Orn, N_δ-(indol-3-ylacetyl)-L-ornithine; IAA-Thr, *N*-(indol-3-ylacetyl)-L-threonine.

Nutrient Medium

The medium contained the mineral salts of Murashige and Skoog (1962) and the following additives (mg/L): thiamine·HCl (1), pyridoxine·HCl (0.5), nicotinic acid (0.5), *myo*-inositol (100), sucrose (30,000), agar (9000), 2-(*N*-morpholino)ethanesulfonic acid (956), benzyladenine (10 μmol/L unless stated otherwise), a source of auxin as specified below, and sufficient KOH solution to adjust the pH to 6.0 (before the addition of agar and autoclaving). Glass-redistilled water was used in all solutions prepared for tissue culture experiments. Tissues were grown in sterile disposable Petri dishes (polystyrene; diameter 4.5 cm, ~15 ml medium/dish). At least five plates were prepared for any particular combination of the growth regulators tested.

Plant Material

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Marglobe) were sterilized by soaking them for 20 min in 0.5% (wt/wt) sodium hypochlorite solution containing 0.01% (wt/wt) of sodium dodecyl sulfate, rinsed with sterile water, planted on water agar (0.8%, wt/vol) containing sucrose (0.5%, wt/vol), and left to germinate in the dark, at 27°C, for 6 days. The hypocotyls were cut into sections about 5 mm in length, excluding the apical and basal 5 mm. Sections were randomly distributed to Petri dishes containing the test media, using four sections per plate. The Petri dishes were sealed with Parafilm and kept at 22°C, under continuous illumination from "cool-white" fluorescent lamps (GE Deluxe) applying 40–60 μmol m^{-2} s^{-1} of photosynthetically active radiation (400–700 nm) at the level of the cultures.

For evaluation of organogenesis, a shoot was defined as containing at least one leaf, possibly vitrified and/or rush-like in shape. Leaves and roots partially transformed into callus were classified according to their general appearance. Fresh weights of shoots and calli were determined separately for each individual explant.

For monitoring growth kinetics, five to 10 plates per treatment were sampled at random from the same lot of explants, in regular time intervals. The explants were weighed and shoots and roots (visible to the naked eye without dissecting the explants) were counted. Chlorophyll was extracted with ethanol:water (8:2, vol/vol) at 85°C, for 30 min, and its concentration (μg/ml) was determined spectrophotometrically as 20.2 (A_{645}–A_{710}) + 8.02 (A_{665}–A_{710}).

When hypocotyl sections were transplanted to a second medium, evaluations were made when the explants had entered the near-stationary phase of growth, which was about 2 months after the last transfer, and up to 3 months after planting. Controls included to assess mechanical effects of transplanting are discussed for each experiment. To ensure comparable results, the experiments which did not involve transfer to a second medium were also evaluated during the third month after planting.

Results and Discussion

Characterization of the Experimental System

The response of tomato hypocotyl explants to free and conjugated IAA depends on the cytokinin level in the medium. For example, in the absence of cytokinin, both IAA and IAA-Ala stimulated root formation. As the benzyladenine concentration increased, differences in the morphogenetic responses to the two sources of auxin began to appear (data not shown), and were distinctly expressed at a cytokinin level of 10 μmol/L: IAA-Ala favored callus formation; IAA more efficiently stimulated shoot and root growth. The interaction of free and conjugated IAA was therefore examined at a benzyladenine concentration of 10 μmol/L, as in the prior studies by Hangarter et al. (1980).

The growth characteristics of tomato hypocotyl explants over a 6-week period are shown in Fig. 1. Hypocotyl segments, weighing 3.2 ± 0.2 mg were planted on media containing IAA (1 and 10 μmol/L), IAA-Ala (1 μmol/L), or no auxin (control). During the first 10 days, the explants gained about 15 mg of weight, regardless of the presence or absence of a source of auxin (Fig. 1a). The growth of the controls then started to lag behind, whereas the weight increase with IAA and IAA-Ala remained about the same up to the end of the third week, when vigorous callus growth began in conjugate-treated tissues.

Shoots formed mostly on the side of the explants in contact with the medium. For clarity, we will distinguish: (1) bud induction, (2) leaf growth, (3) leaf differentiation, and (4) internode elongation. While bud induction responded to auxin treatment, growth kinetics indicated that meaningful shoot counts can only be obtained 3 to 4 weeks after planting (depending on the source of auxin), before various secondary transformations start to occur. As this is well before the effect of free and bound auxin on callus growth and root induction is fully expressed, shoot counts were abandoned as an impractical criterion for comparative studies. It was possible to estimate the extent of shoot formation by measuring the chlorophyll content in the tissues (Fig. 1b). However, the weight of the shoots (comprising the leaves and the stunted internodes) per explant determined at the end of the culture period was found to be a more reproducible criterion permitting comparison of the effects of free and conjugated IAA on shoot growth in tomato hypocotyl explants (Table 1). Differentiated, pinnate tomato leaves were frequently formed in the presence of free IAA, while rush-like structures prevailed when only the conjugates (in particular, the more active ones) were supplied. Internode elongation was (reversibly, as suggested by transfer to hormone-free medium) inhibited by benzyladenine at the routinely used concentration (10 μmol/L).

Although not shown in Fig. 1, root growth was observed to occur predominantly during the early and late stages of culture. Five to 10 days after planting, single roots appeared on about 5% of the explants (on the side *not* in contact with the me-

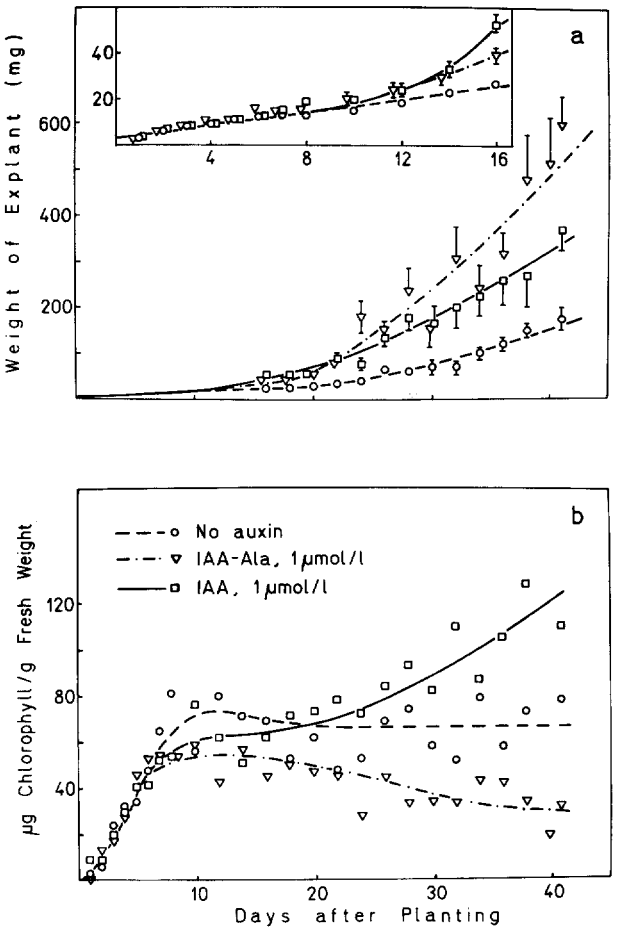

Fig. 1. Time course of growth and differentiation in tomato hypocotyl explants grown on media containing no auxin or 1 µmol/L of free IAA or IAA-Ala. (a) Weights of the whole explants including callus, shoots, and roots; (b) amount of chlorophyll per grams fresh weight. In (a) growth during the first 16 days is presented in an insert using expanded scales on both the abscissa and the ordinate; standard errors are indicated as far as they exceed the margins of the symbols presenting the data points. In (b) the amount of plant material available required analysis of pooled samples only, thus standard errors could not be obtained. The determination of chlorophyll, which is preferentially localized in the newly formed shoots, permitted (approximate) monitoring of shoot growth at the early stages of organogenesis when the shoots were too small to be excised and weighed. Extreme variability of shoot growth (and organogenesis in general) reflected by the widely scattered data points was typical for no-auxin controls. The response to 10 µmol/L of IAA was about the same as obtained with a free IAA level of µmol/L (data not shown).

dium), in the presence or absence of auxin. Auxin-induced root growth was observed after 30 to 40 days in cultures on IAA, and several weeks later for IAA-Ala treated material. In contrast to the situation with shoots, root *numbers* were the better quantitative criterion to monitor auxin effects.

While callus weights depended on the source of auxin applied (Fig. 2a), there appeared to be no consistent morphological differences. Developmental differences reflecting the source of auxin used may, however, exist. For instance, increasing concentrations of *N*-naphthylphthalamic acid (0.01–100 µmol/L) progressively *inhibited* callus growth in response to IAA-Ala (1 µmol/L). The same treatment *stimulated* callus growth in the presence of free IAA (1 and 100 µmol/L). In contrast, the effect of *N*-naphthylphthalamic acid on organogenesis (inhibition at high concentrations) showed no obvious dependence on the source of auxin supplied (data not shown).

Table 1. Callus growth and organogenesis in tomato hypocotyl explants cultured on media containing both free IAA and its amino acid conjugates.[a]

Conjugate	Concentration (µmol/L)	Mean callus weight[b] (mg)	Organogenesis at optimal IAA concentration[c] Shoot weight (mg)	Root number
IAA-Asp	0	459 ± 17	284 ± 45	7.5 ± 0.6
	10	483 ± 18	271 ± 49	7.5 ± 0.6
	100	641 ± 26	193 ± 45	5.6 ± 0.6
IAA-Thr	0	447 ± 18	282 ± 49	7.8 ± 0.7
	10	487 ± 20	261 ± 55	6.2 ± 0.5
	100	788 ± 24	118 ± 23	4.7 ± 0.6
IAA-Orn	0	524 ± 13	275 ± 27	5.5 ± 0.6
	1	580 ± 13	145 ± 23	6.9 ± 0.5
	10	907 ± 20	No shoots	4.2 ± 0.7
IAA-Lys	0	560 ± 16	155 ± 30	5.2 ± 0.5
	1	890 ± 19	No shoots	4.7 ± 0.4
	10	1036 ± 26	No shoots	No roots

Organogenesis in the absence of free IAA (in parentheses: conjugate concentration in µmol/L). Shoot weights (mg/explant): IAA-Asp: 179 ± 32 (10), 144 ± 32 (100); IAA-Thr: 65 ± 23 (10), 48 ± 17 (100); IAA-Orn: 47 ± 19 (1), 0 (10); IAA-Lys: 0 (1, 10). Root numbers: IAA-Asp: 1.5 ± 0.4 (10), 1.7 ± 0.3 (100); IAA-Thr: 0.7 ± 0.2 (10), 1.2 ± 0.4 (100); IAA-Orn: 1.4 ± 0.2 (1), 2.5 ± 0.4 (10); IAA-Lys: 2.6 ± 0.5 (1), 0 (10).

[a] The conjugates were tested in combination with the following series of IAA concentrations: 0, 1, 1.5, 3, 6, 10, 15, 30, 60, and 100 µmol/L using 16–20 explants per treatment. The general shape of the dose–response curves was the same as in Fig. 3. The numbers given are arithmetic means ± standard error of the mean.

[b] Grand mean ± standard error calculated from callus weights at 0–100 µmol/L of free IAA.

[c] Optimal ranges of IAA concentrations were, in the experiments presented in this Table, 6–15 µmol/L for shoot growth and 60–100 µmol/L for root formation (exception: 6–10 µmol/L for IAA-Lys).

Response to IAA, IAA-Ala, and to Mixtures of Both Compounds

The results of a representative experiment showing

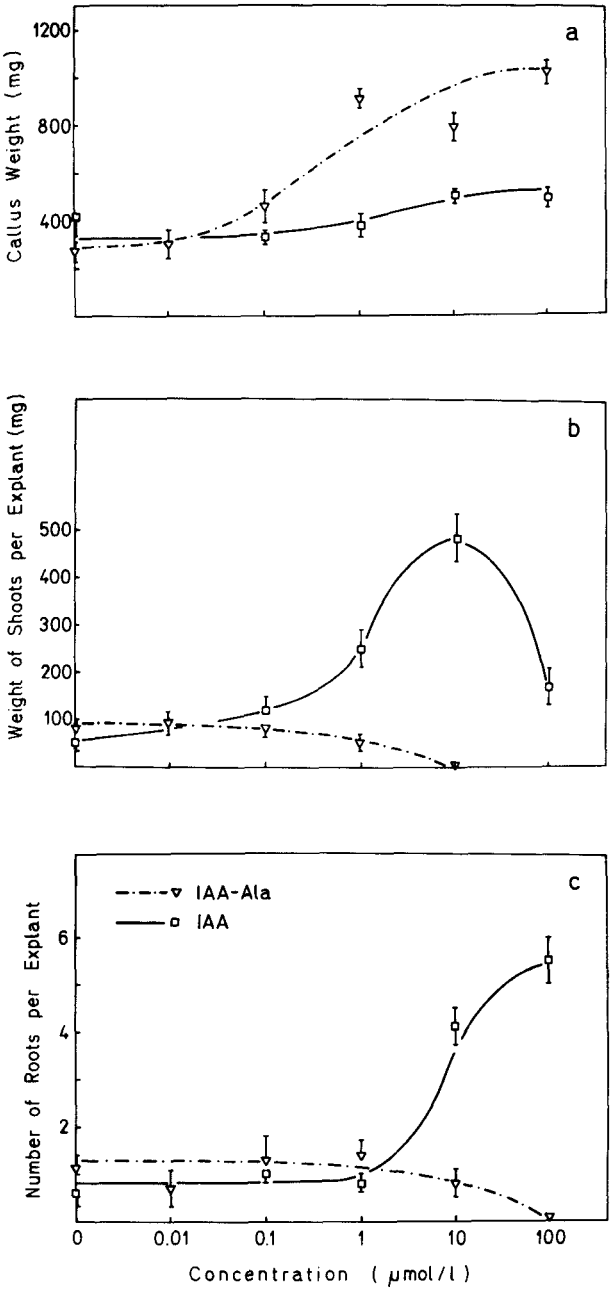

Fig. 2. Callus weights (**a**), shoot weights (**b**), and root numbers (**c**) for tomato hypocotyl explants supplied with various concentrations of either free IAA or IAA-Ala. The data were collected 2 months after planting; bars indicate standard errors exceeding the margins of the symbols used to present data points. The scale on the abscissa is proportional to log(concentration + 0.001). This transformation permits presentation of zero concentration, while deviations from an ordinary log-scale are negligible at higher concentrations.

the effect of IAA and IAA-Ala on growth and differentiation of tomato hypocotyl explants are presented in Fig. 2. Free IAA stimulated maximal shoot growth at a concentration of 10 μmol/L, while 100 μmol/L was optimal for root formation. These values varied somewhat between experiments, ranging from 1–15 μmol/L for optimal shoot growth and from 60–100 μmol/L for maximal root formation. Callus growth was modest at any IAA level examined, whereas IAA-Ala mainly promoted callus proliferation. For concentrations of the conjugate up to 1 μmol/L, shoot and root growth were about the same as in no-auxin controls, but higher concentrations were inhibitory. Auxin concentrations above 100 μmol/L were not examined as they were known from previous studies (Magnus et al. 1992) to be inhibitory. The results presented extend the studies of Hangarter et al. (1980) in which root formation in the presence of IAA-conjugates was not noted due to the shorter culture period (1 month) used.

As previously described by Hangarter et al. (1980), when free IAA and IAA-Ala were applied simultaneously, both the root initiation effect of the free auxin and callus formation in response to the conjugate were expressed. The concentration dependence of this interaction is shown in Fig. 3. Callus growth responded mainly to the concentration of IAA-Ala. Root induction by free IAA was stimulated by intermediate, and inhibited by high levels of the conjugate. Figure 3 further shows that IAA-induced shoot growth can persist in the presence of IAA-Ala. The optimal IAA concentration remained unchanged, in this case, while the entire dose–response curve was gradually attenuated as the background level of the conjugate increased.

Interaction of Free IAA with Other Amino Acid Conjugates

The four other IAA–amino acid conjugates tested for interaction with free auxin could be classified into two phenomenological types: (1) IAA-Lys and IAA-Orn, like IAA-Ala, supported vigorous callus proliferation and suppressed shoot growth at all but the lowest concentrations; and (2) IAA-Asp and IAA-Thr moderately stimulated callus development while shoot growth persisted even at high concentrations. There were no clear differences with respect to root formation. The interaction of these conjugates with free IAA (Table 1) followed a similar pattern as described for IAA-Ala, only the range of concentrations in which such an effect was observable was different. IAA-Lys, the most active compound tested, even at the 1 μmol/L level completely inhibited IAA-induced shoot growth.

Transplanting from Auxin-Containing to Auxin-Free Media

IAA and IAA-Ala interact when administered simultaneously, but the temporal requirements for

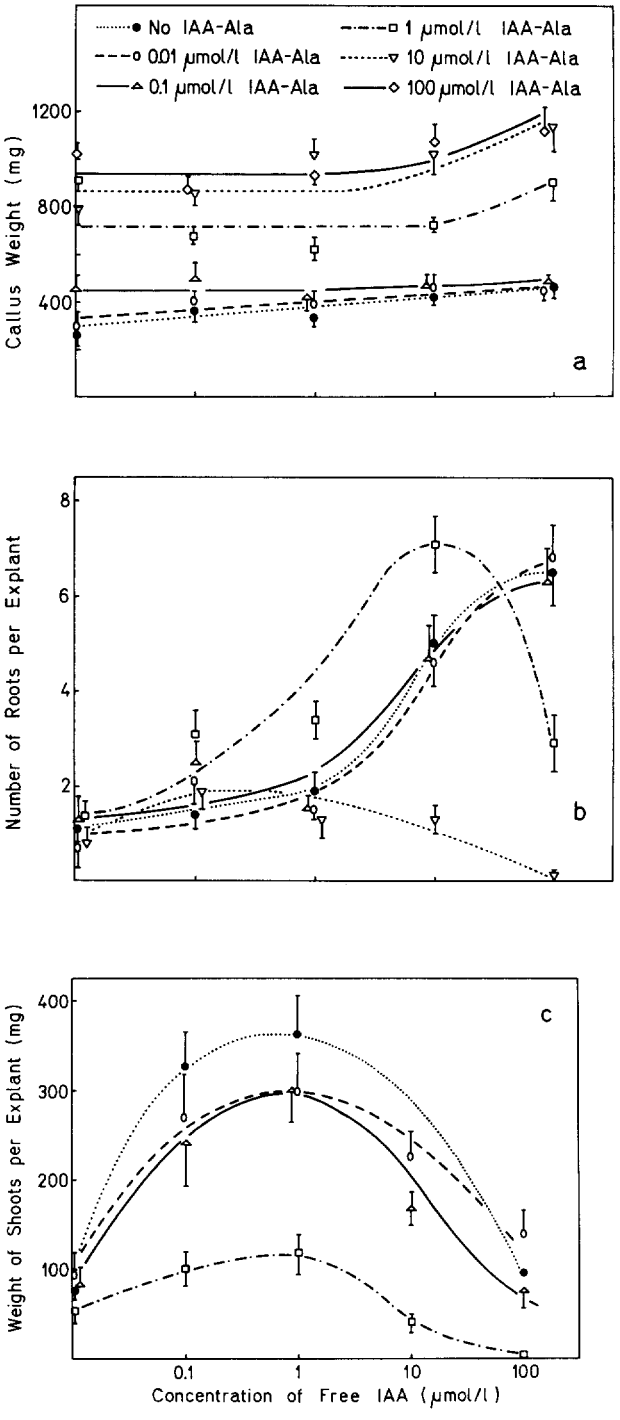

Fig. 3. Effect of free IAA in the presence of various background levels of IAA-Ala on (**a**) callus weights, (**b**) root numbers, and (**c**) shoot weights in tomato hypocotyl explants. The data were collected 2 months after planting; bars indicate standard errors exceeding the margins of the symbols used to present data points. The scale on the abscissa is proportional to log(concentration + 0.01) for reasons explained in the legend of Fig. 2. Shoot weights at an IAA-Ala concentration of 10 μmol/L were too small to be presented in (**c**); neither shoot nor root growth were noted at 100 μmol/L of IAA-Ala. Sample-dependent differences (Fig. 2 *vs.* Fig. 3) in the auxin response are discussed in the text.

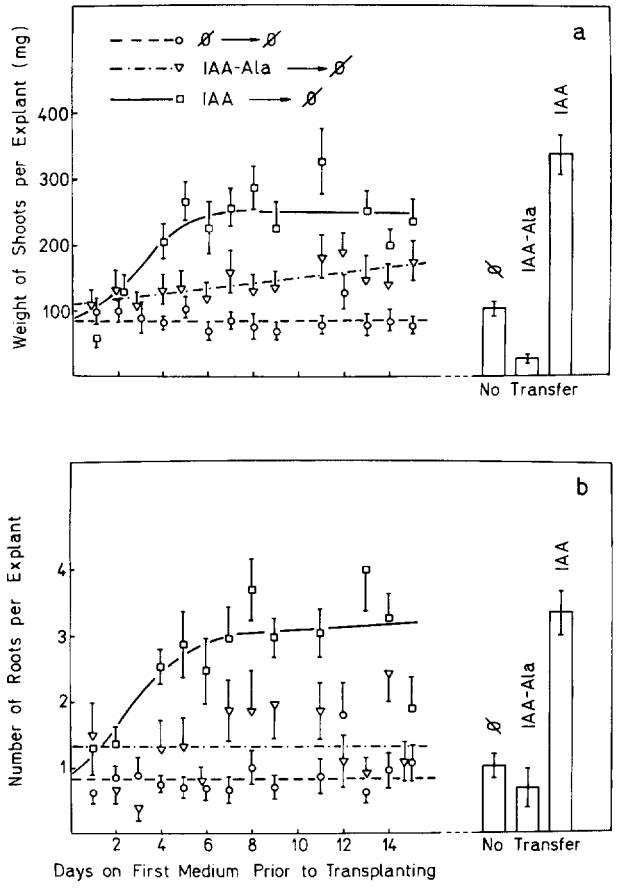

Fig. 4. Effect of transplanting tomato hypocotyl sections from a medium containing IAA (10 μmol/L), IAA-Ala (1 μmol/L), or no auxin (Ø) to an otherwise identical medium devoid of auxins (Ø) on (**a**) shoot weights and (**b**) root numbers. Data were collected 2 months after transplanting. For transfer from IAA-Ala to auxin-free medium in (**a**) linear regression analysis suggested a significant ($p = 0.05$) correlation.

their presence could only be determined by applying them in sequence. To facilitate the interpretation of such experiments, explants were first kept on either IAA or IAA-Ala for 1–14 days, and then transferred to auxin-free medium (Fig. 4). Since transplanting not only deprives the tissues of an external source of auxin, but also exposes them to a fresh supply of cytokinin, vitamins, and nutrients, control samples were grown on auxin-free medium and transferred to medium of the same composition. In the controls, shoot and root growth were not affected by transplanting. In both auxin-pretreated tissues and the controls, the effect of transplanting during the 2-week period examined was small (+10–20%) and inconsistent with callus weights, which reached about 450 mg/explant (data not shown).

Hypocotyl explants transferred from free IAA to auxin-free medium after 5 or more days formed

about the same number of roots and shoots of about the same weight as if left on the original medium (Fig. 4). This indicates an early inductive effect of IAA on organogenesis. Tissues grown on IAA-Ala, on the other hand, showed more shoot growth when transferred to auxin-free medium than when left in the presence of the conjugate (Fig. 4a), but there was no obvious induction period. In the latter experiment, average root formation was also slightly enhanced in tissues transplanted to auxin-free medium, but the data points (Fig. 4b) were too widely scattered to permit more specific conclusions. No marked effect on callus weights was noted for explants transferred from either IAA or IAA-Ala to auxin-free medium within 15 days after planting (data not shown).

Transfer from IAA to IAA-Ala and Vice Versa

Root formation induced by exposing the explants to free IAA for 7 or more days was expressed even though the tissues were transferred to IAA-Ala before these roots started to elongate (Fig. 5c). This was also true for shoot growth (Fig. 5b), although the response was less pronounced than on transplanting to auxin-free medium. Shoot growth induced by free IAA thus appears to be inhibited by subsequent application of the conjugate. The widely scattered values in the respective control experiments (transfer from IAA to fresh IAA-containing medium; Fig. 5b and c) are likely due to orientation changes during transfer. Turning the explants even slightly during that operation will expose a different part of their surface to contact with the medium and this may well be a stimulus for additional induction of shoots and, in particular, roots.

Tomato hypocotyl sections grown on IAA-Ala became increasingly less responsive to free IAA as an inducer of organogenesis (Fig. 5e and f). Comparing the time-course of that inhibition to the kinetics of shoot and root induction by free IAA (Figs. 4 and 5b and c) strongly suggests that IAA and IAA-Ala are antagonists in the early inductive processes. Interestingly, 3 weeks of exposure to IAA-Ala were sufficient to achieve the same level of callus growth (after transplanting to IAA) as in the continuous presence of the conjugate (Fig. 5d). This may suggest that there is an inductive component to callus formation in response to IAA-Ala.

General Discussion

Plant tissues are capable of hydrolyzing IAA–amino acid conjugates to yield the free hormone (e.g., Bialek et al. 1983, Hangarter and Good 1981) and, thus, it may be assumed that hydrolysis is a major factor contributing to the auxin activity of such conjugates. Indeed IAA conjugates that are readily hydrolyzed function as a good source of auxin in tissue culture, whereas those that are poorly hydrolyzed are less capable of stimulating auxin-induced growth effects (Hangarter and Good 1981). Addition of free IAA along with an IAA–amino acid would therefore be expected to simply increase the auxin supply to the tissue and further stimulate callus growth. However, the results reported here and elsewhere (Hangarter et al. 1980) suggest that at least some functions of conjugated IAA may be unique from those of free IAA.

When free IAA is added to nutrient media, it is chemically degraded and metabolized by the cultured tissues at a relatively fast rate (Hangarter and Good 1981). If an amino acid conjugate is used instead, it is slowly hydrolyzed and should thus yield a steady-state level of IAA which is maintained for a longer period of time (Hangarter and Good 1981). These differences in the "pharmacodynamics" of free and conjugated IAA are, for example, reflected by the onset of root growth in tomato hypocotyl tissues. Once roots are induced, their elongation, which is inhibited by all but very low auxin levels, should not occur until the supply of IAA in the medium is depleted. This indeed occurred earlier, and in a more synchronized fashion, for IAA-grown tissues (30–40 days) and, somewhat diffusely, towards the end of the culture period (60–80 days) when IAA-Ala was the source of auxin. Also, transferring tomato hypocotyl sections every 4 days to fresh medium containing 17 μmol/L of IAA was previously shown to mimic the effect of continuous culture in the presence of an equimolar amount of IAA-Ala (Hangarter et al. 1980).

A pharmacodynamic model may also be adequate to explain some of the interactions of free and bound IAA in tomato hypocotyl explants. Applying both sources of auxin together in an appropriate ratio would expose the explants to an initially high IAA concentration, which would then decline to a constant lower level. Moreover, these pharmacodynamic changes may not affect the individual tissues within an explant to the same extent. The mechanisms of uptake and distribution (within the explants) are different for free and bound auxin, as exemplified by the fact that, in contrast to free IAA, IAA-Ala, and IAA-Glycine were *not* basipetally transported through stem sections (Hangarter et al. 1980). The activities of the hydrolases liberating IAA from its amino acid conjugates, and of the IAA-metabolizing enzymes, may also be different in individual tissues of the same explant. Indeed differences in local auxin levels, or even defined concentration gradients, are a likely prerequisite for

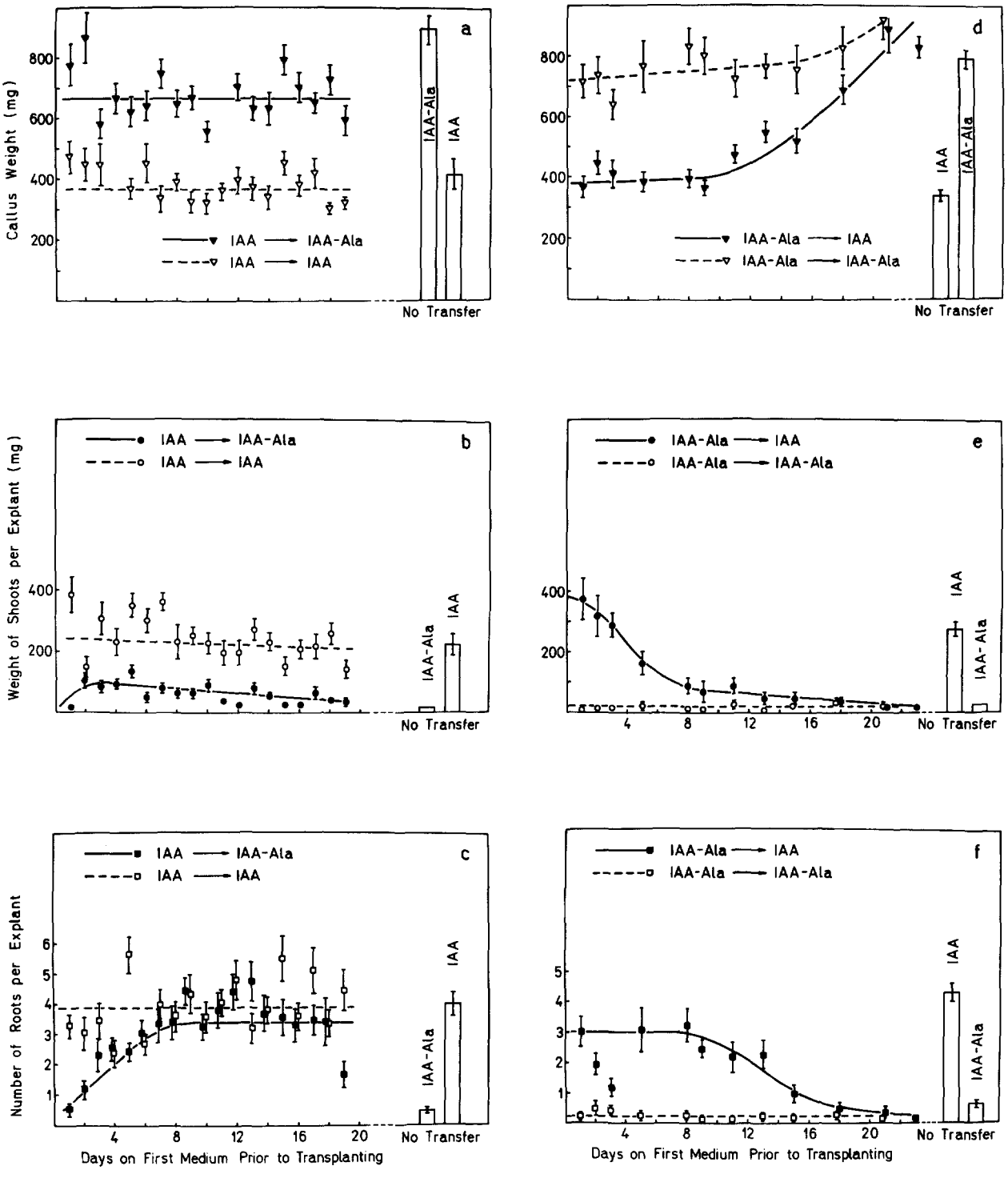

Fig. 5. Effect of transferring tomato hypocotyl explants from a medium containing free IAA (10 μmol/L) to fresh medium containing IAA-Ala (1 μmol/L) (**a–c**) and *vice versa* (**d–f**), on callus growth (**a, d**), shoot development (**b, e**), and root formation (**c, f**). Data were collected 2 months after the last transfer. Data points which represent the effect of transplanting to a medium containing a different source of auxin are presented by solid symbols, while open symbols are used for the controls (transplanting to medium of identical composition).

concerted, organized growth, and thus also for organ induction and development in plant tissue culture. During the responsive phase (first week after planting), transiently high amounts of free IAA imported directly from the nutrient medium may be required to establish the patterns of auxin distribution necessary to induce organogenesis. In contrast, IAA continuously liberated from an amino acid con-

jugate would distribute in a pattern favoring callus growth, while interfering with the development of already induced organs to a limited extent. The results obtained when explants were transferred from IAA to IAA-Ala (Fig. 5b and c) are consistent with such a pharmacodynamic model. Organs that were induced by free IAA continued to develop in the presence of callus-inducing levels of IAA-Ala, although the shoots did not reach the size achieved in continuous culture on IAA-containing medium.

However, several observations are difficult to explain completely within the framework of the pharmacodynamic model. For example, in contrast to the effects noted at high concentrations (see above), at low auxin levels (1.7 μmol/L), explants transferred every 4 days to fresh IAA-containing medium showed less callus and more root formation than those continuously grown on medium supplied with an equimolar concentration of IAA-Ala (Hangarter et al. 1980). Also, the growth kinetics presented here (Fig. 1) did not indicate differences in the amount of free auxin available to explants grown on IAA and IAA-Ala (both 1 μmol/L), during the first 3 weeks after planting. As growth rates and concentrations of available free auxin are often correlated (Bialek et al. 1983), the pharmacodynamic model would predict that explants supplied with free IAA grow faster, during that period, than those cultured in the presence of IAA-Ala, but they grew at approximately the same rates. Moreover, the time-course of IAA-induced shoot growth and root initiation (Fig. 5b and c) and their corresponding inhibition by pretreatment with IAA-Ala (Fig. 5e and f) are so closely correlated that specific competition of free and bound IAA at some critical steps should be considered. In an IAA–amino acid conjugate, although the carboxyl group of IAA is chemically modified, the indole ring remains sterically accessible as demonstrated by x-ray crystallography and NMR methods (Duddeck et al. 1989, Kojić-Prodić et al. 1990, 1991). Moreover, the conformation of the IAA moiety in most amino acid conjugates is the same as in the free hormone. Thus, conjugated IAA may display at least some affinity for the IAA-binding sites of auxin "receptors" and auxin-regulated enzymes. If so, these proteins may (1) execute their normal functions, (2) be inhibited or less efficient, or (3) be completely inactivated. This would lead to complex interactive patterns when free and bound IAA are added in sequence, or simultaneously.

Attributing physiological activity to IAA-conjugates extends classical concepts (Cohen and Bandurski 1982) which have interpreted the function of the bound auxins mainly in terms of reconversion to free IAA. It has recently been shown, however, that IAA-glucose has a positive, and IAA-inositol a negative cooperative effect on the IAA-induced curvature of *Avena* coleoptiles (Wodzicki et al. 1987). Furthermore, IAA-Asp was metabolized to 2-indolone-3-acetyl aspartic acid, 3-hydroxy-2-indolone-3-acetyl aspartic acid, and its 3-*O*-glucoside (Plüss 1987, Tsurumi and Wada 1986) without involving free IAA as an intermediate. The biochemistry and physiology of the bound auxins may thus be more complex than has previously been recognized.

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