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Auxin-Induced Ethylene Production as Related to Auxin Metabolism in Leaf Discs of Tobacco and Sugar Beet[1]

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ABSTRACT

Exogenously supplied indole-3-acetic acid (IAA) stimulated ethylene production in tobacco (*Nicotiana glauca*) leaf discs but not in those of sugar beet (*Beta vulgaris* L.). The stimulatory effect of IAA in tobacco was relatively small during the first 24 hours of incubation but became greater during the next 24 hours. It was found that leaf discs of these two species metabolized [1-^{14}C]IAA quite differently. The rate of decarboxylation in sugar beet discs was much higher than in tobacco. The latter contained much less free IAA but a markedly higher level of IAA conjugates. The major conjugate in the sugar beet extracts was indole-3-acetylaspartic acid, whereas tobacco extracts contained mainly three polar IAA conjugates which were not found in the sugar beet extracts. The accumulation of the unidentified conjugates corresponded with the rise of ethylene production in the tobacco leaf discs. Reapplication of all the extracted IAA conjugates resulted in a great stimulation of ethylene production by tobacco leaf discs which was accompanied by decarboxylation of the IAA conjugates. The results suggest that in tobacco IAA-treated leaf discs the IAA conjugates could stimulate ethylene production by a slow release of free IAA. The inability of the exogenously supplied IAA to stimulate ethylene production in the sugar beet leaf discs was not due to a deficiency of free IAA within the tissue but rather to the lack of responsiveness of this tissue to IAA, probably because of an autoinhibitory mechanism existing in the sugar beet leaf discs.

In vegetative tissues, the rate of ethylene production is thought to be regulated by the endogenous level of free auxin (9, 18). Recently, Yu *et al.* (24, 25) demonstrated that IAA stimulates ethylene production by inducing the synthesis of the ACC[2] synthase, which is the rate-limiting enzyme in the pathway of ethylene biosynthesis (23). Evidence has been presented, showing that the rate of IAA-induced ethylene production was parallel to the level of free IAA retained in the tissue (15, 17, 18). The level of the latter depends on the rate of IAA uptake, decarboxylation, conjugation, and IAA transport out of tissue.

In this study, leaf discs of tree tobacco and sugar beet were investigated. Tobacco leaf discs produce a large amount of ethylene which lasts for several days, and exogenous IAA enhances it. The second system, leaf discs of sugar beet, produces a small amount of ethylene (after the amount of wound-induced ethylene subsidies) and added IAA fails to induce ethylene production. Rates of uptake and metabolism of [^{14}C]IAA in relation to ethylene production were studied.

MATERIALS AND METHODS

Plant Material and Pretreatment. Experiments were conducted with discs taken from fully expanded leaves of sugar beet (*Beta vulgaris* L. cv Saccarifera) and of tree tobacco (*Nicotiana glauca*). Sugar beet was grown in a greenhouse under natural light at temperatures between 20 and 30°C. Tree tobacco was grown outdoors on the campuses of the University of California, Davis, and of the Volcani Center, Rehovot, Israel. Leaves were washed in running tap water, surface-sterilized with 0.5% NaOCl solution for 30 s, and washed several times in sterile distilled H_2O. Discs (1 cm in diameter) were cut from leaf blades with a corkborer and were floated for about 1 h in Petri dishes containing H_2O. In one experiment, leaf discs of sugar beet were floated in Petri dishes on 0.1 mM $AgNO_3$ solution for 40 min.

Incubation Media. Ten leaf discs were incubated in each 25-ml Erlenmeyer flask while floating, abaxial surface down, on 2 ml of incubation medium containing 50 mM Mes buffer (pH 6.1), 2% sucrose, and 50 μg/ml chloramphenicol. Where indicated, additions were: ACC (Calbiochem), [1-^{14}C]IAA (Amersham/Searle Corp., 58 mCi/mmol), 2,4-D and IAA (Sigma). In those experiments in which labeled IAA was employed, a plastic center well containing a filter paper wick wetted with 0.2 ml of 50% KOH was hung in the flask to absorb evolving CO_2. The flasks were sealed with rubber serum caps and incubated in darkness at 30°C.

Measurement of Ethylene Production Rate. Average rates of ethylene production during the incubation of the leaf discs were determined by analysis of a gas sample withdrawn from the Erlenmeyer flasks with a hypodermic syringe. Ethylene was allowed to accumulate as indicated and thereafter the flasks were flushed with fresh air. Ethylene was analyzed by a gas chromatograph equipped with an alumina column and a flame ionization detector.

Measurement of Decarboxylation of [1-^{14}C]IAA. $^{14}CO_2$ absorbed by KOH solution during the incubation was released by acidification with lactic acid, then reabsorbed into 0.5 ml of ethanolamine-ethoxyethanol mixture (1:1, v/v), and assayed by liquid scintillation (17).

Extraction Procedure. At the end of incubation, leaf discs were rinsed with sterile water and grounded by glass homogenizer with 2 ml of 70% (v/v) ethanol. The homogenizer was washed twice with 1 ml of 70% ethanol. The homogenate was centrifuged

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; IAAsp, indole-3-acetyl-aspartic acid; IAGlu, 1-(indole-3-acetyl)-β-D-glucose; SAM, *S*-adenosylmethionine.

at 5000 rpm for 10 min. The pellet was resuspended in 2 ml of 70% ethanol and centrifuged again. The combined extract was concentrated *in vacuo* at 38°C to a final volume of 1.5 ml and a 50-μl aliquot of it was counted for radioactivity.

Measurement of Radioactivity in the Pellet. The pellet was resuspended with 70% ethanol and collected by filtration through filter paper in a Büchner funnel. The filter paper containing the pellet was combusted in a Packard automatic oxidizer, and the $^{14}CO_2$ evolved was absorbed and counted for radioactivity.

Radioactivity of the pellet, incubation medium, extract, and $^{14}CO_2$ evolved from IAA decarboxylation are expressed as dpm after quenching and efficiency corrections.

Chromatography of Extract. A 100-μl aliquot of the extract was chromatographed on a Whatman 3MM paper using different solvent systems. Unlabeled IAA (Sigma), IAAsp (Research Organic Inc.), and IAGlu (kindly provided by Dr. J. Riov) were cochromatographed. After drying, the chromatograms were scanned and visualized under short UV light for location of the unlabeled standards.

Accumulation and Application of IAA Conjugates. In order to collect a high amount of metabolites, five samples of 10 tobacco leaf discs were incubated for 3 d in five flasks, each containing 3 ml of Mes buffer, chloramphenicol, 1.0 mM IAA, and 9 μM [1-^{14}C]IAA (1 μCi). Preliminary experiments revealed that under these conditions, without sucrose in the medium, maximum IAA conjugates are accumulated. The leaf discs were pooled and extracted as described above. The concentrated extract (0.8 ml containing 4×10^6 dpm ^{14}C) was equally loaded on six paper strips and chromatographed with 1-butanol:acetic acid:H_2O (4:1:4, v/v). After drying, each of the scanned chromatograms was divided into 7 R_F sections (Fig. 8). Every three parallel R_F sections were placed in an Erlenmeyer flask and eluted to the 5-ml incubation medium containing Mes buffer, chloramphenicol, and 2% sucrose, by shaking for 24 h. Thereafter, 10 tobacco leaf discs were placed in each of the Erlenmeyer flasks for 3 d incubation, during which rates of ^{14}C uptake, decarboxylation, and ethylene production were daily monitored.

Treatments with each experiment were tested in duplicate or triplicate flasks. The standard errors were generally in the range of 5 to 15% of the means. The experiments were repeated at least twice and gave reproducible results. Representative experiments are presented.

RESULTS

Induction of C_2H_4 Production by IAA and ACC. IAA applied to tobacco leaf discs at concentrations above 10 nM induced C_2H_4 production which increased progressively with increasing concentrations of IAA (Fig. 1). In sugar beet leaf discs, regardless

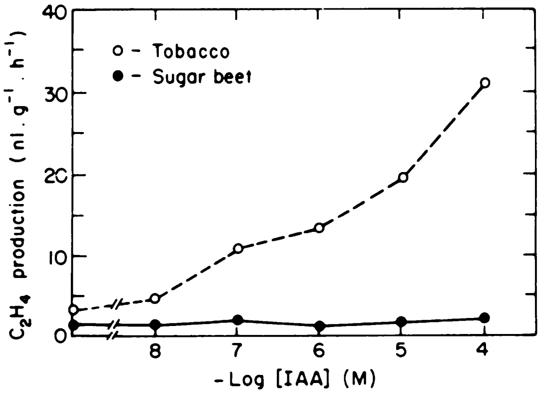

FIG. 1. Effect of increasing concentrations of IAA on average ethylene production rates by tobacco and sugar beet leaf discs. Ethylene was allowed to accumulate for 20 h.

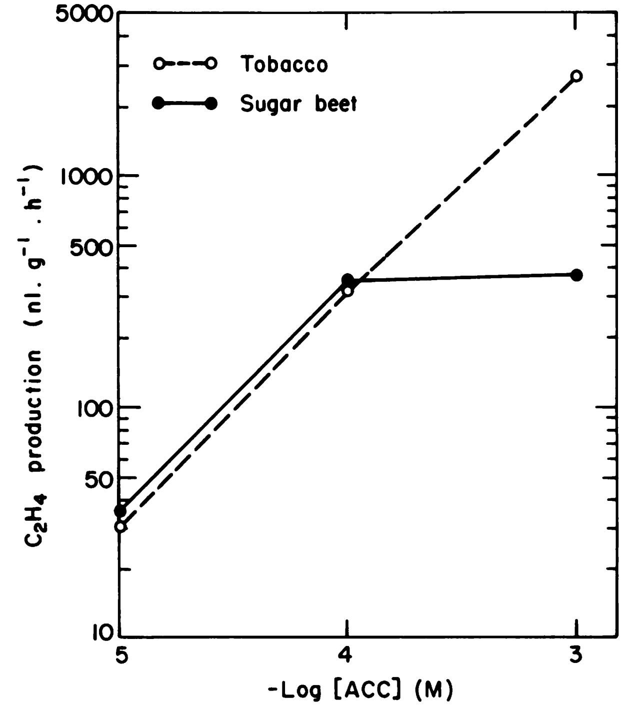

FIG. 2. Effect of increasing concentrations of ACC on average ethylene production rates by tobacco and sugar beet leaf discs. Ethylene was allowed to accumulate for 18 h.

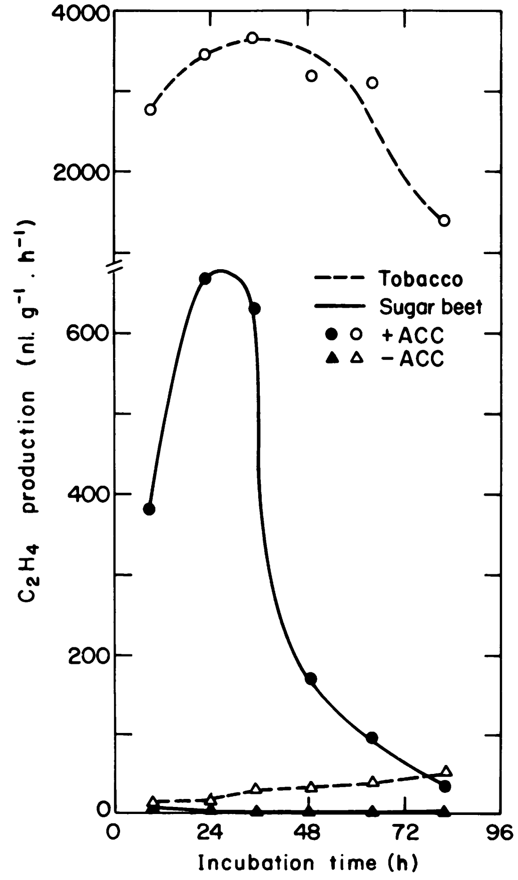

FIG. 3. Time course of average ethylene production rates by tobacco and sugar beet leaf discs incubated with 1 mM ACC.

of either the variety or the age of the leaf used, exogenously supplied IAA did not increase ethylene production. The activity of the enzymic system converting ACC to ethylene was checked by application of exogenous ACC. Both tobacco and sugar beet leaf discs had the capability to convert ACC to C_2H_4 (Fig. 2). It is noteworthy that the ethylene production rate of the tobacco in the presence of 1 mM ACC exceeded 2000 nl g^{-1} h^{-1} (Figs. 2 and 3), which is one of the highest, if not the highest, we have observed in plant tissues. However, the data in Figure 2 show that the capability of the sugar beet leaf discs to convert ACC to C_2H_4 was much lower than that of the tobacco when ACC was applied at 1 mM, and also the rate declined more rapidly with incubation (Fig. 3). In the absence of exogenous ACC, leaf discs of tobacco also had the capability to produce C_2H_4 in the course of prolonged incubation, whereas that of the sugar beet was very low (Fig. 3).

When sugar beet leaf discs were cut and incubated with or without IAA, an immediate surge followed by a sharp decline of wound C_2H_4 production occurred (Fig. 4). The results show that the wound C_2H_4 production in sugar beet leaf discs was not significantly affected by the exogenous IAA. On the other hand, in both IAA-treated and untreated tobacco leaf discs, wounding caused a relatively small effect initially on ethylene production, but the ethylene production rate continued to rise with incubation time. However, the IAA-treated tobacco discs showed after 40 h a 2.5-fold increase in ethylene production as compared with untreated discs.

Effect of Ag^+ on IAA- and 2,4-D-Induced Ethylene Production by Sugar Beet Leaf Discs. In order to study the unexpected irresponsiveness of sugar beet to added IAA, with respect to ethylene production, 2,4-D and Ag^+ were applied to the leaf discs. 2,4-D, which was known to be more stable than IAA in plant tissues, also did not increase significantly the ethylene production by sugar beet leaf discs (Fig. 5). However, a short preincubation of 15 min of both IAA- and 2,4-D-treated leaf discs with 0.1 mM $AgNO_3$, an antagonist of ethylene action, resulted in a great increase of ethylene production which lasted for several days. The 2,4-D was more active than IAA in the induction of ethylene production by the Ag^+-pretreated leaf discs. The stimulatory effect of the Ag^+ on ethylene production by the IAA-treated leaf discs are remarkedly reduced in the presence of 3000 μl/l propylene gas, an analog of ethylene.

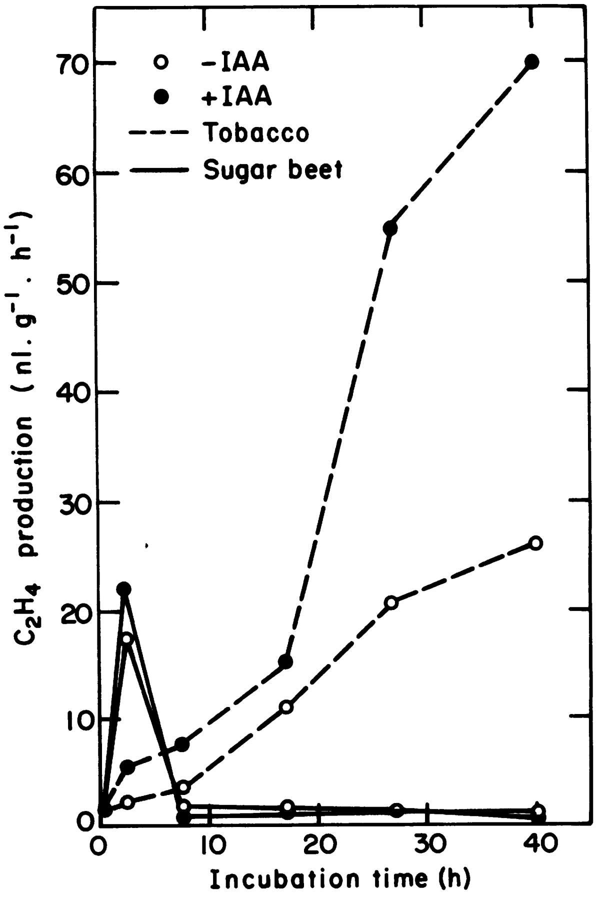

FIG. 4. Time course of average ethylene production rates by tobacco and sugar beet leaf discs incubated with 9 μM [1-^{14}C]IAA (1 μCi).

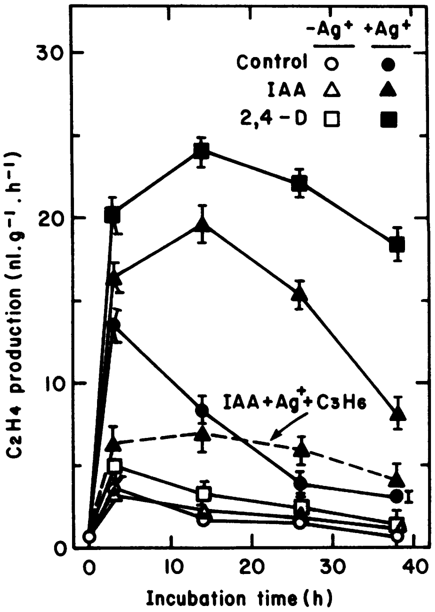

FIG. 5. Time course of average ethylene production rates by sugar beet leaf discs incubated with 0.1 mM IAA or 2,4-D and preincubated for 40 min with or without 0.1 mM $AgNO_3$. Propylene gas (3000 μl/l) was added to the combined treatment of IAA + Ag^+. The bars indicate 2 SE of the means.

Uptake and Metabolism of [1-^{14}C]IAA. Some factors which could affect the level of free IAA in the leaf discs system and thereby the rate of ethylene production, have been studied. Great differences in the rate of decarboxylation between the two species were found when labeled IAA was used (Table I). Although sugar beet leaf discs took up more label from the medium, less ^{14}C was retained in the tissue because of a greater rate of decarboxylation. The maximal difference in the rate of decarboxylation between the two species was observed after 5 h, and the differences became smaller with incubation time. After 50 h of incubation, 74% of the total labeled IAA taken up was decarboxylated by the sugar beet leaf discs, whereas only 41% was decarboxylated by the tobacco discs.

Significant differences between the two species, in the levels of free IAA and its metabolites during incubation, are shown in Figure 6. Three major radioactive peaks were detected on the chromatograms developed with the 2-propanol:8 N NH_4OH (8:2, v/v) solvent system. It seems that the first peak (0.0–0.1 R_F zone) could be of polar IAA conjugates, the second (0.1–0.2 R_F Zone) corresponds to IAAsp, and the third (0.45–0.60 R_F zone) is the free IAA. Extracts from sugar beet contained much less polar IAA conjugates and much more IAAsp and free IAA. No IAGlu was detected in this solvent system. Levels of free IAA in the extracts of tobacco remained about the same during incubation, whereas those of the sugar beet increased significantly. Thus, C_2H_4 production rates (Fig. 4) by the leaf discs, in this particular

Table I. *Uptake and Decarboxylation of [1-^{14}C]IAA by Tobacco and Sugar Beet Discs*

Uptake was calculated from the total radioactivity found in the extract pellet and CO_2. Radioactive IAA employed was 1 μCi.

Leaf Disc	Incubation Time	Total Uptake	Distribution of ^{14}C			Decarboxylation
			Extract	Pellet	CO_2	
	h		*dpm × 10^{-3}*			%
Tobacco	5	193	146	8.0	39	20
	10	592	384	43.1	165	28
	24	1140	632	64.8	443	39
	50	1698	920	74.8	703	41
Sugar beet	5	410	142	10.1	258	63
	10	832	220	27.2	585	70
	24	1429	340	43.1	1046	73
	50	1718	420	34.2	1264	74

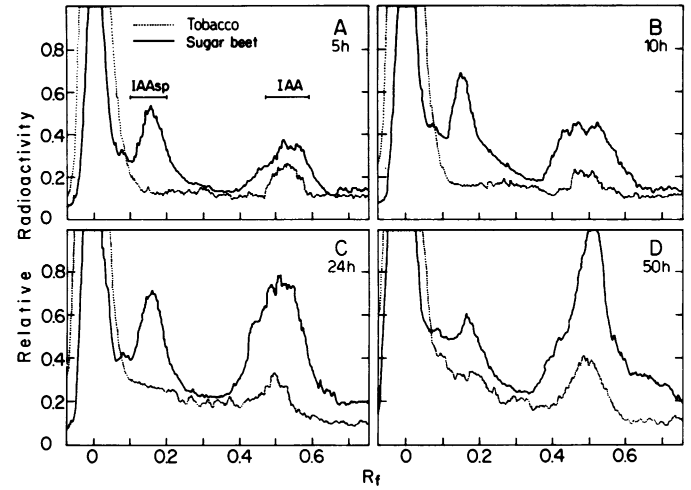

FIG. 6. Radiochromatogram scans of extracts from tobacco and sugar beet leaf discs incubated with 9 μM [1-^{14}C]IAA (1 μCi) for 5 h (A), 10 h (B), 24 h (C), and 50 h (D). Chromatograms were developed with 2-propanol:8 N NH_4OH (8:2, v/v). Locations of authentic IAA and IAAsp are designated by bars. There were no other radioactive zones on the chromatograms. IAGlu is located in this system at the 0.78 to 0.85 R_F zone.

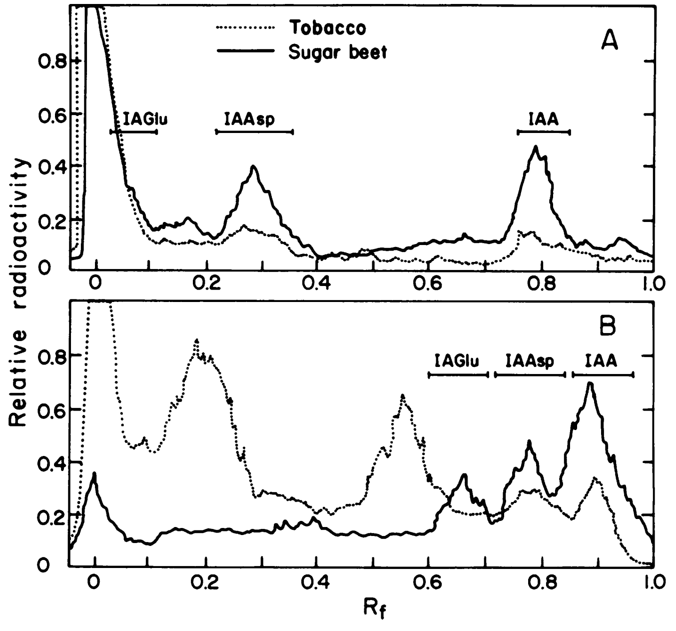

FIG. 7. Chromatographic separation of [^{14}C]IAA conjugates from extracts of tobacco and sugar beet leaf discs incubated with [1-^{14}C]IAA for 50 h. The same extracts as in Fig. 6D were chromatographed on paper developed with chloroform:ethylacetate:formic acid (5:4:1, v/v) (A) or 1-butanol:acetic acid:H_2O (4:1:4, v/v) (B). The R_F zones of authentic IAA, IAAsp, and IAGlu developed on the same chromatograms are designated by bars.

experiment, were not correlated to the level of the free IAA.

Chromatographic Separation of Auxin and Its Conjugates. When ammonia is used in the chromatography solvent system an elevated level of IAA has been reported (16). To verify the results obtained with ammonia (Fig. 6), and also for further separation of the IAA conjugates of the two species, the extracts were also developed with chloroform:ethyl acetate:formic acid (5:4:1, v/v) (Fig. 7A), and with 1-butanol:acetic acid:water (4:1:4, v/v) (Fig. 7B). As shown before, the level of free IAA was higher in the extract of the sugar beet leaf discs than in that of tobacco. The former also contained a higher level of IAAsp.

The developing solvent system of 1-butanol-acetic acid-water (4:1:4, v/v) allowed the separation of polar conjugates, in the extract of the tobacco leaf discs after 50 h of incubation, into three additional peaks (0.0–0.08, 0.10–0.30, and 0.5–0.60 R_F zones). In the sugar beet extracts, only a small peak (0.0–0.08 R_F zone) of a polar conjugate was found.

Further verification of the results, showing a significant difference between sugar beet and tobacco leaf discs to metabolize [1-^{14}C]IAA was obtained by using thin-layer chromatography plates developed with *n*-propanol:methyl acetate:20% NH_4OH (45:45:20, v/v), ethyl acetate:chloroform:formic acid (5:4:1, v/v), or ethyl acetate:butanol:formic acid:water (5:3:1:1, v/v). The latter solvent system (20) was able to separate the polar IAA conjugates similarly to the butanol:acetic acid:water solvent system.

Induction of Ethylene Production by IAA Conjugates in Tobacco Leaf Discs. In order to study possible biological activity of the IAA conjugates as expressed by decarboxylation and induction of ethylene production by the leaf discs, all the R_F sections were eluted from the chromatograms and reapplied to fresh leaf discs. Figure 8A shows the amount of radioactive metabolites taken up by the leaf discs in the course of a 3-d incubation. The per cent of uptake was between 20 and 30% in zones 1, 3, and 4, about 50% in zone 2, and above 60% in zone 5 to 7. Decarboxylation, which could indicate the release of free IAA following hydrolysis of the conjugates, was found in all the zones (Fig. 8B), and its rate was proportional to the amount of the metabolites taken up by the leaf discs. Ten to 20% of decarboxylation of the metabolites taken up was recorded in zones 1, 2,

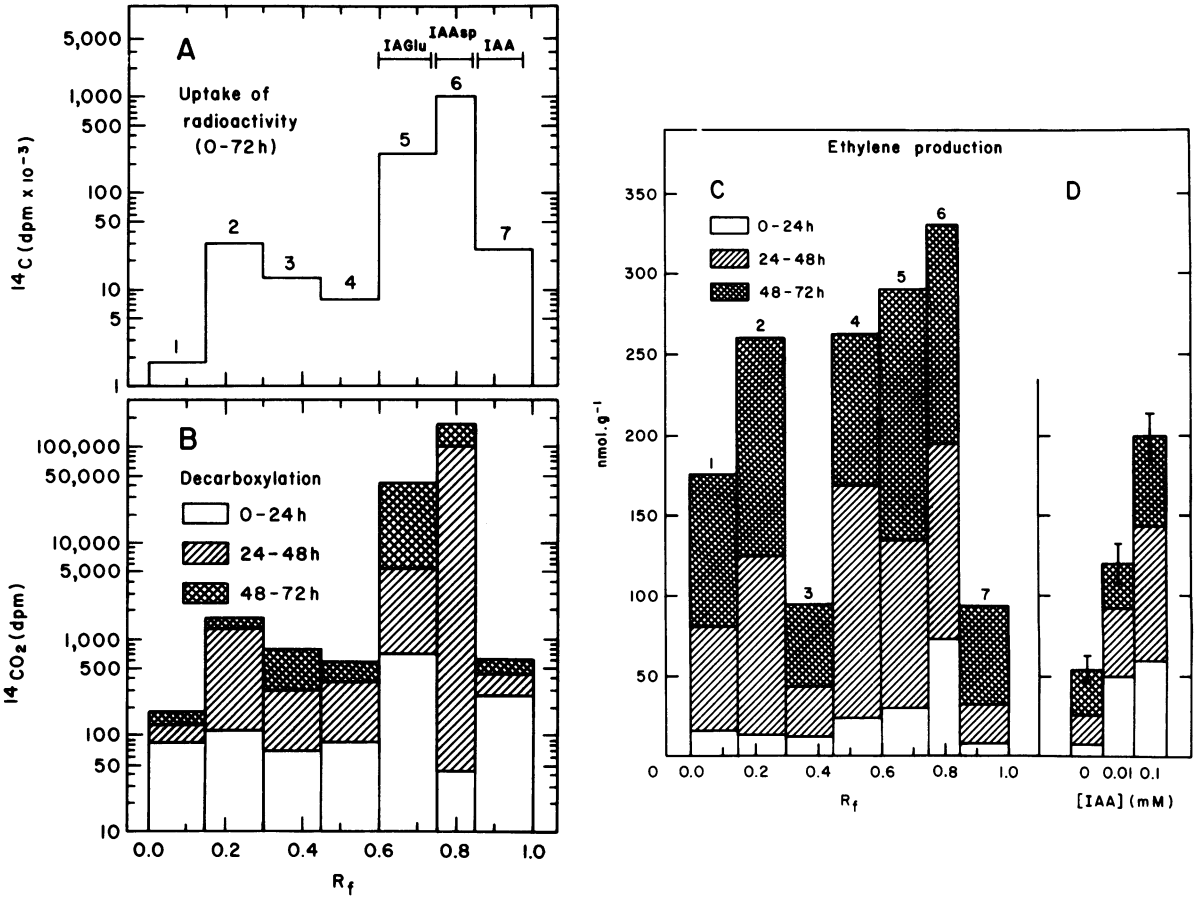

FIG. 8. Biological activity of IAA-conjugates. The metabolites were chromatographed with 1-butanol:acetic acid:H_2O (4:1:4, v/v). The R_F zones of authentic IAA, IAAsp, and IAGlu, developed on the same chromatograms, are designated by bars. Leaf discs of tobacco were incubated for 3 d with R_F sections containing labeled and unlabeled IAA metabolites. A, Radioactive metabolites taken up by the leaf discs during 72 h of incubation; B, rate of decarboxylation; C, amount of ethylene production; D, response of the leaf discs to exogenous IAA. No significant effect on ethylene production was found by the residues of various organic solvents along the chromatograms. The bars indicate 2 SE of the accumulated ethylene.

5, 6, and less than 10% in the others. All the zones increased ethylene production in the tobacco leaf discs above the control level, while zones 2, 4, 5, and 6 increased ethylene production even more than 0.1 mM IAA did (Fig. 8, C and D).

DISCUSSION

Sugar beet leaf discs did not increase their ethylene production in response to exogenously supplied IAA (Fig. 1), but had the capability to produce a relatively high level of ethylene following excision (Fig. 4) and water stress (2). This surge of ethylene production declines sharply, and later on, in an advanced stage of senescence, there is a rise of ethylene production in a climacteric-like pattern (4). Exogenous IAA stimulated ethylene production that lasted for several days in tobacco leaf discs. Although the rate of ethylene production by attached leaves of tobacco decreased with leaf age (unpublished), as found with other leaves (4), excision of either expanding or fully expanded mature leaves resulted in an increase of ethylene production in a climacteric-like pattern.

Ethylene production by vegetative tissues is thought to be regulated by the endogenous auxin (18), and it has been shown that methionine is the biological precursor of ethylene in auxin-treated tissues (9, 22). Adams and Yang (1) established the following biosynthesis pathway in apple tissue: methionine → SAM → ACC → C_2H_4. This sequence was later shown to be operative in other vegetative tissues (23). Yu *et al.* (24, 25) have recently provided evidence that IAA exerts its effect by inducing the ACC synthase, which catalyzes the conversion of SAM to ACC. The finding that exogenously supplied ACC was readily converted to ethylene, but that IAA failed to induce ethylene production in sugar beet leaf discs (Figs. 1 to 3), suggests that the exogenously supplied IAA does not have the ability to induce ACC synthase, which is presumably the main rate-limiting step in the IAA-induced ethylene synthesis (23). Although the induction of ethylene production in vegetative tissues by IAA is a general phenomenon, Bradford and Yang (8) have recently demonstrated that ethylene production in *dgt* tomato plants was readily induced by anaerobic stress in the root (or by floating), despite the plant's relative insensitivity to IAA with respect to ethylene production. The results obtained in the present study show that the ineffectiveness of IAA treatment to induce ethylene production in sugar beet leaf discs could not be due to deficiency of free IAA in the incubated tissue, since sufficient amounts of free IAA have been found in their extracts (Figs. 6 and 7). Furthermore, this conclusion is strengthened by the fact that 2,4-D, which does not undergo oxidation in the leaf tissue, also did not increase significantly the rate of ethylene production (Fig. 5).

The results suggest that irresponsiveness of the sugar beet leaf discs to IAA could be related to an inhibitory mechanism in the ethylene biosynthesis pathway. Since ACC synthase was found to be the rate limiting enzyme in the pathway of ethylene biosynthesis (23), one could suggest that the inability of IAA and 2,4-D to induce ethylene production in sugar beet leaf discs results from inhibition of the step converting SAM to ACC. This inhibition might be partially caused by the high level of wound ethylene produced immediately after excision (Fig. 4). This assumption is supported by the data showing that Ag^+, an antagonist of ethylene, could stimulate ethylene production in IAA-treated leaf discs (Fig. 5), probably because this agent could overcome the autoinhibitory effect of ethylene (3). The existence of an autoinhibitory mechanism in the ethylene biosynthesis pathway in the sugar beet system also can be demonstrated by the effect of propylene, an analog of ethylene. This gas, when applied to sugar beet discs, could nullify the stimulatory effect of the Ag^+ in auxin-treated leaf discs (Fig. 5). Recently, Riov and Yang (21) found that ethylene inhibited the formation of ACC from SAM in the flavedo tissue of citrus fruit, and similar results have been obtained with tobacco leaf discs in our laboratory (unpublished). In addition, we have found that Ag^+ action in overcoming the inhibitory effect of ethylene also occurs in the same biosynthetic step, namely in the conversion of SAM to ACC.

The increased rate of IAA decarboxylation by the sugar beet leaf discs (Table I) is probably associated with the lower efficiency of IAA conjugation in this tissue (Figs. 6 and 7), since conjugates of IAA were found to be protected from oxidative decarboxylation (5, 10). Lau and Yang (17) found in mung bean hypocotyl segments that kinetin decreased formation of IAAsp and increased both the level of free IAA and its rate of decarboxylation. We found that the rate of decarboxylation was very low in tobacco leaf discs and that the level of IAA conjugates was very high. The level of free [^{14}C]IAA in the tobacco leaf discs floated on [1-^{14}C]IAA was relatively low, but constant. In further experiments with tobacco of other *Nicotiana* species (*N. rustica* and *N. tabacum*), we have found very little free [^{14}C]IAA, although these species also produced ethylene at a high rate in response to the IAA applied.

The increased ethylene production by the IAA-treated tobacco leaf discs parallels the accumulation of the polar IAA conjugates (Figs. 6 and 7). The data show that biological activity of IAA conjugates to induce ethylene production correlates well with their decarboxylation. We assume that this biological activity of IAA conjugates stems from their hydrolysis, thereby releasing free IAA. These data indicate that in tobacco leaf the IAA conjugates play a considerable role in the control of ethylene synthesis. Further evidence on the biological activity of the IAA conjugates, in relation to ethylene synthesis, will be published elsewhere. It should be noted that, in pulse experiments in which IAA was applied only for 4 or 8 h, increased ethylene production continued for several days, and a continuous supply of IAA had a relatively small effect (unpublished). These findings are in contrast to those reported with mung bean (17) and with pea seedlings (15), for which a continuous supply of IAA was necessary for increased ethylene production. This different response between the various species could be explained by the type of IAA conjugates that are formed. Exogenously supplied IAA is rapidly converted in vegetative tissues, mainly to IAAsp (5, 7, 15, 26) and to IAGlu (20, 26). The former had little activity in inducing either growth (5) or ethylene production (14) in pea stem segments and also was found to be an immobilized form of auxin (14). Therefore, the conjugation to these compounds was regarded as a rapid detoxification mechanism for supraoptimal concentrations of IAA (5). The rate of uptake of labeled IAA by mung bean hypocotyl segments (17) was much higher than that of the leaf discs (Table I). The former absorbed more IAA in 5 h of incubation than did leaf discs incubated for 50 h. Zenk (26) measured a great amount of IAGlu after feeding leaf tissue with 0.05 or 0.1 mM IAA for 24 h. Riov and Gottlieb (20) have found that a substantial amount of IAGlu was formed in pine tissue only if the concentration of the exogenous IAA in the incubation medium was above the physiological level. Since we used a relatively low concentration of IAA in our experiments (Figs. 6 and 7) and the rate of uptake was very low, one could expect that some of the IAA conjugates accumulated in this system have physiological significance.

Recently, Bandurski and Schulze (6) have demonstrated that most of the endogenous IAA in untreated various tissues exists either as an ester or as an amide, when the type of conjugation depends on the plant species. They, as well as others (5, 10, 19), suggested that some conjugates could serve as a reserve source for free IAA. Epstein *et al.* (11) have recently provided evidence showing an *in vivo* conversion of IAA-*myo*-inositol to free IAA in *Zea mays* seedlings. Feung *et al.* (12) showed biological activity of a great number of L-α-amino acid conjugates in induction of growth of oat coleoptile. Hangarter *et al.* (13, 14) reported that some indoleacetylamino acid conjugates induced sustained ethylene production in pea stems, and recently they have found that the rate of ethylene production was correlated with the hydrolysis of the IAA conjugates. Liu *et al.* (19) proposed that IAA conjugates may play an important role in tumorigenesis in *Nicotiana* hybrids. They also suggested that the hydrolysis of conjugated IAA provides the free auxins for tumor tissues.

In our experiments, the increased rate of ethylene production in IAA-treated or untreated tobacco leaf discs could be a result of hydrolysis of IAA conjugates. In contrast to the conventional view, the results of this work suggest that in leaf discs the internal level of the exogenously supplied IAA is not the most important and indispensable factor in the induction of ethylene production. Other factors, related to the capability of the tissue to respond to free IAA or its metabolites may also be involved.

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