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Metabolism of 1-Naphthaleneacetic Acid in Explants of Tobacco: Evidence for Release of Free Hormone from Conjugates

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Abstract. 1-Naphthaleneacetic acid (1-NAA), required for in vitro flower bud formation, was taken up by pedicel explants of tobacco (*Nicotiana tabacum* L.) in large amounts and rapidly metabolized into various conjugates. These conjugates have been tentatively identified in four thin-layer chromatographic systems using authentic standards as references. The major metabolite formed during the first hours of culture comigrated with 1-NAA-glucoside (1-NAGlu). From the 6th hour on, most 1-NAA had been converted into a yet unidentified metabolite. 1-NAglu was an intermediate in the formation of this metabolite. After 24 h, 1-NAA-aspartate (1-NAAsp) became the second major metabolite. The increase in 1-NAAsp formation was induced by 1-NAA. The inactive analog 2-naphthaleneacetic acid (2-NAA) was metabolized similar to 1-NAA, but was unable to increase the formation of the aspartate conjugate. When explants were fed labeled 1-NAGlu, 1-NAAsp or the major unidentified metabolite, radioactivity became associated with free 1-NAA and all major conjugates, indicating interconversion of conjugates and breakdown to free 1-NAA. A regulatory role of conjugation in maintaining a particular level of free 1-NAA in the tissue is proposed herein.

Auxins are chemically modified in plant tissue (Cohen and Bandurski 1982, Sembdner et al. 1980). Indole-3-acetic acid (IAA) for instance, can be oxidized (Andreae 1967), or conjugated to sugar and amino acid derivatives (Cohen and Bandurski 1982). This results in inactivation, since the free auxin is the only biologically active form (Cohen and Bandurski 1982). Due to this inactivation, the level of active auxin in the tissue is not the sum of synthesis and import or, in tissue culture, synthesis and uptake. Oxidation and metabolism must be taken into account.

Conjugated IAA is hydrolyzed in plants, yielding free auxin (Hangarter and Good 1981). Apparently, conjugated IAA serves as a transport and storage form for IAA. While conjugated, the IAA moiety is protected from enzymatic oxidation (Bandurski et al. 1987; Cohen and Bandurski 1982). Synthesis and hydrolysis of conjugates may very well constitute the homeostatic control mechanism of the free auxin level (Bandurski 1980; Bandurski et al. 1987; Cohen and Bandurski 1982; Grambow 1988; Kopcewicz et al. 1974; Magnus 1987).

In tissue culture systems, the synthetic auxin 1-naphthaleneacetic acid (1-NAA) is often used instead of IAA, because it is not photodestructed or oxidized in the medium before entering the tissue (Dunlap et al. 1986). In the tissue, the substance is not oxidized (Andreae 1967; Zenk 1962), but it is conjugated into a glucose ester and several amino acid amides (Aranda et al. 1984; Goren and Bucovac 1973; Greenwood et al. 1974; Shindy et al. 1973). In contrast to IAA, release of free NAA from its conjugates has hardly been found. 1-NAA-aspartate (1-NAAsp) is hydrolyzed to free 1-NAA very slowly (Kazemie and Klämbt 1969; Vijayaraghavan and Pengelly 1986). Hydrolysis of other conjugates, such as 1-NAA-glucoside (1-NAGlu), has not been investigated. As a consequence, the homeostatic control of the free 1-NAA level by synthesis and breakdown of derivatives has been proposed but not yet confirmed (Caboche et al. 1984; Goren and Bucovac 1973).

Superficial explants from flower stalks of tobacco produce flower buds when cultured in vitro (Tran Thanh Van 1973; Van den Ende et al. 1984). The

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level of regeneration can be regulated by the concentration of 1-NAA in the medium (Smulders et al. 1988a,b). Because of the regulatory role of 1-NAA in flower bud regeneration, we have investigated the relation between the exogenous supply of this hormone in the medium and its conversion within explants. In order to understand what reactions took place, the fate of pulses of 1-NAA was also determined, as well as the fate of 1-NAA metabolites fed to explants. It will be shown that interconversion between free NAA and conjugates takes place.

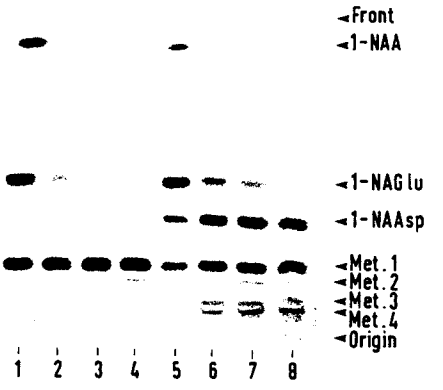

Fig. 1. Metabolism of pulses of 1-NAA. Explants were cultured for 0 h (lanes 1–4) or 24 h (lanes 5–8) on medium with 1 μM 1-NAA, and then transferred for 2 h to medium containing 1 μM (104 kBq) 1-[carboxyl-^{14}C]1-NAA. After the incubation, radioactivity was extracted either immediately (lanes 1 and 5) or after a chase of 2 h (lanes 2 and 6), 6 h (lanes 3 and 7), or 24 h (lanes 4 and 8) on medium without 1-NAA. After TLC separation, an autoradiograph was made. The position of free 1-NAA and the conjugates is indicated.

Materials and Methods

Plant and Tissue Culture

Plants of *Nicotiana tabacum* L. cv. Samsun were raised from seeds in the greenhouse. The procedure for preparing and culturing explants was essentially as described by Van den Ende et al. (1984) and Smulders et al. (1988b). Briefly, flower buds with pedicles attached were harvested at anthesis and surface-sterilized in 2.5% sodium hypochlorite. Superficial explants, 8 × 1 mm and six to eight cell layers thick, were cultured on Murashige-Skoog medium (Murashige and Skoog 1962) supplemented with 1% agar, 125 mM glucose, 1 μM benzylaminopurine (BAP) (basal medium), and different concentrations of labeled or unlabeled 1-NAA or 2-NAA as indicated. The explants were cultured in groups of 10 on a nylon mesh (width 180 μm) on 25-ml medium in a 9-cm plastic Petri dish at 25°C under continuous light.

Chemicals and Radiochemicals

1-NAGlu was generously supplied by Dr. M. Caboche. 1-NAAsp was a gift from Professor N. Amrhein. Agar was obtained from Difco Laboratories, and Murashige-Skoog medium was purchased from Flow Laboratories. 1-[carboxyl-^{14}C]NAA (2.07 GBq mmol^{-1}) and 2-[^{3}H]NAA (651 GBq mmol^{-1}) were purchased from Amersham. The 1-[^{3}H]NAA was custom-synthesized by Amersham, and purified as described previously (Smulders et al. 1988a). The specific activity was approximately 185 GBq mmol^{-1} at a purity of 98%, as checked by high-performance liquid chromatography (HPLC) (Smulders et al. 1987).

Radioactive Determinations

After incubation on medium containing labeled 1-NAA or 2-NAA, followed by the appropriate period of chase on medium without NAA, the explants were thoroughly rinsed. Groups of 5–10 labeled explants were extracted twice with 0.65 ml methanol overnight at −20°C. From the combined extracts a sample of 50 μl was taken to determine total uptake. The remainder of the extracts was dried under nitrogen, dissolved in 50 μl methanol, and separated by thin-layer chromatograph (TLC) on 0.25-mm silica gel plates with a fluorescence indicator (Merck). The plates were routinely developed with chloroform:methanol:acetic acid, 75:20:5 vol/vol/vol (solvent A, Caboche et al. 1984). In addition, chloroform:ethylacetate:formic acid, 5:4:1 vol/vol/vol (solvent B) or 4:5:1 vol/vol/vol (C), and isopropanol:ammonium hydroxide:water 8:1:1 vol/vol/vol (D) were used as solvents for TLC. Along with the samples, 0.1 μmol quantities of authentic 1-NAA, 1-NAAsp, and 1-NAGlu were cochromatographed, as well as—in the case of labeling with 1-[^{3}H]NAA—10 μl of an extract from tissue that had been labeled during 48 h with 1-[^{14}C]NAA. After separation, the standards were visualized under UV light, and the [^{14}C]-labeled compounds by autoradiography on Kodak XAR-5 film. In this way, the positions of [^{3}H]-labeled compounds in the samples were determined. The silica was then scraped off into scintillation vessels.

Radioactivity was measured after the addition of 4 ml Lumagel (Lumac) in a Philips PW 4540 liquid scintillation analyzer. The results were corrected for quenching and expressed as pmol per explant (for the uptake measurements) or as percentage of the radioactivity recovered (for the TLC samples). Through combination of these data, the amounts of free NAA and metabolites present in the explants were calculated.

Results

Putative Identification of the Conjugates

Extracts from explants cultured 48 h on labeled 1-NAA were separated by four different solvent systems (described above). In system A, which gave the best separation (see also Caboche et al. 1984), eight compounds were detected, seven of which contained 5% or more of the radioactivity. Figure 1 shows examples of the separation in solvent system A.

Free NAA, 1-NAGlu, and 1-NAAsp were identified by cochromatography with authentic samples

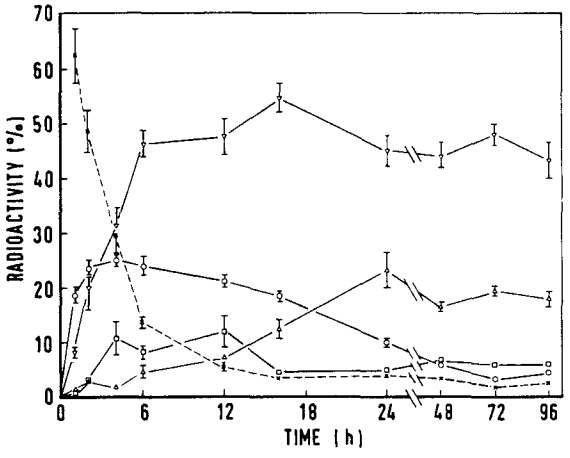

Fig. 2. Metabolism of 1-NAA during culture of tobacco explants. Groups of 10 explants were cultured on 1 μM 1-NAA with 37–370 kBq 1-[^{3}H]NAA for different periods of time. Hereafter, radioactivity was extracted from the explants. After TLC separation, the distribution of the radioactivity over the individual spots was determined and expressed as percentage of total radioactivity recovered. Each point is the mean of five samples of four explants. Standard errors are indicated when larger than the symbol. Symbols represent free 1-NAA (x, dotted line), NAGlu (○), 1-NAAsp (△), and metabolites 1 (▽) and 2 (□).

in the four solvent systems, and compared to R_f values reported in the literature (Aranda et al. 1984; Brenner and Tonkinson 1974; Caboche et al. 1984; Goren and Bucovac 1973; Riov et al. 1979; Veen 1966; Venis 1972; Vijayaraghavan and Pengelly 1986; Zenk 1962). In solvent B, 1-NAGlu cochromatographed with metabolite 1, giving the glucoside compound designated "B" by Caboche et al. (1984), at R_f 0.05–0.09. The latter split up into two compounds after chromatography in solvent A, that is, metabolite 1 at R_f 0.25–0.29 and 1-NAGlu at R_f 0.52–0.56. 1-NAAsp resulted in a separate spot with all solvents. In the lower range, R_f 0.00–0.12 in solvent A, a few additional compounds were found, notably metabolite 4 (also found by Caboche et al. 1984). For metabolites 2 (R_f 0.21–0.24) and 3 (0.12–0.16) no references have been found in the literature.

Metabolism of 1-NAA During Continuous Culture

When extracts from explants cultured for different periods of time on 1-[^{3}H]NAA were separated in solvent A, the distribution of the radioactivity over the spots showed that free 1-NAA declined rapidly during the first 12 h of culture, indicating rapid metabolism (Fig. 2). After 24 h, less than 5% of the 1-NAA taken up was present as free hormone.

During the first hour of culture, 1-NAGlu was the major metabolite, but from 6 h onward, metabolite 1 increased to become the major one. The proportion of radioactivity in 1-NAGlu, about 25% after 4 h, steadily decreased from 6 h onward, whereas that of metabolite 1 remained at a constant level of 45–55%. 1-NAAsp and metabolite 2 were detected only after a lag period of 1–2 h (Fig. 2). 1-NAAsp became more abundant at 24 h of culture and remained at a stable level of ±23% of radioactivity thereafter. Metabolites 3 and 4 (not shown in Fig. 2) became more abundant after several days of culture, but together they never comprised more than 15% of the total radioactivity in the explants.

At different concentrations of 1-NAA in the medium, the same metabolites were formed in similar percentages (Table 1). There was, however, a tendency for a more than proportional conversion rate of free 1-NAA at higher 1-NAA concentrations, both after 2 and 24 h of culture.

Metabolite Turnover After Application of a Pulse of 1-NAA

Since the percentage of 1-NAGlu was found to be high at the early stage of culture, and to decrease in later stages, two possibilities exist: 1-NAA was either conjugated into 1-NAGlu in larger amounts at the onset of culture compared to later times, or 1-NAGlu was converted into other compounds. During continuous culture, no net decrease in the total amount of 1-NAGlu was found (unpublished observations). Since 1-NAA was continuously taken up and metabolized in large amounts, 1-NAGlu might be turned over extensively without any decrease in the actual level.

To investigate conjugate turnover, a pulse-chase experiment was carried out. Explants were labeled for 2 h immediately after excision with radioactive 1-NAA and subsequently metabolite levels were monitored during a chase. Figure 1 (lanes 1–4) shows that 1-NAGlu was synthesized very quickly, but also disappeared rapidly during the chase. The absolute amounts of 1-NAA and the major metabolites after various chase periods are presented in Table 2 (upper half). These data show that the breakdown did not result in more free NAA, since its level also decreased. The radioactivity became for the most part associated with metabolite 1, as shown by the relatively constant level of 1-NAGlu together with metabolite 1 (Table 2).

This conversion, and the formation of the other metabolites, was not affected by the presence of nonlabeled 1-NAA in the medium during the chase (chase without 1-NAA in Fig. 1; chase with 1 μM 1-NAA not shown).

Table 1. Metabolism of 1-NAA at different concentrations of 1-NAA.

Duration of the incubation[a]	Concentration of 1-NAA (μM)	Distribution of radioactivity (%)[b]			
		Free 1-NAA	1-NAAsp	1-NAGlu	metabolite 1
2 h	0.1	46.3 ± 5.1[c]	1.7 ± 0.7	21.9 ± 1.1	24.9 ± 5.3
	1	38.5 ± 3.8	1.5 ± 0.5	26.9 ± 1.8	29.3 ± 6.4
	10	35.9 ± 5.8	1.1 ± 0.0	26.5 ± 2.8	31.8 ± 7.5
24 h	0.1	7.3 ± 1.4	6.8 ± 0.8	10.6 ± 1.0	55.8 ± 3.1
	1	3.1 ± 0.4	14.5 ± 0.4	10.3 ± 0.8	56.9 ± 1.1
	10	2.1 ± 0.5	13.3 ± 1.1	10.6 ± 0.4	59.3 ± 2.0

[a] Groups of 10 explants were cultured on 0.1, 1, or 10 μM 1-NAA with 37 or 185 kBq 1-[^{3}H]NAA. After 2 or 24 h, radioactivity was extracted and separated on TLC.
[b] Expressed as the percentage in a spot of the total amount of radioactivity recovered in the whole lane.
[c] Each value is the mean of three samples of seven explants ± SEM.

Table 2. Distribution of radioactivity over the different compounds during a chase.

Duration of pretreatment, pulse + chase (h)[a]	Amounts present (pmol explant^{-1})[b]					
	Free 1-NAA	1-NAGlu	metabolite 1	1-NAGlu + metabolite 1	1-NAAsp	Others
0, 2 + 0	2.9 ± 0.4[c]	2.9 ± 0.1	3.5 ± 1.5	6.4 ± 0.3	0.1 ± 0.0	0.4 ± 0.0
0, 2 + 2	0.5 ± 0.0	1.0 ± 0.0	7.9 ± 0.1	8.4 ± 0.3	0.2 ± 0.1	0.8 ± 0.0
0, 2 + 24	0.1 ± 0.0	0.2 ± 0.0	8.1 ± 0.1	8.3 ± 0.1	0.2 ± 0.0	1.2 ± 0.1
24, 2 + 0	1.5 ± 0.1	4.0 ± 0.0	1.8 ± 0.1	5.8 ± 0.1	1.6 ± 0.2	0.9 ± 0.0
24, 2 + 2	0.3 ± 0.0	1.4 ± 0.1	3.8 ± 0.2	5.2 ± 0.1	2.8 ± 0.2	1.4 ± 0.1
24, 2 + 24	0.1 ± 0.0	0.2 ± 0.0	4.2 ± 0.0	4.4 ± 0.0	3.2 ± 0.1	2.0 ± 0.1

[a] Explants were cultured for 0 or 24 h on medium with 1 μM 1-NAA, and then transferred for 2 h to medium containing 1 μM (104 kBq) 1-[carboxyl-^{14}C]NAA. After the incubation, radioactivity was extracted either immediately or after a chase of 2 or 24 h on medium without 1-NAA.
[b] A sample of the extract was used to determine the total amount of radioactivity present. The remainder was separated on TLC, the silica was scraped off, and the radioactivity in the spots was determined. From these data, the amounts of chemicals present was calculated.
[c] Each point is the mean of two samples of 10 explants ± SEM.

Changes in Metabolism During Culture

In order to investigate how 1-NAA metabolism changed during culture, a similar pulse-chase experiment was carried out with explants cultured for 24 h on 1 μM 1-NAA (Fig. 1, lanes 5–8). The turnover of 1-NAGlu was similar to that of freshly cut explants, although the sum of 1-NAGlu and metabolite 1 slightly decreased during the chase (Table 2). This suggests interconversion between metabolites, or release of free 1-NAA.

The major change in the pattern of metabolites was the enhanced synthesis of 1-NAAsp, from hardly any synthesis in freshly cut explants to more than 20% of the total amount of radioactivity in 1-day-old explants (Fig. 1, compare lanes 5–8 with 1–4). Presumably, the enzyme for the synthesis of this metabolite is not yet present when the explants are cut.

To check whether exposure to 1-NAA induced these differences in metabolism, explants were precultured for 24 h on either 1-NAA, the inactive analog 2-NAA, or on basal medium without additions, before they received a 2-h pulse with labeled 1-NAA, followed by a 24-h chase (Fig. 3). Only the explants which had been in contact with 1-NAA formed considerable amounts of 1-NAAsp. Preincubation on basal medium, or basal medium supplemented with 2-NAA, resulted in a 1-NAA metabolism comparable to that in freshly cut explants. The only effect brought about by the 24-h preincubation was a 10% rise in the fraction of metabolites 3 and 4. Enhanced synthesis of these metabolites apparently did not depend on the presence of active auxin.

Metabolism of 2-NAA

The question arose whether 2-NAA, which could

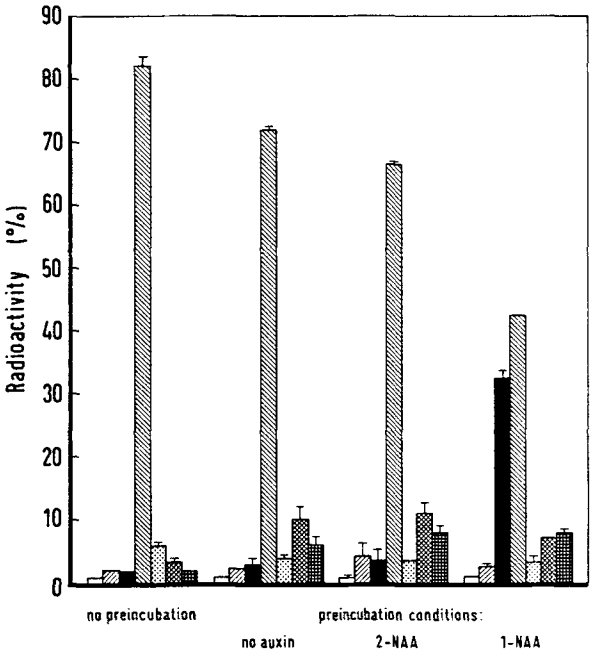

Fig. 3. Metabolism of 1-NAA after different treatments. Freshly cut explants and explants preincubated for 24 h on medium containing no auxin, 1 μM 2- NAA, or 1 μM 1-NAA, were cultured for 2 h on medium containing 1 μM 1-NAA and 185 kBq 1-[^{3}H]NAA. After a 24-h chase on medium without auxin, radioactivity was extracted and separated, and the distribution of the radioactivity was determined. Columns from left to right: 1-NAA, 1-NAGlu, 1-NAAsp, metabolites 1, 2, 3, and 4. Each point is the mean of three samples of seven explants. Standard errors are indicated when larger than 0.25%.

not induce changes in the metabolism of 1-NAA was also metabolized differently. To test this, explants were cultured for 1 or 24 h on 1 μM labeled 1-NAA or 2-NAA, or on labeled 2-NAA in the presence of unlabeled 1-NAA. As shown in Fig. 4, the metabolism of 2-NAA was initially the same as the metabolism of 1-NAA. Later on differences occurred, notably the absence of an increase in the synthesis of 2-NAAsp. The reduced synthesis was not due to a lower affinity of the aspartate-forming enzyme for 2-NAA, since in the presence of unlabeled 1-NAA, the synthesis of labeled 2-NAAsp was at the same level as that of labeled 1-NAAsp. The synthesis of metabolite 3 of 2-NAA was higher than that for 1-NAA.

Conversion of Metabolites and Release of 1-NAA

To obtain conclusive evidence for the release of 1-NAA from conjugates, radioactivity was extracted from explants cultured for 6 h on medium containing 10 μM 1-[^{14}C]NAA. After separation, the labeled metabolites were extracted from the TLC plate and fed to explants. After 2 h of culture on labeled 1-NAGlu, the radioactivity was extracted from explants and again separated on TLC (Table 3). The very large fraction of 1-NAGlu (44%) indicated that the radioactivity had indeed been taken up in the form of the glucoside (compare Table 3 with Fig. 2). Radioactivity was also found in free 1-NAA and metabolite 1 (Table 3). After 3-day incubations on labeled 1-NAGlu, 1-NAAsp, or metabolite 1, all of the radioactivity had been converted to all these metabolites in the same proportions, irrespective of which conjugate had been applied.

Discussion

1-NAA is quickly metabolized in tobacco explants (Figs. 1 and 2). The metabolites were only analyzed by TLC. Their behavior in four solvent systems was considered sufficient proof for identification within the scope of this study. 1-NAGlu is the first major metabolite after feeding of 1-NAA (Goren and Bucovac 1973; Riov et al. 1979; Zenk 1962). In the experiments described herein, it was found that the 1-NAGlu formed is further metabolized, presumably into metabolite 1. This metabolite—probably also a sugar ester (Caboche et al. 1984)—within a few hours becomes the most abundant conjugate. The turnover of 1-NAGlu is indicated by (a) the synthesis and disappearance of 1-NAGlu during a pulse-chase experiment (Fig. 1), (b) the turnover both at the beginning of culture and 24 h later, and (c) the constancy of the sum of 1-NAGlu and metabolite 1 during the disappearance of 1-NAGlu (Table 2). At this moment, two possible mechanisms for this interconversion can be envisaged: direct conversion of 1-NAGlu into metabolite 1, or breakdown to free 1-NAA, from which metabolite 1 is subsequently metabolized.

Direct conversion of IAA-glucose into IAA-*myo*-inositol has been found in maize kernels (Bandurski 1984; Michalczuk and Bandurski 1982). Recently, IAA-*myo*-inositol has also been detected in tobacco (Aharoni and Cohen 1986). The IAA-glucose synthase (UDP-glucose: IAA glucosyl transferase) from maize kernels has been reported to have a high affinity for 1-NAA. The same enzyme may therefore be active in our tobacco tissue. The IAA-*myo*-inositol synthase (indoleacetyl-glucose: *myo*-inositol indoleacetyl transferase) from maize kernels only has a very low affinity toward 1-NAGlu compared to IAA-glucose (Bandurski 1984; Michalczuk and Bandurski 1982). In view of its rapid synthesis (Fig. 2), metabolite 1 would not

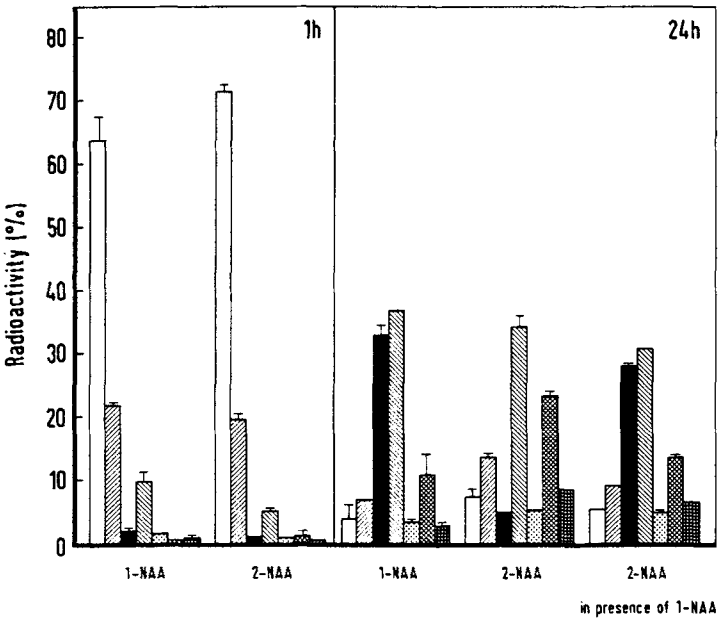

Fig. 4. Comparison of the metabolism of 1-NAA and 2-NAA. Explants were cultured for 1 h on 1 μM 1-NAA and 370 kBq 1-[^{3}H]NAA or 24 h on 1 μM 1-NAA and 37 kBq 1-[^{3}H]NAA. Other explants were cultured for 1 h on 1 μM 2-NAA and 370 kBq 2-[^{3}H]NAA or for 24 h on 1 μM 2-NAA and 37 kBq 2-[^{3}H]NAA. Finally, explants were cultured for 24 h on 1 μM 1-NAA and 37 kBq 2-[^{3}H]NAA. After the incubation, radioactivity was determined. Columns as in Fig. 3.

Table 3. Fate of 1-NAA metabolites fed to explants.

Metabolite fed[a]	Time of incubation (h)	Distribution of the radioactivity (%)[b]				
		1-NAA	1-NAGlu	1-NAAsp	metabolite 1	Others
1-NAGlu	2	23.5 ± 0.3[c]	44.0 ± 1.4	0.9 ± 0.2	26.1 ± 0.5	5.2 ± 0.8
1-NAGlu	24	2.4 ± 0.1	10.3 ± 1.2	3.3 ± 0.8	61.7 ± 0.4	22.1 ± 0.9
1-NAGlu	72	0.3 ± 0.0	2.2 ± 0.2	1.4 ± 0.1	53.1 ± 1.2	42.9 ± 1.8
1-NAAsp	72	0.8 ± 0.8	3.9 ± 1.5	3.5 ± 1.8	60.7 ± 2.0	31.1 ± 0.5
metabolite 1	72	1.3 ± 0.3	2.2 ± 0.1	1.2 ± 0.1	65.0 ± 0.5	30.3 ± 0.1

[a] Labeled 1-NAA metabolites were obtained from groups of 40 explants incubated for 6 h on medium containing 10 μM (1040 kBq) 1-[^{14}C]NAA by extraction and separation on TLC. The conjugates were fed to explants for 2, 24, or 72 h.

[b] Extracts from these explants were separated, and the distribution of the radioactivity over the spots was determined.

[c] Each point is the mean of two samples of five explants ± SEM.

be expected to be 1-NAA-*myo*-inositol. Alternatively, the IAA-inositol synthase in tobacco could have a higher affinity for 1-NAA.

An alternative to a direct conversion of 1-NAGlu into metabolite 1 is that 1-NAGlu is hydrolyzed into free NAA, from which metabolite 1 is subsequently synthesized directly. Hydrolysis of 1-NAGlu into 1-NAA is possible, as shown in explants that were fed 1-NAGlu (Table 3). The synthesis of IAGlu from IAA is reversible (Leznicki and Bandurski 1988). If formation of metabolite 1 were to occur from free 1-NAA, one would expect compartmentation of the conjugation processes because 1-NAA newly entering the cell is first converted to 1-NAGlu and not metabolite 1. 1-NAGlu is possibly hydrolyzed into free 1-NAA in a compartment different from where it is formed, and in this new compartment the formation of metabolite 1 could take place. Compartmentation of conjugation processes was also suggested by Davidonis et al. (1980) for 2,4-dichlorophenoxyacetic acid (2,4-D) conjugation.

When explants were fed 1-NAGlu or other metabolites, both free 1-NAA and all other metabolites were consistently found (Table 3). Conjugates of 1-NAA were considered stable. Hydrolysis at a low rate has only been reported for 1-NAAsp (Kazemie and Klämbt 1969; Vijayaraghavan and Pengelly 1986). Release of free hormone from conjugates has been found for IAA (Hangarter and Good 1981). Also the synthetic auxin 2,4-D is released from its metabolites in plant tissues (Davidonis et al. 1980). In the light of our results, it appears that 1-NAA metabolites are also subject to such degradation.

Release of free 1-NAA from the conjugates (Table 3) indicates that the low level of free 1-NAA

after longer incubations is the result of an equilibrium between conjugate synthesis and degradation. In this way, a low level of 1-NAA is maintained as free hormone—a mechanism also proposed for IAA (Bandurski 1980; Cohen and Bandurski 1982; Leznicki and Bandurski 1988) and 2,4-D (Davidonis et al. 1980). From the results in Fig. 2, it appears that the level of free hormone is 2–4% of the total amount taken up.

The metabolism of 1-NAA changes during the course of culture (Fig. 1), that is, at different stages in culture metabolites are formed in varying amounts (Caboche et al. 1984; Südi 1964, 1966; Zenk 1962). The change in the amount of 1-NAAsp is important. 1-NAAsp is formed at a high rate after 24 h of culture, but hardly at the start of culture (Figs. 1 and 3). The change in metabolism was induced by 1-NAA but not by 2-NAA (Fig. 3). This was not due to the lack of affinity of the aspartate-forming enzyme for 2-NAA, since 2-NAAsp was accumulated in almost the same amount as 1-NAAsp when both substances were supplied to the explants (Fig. 4). Therefore, the difference in effect must be related to the difference in activity as an auxin, 2-NAA being an inactive analog (Smulders et al. 1988a). It has also been found for other chemicals that the capacity to induce the formation of the aspartate-conjugate is dependent on the activity as an auxin (Bandurski 1984; Südi 1964, 1966; Venis 1972). The induction is also concentration-dependent, since high concentrations of 1-NAA lead to more than proportional increase in the NAA conjugation (Table 1). Thus the induction of auxin-aspartate synthase may be a specific and quick response to auxin-receptor binding. However, the physiological function of this induced synthesis remains unclear. From the data on the metabolism of 2-NAA (Fig. 4), it appears that if the aspartate formation is not induced, this does not lead to a higher concentration of free NAA in the tissue.

In conclusion, it appears that conjugation of 1-NAA has more in common with IAA metabolism than generally assumed. In particular, 1-NAA conjugation, as IAA-conjugation, seems to play a regulatory role in maintaining a particular level of free hormone in the tissue.

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