Abscission of Citrus Leaf Explants

NO CORRELATION WITH NAPHTHALENEACETIC ACID CONJUGATION IN THE ABSOLUTION ZONE

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ABSTRACT

The role of α-naphthaleneacetic acid (NAA) in the control of abscission in Citrus (Citrus sinensis L. Osbeck) leaf explants and its conjugation were studied in non-aged and 24-hour-aged explants. Dipping non-aged explants in 1.5 micromolar NAA for 15 minutes immediately after excision did not delay abscission whereas 150 micromolar NAA effectively delayed it. As incubation time was prolonged up to 24 hours after excision, the delaying effect of both concentrations gradually increased. In general, both concentrations did not delay abscission when applied to 24-hour-aged explants held for an additional period of up to 24 hours. The uptake and conjugation of 14C-NAA to glucose and aspartic acid were similar in petiole, abscission zone, and leaf blade of non-aged and aged tissues, for all NAA concentrations. No correlation was established between the kinetics of abscission and the rate of conjugation in the abscission zone.

In Citrus leaf and fruit explants, and in many other plant species, auxin delays abscission when applied during the first few hr after excision (2, 20), but loses its delaying effect—and can even accelerate the separation process—when applied to aged explants (1). This retarding effect could be connected to the delaying effect of auxin on the activity of hydrolytic enzymes in the AZ (17, 22). The mechanism which causes auxin to lose its delaying ability, however, is still obscure. In bean explants this phenomenon is said (8) to be independent of auxin translocation, but is probably mediated by immobility of auxin through the conjugation mechanism.

The ability of plant tissues to conjugate IAA and NAA to glucose and aspartic acid is well established (3, 9, 10). We have found (11) an in situ increase in the level of auxin in AZs sections of isolated Citrus leaf during the first hours of abscission, and a marked decrease in endogenous auxin at later stages. This decrease is correlated with the increased failure of auxin to delay abscission, and we wondered whether it is also correlated with an increase in the conjugation mechanism in the AZ layers. We investigated this question by using NAA—a synthetic auxin which is resistant to peroxidation (5). The data presented in the following are based on kinetic studies of NAA conjugation at various stages of abscission of Citrus leaf explants, which are a sensitive and reliable system for physiological study of abscission, and conjugate NAA effectively. Their performances in abscission studies have been well characterized in this laboratory (11, 12, 18, 19).

Received for publication November 2, 1977 and in revised form April 6, 1978

MATERIALS AND METHODS

Plant Material. Four- to 10-month-old leaves from 44-year-old Shamouti orange (Citrus sinensis L. Osbeck) trees, growing in the coastal area of Israel, were picked and processed as previously described (12, 17).

Abscission. When not otherwise stated, explants consisted of a 10-mm-thick tissue from the blade, distal to the AZ and of the petiole proximal to it (Fig. 1, type II). In some experiments explants consisted of the AZ only (Fig. 1, type III); in these cases 2 mm of the petiole and blade tissue were left on both sides of the separation line. In others, the whole leaf was used (Fig. 1, type I). Explants were washed under running tap water, and treated immediately by immersion for 15 min with either 20 mM phosphate-10 mM citrate buffer (pH 4.2—the pK of NAA), or with a solution of different NAA concentrations in the same buffer. After the termination of the treatment with either NAA or buffer, groups of 10 explants were arrayed on a Saran screen, in chambers lined with moist filter paper, and kept for the whole experimental period in the dark at 25 ± 1 C. When explants (type III) were exposed to the treatment solution for more than 15 min (kinetics studies), they were kept in biological vials containing 1 ml of treatment solution at the above mentioned conditions, and shaken to maintain the appropriate aeration. Each treatment was repeated seven times, namely a total of 70 individual explants. Experiments were repeated at least twice. Per cent of abscission was determined by counting the distal sections which had already abscised and those which abscised following a gentle touch (6, 17).

Conjugation. Experimental units consisted of 30 AZ sections or 30 petiole sections 4 mm long, sampled at a distance of 3 to 4 mm proximal to the separation line, and 20 leaf blade discs, 9 mm in diameter (Fig. 1). Plant material was incubated for the required time in either 1.5 μM 14C-NAA or 1.5 μM 14C-NAA + 148.5 μM NAA (specific radioactivity of 14C-NAA was 56.4 mCi/mmol), prepared in the above mentioned phosphate-citrate buffer.

AZ sections and petiole sections were incubated in the dark at 25 ± 1 C, in small vials containing 1 ml of treatment solutions which were continuously shaken. Leaf discs were floated on 4 ml of treatment solutions in small Petri dishes with their abaxial side touching the solution and at the above mentioned conditions without shaking. Experiments were repeated at least twice, and each experimental unit was repeated three times.

Extraction. Extraction was done by 80% ethanol as previously described (9). Extracts were chromatographed on 0.4-mm-thick Silica Gel G plates, and developed with chloroform-ethylacetate-formic acid (5:4:1, v/v). Ten Rf sections were scraped into scintillation vials containing Bray's solution. Radioactivity was counted in a Packard (model 3255) scintillation spectrometer, equipped with external standardization unit, and data are presented on fresh weight basis as dpm after quenching and efficiency corrections.

RESULTS AND DISCUSSION

NAA Concentration, Aging, and Abscission. The first step was
to find the concentration of NAA which does not delay abscission when applied to the whole explant at excision time, and to compare its metabolism to that of a higher concentration which does delay it. Dipping type II explants (Fig. 2) in 1.5 μM NAA for 15 min did not affect abscission, but was sufficient for conjugation studies (7, 9), and was therefore adopted as one of the two treatments required. Increasing concentrations of NAA gradually delayed abscission, as expected (2); we chose to treat explants in the following experiment with 150 μM.

Incubation of non-aged explants (type III) in 1.5 μM NAA for periods longer than 15 min increased abscission; the delaying effect was much less pronounced in aged explants exposed to the same treatment (Table I). The higher concentration delayed abscission efficiently in non-aged explants, but was almost totally ineffective in aged explants.

Veen (20) could show that NAA conjugation is closely related to its concentration in the media. The question was, therefore, whether the differences observed (Table I) were due to changes in the rate of conjugation in the AZ, or whether the degree of conjugation was adequate to reduce the level of free NAA below a threshold in the 1.5 μM treatment and not adequate to cause the same reduction in the 150 μM treatment of the non-aged explants. The following conjugation experiments were conducted in order to provide an answer to this question.

**Kinetics of NAA Conjugation during Abscission.** Conjugation patterns of the two concentrations were studied in isolated AZ explants (type III) and petiole sections and in leaf blade discs of non-aged and aged tissues. The uptake pattern of 14C-NAA was similar in AZ and petiole sections (Fig. 3), but slower in leaf discs, where it never reached the level obtained in the other non-aged tissues incubated in 150 μM NAA for 24 hr.

In general, kinetics of 14C-NAA conjugation and changes in the level of free 14C-NAA in the tissue were similar in non-aged and aged tissues (Figs. 4 and 5). They were rather similar in the AZ and the adjacent non-abscising tissues. Similar observations were

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**Fig. 1.** Schematic illustration of tissues used for study of abscission and 14C-NAA conjugation.

**Fig. 2.** Effect of different concentrations of NAA on abscission of leaf explants. Explants consisting of petiole and 10 mm of leaf blade (type II) were dipped immediately after excision for 15 min in treatment solution and kept in humid chambers for the required period of time.

**Fig. 3.** Kinetics of 14C uptake after incubation of different leaf tissues in two concentrations of 14C-NAA. Tissues were incubated and either shaken in 1 ml (petioles and AZ sections) of treatment solution or floated on 4 ml (leaf discs) of the solution. One hundred percent radioactivity: 185,000 dpm/1 ml or 760,000 dpm/4 ml in either 1.5 μM or 150 μM of NAA in phosphate-citrate buffer (pH 4.2).

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**Table I.** Effects of two concentrations of NAA on the abscission of non-aged and aged AZ explants

<table>
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<tr>
<th>Incubation time in NAA (hr)</th>
<th>1.5 μM</th>
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<td>96</td>
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*For 24 hr in humid chamber.*

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**Fig. 4.** Kinetics of 14C-NAA uptake and conjugation in non-aged leaf tissues. Petioles, AZ sections, and leaf discs were separated at excision and incubated for the required period of time in the radioactive solution (see legend of Fig. 1).
also reported by Craker et al. (8). The data presented in Figures 4 and 5 indicate that no correlation can be established between the rate of conjugation and the control mechanism of NAA on the abscission of the explants. Furthermore, the differences in the kinetics of abscission in non-aged and aged explants and the variety of responses of such tissues to various concentrations of exogenous NAA (Table I) were not reflected in the conjugation rate of NAA to either glucose or aspartic acid in the AZ (Figs. 4 and 5). It is noteworthy that the specific activity of the 150 μm treatment was 100-fold lower than that of the 1.5 μm but the pattern of conjugation in both cases was similar. On the other hand, it can be argued that the actual level of free NAA in the 150 μm treatment was 100-fold higher than the data presented in terms of radioactivity at the bottom of Figure 4, since it does not indicate the level of "cold" free NAA. If this is the case, it could have been suggested that the higher level of auxin was responsible for the delay of abscission in the 150 μm treatment in non-aged explants (Table I).

There are, however, several points in the results presented so far in Figures 4 and 5 which deserve attention. (a) It is usually assumed (3) that the length of lag time required to induce the conjugation mechanism does not depend on the concentration of the auxin inducing the hypothetical (21) N-acyl aspartate synthetase. However, results indicated (see inset in Fig. 4E) that the lag time is shorter in AZ sections incubated in the higher NAA concentration. (b) The conjugation rate of NAA to glucose was higher in leaf discs than in other types of tissues tested. (c) The level of free 14C-NAA in leaf discs remained high even after 24 hr of incubation and did not decrease in the course of time due to conjugation to both glucose and aspartic acid, but did so in AZ and petiole sections (Figs. 4 and 5) as well as in other plant material (3, 9, 21) whenever a nontoxic concentration of auxin was used. This could not be due to an excessive level of auxin interfering with the conjugation mechanism (3), since the same was true for discs incubated in either high or low (and nontoxic) levels of NAA.

In spite of the above-mentioned results, a more direct experiment was required to confirm our conclusion that NAA conjugation in the AZ does not play any role in the regulation of abscission in Citrus. The rate of conjugation was, therefore, studied in explants which differ from each other in their abscission pattern. In Coleus explants, the kinetics of abscission varies according to the length of the tissue tested (13). This was also true for Citrus. Shortening of the leaf blade tissue of explants not treated with NAA but only with buffer enhanced their abscission rate (Fig. 6), probably due to lack of carbohydrates and auxin supply from the leaf blade to the AZ (13). Moreover, the size of explants used for aging affected abscission rate of AZ explants (type III) which were isolated after an aging period of 24 hr (Table II). When AZ sections (type III) were isolated from aged explants (types I and II) and treated with 1.5 μm 14C-NAA, conjugation rate was similar in all AZ sections (Table II) regardless of the differences in their abscission rate. These results further support the conclusion that at least in Citrus leaf explants, there is no correlation between NAA conjugation at the AZ and the regulation of abscission. Our results, however, do not exclude the possibility that auxin conjugation in the leaf blade may have a role in the regulation of abscission. We were unable to show any inductive effect of ethylene on either NAA or IAA conjugation rate (9, 10). Beyer and Morgan (5) reported that ethylene-induced inhibition of auxin transport is not due to its increased conjugation rate to aspartic acid. We also found recently (Riov and Goren, in preparation) that ethylene inhibited the polar translocation of auxin in the midrib toward the AZ, and that this inhibition is not correlated with increased conjugation. These observations together with the fact that Beyer (4) was able to demonstrate that the leaf is the sensitive tissue when abscission is induced by ethylene, suggest that if conjugation is involved in the regulation of abscission it is in the leaf blade. Additional experiments are required in order to confirm this hypothesis. Further results (Gaspar, Goren, Huberman, and Dubiq, in preparation) indicated that the decrease in situ of endogenous IAA in the AZ along the abscission process in Citrus leaf explants is correlated with an increase in the activity of certain peroxidase isoenzymes, suggesting that auxin degradation by peroxidase and not conjugation may be directly involved in the regulation of abscission in the AZ of Citrus leaves as in cotton

![Figure 5](image-url) Kinetics of 14C-NAA uptake and conjugation in aged leaf tissues. Explants consisting of petiole and 10 mm of leaf blade (type II) were aged for 24 hr. After aging, different tissues were separated and incubated as in Figure 4.

![Figure 6](image-url) Kinetics of abscission of different types of leaf explants. Explants were: whole leaf (type I), petiole and 10 mm of leaf blade (type II), and AZ only (type III). Explants were prepared at excision, dipped for 15 min in phosphate-citrate buffer (pH 4.2), and kept in humid chambers. For explanation of the different types of explants, see Figure 1.
of NAA studies with that their (10) that their conjugation rate is not always uniform. Further studies with IAA should, therefore, be required before determining conclusively that auxin conjugation in the AZ is not directly involved in the regulation of abscission in nature.

Acknowledgment—The authors wish to express their thanks to M. Huberman for his skillful and competent assistance.

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