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Effects of D and L Amino Acids on Foliar Abscission [1]

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Some naturally occurring amino acids have been reported to accelerate abscission of the ovary when applied to unfertilized ovaries of tobacco flowers (18). Methionine was especially active in promoting abscission. Several years ago in this laboratory, Nelson (10) observed that methionine not only promoted abscission of debladed petioles of bean and cotton, but when labeled in the methyl group gave rise to radioactivity in a pectic fraction. IAA which delays abscission (7), suppresses the effects of methionine on abscission of unfertilized ovaries of tobacco flowers (19) and in debladed petioles of cotton (10). Recently several amino acids have been reported to be more effective than methionine in promoting abscission in explants (13). The possible importance of amino acids in the control of abscission has led to a reexamination of their effects on the abscission of debladed petioles under greenhouse conditions.

Materials and Methods

Coleus blumei Benth., *Gossypium hirsutum* L. (Acala 4-42 Family 132), and *Phaseolus vulgaris* L. variety Black Valentine bean were grown under greenhouse conditions during the months November through May with minimum temperatures of 24° during the day and 22° during the night. The age of the experimental plant material at treatment was 2 to 5 weeks for bean plants, 5 to 10 weeks for cotton plants (6 to 8 weeks for cotton plants used in the radioisotope studies), and approximately 2 to 3 months in the case of *Coleus*.

In investigations of the effects of chemicals on abscission of bean petiolules, 0.01 ml droplets of the solution (pH = 6) were placed, immediately after deblading upon the cut surfaces of the lateral petiolules (5 mm in length) of first trifoliate leaves of bean. In the case of debladed petioles of *Coleus* and cotton, the chemicals (with the exception of IAA) were applied in solution in glass capillary tubes of 0.1 ml capacity to the cut surfaces of the petioles immediately after deblading. The capillary tubes were inserted 0.5 cm into the debladed petioles 2 or 4 cm from the abscission zones of cotton and 2.5 cm from the abscission zones of *Coleus*. Complete uptake from the capillary tubes occurred within 24 to 30 hours after treatment. IAA was applied in cavities (0.5 cm deep) made in the ends of debladed petioles, to ensure uniform movement of the auxin to the abscission layer.

Petioles that were treated with 0.4 μc of labeled amino acid per petiole were harvested along with the respective nodal region of the stem when abscission was imminent. At this time a thin hyaline line was apparent at the base of the petiole. The petiole tissue was divided into several samples, 5 mm of tissue from regions 3 cm, 2 cm, and 1 cm distal to the abscission layer; 2 mm of tissue from the abscission layer; 3 mm of tissue from the nodal region adjacent to the abscission layer.

The samples from identical regions of 10 treated

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plants were combined for extraction of pectic substances by a procedure described by Kertesz (6) and used recently by Nance (9). The sample of tissue was homogenized at 5° in a tubular glass mortar by a power-driven Teflon pestle. The homogenate was then filtered through moist glass wool. The cell wall residue trapped in the glass wool was washed with cold water and then extracted 4 times with 3 ml of 0.05 N HCl held at 80° for 30 minutes on a water bath. After each extraction the mixture was filtered through glass wool. The 4 extracts were combined, 3 ml of 10 % HCl were added, and the pooled extract was filtered through glass wool into a stainless steel centrifuge tube. The pectic substance was precipitated by the slow addition of 24 ml of 95 % ethanol. Separation of the precipitated pectic substance was accomplished by centrifugation at 3,000 g and 20° for 30 minutes. The precipitate was resuspended in an ethanol : H_2O mixture (2 : 1 ; v : v) and was recentrifuged 4 times to remove the free acid. Better formation of a pellet during the washings occurred at 4,000 g and 20°.

The washed precipitate was resuspended in 1.5 ml of distilled water and aliquots were placed on tared stainless steel planchets, dried under an infrared lamp at 45°, placed in a desiccator, and reweighed. The amount of radioactivity in the methyl ester form was measured by determining the loss of activity from the pectic substance upon saponification, with subsequent heating to remove the methanol. The planchets were counted under a Micromil window tube in an atmosphere of Q gas.

Results and Discussion

Alanine and glycine, 2 amino acids which have been reported (13) to be more effective in promoting abscission in bean explants than methionine, were compared with the latter compound for their effects on abscission in petiolules of bean. As shown in table I, experiment 1, alanine is more effective in promoting abscission of petiolules with blades in an early stage of expansion than is methionine. However, when the effects of the amino acids on abscission are compared in tissue where leaflets are more fully expanded (table I, experiment 2), methionine is more effective in promoting abscission than either alanine or glycine. The effects of alanine and methionine on abscission in petiolules of young tissue (experiment 1) are similar to the effects on abscission observed in explant experiments (13). Such experiments utilized excised tissue from 13-day-old seedlings. However, results obtained in experiments where petiolules from more fully developed leaves were used (experiment 2), would appear to be more reliable in elucidating the abscission processes since abscission as a natural process is characteristic of mature, if not senescent, tissues and organs.

Table I. *Effects of Alanine, Glycine, and Methionine on Abscission of Debladed Bean Petiolules at Different Stages of Leaf Expansion*

Average area per leaflet for expt. 1 : 3 cm^2; expt. 2 : 34 cm^2. Thirty petiolules for each treatment.

Treatment	Conc M	No. abscised by 33 hr	Hr for 50 % abscission
Experiment 1			
Control	0	9	35
L-Alanine	0.01	30	25
L-Methionine	0.01	11	34
Experiment 2			
Control	0	2	39
L-Alanine	0.005	3	39
	0 01	3	38
Glycine	0.005	2	38
	0.01	3	38
L-Methionine	0.005	7	36
	0.01	14	34

When the effects of treatment with 0.1 ml of 0.05 M solution of glycine, L-alanine, and L-methionine on the abscission of debladed petioles of *Coleus* were compared, only methionine accelerated abscission, the hours required for 50 % abscission being 48 in the case of treatment with methionine and 59 for controls. In other experiments alanine and glycine at concentrations of 0.005 M to 0.15 M did not accelerate abscission, and in some experiments these amino acids actually delayed abscission. On the other hand, 0.10 M methionine would cause 100 % abscission in as little as 24 hours.

When the effects of several amino acids on the abscission of debladed petioles of cotton were examined, marked differences in the effects of some D and L forms were observed. As shown in table II, the D forms of alanine, aspartic acid, glutamic acid, and serine reduced the time for 50 % abscission from

Table II. *Effects of the* D *and* L *Forms of some Amino Acids on Abscission of Debladed Cotton Petioles*

Ten petioles were used per treatment which consisted of an application of 0.1 ml of the respective solution 2 cm distal to the abscission zone of petioles at the second node above the cotyledonary node.

Treatment	Days for 50 % abscission Concentration		
	0.01 M	0.05 M	0.10 M
Control	11	13	12
L-Alanine	11	13	12
D-Alanine	2.4	1.7	
L-Aspartic Acid	11	13	12
D-Aspartic Acid	2.4	1.7	
L-Glutamic Acid	11	13	12
D-Glutamic Acid		1.6	
L-Serine	11	13	12
D-Serine		2.7	
Glycine	11	13	12
L-Leucine	2	2	
D-Leucine	1.7	3	
L-Methionine	2	1.5	
D-Methionine	2.7	4	
L-Phenylalanine	2	1	
D-Phenylalanine	2	1.5	

an average of 12 days for the controls to less than 3 days. The naturally occurring forms of these 4 amino acids had little or no promotive effects on abscission even at concentrations as high as 0.1 M. Glycine was also tested for its effect on abscission in the same plant material, since this amino acid along with the naturally occurring forms of alanine, aspartic acid, glutamic acid, and serine, were the amino acids reported to be the most effective in promoting abscission in bean explants (13). Glycine did not promote abscission in debladed petioles of cotton at concentrations as high as 0.10 M. Similar differences in the effects of the D and L forms of alanine, aspartic acid, glutamic acid, and serine on abscission in debladed petioles of *Coleus* were also observed.

Table II also shows that the naturally occurring forms of leucine, methionine, and phenylalanine have marked promotive effects on abscission in comparison with the amino acids previously discussed. The D forms of leucine, methionine, and phenylalanine also are effective in promoting abscission. Both forms of these 3 amino acids reduce the time for 50 % abscission from 12 days for the controls to 1 to 4 days. L-Methionine brings about 50 % abscission in 1.5 to 2 days as compared to 2.7 to 4 days in the case of D-methionine.

Leucine, methionine, and phenylalanine, whose naturally occurring forms promote abscission, are known (5) to increase in senescent leaves of cotton in an initial stage of abscission, as well as in leaves undergoing abscission after treatment with a defoliating agent. Leucine and phenylalanine have also been reported (12) to increase in senescent leaves of *Acer platanoides* prior to abscission. The failure of glycine and the naturally occurring forms of alanine, aspartic acid, glutamic acid, and serine to promote abscission in debladed petioles of cotton and *Coleus* may be due to the conversion of these amino acids to acids of the Krebs cycle. It has been suggested (12) that such conversion in the senescent leaves of *Acer platanoides* does occur. This would explain the absence of an increase in the level of free amino acids such as alanine, aspartic acid, glutamic acid, glycine, and serine in senescent leaves of cotton and maple, even though they are being liberated through proteolysis. In this view amino acids such as leucine, methionine, and phenylalanine which are less easily oxidized would be translocated out of the senescent leaf and may become involved in the abscission processes upon movement through the region of separation.

The marked effect of D amino acids and certain of the naturally occurring amino acids on abscission may be through their interference in protein synthesis. The D amino acid may be incorporated into peptides and thus block further synthesis, due to the failure to completely fit the pattern of the natural amino acids (16). L-Amino acids, when present at abnormal levels, may also act as antimetabolites by altering the amino acid sequence in synthesis of proteins (17).

In addition to interference in protein synthesis, the amino acids may serve as substrates for metabolic processes in the cell wall and middle lamella. It is logical to believe that D amino acids, upon failure to be converted to the naturally occurring form and utilized in the tissue, would be broken down. Such a break-down could serve as a source of active groups which could then be incorporated into the pectic fraction of the cell wall and middle lamella in the abscission zone. The amino acids whose L forms are effective in promoting abscission, may also give rise to active groups.

Radioisotope Studies. It was of interest to examine an incorporation of methionine or derived groups into the pectic substances of the middle lamellae of cells comprising the abscission region, since the dissolution of middle lamellae has been considered to be the mode of separation for the petiole from the main plant body (3, 8, 14).

An analysis of the pectic substances of the abscission zone of debladed petioles of cotton just prior to abscission shows a high degree of incorporation of radioactivity from methionine-methyl-C-14 in comparison with other regions of the petiole (fig 1). The labeled amino acid was applied 4.5 cm from the abscission zone. The samples at 3, 2, and 1 cm from the abscission zone showed a decline in radioactivity corresponding to a distribution gradient from the source. In the abscission zone, however, the level of activity is about 3.7 times that in the adjacent petiole tissue. Saponification of the pectic substances

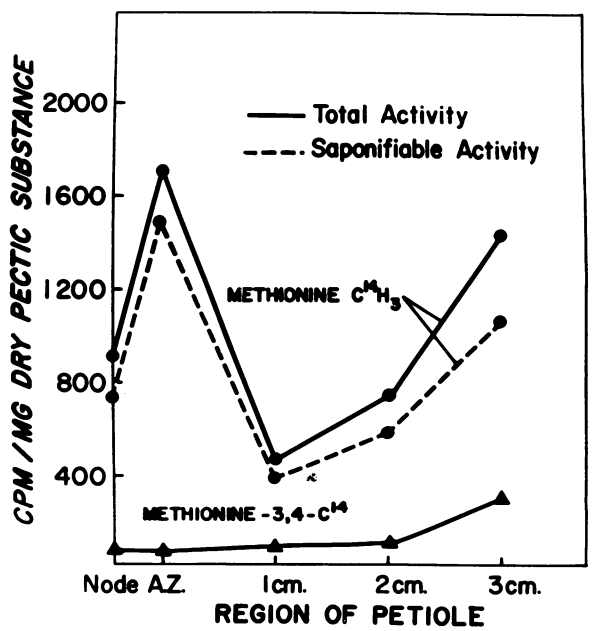

FIG. 1. Distribution of C^{14} labeled methionine in the pectic substances of the cell wall of debladed cotton petioles. Petioles were harvested 70 hours after application of L-methionine-methyl-C^{14} (1.84 mc/mM) or DL-methionine-3,4-C^{14} (1 mc/mM) 4 cm distal to A.Z.

showed that the high amount of radioactivity in the abscission zone was due to the methyl groups transferred to the pectic substances from methionine. As seen in figure 1, the loss of activity upon saponification is approximately 90 % of the activity present in the pectic substances extracted from the abscission zone, indicating that this amount of the original pre-saponification activity is in an ester form. The results where methionine-3,4-C-14 was applied to the debladed petioles (fig 1) show further that methionine does not contribute to the high level of activity present in the abscission zone other than by its terminal methyl group.

Experiments where debladed petioles of *Coleus* were used as the experimental plant material (fig 2) also show a peak of radioactivity in the abscission zone which is present due to methylation of the pectic substances of the abscission zone. In *Coleus*, the activity present in the 3 regions outside the abscission zone is higher than in the case of cotton. This is due to the fact that the labeled amino acid was applied only 3 cm from the abscission zone, thus much closer to the samples taken.

Occasionally, the distribution of activity was found to be different than in the experiments so far described. In this case an increase of activity in the abscission zone over the rest of the petiole was observed, but the amount of activity in the nodal tissue was higher than the amount in the abscission zone. Because added IAA reduces the incorporation of the labeled carbon in both the nodal tissue and abscission zone, as shown in figure 3, a high level of incorporation in the nodal tissue probably results from a low auxin level in the stem. The usual situation in which the radioactivity in the nodal tissue is lower than in the abscission zone is probably the result of a higher (normal) auxin level in the stem.

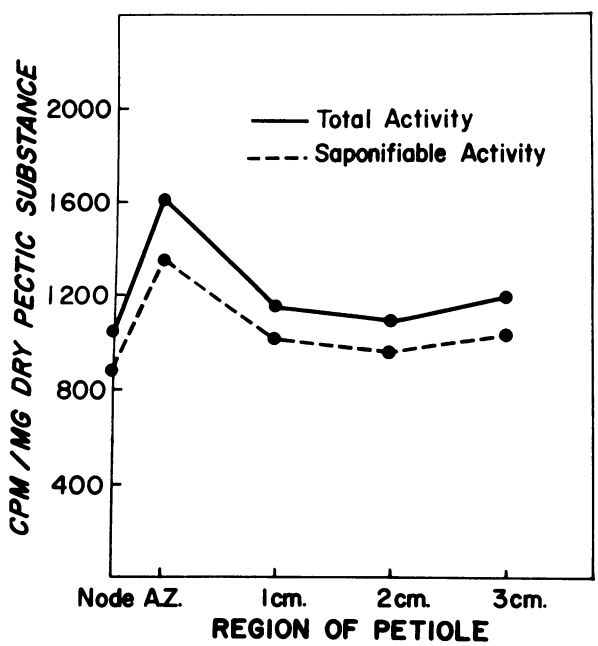

FIG. 2. Distribution of methionine-methyl-C-14 in the pectic substances of the cell wall of debladed *Coleus* petioles. Petioles were harvested 36 hours after application of L-methionine-methyl-C-14 (11.52 mc/mM) 3 cm distal to A.Z.

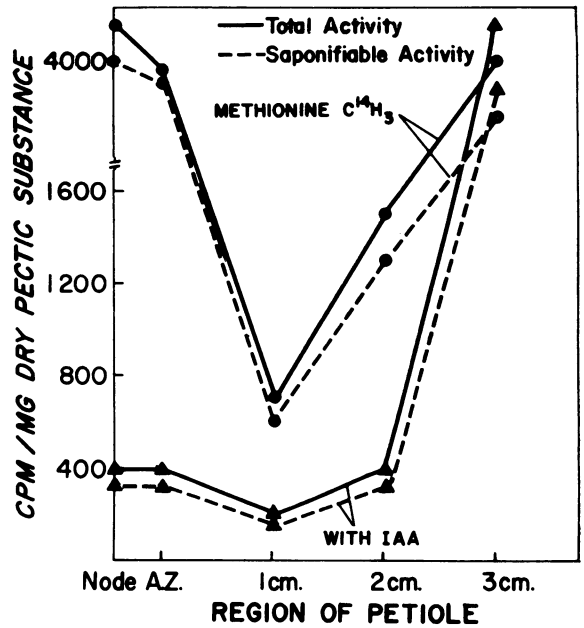

FIG. 3. Influence of IAA on the distribution of methionine-methyl-C-14 in the pectic substances of the cell wall of debladed cotton petioles. Petioles were harvested 65 hours after application of L-methionine-methyl-C-14 (11.52 mc/mM) 4 cm distal to A.Z. Petioles treated with auxin received 0.05 ml 10^{-4} M IAA.

Although methionine has been reported to serve as a methyl group donor in various plant tissues (1, 2, 11, 15) the exact function of methylation of the pectic substances in cell wall metabolism has not been established. A high degree of methylation of the pectic substances of the abscission region would lead to the reduction of possible bonds between polymer chains and fewer chances of bonding with ions. This loss of cementing properties would result in the separation of the organ from the plant body. An increase in the solubility of substances of the middle lamellae upon a shift of pectic acids to pectin has been reported (3) and the present study indicates that the effect of methionine on abscission may be the result of methylation of the pectic substances.

The results of experiments in which IAA was applied to debladed petioles in addition to methionine-methyl-C-14 are shown in figure 3. The high incorporation of radioactivity into the pectic substances of the abscission zone when methionine is applied alone, is almost completely suppressed by the exogenous supply of IAA. Since no visible signs of abscission were apparent in the petioles treated with methonine plus auxin at the time of harvest for analysis of the pectic substances, it would appear that an effect of

auxin in delaying abscission is by its suppression of the methylation of pectic substances in the abscission zone.

Since the naturally occurring form of phenylalanine also promotes abscission, the distribution of phenylalanine-3-C-14 or derived groups in the pectic substances was examined. Experiments in which phenylalanine-3-C-14 was applied to debladed cotton petioles show somewhat similar distribution patterns of radioactivity in the pectic substances extracted from various regions of the petiole. As shown in figure 4, a high incorporation of radioactivity occurs in the abscission region as well as in the nodal tissue. No loss of radioactivity was observed upon saponification, indicating that phenylalanine is either being incorporated directly into the pectic fraction, or is contributing some nonester group which may result in changes in the middle lamella or cell wall, leading to abscission. Although a recent study (20) has suggested that the substance binding leaf cells together is pectic acid with few carboxyl groups esterified, proteins and metals have been reported (4) to serve also as binding agents between cells of the root tip of Alaska pea seedlings. It is clear that any fractionation process for cell wall materials must be considered as somewhat arbitrary; the inclusion of protein components with pectic substances is a distinct possibility and this may account for the role of phenylalanine.

Experiments were conducted in which IAA was applied simultaneously with phenylalanine-3-C-14 to debladed petioles of cotton. The high degree of radioactivity which appears in the abscission zone when phenylalanine is applied alone (fig 4), is almost completely absent in the presence of IAA. Furthermore, petioles which received IAA showed no signs of abscission as compared with the petioles treated with the amino acid only.

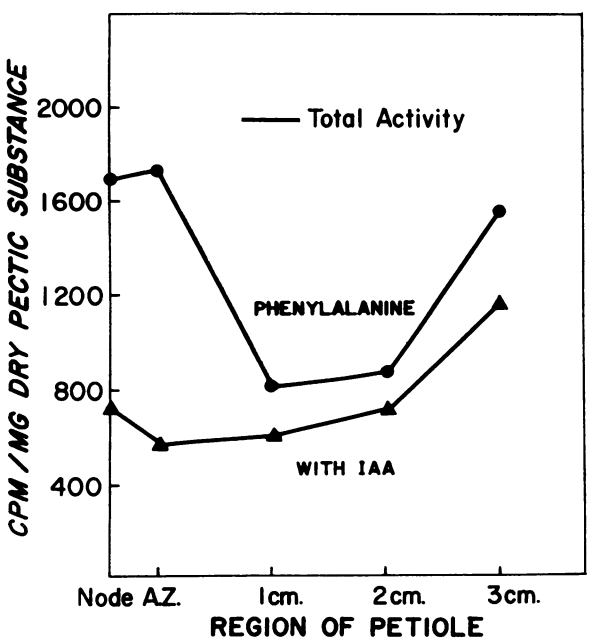

FIG. 4. Influence of IAA on the distribution of phenylalanine-3-C-14 or a derived group in the cell wall of debladed cotton petioles. Petioles were harvested 68 hours after application of DL-phenylalanine-3-C-14 (3–10 mc/mM) 4 cm distal to A.Z. Petioles treated with auxin received 0.05 ml 10^{-3} M IAA.

Summary

Methionine has been found to be an effective promoter of abscission in debladed petioles of cotton and *Coleus*, and in petiolules of bean except in petiolules of lamina in an early stage of expansion. The D forms of alanine, aspartic acid, glutamic acid, and serine are also very effective in promoting abscission in cotton and *Coleus* while the L forms have little or no effect. Both the D and L forms of leucine, methionine, and phenylalanine promote abscission. The response to L-methionine is more rapid than the response to D-methionine.

Experiments using C-14 labeled methionine show high incorporation rates of methyl groups into the pectic fraction of the abscission zone, prior to abscission. Indoleacetic acid which delays abscission, suppresses this incorporation. Similar results have been obtained in experiments using DL-phenylalanine-3-C-14, but it is not established whether this amino acid is donating a nonsaponifiable group or is being incorporated directly into the pectic fraction. These experiments indicate that methionine and phenylalanine may promote abscission by serving as sources of methyl or other groups which are incorporated into the cell wall and middle lamella in the zone of separation.

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Relationship of the Syntheses of Lipid and Water Soluble Acids by Chloroplast Preparations [1, 2]

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Introduction

Acetate metabolism by *Chlorella* rapidly introduces label into a range of compounds, including lipid, organic acids, and amino acids (26). At the point of acetyl CoA, contributions may be made to acids of the tricarboxylic acid cycle, fatty acids and sterols. Various factors influence the pathway taken by acetyl CoA. For example, the type of inorganic nitrogen source influences the lipid content of *Chlorella* (23); the composition of the suspending medium influences the distribution of label in *Chlorella* photosynthesizing in $C^{14}O_2$ (11); illumination causes an apparent inhibition of synthesis of acids of the tricarboxylic acid cycle (6). From a physiological point of view, it is not satisfactory to consider the effect of variation of conditions on one metabolic fate of acetyl CoA without simultaneous consideration of other possibilities.

The chloroplast fraction from leaves of higher plants has been shown to synthesize long chain fatty acids from acetate (17, 22, 24). Cofactor requirements for optimal fatty acid synthesis have been determined, but little account has been taken of the concomitant effects on the synthesis of water soluble compounds. In this paper an attempt has been made to relate the activity of fatty acid synthesis to the synthesis of organic and amino acids.

Materials and Methods

Preparation of Chloroplast Fractions. Spinach was obtained from local wholesalers. Chloroplasts were isolated by the method of Whatley et al. (28). The chloroplasts were washed once in the medium 0.35 M with respect to NaCl and were then resuspended in 0.035 M NaCl ("broken chloroplasts") before addition to the reaction mixture. The chloroplast preparations contained 10 to 15 mg/ml protein and 1 to 2 mg/ml chlorophyll.

Reaction Mixtures and Assays. Standard reaction mixtures contained 100 μmoles Tris buffer pH 8.0, 10 μmoles ATP, 50 mμmoles CoA, 65 mμmoles

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