ETHIONINE AND AUXIN-ACTION IN AVENA COLEOPTILE[1, 2]

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Ethionine has been shown by Schrank (12) to be an inhibitor of auxin-induced elongation in the Avena coleoptile. Since the inhibition can be reversed by methionine, it has been concluded that ethionine acts by affecting some facet of methionine metabolism. Work with animal tissues has shown that there are two ways in which this can occur: ethionine can antagonize the incorporation of methionine into proteins and thus block protein synthesis (14) or it can inhibit transmethylation (13). Recent work has suggested that ethionine may be inhibiting elongation in the Avena coleoptile by interfering with transmethylation. Auxin has been shown to cause an increase in the rate of transfer of methyl groups from methionine to cell wall pectin in this tissue (11). If this transmethylation is obligatory for auxin-action, inhibition of this methylation by ethionine would lead to an inhibition of elongation. This theory is only tenable if ethionine actually does inhibit cell wall methylation and this has not been previously demonstrated. It was the purpose of this investigation to see if such an inhibition could be found.

Schrank has shown that ethionine inhibits total elongation (12). Total elongation (TE) is the sum of reversible (ES) and irreversible elongation (IE). The inhibition of TE by ethionine has now been separated into inhibitions of ES and IE and the rapidity of each inhibition has been determined from time-course studies.

In this paper, the term "cell wall methylation" is used to describe the transfer of methyl groups from methionine to the hot-water-soluble fraction of the cell wall. The effect of ethionine on the methylation of cold-water-soluble pectin has not been examined in this study.

MATERIALS AND METHODS

Seedlings of *Avena sativa* L. var. Victory for all experiments were grown in the manner of McRae and Bonner (9). The effect of ethionine on cell wall methylation was determined by the following technique, based on Ordin et al (11). Coleoptiles 2.75 to 3.25 cm in length were selected and after the central leaf had been removed, a single 10 mm section was cut from each coleoptile. The apical cut was 3 mm below the tip. The sections were divided into lots of 70 and each lot was then incubated for 3 or 3½ hours in 10 ml of 0.0025 M K-Maleate solution (pH 4.8) which contained C^{14}-methyl labeled L-methionine (2×10^{-5} M). The methionine had a specific activity of 0.145 mC/mg. Each solution also contained one of the following sets of additions: 5 ppm 3-indoleacetic acid (IAA), DL-ethionine (0.05 M), IAA and ethionine, or no additions (control). At the end of the incubation, the sections were rinsed with distilled water, ground rapidly in a mortar and the cell wall debris was separated from soluble material by repeated washing with water on a sintered glass funnel. The pectin fraction of the wall was then isolated by extracting the wall twice for 10 minutes with 2 ml of hot water. An aliquot of the combined extract was then plated out and counted. The amount of saponifiable methyl groups in this extract was determined by ascertaining the loss in radioactivity upon saponification of the extract with hot concentrated NH_3.

The material for the elongation experiments consisted of 5 mm Avena coleoptile sections which still contained the central leaf. These sections were obtained in the manner of McRae and Bonner (9). One lot of 20 sections was immediately plasmolyzed by treatment for 90 minutes in 1 M mannitol and the length of the sections was determined (PLi). The remaining sections were placed in 20 ml of a solution which contained K-Maleate (0.0025 M, pH 4.8), sucrose (1 %) and IAA (5 ppm). Some solutions contained no further additions, others contained either DL-ethionine (0.05 M) or ethionine and ethylenediamine tetraacetic acid (EDTA, 0.001 M). After the desired length of time, sections were removed and measured (Lf). They were then plasmolyzed and remeasured (PLf). Irreversible elongation (PLf-PLi) and reversible elongation (Lf–PLf) were determined.

All manipulations were carried out under dim red light. The temperature during growth of the seedlings and during the course of the experiments was 25 ± 2° C.

RESULTS

The effect of ethionine on cell wall methylation can be seen from the results shown in table I. Experiments 1 and 2 were performed at the laboratory of Professor Bennet-Clark in 1959. Experiment 3 is one of three similar experiments carried out in the laboratory of Professor Bonner in 1956. They were identical to the other two experiments except that the total rather than the saponifiable activity of the hot-water extracts was determined. Ethionine caused a distinct but variable inhibition of cell wall methylation

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TABLE I

EFFECT OF ETHIONINE ON INCORPORATION OF RADIOACTIVITY FROM METHIONINE (METHYL-C^{14}) INTO HOT-WATER-SOLUBLE FRACTION OF CELL WALL

		EXPT 1	EXPT 2	EXPT 3
5 *ppm IAA*	0.05 *M ethionine*	*cpm MeOH/ 70 sections*	*cpm MeOH/ 70 sections*	*cpm/10 mg dry cell wall*
+	−	831	740	3,064
−	−	432	630	1,917
+	+	155	60	867
−	+	201	60	874
Incubation time		3½ hr	3½ hr	3 hr
cpm Methionine administered		3.7×10^5	3.7×10^5	3.5×10^5

in all five experiments. Furthermore, in every experiment ethionine completely suppressed the ability of auxin to increase the rate of methyl transfer.

The decrease in methyl transfer induced by ethionine was not due to an inhibition of the uptake of methionine into the tissue. This has been shown in the following way. Sections were incubated for 1 hour in a solution containing methionine-C^{14} but no ethionine. They were then transferred to water, with or without 0.07 M ethionine, and the incorporation of radioactivity into wall pectin was determined after 0, 1, and 2 hours. Cell wall methylation was inhibited by ethionine even under these conditions where ethionine could have had no effect upon the uptake of methionine (fig 1). The inhibition can only be ascribed to an interference with the transmethylation reaction. It should be noted that cell wall methylation was strongly inhibited within 1 hour after application of ethionine.

The inhibition of auxin-induced elongation by ethionine and the reversal of the inhibition by methionine have been confirmed. This is shown in table II. It should be noted that the Avena coleoptile sections used in this experiment were about one-tenth as sensitive to ethionine as were those used by Schrank (12). This may be due in part to the use of DL- rather than L-ethionine.

The effect of ethionine on the time-course of irreversible elongation is shown in figure 2a. In the absence of ethionine, elongation was rapid (due to presence of 5 ppm IAA) and constant. Ethionine had no effect on IE during the 1st hour after its application and produced only a slight inhibition during the subsequent 2 to 4 hours. Thereafter IE was strongly inhibited. EDTA only slightly alleviated this inhibition.

In the absence of ethionine, an increase in IE is accompanied by a proportional increase in ES (5). Upon addition of ethionine, however, there was an immediate decrease in ES (fig 2b). Often the inhibition was even more pronounced than that shown in figure 2b. ES then increased slightly during the next 3 to 4 hours, but thereafter steadily decreased with time. The decrease could be prevented, in part, by addition of EDTA to the medium. If the addition of EDTA occurred at the same time as the addition of ethionine, no inhibition of ES occurred until after 4 to 6 hours. This is even more clearly seen if ES is plotted as a function of IE (fig 2c). This indicates that the immediate decrease in ES was not due to the addition of osmotic solutes to the external medium.

DISCUSSION

Ethionine causes a strong and immediate inhibition of cell wall methylation. Furthermore, ethionine can completely suppress the ability of auxin to cause an increase in the rate of methyl transfer.

The number of radioactive methyl groups in the cell wall at the end of an incubation with methionine (methyl-C^{14}) is equal to the number incorporated by transmethylation minus the number removed from the wall during the incubation period by cell wall demethylation. At the end of the incubation, cell walls of auxin-treated tissues possess a greater number of radioactive methyl groups than do the walls of non-

TABLE II

INHIBITION OF ELONGATION BY DL-ETHIONINE AND ITS REVERSAL BY L-METHIONINE. EXPANSION PERIOD 5½ HRS. ALL SOLUTIONS CONTAINED K-MALEATE (0.0025 M).

TREATMENT	5 PPM IAA	FINAL LENGTH	Δ LENGTH	% INHIBITION
0.05 M mannitol	+	5.47 mm	0.54	...
	−	4.93		
0.03 M ethionine	+	4.99	0.17	69
	−	4.82		
0.03 M ethionine +	+	5.30	0.34	37
	−	4.96		
0.001 M methionine				

auxin-treated controls. This may be due to either an auxin-induced increase in incorporation or an auxin-induced decrease in decorporation of methyl groups. Glasziou (7) and Adamson and Adamson (1) have suggested that it is an auxin-mediated decrease in decorporation which occurs. If this were so, ethionine, which inhibits only the methylation reaction, would not be affecting the auxin-sensitive reaction. Thus while it would cause a decrease in amount of radioactive methyl groups found in the cell wall, it should not eliminate the effect of auxin. The complete elimination of the auxin-effect indicates that ethionine is blocking the auxin-sensitive reaction which must be the methylation process.

Ethionine also inhibits cellular elongation (12). It has been shown by time studies that this inhibition of total elongation occurs in two distinct phases. The first phase consists of a decrease in ES immediately after adding ethionine. This decrease can be reversed by EDTA. After 3 to 4 hours, the second phase sets in. This consists of strong inhibition of both ES and IE. The inhibition of ES can be partially reversed by EDTA; the inhibition of IE is only slightly reversed. The inhibition of total elongation by ethionine is mainly due to this second phase of inhibition.

How does ethionine cause these inhibitions? The fact that both the inhibition of cell wall methylation and the initial decrease in ES occur immediately after

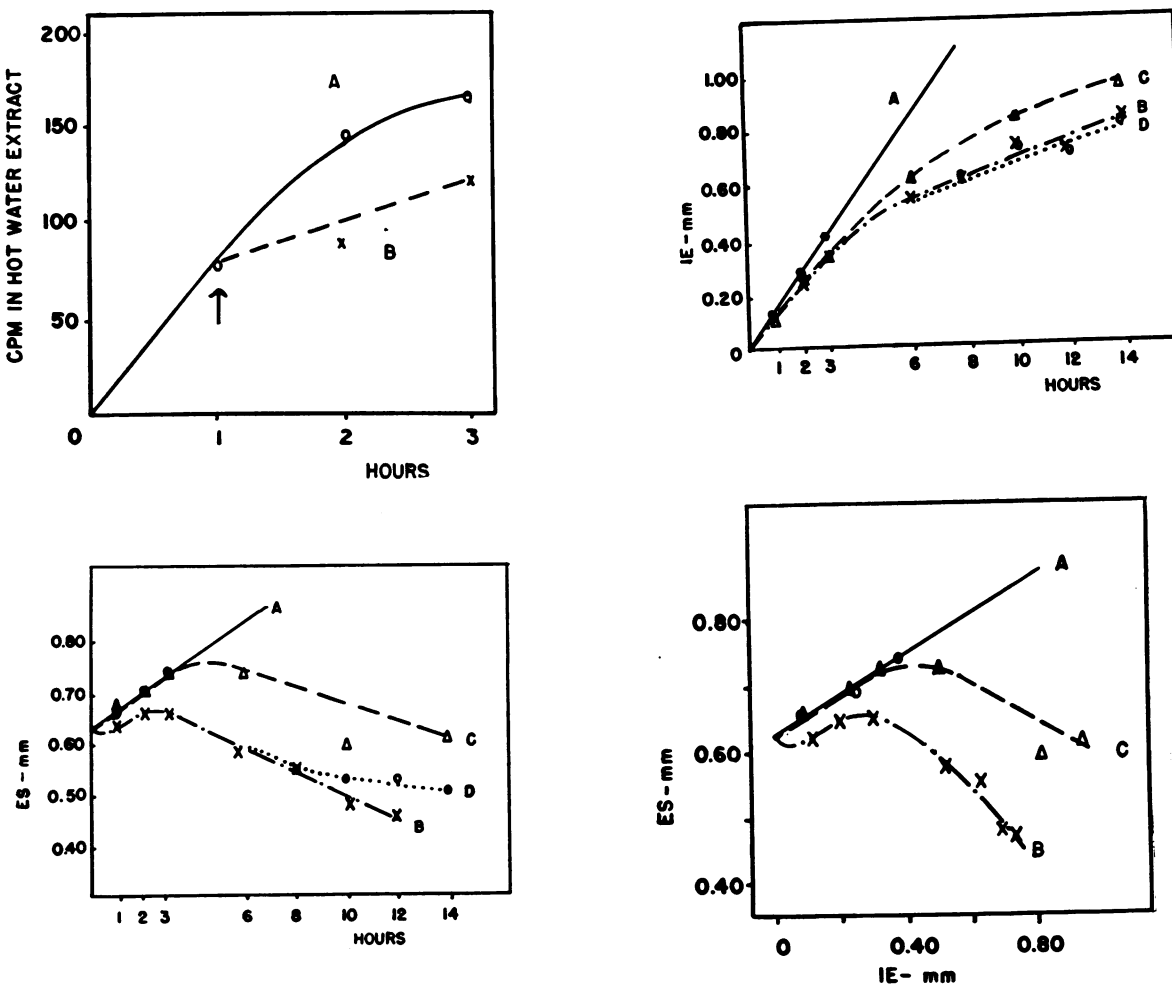

FIG. 1 (*upper left*). A demonstration that the inhibition of cell wall methylation by ethionine is not due to an inhibition of the uptake of methionine into the tissue. Sections incubated with methionine-C^{14} for 1 hour, then placed (at arrow) in water (A) or 0.07 M DL-ethionine solution (B). Incorporation of radioactivity into hot-water-soluble fraction of the wall determined 0, 1, and 2 hours after removal from methionine.

FIG. 2. The effect of ethionine on the time-course of irreversible and reversible elongation. All solutions contained K-maleate (0.0025 M), sucrose (1 %) and IAA (5 ppm). Curve A (-○-), no further additions; B (-×-), 0.05 M ethionine; C (-Δ-), 0.05 M ethionine + 0.001 M EDTA; D (.○.), 0.001 M EDTA added after 6 hours. a) (*upper right*) Irreversible elongation, b) (*lower left*) Reversible elongation, c) (*lower right*) Relation between IE and ES

addition of ethionine suggests that the inhibition of transmethylation is the cause of the decrease in ES. This might occur in the following way.

It has been shown that calcium stiffens cell walls (15). Bennet-Clark (2) has suggested that this occurs through formation by calcium of bridges between the carboxyl groups of adjacent pectin chains. The number of these bridges and thus the rigidity of the wall could be limited by the number of free carboxyl groups. Inhibition of cell wall methylation should cause at least a slight increase in the number of free carboxyl groups. This would lead to more calcium bridges and a decrease in elasticity. EDTA, which can chelate free calcium ions, may be reversing this decrease in ES by removing the newly formed calcium bridges.

There are two objections to this theory. Addition of calcium ions, and thus, presumably, the formation of calcium bridges, reduces the plasticity as well as the elasticity of the Avena coleoptile sections (15). If the inhibition of ES by ethionine involves the formation of new calcium bridges, why does not ethionine inhibit IE at the same time? Secondly, Carr and Ng (4) have shown that with wheat coleoptile tissues, EDTA prevents rather than accelerates the loss of calcium from the cell wall. This is not proof, however, that EDTA does not break calcium bridges.

What is the nature of the second phase of the ethionine inhibition? It is not likely that it results from the inhibition of cell wall methylation, for this phase of elongation-inhibition does not manifest itself until 3 to 4 hours after the inhibition of methylation has occurred. Ethionine has been shown to block protein synthesis in plants (16), animals (14), and bacteria (10). The possibility should be considered that ethionine is inhibiting elongation of Avena coleoptiles by interfering with protein synthesis. Evidence to support this idea is obtained from the fact that another amino acid antagonist, canavanine, inhibits auxin-induced elongation in the Avena coleoptile (3). However, auxin-induced elongation can occur in maize (6) and Avena mesocotyl sections (8) in the absence of any increase in protein level. This suggests that only some small portion of protein synthesis must occur in order for elongation to proceed. This would presumably be some enzyme necessary for auxin-action.

Summary

I. The rate of transfer of methyl groups from methionine to the hot-water-soluble fraction of Avena coleoptile cell walls is strongly inhibited by ethionine. The auxin-induced increase in methylation can be entirely prevented by ethionine. This inhibition occurs within 1 hour after adding ethionine.

II. Ethionine causes an immediate inhibition of the reversible elongation of the tissues. Irreversible elongation is strongly inhibited only after 3 to 6 hours. EDTA will reverse the inhibition of reversible but not irreversible elongation.

III. It is suggested that the inhibition of methylation may be the cause of the inhibition of reversible elongation while an inhibition of protein synthesis may lead to the inhibition of irreversible elongation.

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