THE EFFECT OF 3-INDOLYLACETIC ACID ON THE BINDING OF PECTIN METHYLESTERASE TO THE CELL WALLS OF TOBACCO PITH

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[*Manuscript received March* 20, 1957]

Summary

3-Indolylacetic acid (IAA) was found to promote the binding of pectin methylesterase (PME) to a cell wall fraction prepared from tobacco pith, the effect being increased by increasing concentration of IAA to a peak of activity, after which inhibition occurred.

It is suggested that the immobilization of PME, by binding to the cell wall *in vivo*, would favour methylation of the pectates of the cell wall and hence increase cell wall plasticity.

I. Introduction

The multiplicity of physiological effects of 3-indolylacetic acid (IAA) on the growth of plants has served to develop the view that the primary site of action of the hormone is of very great importance in cellular metabolism. Many investigators have considered that this reaction is closely associated with the pectic substances of the cell walls (van Overbeek 1952; Bennet-Clark 1955), and that the control of cell wall plasticity constitutes the major effect of IAA in the cell (Burström 1953; Levitt 1953).

McRae, Foster, and Bonner (1953), in studying the kinetics of auxin effects on the growth of the oat coleoptile, concluded that each auxin molecule was bound at two points to a cellular component to form a complex active in stimulating growth. At high levels of auxin concentration, a separate molecule was bound at each point causing inhibition of growth. Ordin, Cleland, and Bonner (1955) have shown that the incorporation of methionine-^{14}C, labelled in the methyl group, into the cell wall constituents of oat coleoptiles was enhanced by auxin, and that enhancement of incorporation into pectins occurred even if the growth of the tissues was suppressed by suitable external osmotic concentrations.

Wilson and Skoog (1954) studied the uronide composition of tobacco pith sections which had been stimulated into rapid cell enlargement by IAA, and observed that the changes in uronide and pectic materials which resulted from IAA treatment could be considered as an integral part of auxin-induced growth of cell walls. Bryan and Newcomb (1954) also studying the growth of tobacco pith sections observed that IAA-induced cell enlargement was accompanied by an increase in pectin methylesterase (PME) activity to almost twice the value for the control sections after 120 hr. These authors found that in a cell wall fraction which contained almost all the PME activity of the tissue, the enzyme activity was reduced over a wide range of concentrations by IAA.

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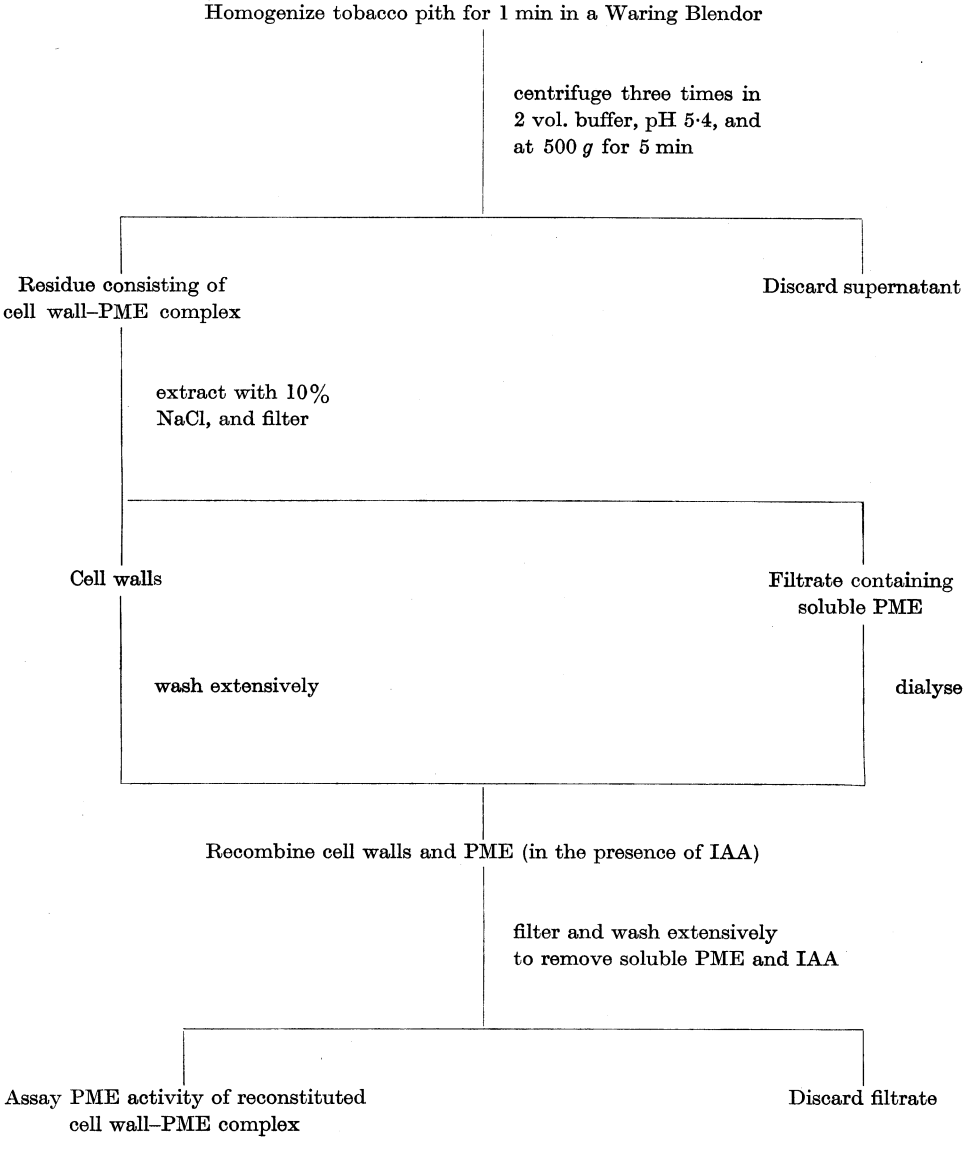
It is pertinent to consider whether IAA functions by promoting the binding of enzymes of cell wall metabolism to loci within the cell. The results presented indicate

TABLE 1

FLOW DIAGRAM OF METHOD OF CLEAVING AND RECONSTITUTING THE CELL WALL–PECTIN METHYLESTERASE (PME) COMPLEX FROM TOBACCO PITH (SEE TEXT FOR FULL DETAILS)



that IAA promotes the binding of one such enzyme (PME) to constituents of the cell wall. To demonstrate this, the enzyme was split from a cell wall preparation and the effect of IAA on the reconstitution of an enzyme–wall complex studied.

II. Experimental

A flow diagram outlining the general method of preparation of the cell wall–enzyme complex is shown in Table 1, and the procedure for one experiment is given.

Tobacco pith (28 g fresh weight), from the stems of large tobacco plants which had flowered, was homogenized in a Waring Blendor for 1 min with 60 ml 0·05M phosphate buffer at pH 5·4. The homogenate was centrifuged at 500 *g* for 5 min and the supernatant discarded. The centrifugation was repeated twice more after resuspension of the residue in 50 ml of the same buffer. To the residue was added 15 ml 0·01M phosphate buffer, pH 7·6, the volume was measured (36 ml), and the appropriate amount of NaCl added (3·6 g). The suspension was stirred at intervals over a 1-hr period and then filtered through Whatman No. 5 filter paper on a Buchner funnel. The filtrate (containing the PME) was transferred to a dialysis sac and dialysed against 5 l. distilled water with rapid mechanical stirring for 4 hr. The dialysate (PME solution) had a final volume of 41 ml.

Table 2

EFFECT OF 3-INDOLYLACETIC ACID (IAA) ON THE BINDING OF PECTIN METHYLESTERASE (PME) TO THE CELL WALL

The complete system contained 1·5 g wall fraction, 7·0 ml enzyme solution, and IAA at the concn. shown. Final vol. 9·5 ml. After 90 min incubation at 25°C, systems containing the cell wall fraction were filtered, washed extensively, and the PME activity of the residue determined by measuring the acid formed during the hydrolysis of methyl ester groups of pectin

System Components	Ml 0·01N NaOH Required to Neutralize the Acid Produced in 30 Min
No enzyme; no IAA	2·0
No IAA	4·0
Complete ($1{\cdot}05 \times 10^{-8}$M IAA)	6·0
Complete ($1{\cdot}05 \times 10^{-10}$M IAA)	7·2
Complete ($1{\cdot}05 \times 10^{-12}$M IAA)	5·3
No wall fraction; no IAA	7·9

To obtain a cell wall fraction with low PME activity, the residue was extracted on a Buchner funnel (Whatman No. 4 paper) with 10 per cent. NaCl buffered at pH 7·2 with 0·01M phosphate buffer. Suction was applied periodically and more NaCl solution added, a total of 100 ml being used over a 3-hr period. The filtrate from this extraction contained residual PME and was discarded. The wall fraction was then washed twice with 0·05M phosphate buffer, pH 5·4, most of the buffer solution removed by suction, and the residue divided into five 1·5-g portions. All operations to this point were carried out in a cold room at 2–4°C.

Six systems were prepared, the complete system containing 1·5 g wall fraction, 7·0 ml PME solution, and IAA at the concentrations shown in Table 2 in a final volume of 9·5 ml. The pH of the incubation mixture was about 5·4, due to the phosphate buffer carried over in the wall fraction.

After 90 min incubation at room temperature (25°C) each system containing the wall fraction was filtered on a Buchner funnel with suction (Whatman No. 4 paper) and washed with 150 ml 0·001M phosphate buffer, pH 5·4, added in portions of 15 ml. After the last washing, the wall fraction was dried by suction and then transferred, complete with filter paper, to the PME assay mixture, and stirred with a glass rod to separate the wall fraction from the paper (which was then removed). System 6 (see Table 2) was added without filtering.

Enzyme assay.—The method used was essentially that described by Bryan and Newcomb (1954) except that the pH of the assay solution was adjusted not to 7·0 but to 7·5 (glass electrode) before and after the addition of the enzyme.

III. Results

The results presented in Table 2 are typical of those obtained in a series of experiments and show that IAA is effective in promoting the binding of PME and the cell wall fraction in such a way that the enzyme cannot readily be washed off with 0·001M phosphate buffer, pH 5·4, the optimal effect being found at an IAA concentration of $1{\cdot}05\times10^{-10}$M. The peak has been noted at 1×10^{-10}M IAA where the range extended from 10^{-6} to 10^{-12}M. However, preliminary experiments in which tobacco pith was taken from the third to the ninth internodes of young growing plants indicate that the optimal concentration of IAA is much higher in this tissue, being of the order of 1×10^{-6}M.

The possibility that the filter paper, which was used during the washing procedure following the recombining of the wall fraction and the enzyme, had affected the results by absorbing some of the enzyme was eliminated by carrying out experiments in which filter paper replaced the wall fraction. The negligible adsorption of PME to the filter paper, either in the presence or absence of IAA, may indicate that the cellulose fibres in the cell wall are not a point of attachment for PME. The effect of IAA on the activity of soluble PME was also examined, no differences from controls being observed when IAA was present in the assay system at final concentrations of 1×10^{-4} to $1{\cdot}6\times10^{-7}$M. Similarly the effect of IAA on the activity of PME when bound to the wall was examined. No inhibition was observed even at concentrations as high as 8×10^{-4}M when added to the assay systems. This latter result is at variance with those of Bryan and Newcomb (1954) who observed reduced activity over a wide range of concentrations and complete inhibition at 1×10^{-4}M.

IV. Discussion

It is well established that PME of higher plants is usually strongly adsorbed to the water-insoluble components (Willaman and Hills 1944, quoted by Kertesz 1951), and in tobacco pith it is almost completely in the cell wall fraction (Bryan and Newcomb 1954). The enzyme is readily desorbed by treatment with salt solutions, suggesting that the bonding between enzyme and wall is of a salt-like nature. The evidence presented here indicates that IAA participates in the bonding of PME to the cell wall. This concept would explain more satisfactorily the auxin effects shown by many synthetic compounds than the hypothesis that such compounds act as the prosthetic group of an enzyme.

It appears possible that the effect of IAA on the binding of PME to the cell wall would lower the activity of the enzyme in the cell, and decrease the rate of hydrolysis of pectins, which would bring about a shift in the balance of the reactions shown in equation (**1**) favouring accumulation of pectins:

$$\text{pectates} + \text{“methyl”} \longrightarrow \text{pectins} \xrightarrow[\text{PME}]{H_2O} \text{pectates} + CH_3OH. \quad ..(\mathbf{1})$$

Such a hypothesis is in accordance with current concepts that methylation of pectates increases cell wall extensibility (Bennet-Clark 1955) and results in plastic extension of the wall under the influence of the turgor pressure of the cell contents.

Similar results have been obtained with 2, 4-dichlorophenoxyacetic acid and will be the subject of a subsequent paper.

V. Acknowledgments

The work described in this paper was undertaken as part of a joint research programme of the Colonial Sugar Refining Co. Ltd., the Plant Physiology Unit, Division of Food Preservation and Transport, C.S.I.R.O., and the Botany School, University of Sydney. The author wishes to thank the management of the Company for permission to publish this paper, Dr. R. N. Robertson for his interest and advice throughout the course of the work, and Professor Crocker, Botany School, University of Sydney, in whose laboratories the work was carried out.

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