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SHORT COMMUNICATION

AUXIN EFFECTS ON THE ACTIVITY OF PECTINTRANSELIMINASE *IN VITRO*

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Abstract—Previously reported auxin inhibition of the *in vitro* activity of pectintranseliminase was shown to be an artifact arising from errors in spectrophotometry. Addition of auxin to a reaction mixture already of high absorbance at the wavelength used for assay prevented the detection of increased absorbancy due to enzyme activity. However, by assaying activity at the absorption minimum for the auxin or by diluting aliquots of the reaction mixture it was shown that auxin had no effect on pectintranseliminase activity *in vitro*.

INTRODUCTION

ALBERSHEIM and Killias[1] reported that the enzyme pectintranseliminase (PTE) could be inhibited *in vitro* by auxin. Further investigations[2] indicated that auxin interacted with the end product of PTE action (Δ4:5 unsaturated uronide) to form a stable but inactive enzyme-product-auxin complex. This postulate for the direct action of auxin on the function of an enzyme implicated in cell extension is attractive as it allows for a rapidity of response not possible when auxin is considered to act through RNA synthesis.

The present results show that auxin inhibition of PTE is an artifact arising from errors in spectrophotometry.

RESULTS AND DISCUSSION

The apparent auxin inhibition of PTE activity reported by Albersheim and Killias[1] could be duplicated using both heated and unheated pectin substrates when assayed at 235 nm. However, as slit width was increased the measured enzyme activity also increased but apparent inhibition by auxin decreased.

Spectra of the components of the assay system revealed that they absorb heavily at wavelengths below 240 nm. Scanning the reaction mixture between 220–270 nm during the assay verified that PTE activity measured at 235 nm largely reflected an increase in absorbancy of a solution already of high absorbance (Fig. 1).

Subsequent assays were made by diluting aliquots from the reaction mixtures to a final concentration which was within the 0 to 1 absorbance range when referred to water. The effects of 5×10^{-4} M 2,4-dichlorophenoxyacetic acid (2,4-D), indole acetic acid (IAA) and tryptophan on PTE activity were assayed at 235, 245, and 251·5 nm (Table 1). With this

[1] P. ALBERSHEIM and U. KILLIAS, *Arch. Biochem. Biophys.* **97**, 107 (1962).
[2] P. ALBERSHEIM, *Plant Physiol.* **38**, 426 (1963).

assay procedure neither IAA nor tryptophan could be shown to inhibit PTE activity. Inhibition of PTE due to 2,4-D was detected only at 235 nm and was an artifact due to the high absorbancy of 2,4-D at this wavelength. When enzyme activity was followed at the absorption

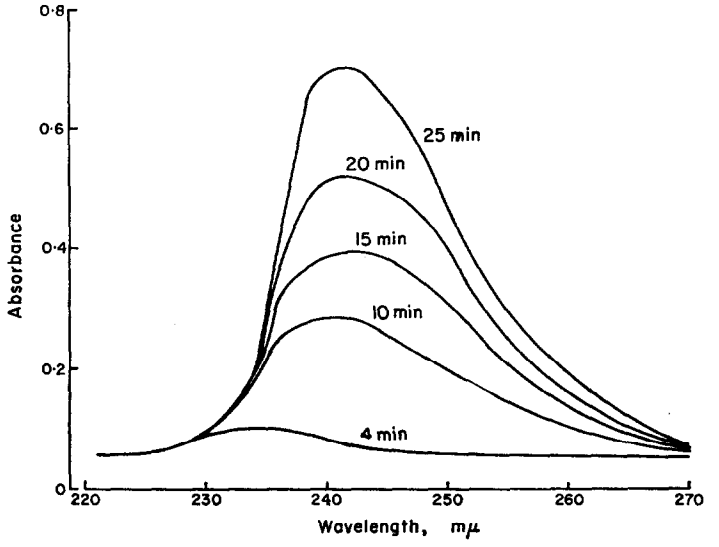

FIG. 1. TIME COURSE OF PTE ACTIVITY SHOWING THE EFFECT OF INCREASED END-PRODUCT CONCENTRATION ON THE ACTIVITY OF PECTINTRANSELIMINASE WHEN MEASURED AT 235 nm. THE OPTICA SPECTROPHOTOMETER WAS BALANCED OVER THE RANGE 220–270 nm AGAINST REACTION MIXTURES, CONTAINING 0·5 PER CENT W/V PECTIN, BOILED ENZYME AND BUFFER. A UNIT OF ENZYME ACTIVITY IS THE AMOUNT CAUSING AN INCREASE OF ONE ABSORBANCE UNIT PER MINUTE.

TABLE 1. EFFECT OF AUXIN-LIKE COMPOUNDS ON PECTINTRANSELIMINASE ACTIVITY MEASURED AT DIFFERENT WAVELENGTHS

Treatment	Enzyme activity (units × 10^{-2})		
	235 nm	245 nm	251·5 nm
Control	1·93	1·75	1·20
2,4-dichlorophenoxylacetic acid (5×10^{-4}M)	0·87	1·76	1·28
Indole-3-acetic acid (5×10^{-4}M)	1·90	1·70	1·20
Trytophan (5×10^{-4}M)	1·90	1·75	1·25

Enzyme activity was measured in reaction mixtures containing 0·5 per cent w/v pectin, 5×10^{-4}M auxin and 0·1 M phosphate-citrate buffer, pH 5·2 by diluting aliquots to a final concentration within the 0 to 1 absorbance range when referred to water. A unit of enzyme activity was the amount which caused an increase in absorbancy of one unit per minute.

minimum for 2,4-D (251·5 nm) there was no evidence of inhibition due to auxin up to pectin concentrations of 1·5 per cent w/v (Fig. 2).

These results indicate that previous reports of an auxin-product inhibition of the *in vitro* activity of PTE can be attributed to errors in spectrophotometry associated with assaying optically dense reaction mixtures.

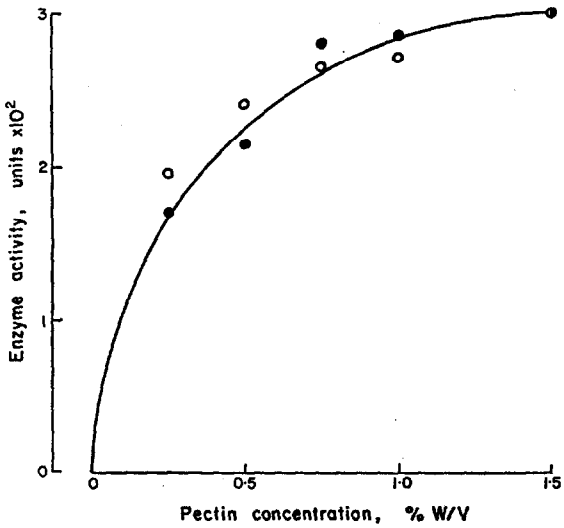

FIG. 2. PECTINTRANSELIMINASE ACTIVITY IN THE PRESENCE (○) AND ABSENCE (●) OF 5×10^{-4}M 2,4-DICHLOROPHENOXYACETIC ACID AT SEVERAL PECTIN CONCENTRATIONS. ENZYME ACTIVITY WAS MEASURED AT 251·5 nm BY DILUTING ALIQUOTS FROM THE REACTION MIXTURE WITH BUFFER TO A FINAL PECTIN CONCENTRATION OF 0·3 PER CENT W/V.

EXPERIMENTAL

PTE was purified from commercial pectinase (General Biochemicals Inc., Ohio) by the method of Albersheim and Killias.[1] Enzyme activity was measured as the rate of increase in absorbance of reaction mixtures containing 0·1 ml of enzyme, 2·0 ml of pectin (D.H.A. medicinal grade) in 0·1 M phosphate-citrate buffer, pH 5·2, and 0·1 ml of auxin or water. A unit of PTE activity was defined as that amount of enzyme causing an increase of one absorbance unit per minute.

Initial assays were made with a single beam Hitachi spectrophotometer (model EPU-2A) and later observations with an Optica split beam ratio recording spectrophotometer, (model CF4R). Quartz cuvettes with 1-cm path lengths were used in both instruments. A cuvette containing all reactants except the enzyme or with boiled enzyme added was used to obtain full scale deflection (100 per cent transmission) for reference during the assay on the Hitachi. Two such cuvettes were used to balance the Optica for difference spectrophotometry.

Below 230 nm the solutions were highly absorbant and transmitted insufficient energy to drive the recorder pen of the Optica.