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Lack of Effect of Plant Growth-Regulators on the Action of Alpha-Amylase Secreted by Virus Tumor Tissue

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LACK OF EFFECT OF PLANT GROWTH-REGULATORS ON THE ACTION OF ALPHA-AMYLASE SECRETED BY VIRUS TUMOR TISSUE[1]

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Introduction

This report deals with the effect of certain synthetic plant growth-regulators on the activity of alpha-amylase (**1**) secreted by virus tumor tissue grown *in vitro*. Previous studies have lacked adequately controlled conditions and have been carried out with enzyme preparations obtained through the usual methods of destruction of cells. In this particular study (*a*) the assay employed to measure enzyme activity includes control of pH and temperature and (*b*) this enzyme, being secreted, is not subject to the drastic preparative procedures involving destruction of cellular structure usually necessary to obtain enzymes.

Material and methods

The tumor tissue used was induced by the wound-tumor virus (*Aureogenus magnivena* Black) in the roots of the sorrel plant (*Rumex acetosa* L.) and was the same R_1 isolate used in other studies (**2**, **7**–**9**). Enzyme activity was assayed (**1**) by following spectrophotometrically the decrease in optical density of the starch-iodine complex. This method is sensitive to low levels of amylase, but, because of the many factors affecting the color of the complex, the reproducibility is only about $\pm 5\%$. The growth-regulators tested and the concentrations used are given in table 1.

Three different enzyme preparations consisting of unpurified spent liquid medium no. 24 (**2**) were used. The medium in each case had been inoculated with approximately one-tenth its weight of tumors 2–10 days earlier. The medium was used immediately after the tumors were removed and after storage overnight in the refrigerator. Enzyme activity was assayed in 1% soluble starch and 0.02 *M*, pH 4.6, acetate buffer at 40° C. Growth-regulator solutions were adjusted to pH 4.6 before being added to the reaction mixture.

Results

It is apparent that these compounds have no significant effect on the activity of the alpha-amylase (table 1). The greatest effect (10% decrease by beta-naphthoxyacetic acid at 100 p.p.m.) approximates the limit of experimental error for the comparison of two runs. The low levels of activity at all growth-regulator concentrations used in this particular experiment indicate that the control may have been high. For this tumor tissue these same growth-regulators at 100 p.p.m. cause a growth inhibition of between 90% and 100% and an inhibition

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of respiration amounting to 37% for indoleacetic acid, 10% for 2,4-dichlorophenoxyacetic acid, and 70% for beta-naphthoxyacetic acid (7).

Discussion

EYSTER (3) reported that indolepropionic acid retarded the action of malt diastase on soluble starch but accelerated the action of the enzyme in the presence of charcoal, presumably by releasing the adsorbed enzyme from the charcoal. The same author later (4) mentioned similar action by indolebutyric, indoleacetic, and alpha-naphthaleneacetic acids at concentrations of 25 and 50 p.p.m. and reached the conclusion that the effectiveness of these compounds was due entirely to a pH effect (4, 5). However, SMITH *et al.* (10) pointed out the lack of pH control in EYSTER's experiments. These workers were unable to duplicate EYSTER's results under controlled conditions. They could find no effect of indoleacetic acid on diastatic activity in the presence or absence of charcoal.

Still other reports indicate effective inhibition of salivary amylase by many plant growth-regulators: indoleacetic, indolebutyric, indolepropionic, alpha-naphthaleneacetic, 2,4-dichlorophenoxyacetic, beta-naphthoxyacetic, and 2,3,5-triiodobenzoic acids (11, 12). In these instances the growth-regulators were used at concentrations as low as 100 p.p.m., but no figures were given to indicate what constituted effective inhibition. NEELY *et al.* (6) have reported reduced levels of alpha- and beta-amylase activity in the stems and leaves of bean plants treated with 2,4-D.

TABLE 1

EFFECT OF PLANT GROWTH-REGULATORS ON SECRETED α-AMYLASE

GROWTH-REGULATOR	ACTIVITY OF CONTROL*	ACTIVITY (CONTROL=100%) IN PRESENCE OF GROWTH-REGULATOR† AT		
		1 p.p.m.	10 p.p.m.	100 p.p.m.
2,4-Dichlorophenoxyacetic acid.	0.338	96	99	93
	.085	100	97	91
Indoleacetic acid.....	.090	98	101	104
	.130	100	96	98
Beta-naphthoxyacetic acid.....	.325	93	98	97
	0.135	94	91	90

* Milligrams of starch hydrolyzed per hour per milliliter of enzyme solution.
† Based on activity of control as 100%.

Summary

1. Plant growth-regulators representing the indole, phenoxy, and naphthoxy types of structure were tested to determine their effect on the activity of an alpha-amylase secreted by virus tumor tissue from the roots of *Rumex acetosa* grown *in vitro*.

2. At concentrations up to and including 100 p.p.m. they had no significant effect on its activity. The greatest effect was within the limit of experimental error.

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