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ENDOPOLYGALACTURONASE FROM TOMATO FRUIT*

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Abstract—A polygalacturonase was extracted from ripening tomato fruit A four step procedure was developed producing a 44-fold increase in specific activity with 9% recovery The enzyme was found to rapidly degrade pectic acid but not pectin No transeliminase activity was detected Viscosity and per cent hydrolysis studies formed a basis for suggesting that this enzyme cleaves its substrate in a random manner and is likely to be an endopolygalacturonase

INTRODUCTION

DURING ripening, the intercellular pectin material of the tomato fruit is weakened by pectic enzymes. Pectinesterase de-esterifies the pectin material, converting it to pectic acid.[1] Pectic acid is then depolymerized by the action of hydrolytic polygalacturonases.[2,3] Two polygalacturonases appear to be involved in the process. One produces a more rapid reduction in viscosity compared with percentage hydrolysis of the substrate than the other; the former is characteristic of an endopolygalacturonase and the latter of an exopolygalacturonase.[4]

The present investigation concerns the purification of tomato endopolygalacturonase. Some time ago Patel and Phaff[5] obtained a 5·5 × increase in specific activity of tomato polygalacturonase.

RESULTS AND DISCUSSION

The current work obtained a higher degree of purification. A 44-fold increase in specific activity was achieved, with a 9% recovery. The procedure involved four major steps which are summarized in Table 1. Column chromatography, the last step in the purification procedure, yielded three protein peaks The first peak eluted with or near the void volume, a second peak came off shortly thereafter, and a third peak trailed. Polygalacturonase activity corresponded with the second peak.

Characterization studies suggest that this enzyme is a hydrolytic endopolygalacturonase. The substrate specificity indicates that this enzyme rapidly degrades pectic acid but

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[1] HOBSON, G E (1963) *Biochem J* **86,** 358
[2] DEMAIN, A L and PHAFF, H J (1957) *J Agr Food Chem* **5,** 60
[3] BESFORD, R T and HOBSON, G E (1972) *Phytochemistry* **11,** 2201
[4] PRESSEY, R and AVANTS, J K (1973) *Biochim Biophys Acta* **309,** 363
[5] PATEL, D S and PHAFF, H J (1960) *Food Res* **25,** 37

TABLE 1. EXTRACTION OF TOMATO ENDOPOLYGALACTURONASE

Fraction	Volume (ml)	Enzyme* (units/ml)	Protein† (mg/ml)	Specific activity	Recovery (%)	Purification
(1) NaCl buffer extract	3000	35	1.3	27	100	none
(2) 50–80% $(NH_4)_2SO_4$ ppt	100	540	5.9	91	51	3
(3) Concentrated dialysis ppt	8	1800	3.4	530	14	20
(4) Sephadex G-100 column	25	360	0.3	1200	9	44

* Activity followed by changes in substrate viscosity. Enzyme assays were performed with fractions diluted to produce between 15–30% decrease in viscosity in 1 hr. Over this activity range the % decrease in viscosity-time relationship is essentially linear. With this system the major measure of enzyme is the dilution required to bring its activity into this 15–30% range. A unit of enzyme activity equals a 25% decrease in viscosity in 1 hr.

† Determined by absorbance at 280 and 260 nm.

not pectin. Pectic acid degradation proceeded with no detectable increase in the absorbance at 235 nm. This suggests that the enzyme is hydrolytic[6]. Activity was such that an increase in A235 nm of 1.5 units would have been expected if the enzyme cleaved by a trans-eliminase mechanism. Studies comparing per cent decrease in viscosity suggest that this enzyme cleaves its substrate in a random, rather than sequential, manner. These results are presented in Table 2. Random cleaving enzymes produce a low rate of hydrolysis, about 2–3% at 50% decrease in viscosity[7–9]. With a sequential attack the rate of hydrolysis is higher, about 20–25% at 50%[10] decrease in viscosity.

EXPERIMENTAL

Medium sized, yellow orange vine ripened Homestead tomatoes were used as an enzyme source. (Step 1) Enzymes were extracted by adding one vol. cold NaCl buffer soln (0.05 M $Na_3C_6H_5O_7$, 1.7 M NaCl, pH 5.5) to tomato slices and homogenizing in a blender for 1 min. The resulting fluid was allowed to stand overnight.

TABLE 2. RELATION BETWEEN PER CENT DECREASE IN VISCOSITY AND PER CENT HYDROLYSIS

Reaction time (min)	Decrease in viscosity (%)	Hydrolysis* (%)
0	0	0
5	23	−0.1
10	40	0
15	49	0.1
30	72	0.3
60	82	0.5
120	91	1.0
300	94	2.2

* Followed by increase in reducing units.

[6] ALBERSHEIM, P., NEUKOM, H. and DEUEL, H. (1960). *Arch. Biochem. Biophys.* **43**, 1422.
[7] NAGEL, C. W. and VAUGHN, R. H. (1961). *Arch. Biochem. Biophys.* **93**, 344.
[8] NAGEL, C. W. and VAUGHN, R. H. (1961). *Arch. Biochem. Biophys.* **94**, 328.
[9] VORAGEN, A. G. J. (1972). Characterization of pectin lyases on pectins and methyl oligogalacturonates. Agric. Res. Rep. 780. Centre for Agric. Pub. and Doc. Wageningen, Netherlands.
[10] MACMILLAN, J. D., PHALL, H. L. and VAUGHN, R. H. (1964). *Biochemistry*, **3**, 572.

at 4°, then centrifuged (15000 *g* for 30 min) to remove insoluble material, and filtered (Whatman No 1) (Step 2) The fluid from Step 1 was cooled to 0° made 50% saturated by the slow addition of granular $(NH_4)_2SO_4$, and centrifuged (15000 *g* for 30 min) The supernatant was made 80% saturated with $(NH_4)_2SO_4$ and recentrifuged The resulting pellet was solubilized in a minimum vol of buffer (0 05 M Na citrate, pH 5 5) (Step 3) The fluid from Step 2 was dialyzed against 4 changes of H_2O and centrifuged (15000 *g* for 30 min) The pellet was suspended in 25 ml cold buffer and placed on a stirrer at 4° overnight. Insoluble material was removed by centri-PM-30 filter (Step 4) This concentrate was placed on a Sephadex G-100 column and eluted with buffer at 4°

Enzyme activity was measured by a viscosity method,[11] by following the increase in reducing units,[12] or A235 nm [6] Size 300 Cannon-Fenske viscometers were used to follow viscosity changes Substrates were 1% soln of pectic acid (Na Polypectate No 6024) and pectin (Pectin, L M No 3446) obtained from Sunkist Growers, Ontario, California Buffer was used to suspend the substrates Merthiolate was added to a final concentration of 0 002% to prevent bacterial growth

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[11] LILLICH, T T and ELKAN, G H (1968) *Can J Microbiol* **14,** 617
[12] NELSON, N (1944) *J Biol Chem* **153,** 375