Pectinesterase from carrot (*Daucus carrota* L.)

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Summary. 3 multiple forms of pectinesterase were found in extracts and purified products from carrot, with a mol. wt near 27,000, similar in properties to the tomato pectinesterase.

More than 100 years ago, Frémy[1,2] described the presence of 'pectase' - pectinesterase (pectinpectyl-hydrolase, E.C. 3.1.1.11) in carrot. Since that time the carrot exo-D-galacturonanases[3-5] have been studied but no papers have appeared on carrot pectinesterase. The carrot pectinesterase was studied by methods used for the purification and characterization of the tomato pectinesterase[6].

Material and methods. Ripe carrots were extracted after homogenization with 5% NaCl and passed through a fruit press. The first extract containing no pectinesterase activity, was used for the preparation of exo-D-galacturonanase[5]. The press residue was extracted with 2 M NaCl adjusted to pH 7.8 during 12 h at 4 °C by stirring and maintaining the pH-value at 7.8. The extract was saturated (after centrifugation at 6000× g) with ammonium sulfate to 90% and the precipitate dissolved in a small volume of 0.005 M NaCl and dialyzed against 0.005 M NaCl. The crude carrot pectinesterase was obtained after concentrating the dialyzate by ultrafiltration (AMICON apparatus) using UM 10 membrane, desalting on a Sephadex G 25 column and freeze-drying. This product was purified by ion-exchange chromatography on a DEAE-Sephadex A 50 column equilibrated with 0.05 M phosphate-NaCl buffer, pH 7.6, using continuous concentration (up to 0.2 M phosphate-NaCl) and pH (up to pH 5.6) gradient elution (figure 1). The main portion of the activity of pectinesterase was present in the first peak; this fraction was concentrated by ultrafiltration, desalted on Sephadex G 25 column and freeze-dried. This product was further purified by chromatography on a Sephadex G 75 column equilibrated with a 0.1 M Tris-NaCl buffer pH 7.8 (figure 2). Pectinesterase activity was found in the 2nd peak of this separation.

Enzyme activity was determined by continuous titration in an automatic titrator (TTT 11, Radiometer Copenhagen, Denmark), in a thermostatized vessel under nitrogen. The activity of pectinesterase is expressed in moles of ester groups hydrolyzed in 1 sec at pH 7.5 and 30 °C.

Gel electrophoresis was carried out in the horizontal arrangement, using hydrolyzed crosslinked starch[7] and Tris-HCl buffer, pH 7.5. After the run, the gel was sliced horizontally and one layer was used for detection of pectinesterase activity by the print technique with the paper impregnated with pectin and Bromothymol Blue[8]. The other layer of the gel was stained for proteins with Amido Black 10 B.

Mol. wt was determined by TLC on Sephadex G 150 superfine, equilibrated with 0.9% NaCl in a TLC-apparatus (Pharmacia, Uppsala, Sweden), using ribonuclease A, chymotrypsinogen, ovalbumin, human serum albumin and isolated tomato pectinesterase[6] (mol. wt 27,500) as standard

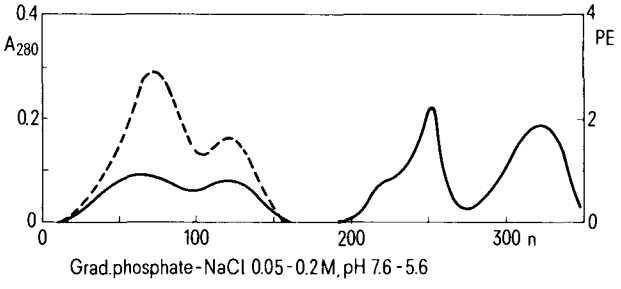

Fig. 1. Chromatography of the crude carrot pectinesterase on DEAE Sephadex A 50 column. ——A_{280}; - - - -PE (pectinesterase activity in μmoles sec^{-1} ml^{-1}); *n* number of fractions.

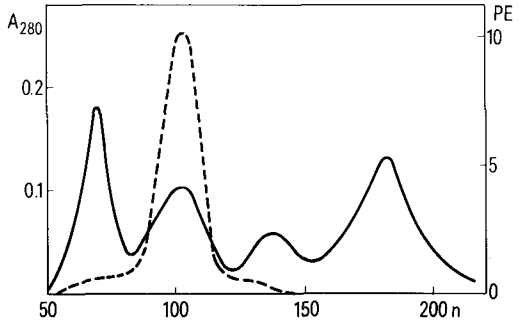

Fig. 2. Chromatography of purified carrot pectinesterase (first peak from DEAE Sephadex A 50 column) on a Sephadex G 75 column. ——A_{280}; - - -PE (pectinesterase activity in μmoles sec^{-1} ml^{-1}); *n* number of fractions. Elution with 0.1 M Tris-HCl buffer, pH 7.8.

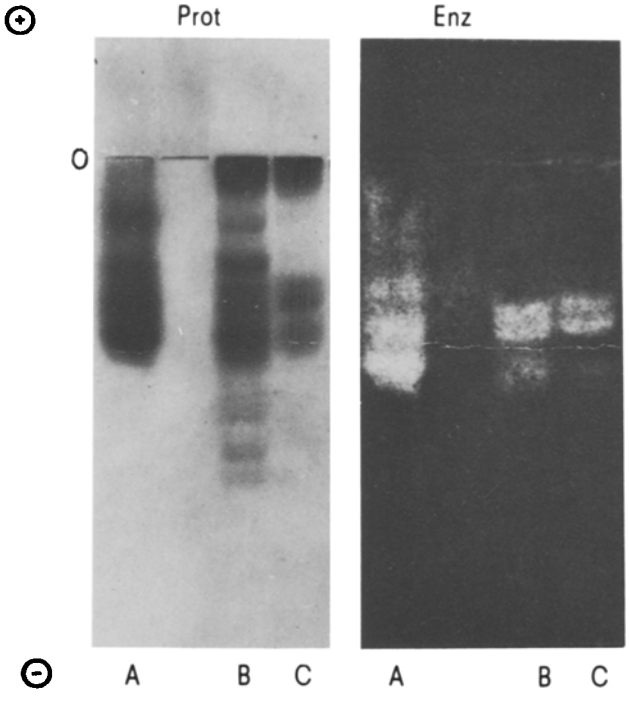

Fig. 3. Starch gel electrophoresis of the carrot pectinesterase. Prot, Detection of proteins with Amido Black 10 B; Enz, detection of pectinesterase by paper print with pectin and Bromothymol Blue; O, origin; A, purified tomato pectinesterase; B, carrot pectinesterase - first peak from the DEAE Sephadex A 50 column; C, carrot pectinesterase - 2nd peak from the Sephadex G 75 column.

samples. Pectinesterase activity was detected by the print technique[8] and proteins were stained with bromophenol blue on a paper replica.

Iodine resublimated (Merck, Federal Republic of Germany) dissolved in sodium iodide in concentrations of 10^{-2} to 10^{-5} M I_2 was used for inhibition. Products of carrot pectinesterase were preincubated with iodine at room temperature and inhibitory effect was stopped by addition of an excess of 0.1 M $Na_2S_2O_3$.

Results and discussion. The crude carrot pectinesterase exhibited a specific activity of 1.1 moles sec^{-1} kg^{-1} and the electrophoretic pattern of pectinesterase was similar to that obtained from the first peak of the DEAE-Sephadex A 50 column - 3 enzyme bands, all moving to the cathode (figure 3, sample B). This peak had a specific activity of 2.8 moles sec^{-1} kg^{-1} and on starch gel electrophoresis showed 8 protein and 3 pectinesterase bands (figure 3, sample B).

The purified product from the Sephadex G 75 column had a specific activity of 5.2 moles/sec^{-1} kg^{-1} and was separated by electrophoresis into 4 protein and 3 pectinesterase bands (figure 3, sample C).

All 3 pectinesterase forms from carrot moved to the cathode with the same rate as those of the tomato pectinesterase (figure 3, sample A). On TLC on Sephadex G 150 superfine, both purified products from carrot showed only one spot of pectinesterase activity and moved with a mobility identical to that of the tomato pectinesterase standard; their mol. wt was found to be near 27,000.

The pH-optimum of the purified carrot pectinesterase was estimated as being 7.8–8.0, the K_m-value 1.5×10^{-6} M methyl D-galactopyranuranosyl residues and this enzyme was activated by sodium chloride (optimum around 200 mM NaCl).

The heat stability of carrot pectinesterase (total inactivation at 70 °C) was similar to that of the tomato pectinesterase[9]. Iodine showed an inhibitory effect on carrot pectinesterase, which increased in relation to the purity of the product, similarly as found with the tomato pectinesterase[10].

From the results obtained, the carrot pectinesterase can be characterized as an enzyme very close to the tomato pectinesterase with regard to molecular and enzyme properties.

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Studies on plasma fibrinogen level in pre-eclampsia and eclampsia

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Summary. The plasma fibrinogen level of maternal blood has been estimated in 30 cases of pre-eclampsia, 60 cases of eclampsia and 35 cases of normal pregnancy of 3rd trimester. The plasma fibrinogen value increased by about 70% and 145% in pre-eclampsia and eclampsia, respectively. In essential hypertension, the fibrinogen level remains more or less the same as in normal pregnancy.

Post-mortem examination of patients who died due to eclampsia, revealed the presence of thrombi in small blood vessels[1,2]. Previous investigators[3,4] detected a material in the kidney glomeruli of normal pregnant women which was immunologically identical with fibrinogen/fibrin, and suggested that the deposition of the same may lead to the development of the endothelial cytoplasmic changes, hypertension and proteinuria which are the generalized features of eclampsia or pre-eclampsia[4]. Examination of 19 out of 21 eclamptic patients indicated the presence of blood in the cerebrospinal fluid and fibrin thrombi in the periportal areas of the liver, adrenal cortex and spleen[5]. All these findings indicated a possible change in the plasma fibrinogen level in eclampsia and pre-eclampsia. With this idea, we measured the plasma fibrinogen level of eclamptic patients and normal pregnant women, and the results are reported in this communication.

Materials and methods. Pre-eclamptic patients were selected from a group of pregnant women of blood pressure reading of 120/90 mm Hg (approximately) with generalized oedema. Eclampsia was characterized by blood pressure reading of 180/100 mm Hg and above, generalized oedema, proteinuria and convulsions in the 3rd trimester. Plasma fibrinogen was measured by clotting the same with thrombin or calcium as described by Raymond and Wilkinson[6]. The incubation mixture consisted of 0.1 ml plasma, 2 NIH units of thrombin (Sigma) or 0.5 ml calcium chloride (0.25%) and 0.5 ml sodium chloride solution (0.154 N) in a final volume of 1.1 ml. The solutions were mixed properly and incubated at 37 °C for 30 min. The clot formed was removed carefully and washed with normal saline; 0.5 ml of sodium hydroxide (3%) was added to the washed clot and kept at room temperature till the clot was completely dissolved. To the above solution, biuret reagent (0.5 ml) was added and allowed to stand for 30 min at room temperature followed by 5 min at 37 °C. The absorbance was measured at 560 nm

Table 1. Plasma fibrinogen in normal pregnancy, pre-eclampsia and eclampsia

Group	Number of cases	Plasma protein g/100 ml**	Fibrinogen* mg/100 ml	Increase (%)
Normal	35	6.25	605 ± 15.6	-
Pre-eclampsia	30	-	1030 ± 30.3	70
Eclampsia	60	8.23	1473 ± 107	143
Essential hypertension	10	-	670 ± 80	-

* Mean value ± SE. ** Average value of 5 patients.