Phytochemistry, Vol. 30, No. 6, pp. 1753–1755, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00
Pergamon Press plc

POLYGALACTURONASE IN TREE POLLENS

RUSSELL PRESSEY

United States Department of Agriculture, Agricultural Research Service, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30613, U.S.A.

(*Received in revised form* 24 *September* 1990)

Key Word Index—*Populus deltoides*; Salicaceae; eastern cottonwood: *Quercus alba*; Fagaceae; white oak; pollen; trees; polygalacturonase; pollination.

Abstract—The pollens from 38 species of trees were analysed for polygalacturonase activity. All of the pollens contained polygalacturonase but the range of activities was great. Eastern cottonwood pollen contained the most polygalacturonase, with a level about 12 times higher than that usually found in ripe tomato fruit, which has been the highest known plant source of this enzyme. Pollens from other members of *Populus* were generally high in polygalacturonase although some were relatively low. Pollens from the oak family contained high activity, with the largest amount in white oak pollen. Pollens from pecan, willows, birch and hickories contained moderate levels of polygalacturonase. The smallest activities were found in pollens from beech, sycamore and conifers. The polygalacturonases from eastern cottonwood and white oak pollens were partially purified and characterized. Both enzymes were found to be *exo*-polygalacturonases that require Ca^{2+} for activity, but they possessed different M_rs, indicating that they are not identical. Exopolygalacturonase may be involved in some function related to pollination, but an explanation for the wide range of activities in different pollens is not obvious.

INTRODUCTION

Polygalacturonase (PG) (EC 3.2.1.15) in higher plants is generally considered to be associated with the processes responsible for softening of ripening fruits [1]. There is ample evidence that this enzyme hydrolyses pectin in middle lamellae and cell walls during ripening and senescence of many fruits [2, 3]. Some ripe fruits contain only *endo*-PG and others contain only *exo*-PG, while a few contain both enzymes [1]. But it is now known that PG is not restricted to fruit tissues with the discovery of activity in foliage [4] and storage organs [5]. Whereas *endo*-PG is the common form in fruits, the enzyme in other tissues appears to be exclusively *exo*-PG [1]. Furthermore, the activities of PG in non-fruit tissues studied earlier were relatively low. Thus we were surprised to find that some grass pollens contained very high levels of PG [6]. Johnsongrass pollen contained more PG than ripe tomato fruit which is usually considered the richest plant source of this enzyme. This observation prompted a comparative study of PG activity in other pollens with emphasis on those from trees.

RESULTS AND DISCUSSION

The levels of PG found in the pollens are presented in Table 1. All of the pollens analysed contained the enzyme but the range of the activities was great. Eastern cottonwood pollen contained the highest level of PG. The amount of PG in this pollen was about 12 times more than that usually found in ripe tomato fruit and about eight times more than in Johnsongrass pollen [6]. Thus eastern cottonwood pollen is the richest known source of PG among tissues from higher plants on a fresh weight basis. Other members of *Populus* were generally high in PG (Table 1) but some such as black cottonwood and lombardy poplar were quite low. Pollens from the oak family also contained high levels of PG, with the highest activity in pollen from white oak (Table 1). The amount of PG in white oak pollen was only slightly less than that in eastern cottonwood. Pollens from the oaks were more consistently high in PG, in contrast to the variation in *Populus*.

The pollen from several hickories, pecan, English walnut, willows, elm and birch contained moderate levels of PG (Table 1). Smaller amounts of PG were found in pollens from maple, ash, black walnut, hazelnut and mulberry. The pollens from American beech, sycamore, ironwood, pines, cedar and alder contained the lowest levels of PG.

The two richest sources of PG (eastern cottonwood and white oak pollens) were selected for partial purification and characterization of the enzymes. The elution profiles of the extracts of these pollens on Sephadex G-100 and Mono S columns were very similar (data not shown). PG from eastern cottonwood pollen was purified to a specific activity of 870 units mg^{-1} protein with a recovery of 74%. The enzyme from white oak pollen was purified to a specific activity of 790 units mg^{-1} protein with a recovery of 81%.

The M_rs of the enzymes were determined by gel filtration on Sephadex G-100 to be 58 000 and 69 000 for eastern cottonwood and white oak, respectively. The enzymes hydrolysed polygalacturonic acid in reaction mixtures at pH 3 to 7.5 but optimally at pH 5.4. Both enzymes required Ca^{2+} for activity with optimal concentrations of 0.5 mM. The reaction rates were highest for a substrate with a DP of 13. The rates were slower for both larger and smaller substrates, with very slow hydrolysis of digalacturonic acid.

Table 1. Concentrations of polygalacturonase in tree pollens

Common name	Species	Polygalacturonase (units g^{-1})
Cottonwood, eastern	*Populus deltoides*	1660
Oak, white	*Quercus alba*	1470
Cottonwood, western	*Populus sargenta*	1150
Oak, water	*Quercus velutina*	1150
Oak, black	*Quercus nigra*	829
Oak, red	*Quercus rubra*	766
Aspen	*Populus tremula*	702
Hickory, shellbark	*Carya laciniosa*	517
Walnut, English	*Juglans regia*	498
Pecan	*Carya illinoensis*	459
Elm, American	*Ulmus americana*	421
Cottonwood, Fremont	*Populus fremontii*	408
Hickory, shakbark	*Carya ovata*	363
Hickory, white	*Carya alba*	313
Willow, black	*Salix nigra*	306
Birch, river	*Betula nigra*	255
Box elder	*Acer negundo*	236
Willow, pussy	*Salix discolor*	220
Ash, white	*Fraxinus americana*	166
Poplar, lombardy	*Populus nigra*	147
Oak, English	*Quercus robur*	134
Hazelnut	*Corylus americana*	121
Gum, sweet	*Liquidambar styraciflua*	115
Mulberry, white	*Morus alba*	89
Maple, sugar	*Acer saccharum*	77
Cottonwood, black	*Populus trichocarpa*	70
Privet	*Ligustrum vulgare*	70
Maple, red	*Acer rubrum*	57
Fir, Douglas	*Pseudotsuga taxifolia*	55
Walnut, black	*Juglans nigra*	25
Olive	*Olea europea*	19
Cedar, red	*Juniperus virginiana*	13
Alder, red	*Alnus rubra*	13
Pine, white	*Pinus strobus*	9
Pine, slash	*Pinus elliottii*	7
Sycamore	*Platanus occidentalis*	7
Ironwood	*Ostrya virginiana*	6
Beech, American	*Fagus americana*	4

The release of reducing groups from pectate, a relatively large substrate, by the pollen enzymes corresponded with small changes in the viscosity of the reaction mixtures. Solutions of pectate and the enzymes were incubated for 1 hr and the residual substrate precipitated by addition of one volume of ethanol and removed by centrifugation. The alcohol-soluble products were analysed by chromatography on a CarboPac PA1 column in a Dionex system. The only product detected was galacturonic acid, and oligogalacturonides of DP between 2 and at least 10 were not found. The small changes in the substrate chain length according to the viscometric assay and the sole release of galacturonic acid suggested that both pollen enzymes are *exo*-PGs.

The *exo*-cleavage of polyuronides by the pollen PGs was confirmed by monitoring the products released from pentagalacturonic acid. Reaction mixtures of this substrate and the enzymes were analysed after various periods using the Dionex system. For both enzymes, the initial products were galacturonic acid and tetragalacturonic acid. As the pentamer was exhausted, the concentration of the tetramer began to decrease with the appearance of trigalacturonic acid and further increase of the monomer. The dimer appeared late in the reaction and accumulated as the trimer was hydrolysed. Application of the procedure described earlier [4] using polygalacturonic acid reduced with sodium borohydride as a substrate demonstrated that the pollen PGs cleave the substrate molecules at the nonreducing ends.

The occurrence of *exo*-PG in eastern cottonwood and white oak pollens is consistent with the earlier observation of this enzyme in corn pollen [6]. Brown and Crouch [7] recently characterized a gene family abundantly expressed in *Oenothera organensis* pollen that showed sequence similarity to tomato polygalacturonase. They neglected to test the pollen or the proteins encoded by the cDNAs in this family for PG activity. Tomato polygalacturonase is a very basic protein that cleaves galacturonans randomly and does not require Ca^{2+} for activity [8]. The implication that pollen may contain or

develop an *endo*-PG similar to the tomato enzyme prompted an examination of crude extracts of the pollens for such an enzyme. The crude extracts were assayed by the viscometric and reductometric methods at various reaction conditions. Very small changes in viscosity relative to the formation of reducing groups indicated the absence of *endo*-PG in eastern cottonwood, white oak and several other pollens.

EXPERIMENTAL

Pollens were obtained from Greer Laboratories Inc., Lenoir, North Carolina and from Sigma. Extracts for assaying PG were prepared by suspending 1 g of pollen in 10 ml of 0.2 M NaOAc, pH 5, containing 20 mM $CaCl_2$ and grinding with a mortar and pestle. The homogenate was stirred for 30 min and centrifuged at 20 000 *g* for 20 min. The pellet was re-extracted with 5 ml of the acetate buffer and the supernatant solns combined. The crude extract was dialysed against 0.15 M NaCl containing 5 mM $CaCl_2$ for 16 hr. All of these steps were conducted at *ca* 4°.

Polygalacturonase was assayed by adding 0.1 ml of dialysed pollen extract to a reaction mixt. consisting of 0.4 ml of 0.05 M NaOAc, pH 5.25 and 0.5 ml of 1% polygalacturonic acid, pH 5.25 [6]. Blanks were prepared with extracts heated for 5 min at 100°. After incubation at 37° for 30 min, the samples were analysed for reducing groups [6]. A unit of polygalacturonase is defined as the amount that catalysed the release of 1 μmol of reducing groups in 30 min.

Pollen PG was partially purified after extracting 5 g of pollen with 50 ml of 0.2 M NaOAc, pH 5, containing 20 mM $CaCl_2$. The crude extract was concd to 10 ml by ultrafiltration using a PM-10 membrane (Amicon Corp.) and applied to a 2.5 × 90 cm column of Sephadex G-100 in 0.15 M NaCl. Elution was conducted with 0.15 M NaCl at a flow rate of 30 ml hr^{-1}. The frs containing PG were pooled, concd to 2 ml and dialysed against 0.02 M NaOAc, pH 4.75. The soln was then chromatographed on a Mono S HR5/5 column (Pharmacia) using a FPLC system. Buffer A consisted of 0.02 M NaOAc, pH 4.75, and buffer B consisted of 0.02 M NaOAc, pH 4.75, containing 1.0 M NaCl. The system was programmed for linear gradient segments of 0–2 min (0% B) and 2–30 min (0–100% B) at a flow rate of 1 ml min^{-1}. The frs containing PG were pooled, ultrafiltered to 1 ml and dialysed against 0.15 M NaCl.

Oligogalacturonides were analysed by separation on a CarboPac PAI column in a Dionex HPLC system equipped with a pulsed amperometric detector. Elution was conducted with a linear gradient of 0.2 to 0.96 M NaOAc in 65 mM NaOH. Standard oligogalacturonides were obtained from Sigma or prepared as described previously [4, 8].

REFERENCES

1. Pressey, R. (1986) in *Chemistry and Function of Pectins*, (Fishman, M. L. and Jen, J. J. eds), ACS Symposium Series 310, 157. American Chemical Society, Washington, DC.
2. Hobson, G. E. (1965) *J. Hort. Sci.* **40**, 66.
3. Crookes, P. R. and Grierson, D. (1983) *Plant Physiol.* **72**, 1088.
4. Pressey, R. and Avants, J. K. (1977) *Plant Physiol.* **60**, 548.
5. Puri, A., Solomos, T. and Kramer, A. (1981) *Lebensm. Wiss. Technol.* **14**, 118.
6. Pressey, R. and Reger, B. J. (1989) *Plant Science* **59**, 57.
7. Brown, S. M. and Crouch, M. L. (1990) *The Plant Cell* **2**, 263.
8. Pressey, R. and Avants, J. K. (1973) *Biochim. Biophys. Acta* **309**, 363.