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SHORT COMMUNICATION

β-GLUCOSIDASE FROM 'MARIANNA' PLUM*

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Key Word Index—*Prunus cerasifera* × *P. munsoniana*; Rosaceae; 'Marianna' plum; β-glucosidase; β-galactosidase; specificity.

Abstract—A β-glucosidase preparation from 'Marianna' plum was characterized. Only monoglycosides with a β-linkage to aromatic aglycones were hydrolyzed. A single pH optimum at 5·3 was found. The highest specific activity occurred with the substrate prunasin (D(−)-mandelonitrile-β-glucopyranoside). β-Galactosidase activity was also detected.

INTRODUCTION

THERE is no clear understanding of the basic cause(s) of graft incompatibility in plants. Recent studies with pear quince grafts have shown a positive correlation between the degree of rejection and the ability to hydrolyze prunasin D(−)-mandelonitrile-β-glucopyranoside), the cyanogenic glucoside in quince.[1] In the interspecies graft of peach (*Prunus persica*) on 'Marianna' plum (*P. cerasifera* × *P. munsoniana*?),[2] the scion either dies or fails to develop normally.[3] The reverse graft, 'Marianna' plum on peach, is successful, suggesting the translocation of a toxic compound from the peach shoots into the 'Marianna' plum. Peach shoots are known to have a high concentration of the cyanogenic glucoside prunasin.[4] In the present study 'Marianna 2624' plum shoot tissue was investigated for β-glucosidase and its hydrolytic activity toward prunasin was determined.

RESULTS

Properties of the Enzyme Preparation

Stem tissue yielded a crude protein fraction with high β-glucosidase activity which showed little loss in activity after Sephadex column chromatography when held in the cold 3 days. Only slight loss in activity after freezing and thawing was found. Activity was optimal at pH 5·3 when assayed with *p*-nitrophenyl-β-D-glucopyranoside. Half maximal activity was found at pH 4 and 6·5. Hydrolytic activity against prunasin also showed a peak at 5·3. Preliminary results showed the presence of β-galactosidase activity in the protein fraction. The β-galactosidase activity showed a pH peak (5·3) identical to β-glucosidase activity, with half maximal activity at pH 4·4 and 6·5.

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[1] A. GUR, *Spec. Bul. Agr. Res. Sta. Rehovot* **10**, 1 (1957).
[2] V. P. HENDRICK, *The Plums of New York*, N.Y. (Geneva) Agr. Expt. Sta. Ann. Rpt. 18, Vol. 111 (2), p. 273 (1910).
[3] J. A. MCCLINTOCK, *J. Agric. Res.* **77**, 253 (1948).
[4] A. R. TRIM, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), Vol. II, p. 295 Springer, Berlin (1955).

Differences between β-glucosidase and β-galactosidase activity were not established with heat inactivation or inhibition by glucose, galactose or glucono-1,5-lactone. A similar loss in activity for both enzymes on heating at 60° was found when assayed at 25°. Activity was reduced to half maximal on heating at 60° for 80 min. Glucose and galactose at 10 mM did not show any inhibition of β-glucosidase or galactosidase activity. Glucono-1,5-lactone at 0·1 mM, however, reduced the hydrolytic activity 55% with prunasin and 63% with *p*-nitrophenyl-β-D-glucopyranoside. β-Galactosidase activity was inhibited 71% with glucono-1,5-lactone.

Activity Against Various Glycoside Substrates

There appears to be a requirement for an aromatic ring in the aglycone, since no substrate hydrolysis was detected against compounds lacking this group (e.g. α- or β-methyl-glucoside). The highest specific activity was found with prunasin (32 μmol/hr/mg protein) and the lack of activity toward amygdalin (D-(−)-mandelonitrile-β-gentiobioside) suggest specificity for a monoglycoside substrate. The enzyme preparation showed a requirement for the β-linkage; no activity was found with *p*-nitrophenyl-α-glucopyranoside. The enzyme was active against both glucose and galactose residues (*p*-nitrophenyl-β-D-glucopyranoside and galactopyranoside; 9·5 and 0·9 μ mol/hr/mg protein respectively). No activity could be detected with arbutin (hydroquinone-β-D-glucoside) as substrate. With salicin (saligenin-β-D-glucopyanoside) activity was 2·1 μmol/hr/mg protein.

DISCUSSION

'Marianna' plum seemed an ideal understock for peach because of its ease of propagation from hardwood cuttings and its resistance to the peach rosette virus, but studies showed that an incompatible union was produced.[3] The reverse combination of 'Marianna' plum grafted on peach, however, produces a successful union, and this success suggested a downward movement of a toxic compound from the peach shoots. Microscopic studies showed that the incompatibility resulted from a failure of the plum rootstock and peach scion phloems to unite.[3] The lack of phloem continuity prevents metabolites from moving down to the rootstock and subsequently results in death of the rootstock. Work with the pear-quince incompatibility problem has shown a negative correlation between a good graft union and the level of prunasin in the quince understock.[5]

Peach shoots are a known rich source of prunasin but in 'Marianna' plum shoots cyanide compounds could not be detected. 'Marianna' plum shoots contained β-glucosidase activity, and tests with various substrates showed that prunasin was the most active substrate (3·4 times more active than *p*-nitrophenyl-β-D-glucopyranoside and 15·3 times more active than salicin, another commonly used substrate for testing β-glucosidase activity). After hydrolysis by a β-glucosidase, prunasin produces mandelonitrile, which is dissociated to HCN and benzaldehyde by an oxynitrilase.[6] The released HCN then may cause the incompatibility as found in pear.[1]

In almond emulsin, β-glucosidase and β-galactosidase activity seems associated with one enzyme.[7,8] Heat inactivation, pH and inhibitor studies suggest that the β-glucosidase present in 'Marianna 2624' plum also has β-galactosidase activity.

[5] R. M. SAMISH, in *Advances in Horticultural Science and their Applications* (edited by J. GERNAUD), Vol. II, p. 12, Pergamon Press, Oxford (1962).
[6] C. BOVE and E. E. CONN, *J. Biol. Chem.* **236**, 207 (1961).
[7] R. HEYWORTH and P. G. WALKER, *Biochem. J.* **83**, 331 (1962).
[8] J. CONCHIE, A. L. GELMAN and G. A. LEVVY, *Biochem. J.* **103**, 609 (1967).

EXPERIMENTAL

Enzyme extraction. Terminal defoliated stems of 'Marianna 2624' plum, 6–8 cm long, were chopped and thoroughly macerated with a mortar and pestle in 2 × their wt of cold 0·1 M citric acid–0·2 M Na_2HPO_4 buffer, pH 5·3. The macerated tissue was left for 15 min with intermittent agitation before filtration through 4 layers of cheesecloth and then centrifuged at 12 000 *g* for 30 min. The clarified supernatant was desalted on a Sephadex G25 column, and the enzyme eluted with citric acid–Na_2HPO_4 buffer, pH 5·3. This Sephadex fraction was used without further purification.

Enzyme assays. For the Sephadex G25 col mn fractionation, the fractions were assayed with *p*-nitrophenyl-β-D-glucose. The pH, heat inactivation and inhibitor studies were assayed with *p*-nitrophenyl-β-D-glucose, *p*-nitrophenyl-β-D-galactose and prunasin. Assays were accomplished by measurement of the *p*-nitrophenol liberated or the analysis of released glucose. In the *p*-nitrophenol assay, 0·1 ml of enzyme, 0·1 ml of substrate, and 0·8 ml of 0·1 M citric acid–0·2 M Na_2HPO_4 buffer at 25° were used. The reaction was terminated by the addition of 2 ml of 1 M Na_2CO_3, and the reaction was read at 400 nm. Released glucose was measured by the Somogyi-Nelson method.[9] Protein was determined by the method of Lowry *et al.* using bovine serum albumin standard.[10] For the pH studies, pH values of 3·0, 3·5, 4·0, 4·6, 4·8, 5·0, 5·2, 5·3, 5·4, 5·6, 5·8, 6·0, 6·5 and 7·0 (citrate and phosphate buffers) were used. Prunasin and *p*-nitrophenyl-β-D-glucose were incubated for 30 min, and *p*-nitrophenyl-β-D-galactose was incubated for 1 hr. For the heat inactivation study, enzyme preparations were kept at 60° (pH 5·3) for varying lengths of time, then cooled with ice. For the inhibitor studies glucono-1,5-lactone was prepared fresh immediately before use.

Substrate preparation. Prunasin was isolated from young peach shoots according to Trim.[4] All other substrates were purchased from commercial sources.

[9] J. E. HODGE and B. T. HOFREITER, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER and M. L. WOLFROM), Vol. I, p. 380, Academic Press, New York (1962).

[10] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193,** 265 (1951).