PARTIAL PURIFICATION AND CHARACTERIZATION OF β-D-GALACTOSIDASE FROM PLANTAIN FRUIT (*Musa paradisiaca* L.)

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ABSTRACT
β-D-Galactosidase (EC 3.2.1.23) from ripe plantain fruit was partially purified and characterized. Purification was carried out using ammonium sulphate precipitation and gel-filtration on Sephadex G25-150 and G-25M. The enzyme displayed activity against *p*-nitrophenyl-β-D-galactopyranoside, with a Km of 1 mM. It was inhibited by galactose and mercuric chloride. Galactose was a noncompetitive inhibitor, while mercuric chloride was an
uncompetitive inhibitor.

Keywords: *Musa paradisiaca*, plantain, galactosidase/β-D-Galactosidase in plantain

INTRODUCTION

During ripening, partial disassembly of the fruit cell wall is largely responsible for softening and textural changes (Crookes and Grierson, 1983).

These apparent changes in cell wall during ripening, implicate the action of a variety of cell wall modifying enzymes, capable of degrading specific cell wall components (Huber, 1983; Rose and Bennett, 1999; Cosgrove, 2001). The large changes in cell wall's pectin structure, due to a dramatic increase in their degradation; that accompany the ripening of many fruit, have been attributed to the action of polygalacturonase (Cooper *et al.,* 1998; Chun and Huber, 2000; Brummell and Harpster, 2001). However, evidence indicates that polygalacturonase (PG) is not the major determinant of fruit softening, although transgenic fruit with low PG are slightly firmer, more resistant to splitting, mechanical damage and pathogen infection (Grierson and Schuch, 1993; Langley *et al.,* 1994; Wang *et al.,* 2005).

Also, during ripening, pectin methylesterase (PME) is responsible for de-esterification of the highly methyl-esterified polygalacturonans (pectin) in the cell wall; this makes pectin susceptible to degradation by PG (Koch and Nevins, 1989; Carpita and Gibeaut, 1993). Although, PME-suppressed transgenic fruits did not exhibit altered fruit softening during ripening, but pectin fragments extracted from their cell walls showed an increase in fragment size, due to reduced pectin depolymerization and methyl de -esterification (Tieman et al., 1992; Hall et al., 1993). However, suppression of PME in over -ripe fruit resulted in reduced loss of tissue integrity, therefore PME plays little role in ripening but does affect fruit senescence (Tieman and Handa, 1994).

Softening accompanying ripening proved to be significantly reduced in transgenic tomato fruit with suppressed β-D-galactosidase, an enzyme that serves to remove pectic galactan side chains and modify pectin (Carey *et al.,* 1995; Carrington and Pressey, 1996; Smith and Gross, 2000; Smith *et al.,* 2002). Moreover, the loss of neutral sugars, especially galactose; due to the activity of β-D-galactosidase is quantitatively the largest ripening-associated change in cell wall composition in fruit (Gross, 1984).

Information about b-D-galactosidases could be especially important because, there

are now several applications for these enzymes in the food industries. The various industrial uses for b-D-galactosidases include removal of lactose from milk and whey (Loveland *et al.,* 1994) and removal of plant saccharides from fruit beverages (Whitaker 1990; Brenchley, 1996).

β-D-Galactosidases have been purified and partially characterized from fruits such as tomato (Pressey, 1983; Carey *et al.,* 1995), apple (Dick *et al.,* 1990; Ross *et al.,* 1994), muskmelon (Ranwala et al., 1992), coffee berries (Golden *et al.,* 1993), avocado (de Veau et al., 1993), kiwifruit (Ross *et al.,* 1993), Persimmon (Kang et al., 1994), mango (Ali *et al.,* 1995), Japanese pear (Kitagawa *et al.,* 1995) and papaya (Ali *et al.,* 1998). In the present paper, we report the partial purification and characterization of β-D-galactosidase from ripe plantain fruit.

MATERIALS AND METHODS

Plant material

Plantain (Musa paradisiaca L. cv. French) was purchased from a local market in Papine, St. Andrew, Jamaica.

Chemicals

All reagents were of analytical grade and were obtained from Sigma Chemical Company, St. Louis, Missouri, USA.

Enzyme extraction

The enzyme was extracted from mature plantain (fully ripe stage), by the method of Golden *et al.* (1993) with slight modifications. One hundred grammes (100g) of plantain pulp were homogenized in 100 ml of 0.05 M sodium citrate buffer pH 4.5 to produce slurry. The slurry was filtered through two layers of cheesecloth and centrifuged in a Beckman centrifuge (Model J2-21) at 10 000 x g for 10 min. After centrifugation, the pellet was discarded and the supernatant brought to 40% ammonium sulphate saturation (w/v). The supernatant was centrifuged at 20 000 x g for 20 min. The pellet collected from this step was resuspended in a minimum volume of extraction buffer (20 ml).

Chromatographic separations

A column (30 x 3.6 cm) containing Sephadex G25-150 was equilibrated with extraction buffer and the sample (20 ml) was applied to the column. The column was eluted with the same buffer and 24 fractions (5 ml each) were collected. Fractions were assayed and those fractions with enzyme activity were pooled. The pooled fractions (65 ml) were concentrated by dialyzing against extraction buffer containing 15% (w/v) polyethylene glycol for 48 h. The new volume (8 ml) was assayed for activity and then applied to Sephadex G-25M column (30 x 3.6 cm) equilibrated with extraction buffer. The column was eluted with the same buffer until 16 fractions (2 ml each) were collected. Fractions containing enzyme activity were pooled (8 ml) and used in subsequent studies as the enzyme extract.

Steps involving chromatographic separations were performed at room temperature (28 ± 2^{o} C) with no effect on enzyme activity; however, dialysis and centrifugation were performed at 4^{o} C.

Enzyme assay

Enzyme activity was also assayed for by the method of Golden et al. (1993) with slight modifications. Assay mixture containing 1 ml of extraction buffer (pH 4.5), 0.4 ml of 4 mM *p*-nitrophenyl-β-D-galactopyranoside (PNPG) and 50µl of the enzyme extract was incubated at 37^{o} C for 10 min. After 10 min. of incubation, 1 ml of 0.3 M Na_2CO_3 was added and the resulting absorbance was determined at 405 nm with Cecil CE 9000 series spectrophotometer (Model No. CE 9050).

Inhibition studies

PNPG at varied concentrations (0.146mM to 0.730 mM) were used as substrate, with inhibition concentration fixed at 1 mM for $HgCl_2$ and 2 mM for galactose. The type of inhibition was determined from Lineweaver-Burk plots (1/v vs 1/S).

Protein determination

This was determined by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Enzyme purification

Purification of β-D-galactosidase from ripe plantain on Sephadex G25- 150 is shown in Fig 1. Chromatography of the enzyme extract on Sephadex G 25-150 after ammonium sulphate precipitation yielded a peak of β-D-galactosidase activity. Fractions 3 through 7 were pooled and concentrated by dialysis. Fig. 2 shows the chromatography of the concentrated enzyme on Sephadex G-25M. Fractions 2 and 3 were pooled and used as enzyme extract, for characterization studies. Ion-exchange chromatography on cellulose phosphate resins and CM-Sephadex resulted in lost of activity of β-D-galactosidase. Results of the purification of β-D-galactosidase are summarized in table 1. Ammonium sulphate precipitation afforded a reduction in sample volume with a purification fold of 2.27. While gel-filtration chromatography on Sephadex G 25-150 and G-25M gave a purification fold of approximately 3; with a specific activity of 0.029 μmole/min/mg protein and 3.3% recovery.

Characterization of β-D-galactosidase

Characterization of β-D-galactosidase

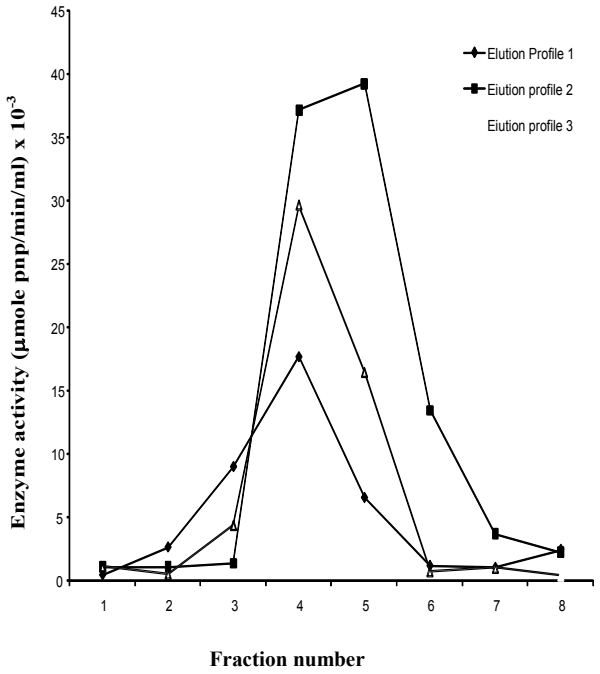

Fig. 1: Elution profile of b-D-galactosidase on Sephadex G25-150

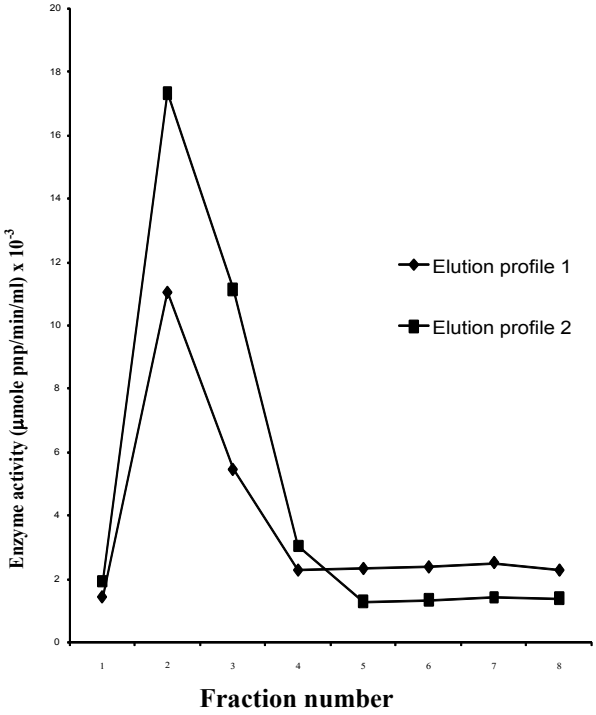

Fig. 2: Elution profile of b-D-galactosidase on Sephadex G-25M

Table 1. Purification of β-D-galactosidase from ripe plantain.

Fraction	Total protein (mg)	Total activity (μmole/min)	Specific activity (μmole/ min/mg protein)	Purification Fold	Recovery (%)
ude extract	152.8	1.680	0.011	1.0	100
$H_4)_2SO_4$ pellet	38.6	0.956	0.025	2.27	56.9
phadex G 25-150	25.4	0.650	0.026	2.36	38.7
phadex G-25M	1.92	0.0549	0.029	2.64	3.3

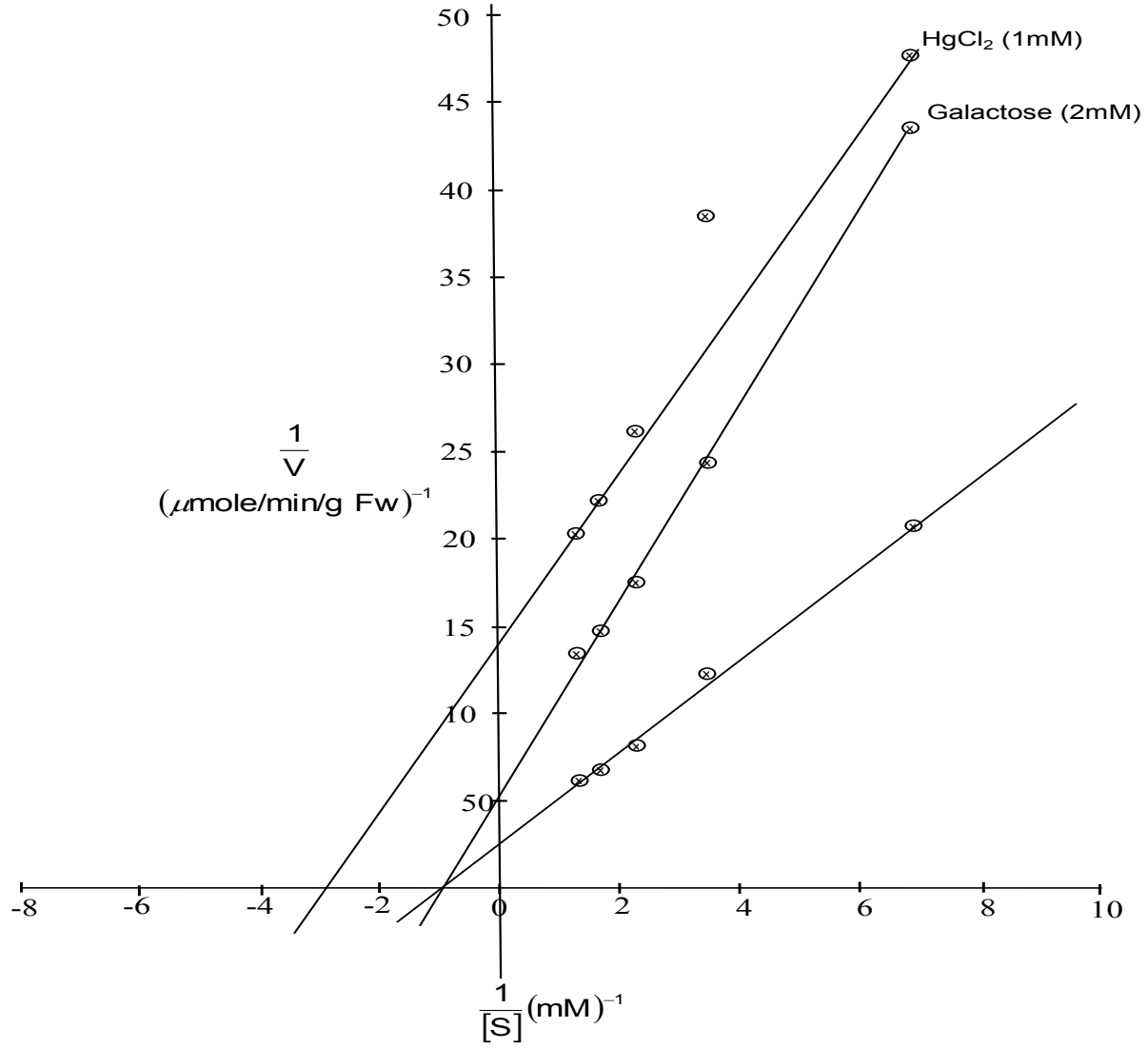

Fig. 3: Lineweaver-Burk plot of β-D-galactosidase activity using PNPG as substrate; for the determination of Km and Vmax

from plantain fruit gave a Km of 1 mM (Figure 3) which is quite similar to 2 mM in carambola fruit (Balasubramaniam et al., 2005) and 0.33 mM in coffee berries (Golden *et al.,* 1993). The Vmax value is 0.04 µmole/ min/g Fresh weight.

Enzyme inhibition

Inhibition studies showed that the enzyme was inhibited by galactose (2 mM) and mercuric chloride (1 mM) as shown in Fig. 3. Galactose was a noncompetitive inhibitor (Km, 1 mM; Vmax, 0.02 µmole/min/g Fresh weight). While mercuric chloride was an uncompetitive inhibitor (Km app., 0.35 mM; Vmax, 7.14 x 10^{-3} µmole/min/g Fresh weight). All these properties are in general, similar to those reported for β-D-galactosidase from other plant sources (Pressey, 1983; Golden *et al.,* 1993; Biles *et al.,* 1997; Li *et al.,* 2001).

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