ARTICLES

Cassava Latex as a Source of Linamarase for Determination of Linamarin

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A major constraint in the enzymatic assays for determination of linamarin in cassava is the preparation of purified linamarase. Cassava latex, which exhibits high linamarase activity, was tried as an alternate source of the enzyme. Enzyme yield from latex was compared with that from rind and leaf. Preparations from latex had significantly higher linamarase activity (~300-fold) compared to leaf and rind. The purification of the enzyme was easier since homogenization of large quantities of tissues could be avoided. A 1 g amount of latex could yield enough enzyme for >3000 assays.

Keywords: *Cassava; latex; linamarase; linamarin determination*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop whose tubers and leaves are widely used for food and feed purpose. Cassava contains two cyanoglucosides linamarin and lotaustralin which are distributed in all the plant tissues in the ratio 93:7. These compounds are hydrolyzed by an endogenous β-glucosidase linamarase which is also present in the plant tissues. Linamarin is hydrolyzed to acetone cyanohydrin and glucose and lotaustralin to 2-butanone cyanohydrin and glucose. The unstable cyanohydrin, which itself is toxic, dissociates forming acetone/2-butanone and hydrogen cyanide (HCN). Its dissociation is also promoted by an endogenous enzyme, α-hydroxynitrile lyase. Under normal physiological conditions, there is no formation of HCN, since the substrates and enzyme are separately compartmentalized, but when the plant tissues are disrupted, the cyanoglucosides are hydrolyzed, with liberation of HCN. The content of linamarin (which is the major glucoside) varies widely in tubers of different cassava varieties. Since cassava is a staple food crop, it is very important to determine the content of cyanoglucosides in the edible tuber.

Several sensitive spectrophotometric methods are available for estimation of cyanoglucosides in fresh and processed cassava. All these methods involve extraction of cyanoglucosides from tissues using an appropriate solvent, hydrolysis of cyanoglucosides to acetone cyanohydrin by addition of purified linamarase, alkali degradation of the cyanohydrin to cyanide, and estimation of cyanide spectrophotometrically. A major constraint in these enzymatic assays is the preparation of pure linamarase. The enzyme is most commonly purified from cassava leaf and rind, both of which are good sources of the enzyme (Cooke, 1978; Yeoh, 1989). Immobilization of linamarase onto surfaces of nylon filters, microcentrifuge tubes, microplates, etc., helps in using the enzyme more effectively, especially when analyzing a large number of samples (Yeoh, 1993). An acid hydrolysis method for assay of linamarin was developed by Bradbury et al. (1992) in order to overcome the difficulty in preparation of linamarase. Purification of linamarase from rind and leaf involves homogenization of large quantities of tissue and handling large volumes. The low protein content in rind and the poor extractability of protein from leaf tissues affects the recovery of the enzyme, resulting in low yield of linamarase in some preparations. Therefore, an alternate source of linamarase was investigated. Cassava latex has been shown to have very high linamarase activity (Pancora and Hughes, 1992; Elias et al., 1997). The possibility of using latex as a source of linamarase was explored. The yield of the enzyme from latex was compared with that from rind and leaf.

EXPERIMENTAL PROCEDURES

Young leaves, rind (root cortex), and latex, collected from cassava variety M4, grown on the CTCRI farm, were used for purification of linamarase. Triplicate samples of each tissue were processed separately. Fresh latex was obtained by cutting the base end of petioles from young and mature leaves and allowing the latex exudate from both cut ends to collect into an eppendorf tube. About 1 g of latex can be obtained from 10 leaves.

Purification of Linamarase from Rind/Leaf. Linamarase was purified from cassava rind using the procedure of Cooke et al. (1978). Cassava rind (25 g) was homogenized in a blender with 200 mL of 0.1 M acetate buffer at pH 6.0. The homogenate was squeezed through muslin cloth and the resulting filtrate centrifuged at 10000*g* for 15 min. The enzyme was precipitated with 60% ammonium sulfate overnight. After centrifugation at 10000*g* for 30 min, the precipitate was dissolved in 5 mL of 0.01 M phosphate buffer at pH 6.0 and dialyzed against the same buffer. This preparation of partially purified linamarase was used for measurement of enzyme activity against linamarin as substrate.

In the case of leaf tissue, acetone powder was first prepared by homogenizing 25 g of leaf tissue with chilled acetone. The dried acetone powder was extracted with 50 mL of phosphate buffer (0.1 M, pH 6.0), filtered through muslin cloth, and

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Table 1. Comparison of Linamarase Activity in Cassava Rind, Leaf, and Latex[a]

tissue	wt of tissue (g)	vol of enzyme extract (mL)	activity (units)
rind	25	5	4 ± 1.2
leaf	25	5	3 ± 1.0
latex	1	5	40 ± 5.0

[a] Values are average of three samples assayed in duplicate ± SE.

centrifuged. The subsequent steps were similar to those described for rind tissue.

Purification of Linamarase from Latex. About 1 g of latex was diluted with 50 mL of 0.1 M phosphate buffer at pH 6.0, kept for 5 min, and centrifuged at 5000*g* for 15 min. Linamarase was precipitated by 60% ammonium sulfate as described earlier. All the above operations were carried out at 4 °C.

Determination of Linamarase Activity. The activity of linamarase was estimated as given in Nambisan and Sundaresan (1994). The reaction system contained 0.2 μmol of linamarin and an aliquot of the suitably diluted enzyme in a total volume of 1 mL of 0.1 M phosphate buffer at pH 6.0. After 15 min of incubation, the reaction was stopped by the addition of 0.1 M NaOH and then neutralized with 0.1 M HCl. A 1 mL volume of 1% Chloramine T was added, followed by 3 mL of pyridine−barbituric acid reagent, and the absorbance was measured at 580 nm after 15 min.

Linamarase activity of 1 unit was defined as the amount of enzyme which released 1 μmol of cyanide/min.

RESULTS AND DISCUSSION

The enzyme activity in rind, leaf, and latex extracts was assayed using linamarin as substrate, and the activities were compared. As seen in Table 1, enzyme preparations from latex had about 300-fold higher activity (per g of tissue basis) compared to leaf and rind, indicating that a significantly higher yield of linamarase could be obtained from latex.

The enzymatic assay for linamarin in fresh and processed cassava normally requires the addition of 0.01 unit of enzyme. A 1 g amount of latex can yield enough enzyme for >3000 assays. To obtain a comparable yield of enzyme from rind and leaf, it would be necessary to use large quantities of tissue, which makes the process of enzyme isolation more cumbersome. Another advantage in using latex is that the concentration of the enzyme is always high, irrespective of the cultivar or growth stage of the plant, while, in the case of rind and leaf tissues, variation in linamarase activity is observed between cultivars (Nambisan and Sundaresan, 1994). Poor extractability of the enzyme from leaf tissues also results in low yields.

Therefore, the major advantages in using latex as a source of limarase are (1) ease of enzyme preparation, since handling of large quantities of tissue and subsequent homogenization can be avoided, and (2) a significantly higher yield of enzyme obtained from latex as compared to other tissues.

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