Purification and Application of β-Galactosidase from Jack Bean (*Canavalia ensiformis* L.)

Yee Yee Myint

Abstract

In this research, β-galactosidase (E.C 3.2.1.23) was isolated from jack bean (*Canavalia ensiformis* L.) seed using successive ammonium sulphate precipitation method. Further purification was carried out using Sephacryl S-200 gel chromatography. The product glucose liberated from enzymic hydrolysis on lactose substrate was determined by using glucose oxidase enzyme reagent method. The fractional numbers from Sephacryl S-200 gel column were analyzed for the determination of β-galactosidase activity and protein content (absorbance at 280 nm). The fraction numbers (20-30) showing the highest β-galactosidase activity were pooled and further experiments were carried out using the pooled fraction. The relative purity of the β-galactosidase enzyme increased about 8 folds from crude to final purification step. The β-galactosidase activity was found to be 45.8 EU per gram of jack bean seeds at final purification step. The purity of the enzyme was confirmed by non SDS-PAGE electrophoresis as a single band. The molecular weight of the purified enzyme was determined to be 75,850 dalton.

Key words: β-galactosidase, jack bean, Sephacryl S-200, non SDS-PAGE, molecular weight

Introduction

Every organism contains thousands of different proteins with a variety functions. One of the functions is as catalysts. Nearly all chemical reactions in biological systems are catalyzed by specific macromolecules called enzyme (Garrett and Grisham, 1992). Enzymes are proteins which may consist of more than 20 types of amino acids with wide-ranging compositions and structural complexities (Gemeiner, 1992).

The ability of β-galactosidase to hydrolyse lactose into galactose is applied in food industry, particularly in the field of dairy products because of the nutritional (lactose intolerance), technological (crystallization) and environmental (pollution) problems associated with lactose (Blakebrough,
The added value gained by the hydrolysis of lactose to its constituent monosaccharides glucose and galactose, lies in the increased usefulness of hydrolysed lactose as a food carbohydrate.

The botanical aspect of jack bean (Figure 1) is as follows:

<table>
<thead>
<tr>
<th>Family</th>
<th>Fabaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
<td>Canavalia</td>
</tr>
<tr>
<td>Species</td>
<td>ensiformis</td>
</tr>
<tr>
<td>Botanical Name</td>
<td>Canavalia ensiformis (Linn) Do</td>
</tr>
<tr>
<td>English Name</td>
<td>Jack bean</td>
</tr>
<tr>
<td>Myanmar Name</td>
<td>Pe-dalet</td>
</tr>
</tbody>
</table>

![Figure 1. Photograph of Jack Bean (Canavalia ensiformis L.) plant](image)

It is an annual, upright and bushy plant, growing to the height of 2 to 5 ft and bearing sword-shaped pods, 8-5 in long and about 1 in broad. The pod contains 10-12 white seeds each having a brown hilum extending to half the length of the seed (Bhatnager, 1950).

The β-galactosidases are mainly used in the applications of biochemistry and biotechnology (Blakebrough, 1981). These are in the production of low lactose milk and utilization of whey lactose and the synthesis of disaccharides by reversal of hydrolysis. The ability of β-galactosidase to hydrolyse lactose into galactose is applied in food industry, particularly in the field of dairy products.

It can also be used to study the structure of complex carbohydrate chains and as a reagent to determine the lactose in the blood and other biological fluids (Byrne and Johnson, 1975).
Materials and Methods

Isolation of β-Galactosidase from Jack Bean

Jack bean seeds (Canavalia ensiformis) were purchased from Nay Pyi Taw Pyinmana Township. Jack bean seeds (200 g) were blended in the blender (Li et al., 1975). A 100 g of jack bean meal was suspended in 600 mL of distilled water and stirred for 2 hours at room temperature. The suspension was filtered through cheesecloth. The turbid filtrate was adjusted to pH 5.5 at room temperature with 1.5 M sodium citrate (pH 2.7) and centrifuged to obtain 450 mL of extract. Solid ammonium sulphate (79.07 g) was added to this extract to obtain 30% saturation (Rosenberg, 1996).

After standing for 2 hr, the precipitate was removed by centrifugation for 20 min at 5000 rpm and was discarded. β-Galactosidase in the supernatant (400 mL) was precipitated by adding solid ammonium sulphate (76.05 g) to 60% saturation. After standing overnight, the precipitated protein containing β-galactosidase was collected by centrifugation for 20 min at 5000 rpm and dissolved in 50 mL of 0.1 M sodium phosphate buffer (pH 7.0) to obtain an opaque solution.

Determination of β-Galactosidase Activity

The reaction mixture consisted of 0.1 mL of enzyme fraction solution and 0.25 mL of lactose (substrate). The mixture was shaken well and incubated at 37 °C for 30 min. After incubation time, the mixture was heated on a boiling water bath for 10 min in order to stop the enzyme reaction. Then this solution was cooled under tap water for 10 min.

Finally, 0.5 mL of glucose oxidase enzyme reagent was added to the reaction mixture. The mixture was incubated at 37°C for 10 min. The filtrate was measured for absorbance at 500 nm by using UV-visible spectrophotometer. The amount of glucose liberated was calculated from a calibration curve, which relates the measured absorbance to an equimolar mixture of glucose and galactose.

Purification of β-Galactosidase Enzyme Using Gel Chromatography

Gel filtration was carried out in a column (2.5x27 cm) packed with pre-swollen Sephacryl S-200 using 0.1 M phosphate buffer (pH 7) and equilibrated with the same buffer (500 mL). The enzyme was eluted from the column with the same equilibrated buffer.
Crude β-galactosidase (0.04 g) was dissolved with 2 mL of pH 7.0 phosphate buffer. This solution was applied to a Sephacryl S-200 gel filtration column previously equilibrated with the same buffer. The flow rate was adjusted to 15 mL/hr by a mini pump and 2.5 mL fractions were collected per tube using a fraction collector. After collection, the protein content of each tube was checked by measuring the absorbance at 280 nm wavelength using a UV-visible spectrophotometer. Each tube was also measured for β-galactosidase activity. The fractions that had the highest β-galactosidase activity were pooled. The pooled β-galactosidase fraction was measured for protein content by the modified Lowry’s method and β-Galactosidase activity. The pooled highest β-Galactosidase fraction was concentrated with acetone, 1:9 ratio (Rosenberg, 1996).

**Purification by Ion Exchange Chromatography**

The ion exchange chromatography was packed in a column with DEAE Sephadex A-50 using 0.02 M sodium phosphate buffer (pH 8.0) and equilibrated with the same buffer.

The concentrated β-galactosidase fraction obtained by Sephacryl S-200 gel filtration column was applied to a DEAE Sephadex A-50. The flow rate was adjusted to 15 mL/hr using a mini-pump and 2.5 ml fraction was collected per tube using a mini-collector. During collection, the protein content of each tube was continuously monitored at 280 nm wavelength by a bio-mini UV-monitor.

**Determination of Molecular Weight of the Purified β-Galactosidase Sample**

The procedure is according to the method of Halim and Smith (Smith, 1975).

**Determination of Lactose Content**

A 1 mL of the clear milk solution was mixed with 1 mL of phosphate buffer (pH 5.6) and enzyme reaction was started by adding 3 beads of immobilized β-galactosidase. After 30 min the solution was filtered and determination of glucose content was carried out by using glucose oxidase enzyme reagent method.
Results and Discussion

Isolation of $\beta$-Galactosidase from Jack Bean

In this research, the jack bean meal was suspended in water (Li, 1975). After being stirred for 2 hr at room temperature, filtering with cheese cloth, ultracentrifuging the suspension was filtered and finally the crude extract was obtained. Extraction steps involved the 30% $(\text{NH}_4)_2\text{SO}_4$ and 60% $(\text{NH}_4)_2\text{SO}_4$ precipitation. By adding the appropriate solid ammonium sulphate, the enzyme protein was obtained. Since ammonium sulphate has little effect on enzyme activity and in some cases stabilizes the enzymes, it is useful as salt of choice in most cases. Thus it was employed in the present work.

$\beta$-Galactosidase Activities, Protein Contents and Specific Activities of the Enzyme Solutions at Different Purification Steps

The purification step involved ammonium sulphate fractionation followed by gel filtration on Sephacryl S-200. Sephacryl S-200, superfine is a new kind of gel filtration medium which combines a highly porous gel structure with excellent chemical and physical stability (Wiseman, 1985). Sephacryl is preswollen and ready to use in both analytical and preparation applications.

In the present research, Sephacryl S-200 was used in glass column 2x40 cm, that will fractionate proteins in the molecular weight range of 5 kDa to 250 k Da (Wiseman, 1985). Figure 2 shows the fractions of highest specific activity 20-30 and these were pooled and concentrated with acetone (cold acetone: enzyme solution = 1:9). It was kept at 4 °C for 15 min, centrifuged 2000 rpm for 20 min, the enzyme precipitate was obtained. The specific activity of the $\beta$-galactosidase was increased by 8.345 fold over crude extract in Table 1. The $\beta$-galactosidase enzyme activity can be defined as micromole of glucose liberated from lactose substrate per minute of enzyme solution. The EU of $\beta$-galactosidase from jack bean was determined as 45.8 EU per gram of jack bean seeds.
β-Galactosidase activity (µmol/min/ml)

0 0.05 0.1 0.15 0.2 0.25 0.3

Fraction number

Figure 2  Purification of crude β-galactosidase enzyme by Gel (Sephacryl S-200) filtration chromatography

Table 1.  β-Galactosidase enzyme activities, protein contents and specific activities of the enzyme solution at different purification steps

<table>
<thead>
<tr>
<th>No</th>
<th>Fractions</th>
<th>Enzyme activity (µmol ml⁻¹ min⁻¹)</th>
<th>Protein (mg)</th>
<th>Specific Activities</th>
<th>Degree of Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Supernantant I</td>
<td>29.80</td>
<td>3.841</td>
<td>7.758</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>Supernantant II</td>
<td>24.59</td>
<td>2.342</td>
<td>10.500</td>
<td>1.353</td>
</tr>
<tr>
<td>3</td>
<td>Crude enzyme</td>
<td>19.40</td>
<td>0.698</td>
<td>27.790</td>
<td>3.582</td>
</tr>
<tr>
<td></td>
<td>solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>After passing</td>
<td>13.66</td>
<td>0.211</td>
<td>64.740</td>
<td>8.345</td>
</tr>
<tr>
<td></td>
<td>S-200Sephacryl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Molecular Weight of Purified β-Galactosidase**

In this work, proteins from the pharmacia high molecular weight (HMW) calibration kit: thyroglobulin (669,000), ferritin (440,000), catalase (232,000), lactate dehydrogenase (140,000) and bovine serum albumin (67,000) were used for molecular weight determined by non SDS-PAGE. The homogeneity of the purified β-galactosidase was confirmed by non-sodium dodecyl sulphate-poly acrylamide gel electrophoresis (non SDS-
The purified β-galactosidase enzyme showed a single band on non SDS-PAGE where the molecular weight of purified β-galactosidase was located near the standard protein (mol wt. 67,000) (Figure 3) after the final step purification (Table 1).

![Image of SDS-PAGE gel](image_url)

**Figure 3** Photograph of non sodium dodecyl sulphate polyacrylamide gel electrophoresis*

*Lane (a) High molecular weight marker proteins
  M I = Bovine Serum Albumin, M II = Lactate Dehydrogenase
  M III = Catalase, M IV = Ferritin, M V = Thyroglobulin
(b) Crude enzyme solution
(c) Purified β-galactosidase fraction obtained from sephacryl S-200 gel

An estimated molecular weight of purified β-galactosidase from jack bean sample was 75,850 dalton from the log of known HMW marker proteins vs. Rf values for non SDS-PAGE.

**Lactose Content in Commercial Milk Powder Products Using Immobilized β-Galactosidase Enzyme**

In this research, the rapid analytical method was developed for the determination of lactose in milk powder sample. After enzymic hydrolysis on lactose in the solution by immobilized β-galactosidase, lactose content was calculated (Table 2). The glucose content in deproteinized milk powder sample was determined using glucose oxidase enzyme reagent methods.

The precision and accuracy (error percent) of the lactose determination in PEP milk powder samples were 1.06% and 2.16%. The
precision and accuracy (error percent) of the lactose determination in Dumex milk powder sample were 0.39% and 4.30%.

Table 2. Lactose Contents in Milk Powder Samples

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Absorbance at 500 nm</th>
<th>Lactose Content(%)</th>
<th>Average</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PEP*</td>
<td>0.816</td>
<td>27.83</td>
<td>28.47</td>
<td>±0.3216</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.847</td>
<td>28.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.840</td>
<td>28.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Deng Chuan Instant Whole Sweet Milk Powder</td>
<td>0.570</td>
<td>19.46</td>
<td>19.13</td>
<td>±0.1123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.561</td>
<td>19.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.551</td>
<td>18.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dumex*</td>
<td>1.143</td>
<td>39.01</td>
<td>38.76</td>
<td>±0.0582</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.135</td>
<td>38.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.129</td>
<td>38.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PEP* certified value = 29.1%, Dumex* certified value = 40.5%

**Conclusion**

In this research, β–galactosidase (EC 3.2.1.23) was isolated from jack bean (*Canavalia ensiformis* L.) seed using successive ammonium sulphate precipitation method. Further purification was carried out using Sephacryl S-200 gel chromatography. The product glucose liberated from enzymic hydrolysis on lactose substrate was determined by using glucose oxidase enzyme reagent method.

The fractional numbers were analyzed for the determination of β-galactosidase activity and protein content (absorbance at 280 nm). The fraction numbers (20-30) showing the highest β-galactosidase activity were pooled and further experiments were carried out using the pooled fraction. The relative purity of the β-galactosidase enzyme increased about 8 folds from crude to final purification step. The purity of the enzyme was confirmed by Sephacryl S-200 gel as a single band. The molecular weight of the purified enzyme was determined to be 75,850 dalton.

In this research, a rapid determination of lactose in milk powder samples were carried out using immobilized β-galactosidase. The results obtained were not much differed from the certified values.
Acknowledgements

The author would like to thank the Department of Higher Education (Lower Myanmar), Ministry of Education, Myanmar, for allowing to carry out this research programme. Sincere thanks are due to Dr Nyunt Pe, Rector, Dr Than Soe Pro-Rector and Professor and Head Dr Khin Aye Kyu, Department of Chemistry, Pathein University for giving the opportunity to present this paper. Grateful thanks are due to Dr Kyaw Naing, Professor, and Dr San San Myint, Lecturer, Department of Chemistry, University of Yangon for their close supervision.

References