Partial purification and characterization of amylase and starch from Elephant foot yam [Amorphophallus campanulatus (Roxb.) Blume]

C Preethi* and T Veerabasappa Gowda
PG Department of Biotechnology, J S S College of Arts, Commerce and Science, Mysore 570 025, India

Received 17 January 2013; revised 29 May 2013; accepted 21 June 2013

Amylase (EC 3.2.1.2) and starch from Amorphophallus campanulatus (Roxb.) Blume (Elephant foot yam) were partially purified. Kinetic characterization of yam amylase at its optimum conditions of activity was carried out. Its activity profile was compared with that of salivary amylase upon yam and commercial potato starch as substrates. A differential behaviour in the activity of yam amylase compared to salivary amylase was observed. Presence of isoforms of amylase in yam was detected. The partially degraded yam starch appeared to be better substrate compared to native starch. Ca\(^{2+}\) ions stimulated the yam amylase activity upon endogenous starch than Na\(^+\) and K\(^+\) ions.

Keywords: Amylase, endogenous starch, isoforms, kinetic characterization, Zymogram

Most of the carbohydrates found in nature are polysaccharides of high mol wt. On hydrolysis with acid or specific enzymes, these polysaccharides yield monosaccharides or simple monosaccharide derivatives. Among the storage polysaccharides, starch is most abundant in plants and is usually deposited in the form of large granules in the cytoplasm of cells. Starch is composed of amylose and amylopectin. Amylases, the starch degrading enzymes, are widely distributed in microbial, plant and animal kingdom. Historically, the term amylase was used to designate enzymes hydrolyzing α-1, 4-glycosidic bonds of amylose, amylopectin and glycogen. They act by hydrolyzing bonds between glucose units yielding characteristic products of the particular enzyme involved. A number of enzymes associated with degradation of starch and related polysaccharide structures have been detected and studied. In the present study, partial purification and kinetic characterization of amylase from yam [Amorphophallus campanulatus (Roxb.) Blume] was carried out and it was compared with the activity of salivary amylase to understand the differential behaviour of yam amylase upon endogenous starch, i.e., yam starch.

The yam was obtained from Mysore district of Karnataka state (India). Sephadex G-75 from Sigma Chemicals, USA; acrylamide, bis-acrylamide; Tris, commercial starch, Glycine from Sisco Research Laboratory, Pvt. Ltd., Mumbai; potassium iodide, glacial acetic acid, ammonium sulphate, iodine, copper(II) sulphate, sodium acetate, di-sodium hydrogen phosphate, sodium di-hydrogen phosphate were from commercial sources of analytical grade.

Amylase was extracted from the peel of yam; 100 g peel in 50 mL of water was centrifuged at 5000 rpm for 5 min at 4°C to remove debris. Supernatant was used as crude source for amylase. The aqueous extract of enzyme was subjected to ammonium sulphate precipitation (25%) and was allowed to stand at 4°C for a minimum of 6 h. The pellet was separated by centrifugation at 4°C. Saturation of the supernatant was raised to 30%. Again the pellet was obtained by centrifugation and the supernatant obtained was further raised up to 45% saturation with ammonium sulphate. Pellets were then separately suspended in 0.1 M acetate buffer, pH 5. The enzyme activity (activity/mL) and specific activity (activity/mg of protein) was calculated. The fraction III (6 mg) was loaded on to a Sephadex G-75 column (length 75 cm, radius 0.5 cm, with bed volume of 60 mL). The protein was eluted with 0.1 M acetate buffer, pH 5. The fractions were screened at 280 nm in a spectrophotometer. Amylase activity was estimated for peak tubes and the specific activities were calculated. The protein in tubes with significant activity was concentrated by lyophilization.

*Author for correspondence:
Tel: +91-821-2480117
Mobile: +91-9739482355
E-mail: preethic_25@yahoo.co.in
The time kinetics was followed for salivary amylase and yam amylase (Table 1) on both yam and commercial starch as substrates. Salivary amylase sample was prepared by diluting 200 times the freshly collected saliva from a healthy individual before lunch time. Diluted saliva served as a source of salivary amylase.

a) Colorimetric Method: The activity of salivary amylase and yam amylase were determined separately by incubating amylases with gelatinized starch (1%), in sodium acetate buffer 0.1 M, pH 5 for Yam amylase and phosphate buffer 0.1 M, pH 7.0 for salivary amylase and were incubated at their optimum temperature (50°C & 37°C, respectively). The reaction was stopped at regular time intervals.

b) Turbidometric Method: The activity of salivary amylase and yam amylase on yam starch were estimated separately by incubating amylases with gelatinized yam starch (1%), in sodium acetate buffer, 0.1 M, pH 5 for yam amylase and phosphate buffer, 0.1 M, pH 7 for salivary amylase and were incubated at their optimum temperature of activity. The turbidity clearance at regular time intervals were recorded using a turbidimeter.

Effect of substrate concentration on yam amylase was estimated by keeping the amount of enzyme constant and substrate concentration was gradually increased. The fraction III (200 µL) was incubated with sodium acetate buffer, 0.1 M, pH 5 and with gelatinized starch (0, 0.03, 0.06, 0.3, 0.6, 0.9, 1.21 × 10^{-3} µmol) at the optimum temperature of activity for 30 min. To determine the effect of metal ions on yam amylase activity, ammonium sulphate fraction III (200 µL) was incubated with 500 µL of 5 mM solution of the following metal chlorides: Ca^{2+}, Ba^{2+}, K^{+}, Na^{+}, Fe^{3+}, Hg^{2+} with gelatinized starch, 1%, 500 µL in 0.1 M sodium acetate buffer, pH 5 at its optimum temperature for 60 min and the activity was estimated as before. The enzyme activity without metal ions was taken as 100%. The relative activities were calculated for each metal ion.

Polyacrylamide gel electrophoresis (PAGE) with 7.5% gel under native basic conditions was carried out. Samples (150 µg) of crude along with ammonium sulphate fractions I, II, and III were run simultaneously and stained for both protein and enzyme activity. The gels were stained for protein with CBB (Coomassive brilliant blue) staining and enzyme activity staining for Zymogram, the gel after electrophoresis was incubated in gelatinized starch (2%) prepared in sodium acetate buffer 0.1 M, pH 5 for 1 h. Subsequently, the gel was stained with iodine reagent (3%).

Yam tubers are rich sources of starch. The starch was isolated from yam according to the mentioned procedure and its purity as per acid hydrolysis was 95%. The percentage of starch in wet tuber was 10%. The peel water extract showed amylase activity. The crude extract upon ammonium sulphate fractionation yielded fractions I, II and III. Supernatant showed diminished activity for amylase. The activity of fractions along with the crude is represented in (Table 2). Fraction III that exhibited highest activity yielded several peaks upon gel filtration on Sephadex (Fig. 1). The peaks 2, 3, 4 and 6 showed amylase activity, which may represent the presence of isozymes. Time kinetics graph (Fig. 2a) though showed similar type of activity for both the amylases on commercial starch, salivary amylase had more efficiency towards the product release than yam amylase. Also, salivary amylase had similar activity pattern on both commercial and yam starch (Figs 2a & b). Yam amylase showed difference in its time kinetics pattern on yam starch (Fig. 2b) compared to potato starch. Initially (up to 60 min), the degradation of yam starch

### Table 1—Physical characteristics of salivary and yam amylase

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Yam amylase</th>
<th>Salivary amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum pH</td>
<td>5.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>50°C</td>
<td>37°C</td>
</tr>
</tbody>
</table>

### Table 2—Summary of purification of yam amylase and its activity

<table>
<thead>
<tr>
<th>Steps</th>
<th>Enzyme activity (µmol/min/mL)</th>
<th>Total protein (mg/mL)</th>
<th>Specific activity (µmol/min/mg)</th>
<th>Enzyme unit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>0.28</td>
<td>1.325</td>
<td>0.21</td>
<td>4.8</td>
</tr>
<tr>
<td>Ammonium sulphate precipitates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25%</td>
<td>0.07</td>
<td>1.5</td>
<td>0.05</td>
<td>20</td>
</tr>
<tr>
<td>25-30%</td>
<td>0.09</td>
<td>1.0</td>
<td>0.09</td>
<td>11.1</td>
</tr>
<tr>
<td>30-45%</td>
<td>0.35</td>
<td>3.35</td>
<td>0.11</td>
<td>9.1</td>
</tr>
<tr>
<td>Sephadex G-75 fractions (Peaks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.25</td>
<td>0.20</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>0.027</td>
<td>0.1</td>
<td>0.27</td>
<td>3.7</td>
</tr>
<tr>
<td>4</td>
<td>0.049</td>
<td>0.5</td>
<td>0.098</td>
<td>10.2</td>
</tr>
<tr>
<td>6</td>
<td>0.060</td>
<td>0.125</td>
<td>0.48</td>
<td>2.08</td>
</tr>
</tbody>
</table>

*One unit of amylase activity was defined as the amount of enzyme producing 1 µmol reducing sugar per min under standard assay conditions
was low. Then the velocity picked up slowly. However, it was not comparable with its action on potato starch. It may be due to the incompatibility between the substrate (yam starch) and the enzyme. Further, the partially degraded yam starch appeared to be the better substrate compared to the native starch.

This fact was evident from the time kinetic curve. The turbidometric measurement of time kinetics for yam amylase was also similar to that of colorimetric measurement of activity for its endogenous substrate (Fig. 3). The results of PAGE and Zymogram (Fig. 4) confirmed the presence of amylase activity and presence of isozymes of amylase.

We propose to explain this as a requirement of the sprouting yam. The rate of degradation should match the rate of growth. Since, the degradation products are expected to serve the supply of energy source and precursor to the building structural blocks of the growing plant. Hence, slow degradation of reserve nutrients is probably desired to sustain slow growth.
pattern of the plant. On the other hand, salivary amylase is a digestive enzyme it is required to degrade the substrates immaterial of the source. The estimated $k_m$ value (0.11 $\mu$mol) for yam amylase was lowest when compared to $k_m$ values for other amylases (Table 3). The size of $k_m$ indicates the tightness with which the enzyme binds to the substrate. Lower the $k_m$ value, higher is the affinity of the enzyme for the substrate. The process of enzyme action for such enzymes is slow. Therefore, the rate of release of products is slow for such pairs. On the contrary higher the $k_m$ value, faster is the release of products. The enzyme substrate combination is evolved to regulate metabolic requirements.

Various metal ions, such as, Ca$^{2+}$, Ba$^{2+}$, K$^+$, Na$^+$, Fe$^{3+}$ and Hg$^{2+}$ at 5 mM concentration influenced the enzyme activity (Table 4). Ca$^{2+}$ and Ba$^{2+}$ were found to have both activating and stabilizing effect as indicated by increased activity. The metal ions Hg$^{2+}$, Fe$^{3+}$, Na$^+$ inhibited the activity of amylase perhaps indicating the involvement of SH-group/cysteine in the catalytic function of the enzyme.

The present investigation reveals the regulation of enzyme activity by evolving enzyme substrate combination to meet the metabolic requirement of the growing plant. Stored nutrient starch is degraded by amylase to release the energy providing products and precursors for the building blocks of large molecules at a required rate for the plant.

**Acknowledgement**

Authors are grateful to Post Graduation Department of Biotechnology, J S S College of Arts, Commerce and Science, Mysore, India for providing facilities and assistance to carry out this project.

**References**


