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Purification and Characterization of Midgut *α*-Glucosidase from Larvae of the Rice Green Caterpillar, *Naranga aenescens* Moore

N. Memarizadeh[1], P. Zamani[2], R. H. Sajedi[2], and M. Ghadamyari[1*]

ABSTRACT

Application of chemical pesticides has increased significantly worldwide and has raised serious concerns about environmental pollutions. One of the encouraging trends to minimize pesticide risk is production of resistant plants containing toxic proteins against insect pests. Considering the importance of purification and characterization of digestive enzymes in the production of resistant plants, in this study an *α*-glucosidase from the *Naranga aenescens* Moore's midgut was purified by ammonium sulfate precipitation, ion exchange chromatography on DEAE-sepharose, and concentrating through ultrafiltration. The apparent molecular mass of the enzyme was 48 kDa determined by SDS-PAGE. The optimum pH and temperature of the enzyme were 6.0 and 45°C, respectively. The irreversible thermoinactivation of the enzyme showed that it was highly stable at 35ºC but moderately stable at 40 and 45ºC. Zn^{2+}, Hg^{2+}, Co^{2+} at 10 and 20 mM, and Ba^{+2} only in 20 mM strongly inhibited the *α*-glucosidase activity. Ba^{2+} and Ca^{2+} only at 10 mM, EDTA and Hg_2^{2+} only at 20 mM and Mg^{2+} at 10 and 20 mM significantly increased the enzyme activity. The K_m and K_{cat} values for the *α*-glucosidase were 0.54 mM and 3.62 min^{-1}, respectively, when *p*-Nitrophenyl-α-D-glucopyranoside (pNαG) was used as a substrate.

Keywords: Ion exchange chromatography, Enzyme activity, Kinetic parameters, Thermostability Kinetic parameters, Toxic proteins.

INTRODUCTION

The *Naranga aenescens* Moore (Lepidoptera: Noctuidae), known as rice green caterpillar, is a defoliator pest of the rice crop in Northern provinces of Iran. This pest also feeds on developing panicle rachis near the developing kernels resulting in huge loss of the crop. In 1986, this pest was reported from Iran at Guilan and Mazandaran provinces and was widely distributed in all paddy fields. "*N. aenescens* outbreak in paddy fields in Guilan province and 30,000 hectares of paddy fields were sprayed with synthetic insecticides against this pest annually" (Abivardi, 2001; Asadi *et al*., 2010). Chlorpyrifos and fenitrothion are widely used to control this pest. Since the Guilan and Mazandaran provinces have a very high water table, application of pesticide against *N. aenescens* is extremely hazardous not only to farmers and consumers via drinking polluted water, but also to the environment. Therefore, characterization of purified digestive enzymes can be first step of production of resistant plants as an encouraging research field to decrease usage of chemical pesticides.

[1] Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Islamic Republic of Iran.
[*] Corresponding author: e-mail: ghadamyari@guilan.ac.ir
[2] Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

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Carbohydrates are normally converted into monosaccharides, which can be absorbed through the midgut of insects. Glucosidases have crucial role in final stages of carbohydrates digestion. These enzymes constitute a group of glycoside hydrolase that are involved in the metabolism of oligosaccharides. Furthermore, glucosidases were used for biosynthesis and modification of glycoproteins (Melo *et al*., 2006). Alpha-glucosidase (EC 3.2.1.20) is an enzyme that acts upon α(1→4) glycosidic bonds and liberates the glucose from non-reducing ends of *α*-glucosides, *α*-glucans and α-linked oligosaccharides. In some insects, *α*-glucosidases seems to be an essential tool for the partitioning of carbohydrates from the diet into carbon nutrition and osmo-regulation (Ashford *et al*., 2000). Alpha-glucosidases show diverse substrate specificities in plants, fungi and insects (Frandsen and Svensson, 1998; Wongchawalit *et al*., 2006; Carvalho *et al*., 2010). Some *α*-glucosidases preferentially hydrolyze α-linked di-, oligo-, and/or polyglucans as substrate, while others preferentially cleavage heterogeneous substrates such as aryl glucosides and sucrose (Chiba, 1988; Frandsen and Svensson, 1998). This enzyme was also capable of catalyzing transglycosylation to produce alpha-1,4 linked maltotriose and alpha-1,6 linked isomaltooligosaccharides (Johnson *et al*., 2010) and some *α*-glucosidases show clear transglycosylation activity (Kato *et al*., 2002).

This enzyme can be found in the midgut and salivary glands of insects (Lagadic and Chararas 1988; Ghadamyari *et al*., 2010; Ramzi and Hosseininave 2010; Saberi *et al*., 2012; Asadi *et al*., 2012) as well as hypopharyngeal glands of *Apis melifera* L. (Baker and Lehner 1972; Terra *et al*. 1996). Characterization of *α*-glucosidases from the crude of alimentary canal, salivary glands and hemolymph of *N. aenescens,* which was carried out by Asadi *et al*. (2012), showed that the specific activity of *α*-glucosidases in the alimentary canal was more than the other segments. Till now, *α*-glucosidases have been isolated and characterized from many insects including *Dysdercus peruvianus* Guerin-Meneville (Hemiptera: Pyrrhocoridae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *A. mellifera*; *A. cerana japonica*; *A. cerana indica* (Hymenoptera: Apidae), *Drosophila melanogaster (*Diptera: Drosophilidae), *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae) and *N. aenescens* (Huber and Mathison 1976; Tanimura *et al.* 1979; Baker 1991; Silva and Terra 1995; Ghadamyari *et al.* 2010; Asadi *et al*., 2012).

There are few researches on the purification of *α*-glucosidases in the digestive system of insects and our knowledge about this enzyme in the digestive system of lepidopteran insects is still rudimentary. Moreover, representation of a universal protocol for purification of different isoforms of *α*-glucosidase from different insect sources may require affinity of substrate (ligand) based chromatography to the target site (Chanchao *et al*., 2008). In *A. mellifera,* the purification of *α*-glucosidase has involved CM-cellulose and on Sephadex G-100 (*α*-glucosidase I), DEAE-cellulose, CM-cellulose, and Bio-Gel P-150 (*α*-glucosidase II), or DEAE-sepharose CL-6B, Bio-Gel P-150, and CM-Toyopearl 650M (*α*-glucosidase III) (Takewaki *et al.*, 1980; Nishimoto *et al.*, 2001). In addition, Kubota *et al.* (2004) purified *α*-glucosidase from honey of *A. mellifera* using salting-out chromatography, CM-cellulose, Bio-Gel P-150, and DEAE-Sepharose CL-6B.

Glucosidases inhibitors can serve as plant defense mechanisms, particularly against attack by insects and *α*-glucosidase inhibitors can be used as new insecticide by preventing the digestion of carbohydrates. Therefore, in this study, as the first step of this encouraging field, a novel *α*-glucosidase was purified from larval midgut of *N. aenescens* and then it was characterized.

MATERIALS AND METHODS

Chemicals

p-Nitrophenol and bovine serum albumin were purchased from Merck (Darmstadt, Germany). *p*-Nitrophenyl-α-D-glucopyranoside (pNαG) and 4-methylumbelliferyl-α-D-glucopyranoside (4-MUαG) were obtained from Sigma (St. Louis, USA). DEAE-sepharose was purchased from GE healthcare (UK).

Insect and Enzyme Preparation and Purification

N. aenescens's larvae were collected from rice seedling *Oryza sativa* L. 'variety of Hashemi' in the northern provinces of Iran. The 5^{th} instar larvae were randomly selected for purification and enzyme characterization. Last larval instars were immobilized on ice, dissected under a stereoscopic microscope, and their alimentary canals were removed. Then, the alimentary canals were cleaned of the malpighian tube and adhering lipids. Finally, midgut was separated from foregut and hindgut and used for *α*-glucosidase purification.

For glucosidase isolation from midgut of *N. aenescens*, 300 midgut were homogenized in 6 ml cold phosphate buffer 0.02M (pH 6.0) using a hand-held glass homogenizer and centrifuged at 15,000×g for 15 minutes at 4°C. The supernatants were passed through a filter paper and used in purification process. Ammonium sulfate was added to the crude extract to 85% saturation at 4°C for 4 hours. Ammonium sulfate was added gradually to the raw extract in Erlenmeyer flasks with a magnetic stirrer at low speed. The precipitate was centrifuged at 7,000×g for 30 minutes at 4°C; dissolved in 20 mM phosphate buffer (pH 7.0); and dialyzed overnight against in 50 mM Tris-HCl buffer (pH 7.0). The concentrated protein solution was applied onto a DEAE-Sepharose column previously equilibrated with 50 mM Tris, pH 7.0. Proteins were then eluted with a step wise gradient of 0 to 0.5M NaCl in the same buffer. The active fractions were pooled and concentrated by ultrafiltration (Amicon, Beverly, MA). All of these works were performed in a cold room maintained at 4±1°C.

Measurements of Enzyme Activity and Protein Concentration

The enzymatic activity was determined by measuring the increase in absorbance at 405 nm caused by the hydrolysis of pNαG. 10 μL of homogenate were incubated for 20 minutes at 37°C with 45 μL of substrate solution (20 mM) and 115 μL of 20 mM phosphate-acetate-citrate mixed buffer (Ghadamyari *et al*., 2010). The reaction was stopped by addition of 600 μL of NaOH (0.25 M). Optical density was measured at 405 nm using microplate reader (Stat Fax 3200, Awareness Technology, USA) after 10 minutes. Controls without enzyme or without substrate were included. A standard curve of absorbance against amount of *p*-Nitrophenol was constructed to enable calculation of the amount of *p*-Nitrophenol released during the *α*-glucosidase assays. One unit enzyme is defined as the amount of the enzyme that catalyzes the production of 1 micro mole of *p*-Nitrophenol per minute. The protein concentration was measured by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (1970), using a 10% (w/v) polyacrylamide gel and the gel was stained with Coomassie Brilliant Blue R-250. For zymogram analysis, the above procedure was performed but the samples were loaded onto the gel without heating according to the semi-denaturing procedure (Gabriel and Wang, 1969). Briefly, the gel was immersed in 3 mM 4-MUαG in 0.1M sodium acetate (pH 5.0) for 10 minutes at room temperature to develop bands showing *α*-glucosidase activity. The blue-fluorescent bands appear in a few minutes under UV.

Effect of pH and Temperature

The activity of α-glucosidase was determined at several pH values using 20 mM glycine-phosphate-acetate-citrate buffer, adjusted to various pHs. The activity of the enzyme at different temperatures was determined by incubating the reaction mixture at different temperatures in this buffer (pH 6.0). Thermal stability of the enzyme was examined by incubating the enzyme at 35, 40 and 45°C in the buffer, pH 6.0 for a series of time intervals, followed by cooling on ice, and determining residual activity under standard assay conditions.

Kinetic Parameters and the Effects of Metal Ions and EDTA

The enzyme activities were determined at different substrate concentrations under optimum conditions. K_m, V_{max} and K_{cat} values were determined by Lineweaver–Burk plots.

The effects of chloride salts of various metal ions and EDTA on the activity of the enzyme were evaluated at concentrations of 10 and 20 mM of each in the reaction mixture.

Statistical Analysis

Three replicates were conducted for all the biochemical assays and data were subjected to analysis of variance (ANOVA). Statistical analyses were performed at P= 0.05 by Tukey's test using the SAS software.

RESULTS

Purification of the α-glucosidase and Determination of Its Molecular Mass

A crude extract of *N. aenescens* containing the α-glucosidase specific activity (1.675 U ml^{-1} or 1.675 μmol min^{-1} mg^{-1} protein) was purified through a three-step purification procedure. At first, homogenized and filtered sample was subjected to ammonium sulfate precipitation and then it was dialyzed and resulted in an increase of 1.25-fold in enzyme purity (Table 1). Precipitation was then followed by fractionation through a DEAE-sepharose anion exchange resin, in which six protein peak were visualized, one peak with each salt concentration. The fifth peak, eluted around 0.4M salt, corresponded to the α-glucosidase activity (Figure 1). After this step, a single band of protein was detected in SDS-PAGE. The molecular mass estimated on the gel was 48 kDa. Moreover, a single band was also detected in zymogram obtained by SDS-PAGE (Figure 2).

Kinetic Parameters

The K_m and V_{max} values for the α-glucosidase when pNαG was used as a substrate were estimated to be 0.54 mM and 3.55 mM min^{-1}, respectively. The K_{cat} was calculated at 3.62 min^{-1} from the V_{max} and purified enzyme concentration.

Table 1. Summary of the purification of *N. aenescens* α-glucosidase.

Step	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification fold
Crude enzyme	2314.88	1.675	100	1
Ammonium sulphate	884.26	2.095	38.198	1.25
(DEAE)-sepharose	348.83	24.67	15.06	14.72

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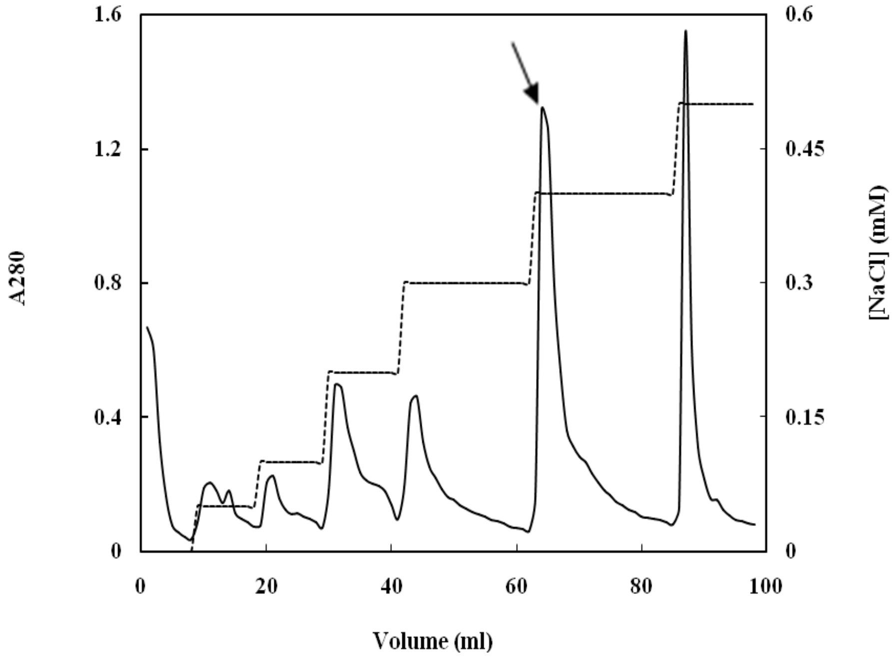

Figure 1. Elution profile of *N. aenescens* α-glucosidase on DEAE-sepharose column. The active peak is indicated. Arrow is pointing to the fifth peak, eluted around 0.4M salt, corresponding to the α-glucosidase activity.

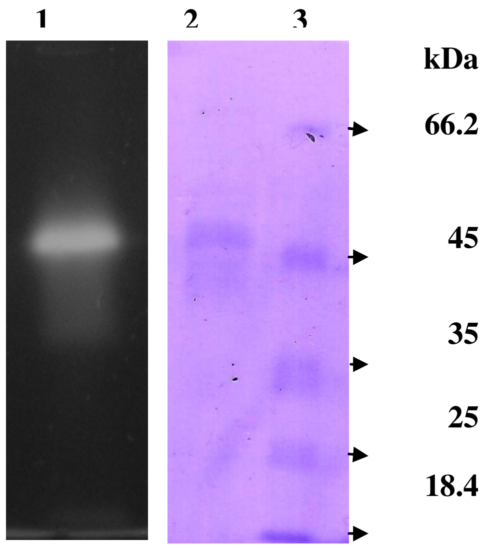

Figure 2. Analysis of purified α-glucosidase by SDS-PAGE. Lanes 1 and 2: Active fraction after ion exchange chromatography stained with histochemical and general staining, respectively; Lane 3: Molecular weight markers.

Effect of pH and Temperature on the Enzyme Activity

A typical bell-shaped pH activity curve was obtained for the α-glucosidase hydrolyzing pNαG substrate in a 40 mM glycine-phosphate-acetic-citric mixed buffer system. Maximum activity was observed at pH 6.0 (Figure 3-a). The optimum temperature for α-glucosidase purified from *N. aenescens* was obtained as 45°C (Figure 3-b).

Thermostability

The irreversible thermoinactivation of the enzyme was recorded in 20 mM phosphate-acetate-citrate buffer, pH 6.0, at 35, 40 and 45°C. As shown in Figure 4, *N. aenescens* α-glucosidase retained more than 80 and 75% of its original activity after 30 and 60

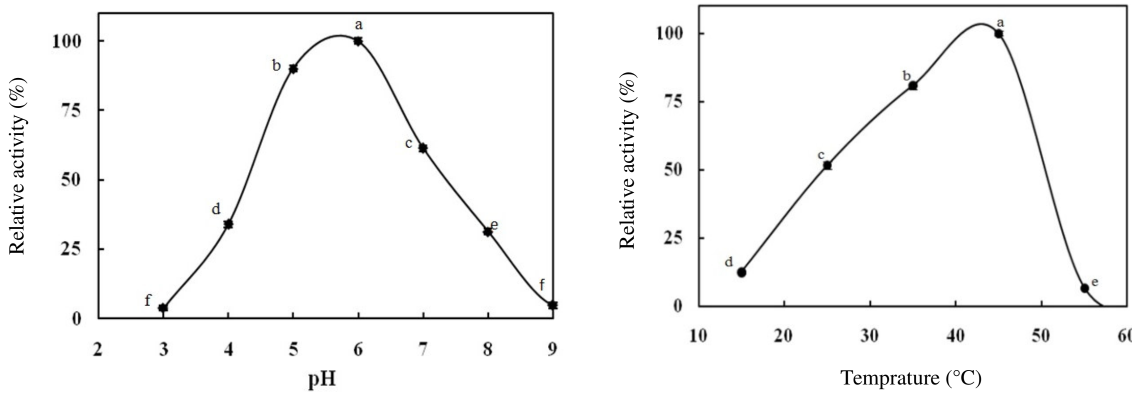

Figure 3. Effect of pH (a) and (b) temperature on the activity of *N. aenescens* α-glucosidase.Different letters indicate that the relative activity of enzymes is significantly different from each other by Tukey's test (P< 0.05).

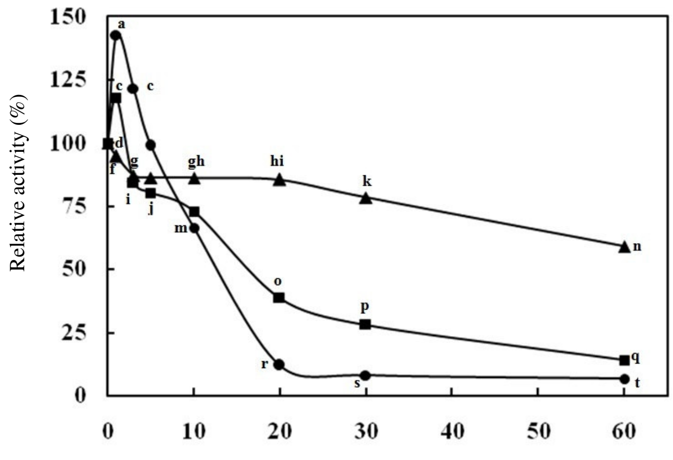

Figure 4. Irreversible thermoinactivation of the *N. aenescens* α-glucosidase at 35 (▲), 40 (■) and 45ºC (●). Different letters indicate that the relative activity of enzymes is significantly different from each other by Tukey's test (P< 0.05).

minutes of incubation at 35°C, respectively. However, the enzyme activity lost 40 and 10% of its original activity after 20 minutes of incubation at 40 and 45°C, respectively, as depicted in Figure 4; and after 30 minutes, a complete inactivation of the α-glucosidase occurred, but the enzyme was still active at 40°C after 60 minutes. Furthermore, activation was obtained for the enzyme in the initial times of incubation, particularly at 45°C.

Effect of Metal Ions and EDTA on Enzyme Activity

The α-glucosidase activity was measured at optimum pH in the presence of various metal ions and EDTA. As shown in Table 2, Zn^{2+}, Hg^{2+}, Co^{2+} in each concentrations of 10 and 20 mM and Ba^{+2} only in 20 mM strongly inhibited the α-glucosidase activity. Enzyme activity could not be affected by K^{+}

Table 2. Effects of metal ions and EDTA on the activity of *α*-glucosidase from *N. aenescens.* All metal ions were added as chloride salts.

Metal ion (mM)	Relative activity (%)
Control	100[fgh]
Ba^{2+}	
10	128.95±2.78[cd]
20	62.75±2.40[i]
K^{+}	
10	141.94±2.85[efgh]
20	113.32± 1.91[h]
Mn^{2+}	
10	121.21±0.01[def]
20	121.70±2.92[gh]
Co^{2+}	
10	21.76±1.19[jk]
20	31.17±0.67[j]
Mg^{2+}	
10	150.66±2.31[ab]
20	138.34±1.54[bc]
Hg^{2+}	
10	12.28±1.22[jkl]
20	0[l]
Ca^{2+}	
10	138.38±5.71[bc]
20	107.06±0.92[efg]
Zn^{2+}	
10	11.49±0.27[kl]
20	0.91±0.3[l]
Hg_2^{2+}	
10	95.81±4.5[gh]
20	166.37±1.72[a]
EDTA	
10	113.96±3.83[defg]
20	119.86±1.56[cde]

and Mn^{2+}. However, Ba^{2+} and Ca^{2+} only in 10 mM, EDTA and Hg^{2+} only in 20 mM, and Mg^{2+} in each concentration of 10 and 20 mM increased the enzyme activity significantly.

DISCUSSION

Purification of *α*-glucosidase from *N. aenescens* by DEAE-cellulose chromatography, showed a relatively high specific activity of 24.67 U mg^{-1} with a 14.72-fold purification. The specific activity of *α*-glucosidase was higher than those obtained for two *α*-glucosidases purified previously from *A. cerana indica* larval midgut (Chanchao *et al*., 2008). Purification of *α*-glucosidase from *A. cerana indica* by DEAE-cellulose and Superdex 200 columns resulted in a relatively high specific activity of 2.2 and 1.8 U mg^{-1} and with a purification fold of 3.1 and 2.6, respectively (Chanchao *et al*., 2008). The specific activity of crude *α*-glucosidase from whole alimentary canal of *N. aenescens* reported by Asadi *et al.* (2012) was 3.08 U mg^{-1} compared to 1.675 U mg^{-1} which was obtained in the present work. Considering that *α*-glucosidase in whole alimentary canal of *N. aenescens* showed two isoforms (Asadi *et al*., 2012), we succeeded to purify one isoform by DEAE-cellulose chromatography. Zymogram analysis in the present study showed homogeneity and revealed only a single band (Figure 2) for the fraction corresponding to the active peak of ion-exchange chromatography (Figure 1). This means that under purification processes, only one isoform could be purified. The molecular mass of *N. aenescens α*-glucosidase estimated by SDS-PAGE was 48 kDa which is different from that reported for purified *α*-glucosidases from *A. cerana indica* (68 kDa) (Chanchao *et al*., 2008), *A. mellifera* L (98 kDa) (Nishimoto *et al.*, 2001) and *A. cerana japonica* (76 kDa) (Wongchawalit *et al*., 2006), but it is within the range of the majority of other *α*-glucosidases (22-120 kDa) (Anindyawati *et al*., 1998; Kashiwabara *et al*., 2000; Nashiru *et al*., 2001; Nishimoto *et al*., 2001; Kato *et al*., 2002; Faridmoayer and Scaman, 2004; Torre-Bouscoulet *et al*., 2004; Yamamoto *et al*., 2004; Okuyama *et al*., 2005; Ezeji and Bahl, 2006; Naested *et al*., 2006; Wongchawalit *et al*., 2006; Chanchao *et al*., 2008; Carvalho *et al*., 2010). Alpha-glucosidase purified from whole *A. mellifera* adult by ammonium sulfate gave two *α*-glucosidase fractions with different solubility considered as *α*-glucosidase-I (highly soluble) and *α*-glucosidase-II (less soluble) (Huber and Mathison, 1976). SDS-

PAGE of these purified α-glucosidases showed relative molecular masses of 93 and 78 kDa for α-glucosidase-I and α-glucosidase-II, respectively. Our result showed that the relative molecular mass of α-glucosidase purified from *N. aenescens* midgut was less than those obtained for α-glucosidases purified previously from different species of *Apis* larval midgut (Nishimoto *et al*., 2001; Wongchawalit *et al*., 2006; Chanchao *et al*., 2008). An α-glucosidase was isolated from the midgut of larval sugar cane stalk borer, *Diatraea saccharalis* using mild-denaturing electrophoresis and it was further purified to near homogeneity by gel filtration (Carneiro *et al*., 2004). The results showed that this α-glucosidase appeared to have a relative molecular mass of 54 kDa. The membrane bound α-glucosidase in *Quesada gigas* (Hemiptera: Cicadidae) was solubilized using Triton X-100 and purified to homogeneity by means of gel filtration and ion-exchange chromatography. The results showed that the purified α-glucosidase was a protein with a pH optimum of 6.0 against the synthetic substrate pNαG and molecular weight of 61 kDa (Fonseca *et al*., 2010).

The K_m value of crude α-glucosidases in the *N. aenescens*'s alimentary canal was 3.96 mM (Asadi *et al*., 2012) and α-glucosidase in the whole alimentary canal of *N. aenescens* showed two isoforms. However, the K_m value of purified α-glucosidase from midgut for pNαG substrate was calculated at 0.54 mM and it showed much higher affinity of purified enzyme to substrate over crud enzyme. Furthermore, the K_m value is within the range of the majority of other α-glucosidases. For instance, the K_m values of purified α-glucosidases from *A. mellifera* L. and *A. cerana japonica* were 0.31 and 1 mM, when pNαG was used as a substrate (Nishimoto *et al*., 2001; Wongchawalit *et al*., 2006).

Alpha-glucosidase purified from *N. aenescens* midgut showed appropriate activity at acidic pH conditions and maximum activity was observed at pH 6.0 for this α-glucosidase (Figure 3-a). Optimum pH for activity of crude α-glucosidase from *N. aenescens*'s alimentary canal was also at pH 6.0 (Asadi *et al*., 2012). The pH of 5.0 was reported as optimal pH for the purified α-glucosidase from *A. cerana indica* (Chanchao *et al*., 2008). The effects of pH on the activity of α-glucosidase III from *A. mellifera* using maltose as the substrate showed that the pH optimum of the enzyme was 5.5, which did not differ greatly from α-glucosidase I and II showing pH optima of 5.0 (Nishimoto *et al*., 2001). Most of α-glucosidase, extracted from insects, exhibits pH optima ranging from 4.5 to 7.0 (Frandsen and Svensson, 1998; Ghadamyari *et al*., 2010; Ramzi and Hoseininaveh, 2010; Saberi *et al*., 2012). Nakonieczny *et al.* (2006) reported that optimal pH for α-glucosidase in the larvae of Apollo butterfly, *P. apollo* ssp. *Frankenbergeri* was between 4.9 and 5.6.

The optimum temperature for activity of purified α-glucosidase was obtained as 45˚C (Figure 3-b) which was equal to crud α-glucosidase (Asadi *et al*., 2012). The irreversible thermoinactivation of the enzyme showed that it was highly stable at 35ºC but moderately stable at 40 and 45ºC (Figure 4). Wongchawalit *et al.* (2006) showed that α-glucosidase from Japanese honeybee was stable in a temperature-range up to 40°C. The optimal temperature for the purified α-glucosidase from *A. cerana indica* was 50°C (Chanchao *et al*., 2008). *A. mellifera* α-glucosidase III was stable up to 40°C, but it lost the activity completely by incubation at 60°C for 15 minutes (Nishimoto *et al*., 2001). Several purified α-glucosidases from various sources showed optimum temperature ranging from 50 to 70°C (Anindyawati *et al*., 1998; Martino *et al*., 2001; Tanaka *et al*., 2002; Zdzieblo and Synowiecki, 2002; Iwata *et al*., 2003; Bravo-Torres *et al*., 2004; Yamamoto *et al*., 2004; Okuyama *et al*., 2005; Ezeji and Bahl, 2006; Giannesi *et al*., 2006; Zhou *et al*., 2009).

Results of the present study indicated that Zn^{2+}, Hg^{2+}, and Co^{2+} in each concentrations of 10 and 20 mM and Ba^{2+} only in 20 mM strongly inhibited the α-glucosidase activity and K^+, Mn^{2+}, Mg^{2+} and EDTA in each concentrations of 10 and 20 mM, Ba^{2+} and Ca^{2+} only in 10 mM and Hg_2^{2+} only in 20 mM significantly increased the enzyme activity. Asadi *et al*. (2012) showed that in the presence of Fe^{2+}, Mn^{2+}, Hg^+, and Zn^{2+} (10, 20 mM) and Hg^{2+} (20 mM), crude *α*-glucosidase from alimentary canal were completely inactivated. Carvalho *et al*. (2010) reported that in the presence of Na^+, Ba^{2+}, Co^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Al^{3+}, Zn^{2+} and Ca^{2+}, *α*-glucosidase from *Thermoascus aurantiacus* CBMAI-756 maintained 90-105% of its maximum activity and was inhibited by Cr^{3+}, Ag^+, and Hg^{2+} (Carvalho *et al*., 2010). Alpha-glucosidase Purified from *Chaetomium thermophilum* var. coprophilum was completely inhibited by 1 mM Hg^{2+} and Ag^{2+}, while Al^{+3}, Zn^{2+}, Co^{2+} and Cu^{2+} inhibited 35, 70, 51, and 46%, respectively. Most of other ions, such as Mg^{2+}, Ca^{2+}, Ba^{2+}, K^+, Fe^{2+}, NH^{4+} and Mn^{2+}, tested at 1 mM concentration, or EDTA were without effect (Giannesi *et al*., 2006). The effect of metal ions and other chemical reagents were examined on the *G. pyloalis* *α*-glucosidase activity. The results showed that $CaCl_2$ (40 mM) increased *α*-glucosidase activity and, also, the *α*-glucosidase activity was enhanced with increase in concentration of EDTA. Different concentrations of $MgCl_2$ and KCl (5, 10, 20 and 40) did not have any effect on *α*-glucosidase activity in this insect (Ghadamyari *et al*., 2010).

In conclusion, this work was done on the purification and characterization of glucosidases in phytophagous insects and research in this field may enable us to control pests by inhibiting or by disturbing these enzymes in the insect pests. Glucosidase inhibitors are attractive candidates for pest control and these inhibitors are widely distributed in plants and can be synthesized as pesticides. Using insect specific toxic proteins expressed in genetically modified crops is a safe method in integrated pest management. The first step in the production of resistant plants containing toxic proteins against insect digestive enzymes is the purification and characterization of digestive enzymes and it is possible to create plants that are resistant to pest.

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خالص سازی و تعیین ویژگی های بیوشیمیایی آنزیم آلفا-گلوکوزیداز روده میانی لاروهای کرم سبز برنج، *Naranga aenescens* Moore

ن. معماری زاده، پ. زمانی، ر. ح. ساجدی، و م. قدمیاری

چکیده

کاربرد آفت کش های شیمیایی به طور چشمگیری در کل جهان در حال افزایش است که می‌تواند منجر به افزایش نگرانی‌های زیست محیطی شود. یکی از رویه های امیدوارکننده در جهت به حداقل رساندن آلودگی‌های زیست محیطی، تولید گیاهان حاوی پروتئین‌های سمی و مقاوم در مقابل حشرات آفت است. با توجه به اهمیت خالص سازی و تعیین ویژگی های آنزیم های گوارشی در تولید گیاهان مقاوم، در این مطالعه آلفا-گلوکوزیداز تولید شده توسط روده میانی *Naranga aenescens* Moore به واسطه ی رسوب گذاری با سولفات آمونیوم، کروماتوگرافی به روش تعویض یونی با DEAE-sepharose و تغلیظ با اولترافیلتراسیون صورت گرفت. وزن مولکولی مشهود آنزیم به وسیله ی SDS-PAGE، ۴۸ kDa تخمین زده شد. pH و دمای بهینه ی آنزیم به ترتیب ۶ و ۴۵ درجه سانتیگراد بود. غیرفعال کننده گی برگشت ناپذیر حرارتی آنزیم نشان داد که این آنزیم در دمای ۳۵ درجه سانتیگراد بسیار پایدار ولی در دماهای ۴۰ و۴۵ درجه سانتیگراد پایداری متوسط دارد. Zn^{2+}، Hg^{2+}، Co^{2+} در ۱۰ و۲۰ میلی مولار و Ba^{+2} تنها در ۲۰ میلی مولار به شدت فعالیت آلفا-گلوکوزیداز را بازداشتند. Ba^{2+} و Ca^{2+} تنها در ۱۰ میلی مولار، EDTA و Hg_2^{2+} تنها در ۲۰ میلی مولار و Mg^{2+} در ۱۰ و ۲۰ میلی مولار به طور معنی دار فعالیت آنزیم را افزایش دادند. مقادیر K_m و K_{cat} برای آلفا گلوکوزیداز، زمانی که *p*-Nitrophenyl-α-D-glucopyranoside (pNαG) به عنوان سوبسترا استفاده شد، به ترتیب ۰/۵۴ میلی مولار و ۳/۶۲ بر دقیقه بود.