IDENTIFICATION AND CHARACTERIZATION OF ALPHA-AMYLASE IN THE ITALIAN LOCUST, *CALLIPTAMUS ITALICUS* (LINNAEUS, 1758) (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT: *Calliptamus italicus* (linnaeus, 1758) (Orthoptera: Acrididae) is a polyphagous locust which widely distributed throughout of Europe, Middle East and North Africa. Adults and nymphs are pests of great number of plants including cereals, fabaceous, red and sugar-beet, many solanaceous, cruciferous, sunflower, vegetable and young plants of various fruit plants. The present study for the first time showed that α-amylase is present in the *C. italicus*. Alpha-amylases have been found to be active in different insect species. Italian Locust α-amylase activity pH was determined to be around 8. Generally, optimal pH is corresponding to the pH prevailing in the midgut from which the enzyme has been extracted. The effect of temperature on the enzyme activity was determined showing that the enzyme was active from 20°C to more than 70°C. Optimum temperature was determined to be 40°C. Sharp decrease in enzyme activity was seen at low temperature e.g. temperature lower than 30°C. It seems that the enzyme was more active at high temperatures than low ones. The enzyme was active on the wide range of the temperatures from 30 to 60°C. Even at 60°C more than 50% of the enzyme activity was present. However, at 70°C only about 30% of the enzyme activity was left and more than 70% of the enzyme activity was lost. Native gel electrophoresis analysis showed that a mixture of different iso-enzyme was present in the gut homogenate. The analysis was shown that 9.0 amylase bands were seen in the gel thus the insect use an array of different alpha amylases for digestion of their food in the midgut. In conclusion it should be mentioned that because the Italian locust feed on different plant species, it needs different α-amylases for the digestion of some carbohydrate the insect encounter during feeding.

KEY WORDS: Italian locust, α-amylase, gut, temperature, pH.

Calliptamus italicus (linnaeus 1758) (Orthoptera: Acrididae) is a polyphagous species that widely distributed throughout Europe, Middle East and North Africa. *C. italicus* have migratory behavior and move short distances. Some years, due to changes in weather conditions and dry vegetation in the permanent center, they attack and damage the adjacent fields. This pest is considered as the important pests of cereals, cotton and grass.The insect lays eggs in soils of varying densities in stations with a sharp xerophytic nature and with rather sparse vegetation, in depth of 5-10 mm from soil surface. Dry steppes and semi-deserts with mosaic sward are optimum for the Italian Locust. The localities are characterized by a circle of preferable plants, drought-resistant dicotyledonous.

The locust density in undisturbed herbaceous localities in the south of Europe is usually insignificant (to 1 individual per sq. mile), but it is much more widespread there. In the north central part of its area it is sparsely distributed. The main regions of mass outbreaks are located in Kazakhstan and in the south of Western Siberia (Sergeev et al., 1986). They sometimes change their location for several kilometers to feed, but the inclination of the Italian Locust to wormwood sites is rather clear. Only 5th instar larvae and imagoes start to settle other types

of localities. After completion of the insect feeding and maturation periods, they come back to permanent inhabitants. This pest has one generation per year and the damage occurs more in mountainous areas and hills (Poppov, 1987).

The larvae and adults greatly harm sowings of grain cereals, fabaceous, red and sugar-beet, many solanaceous, cruciferous, gourds, poppy, sunflower, poligonaceous and medicinal cultures, cotton, flax, Palma Christi, vegetable and volatile-oil-bearing plants, hemp, sesame, ambary, young plants of various fruit, berrylike and forest arboreal and bushy species, grapes, and also pastures and hay lands. In the south of Europe mass outbreaks occur in arid years. In the south of Kazakhstan and Kyrgyzstan, in plain parts of Uzbekistan and Turkmenistan the species can reproduce greatly along river valleys and oasis, being quite often serious pests of cotton and alfalfa (Lachininskii et al., 2002).

a-Amylases (a-1,4-glucan-4-glucanohydrolase; EC 3.2.1.1) constitute a family of hydrolases that cleave a-D (1,4)- glucan linkages in starch components, glycogen and various other related carbohydrates. They are the most important digestive enzymes of many insects that feed exclusively on plants during larval and/or adult life. They convert starch to maltose, which is then hydrolyzed to glucose by α-glucosidase. In insects, only α-amylases that hydrolyse α-1, 4- glucan chains such as starch or glycogen have been found (Terra et al., 1999). Locust feeds on grasses and the other plants which are rich in carbohydrate thus α-amylases should be important enzyme in their digestion process. So far, no studies have been done to characterize α-amylase activity in the gut system of the locust. The aim of the current study was to identify and characterize α-amylase activity in the locust gut and determine their isozyme conditions.

MATERIALS AND METHODS

Insect

Insect were collected from cultivated farmlands in the College of Agriculture and Natural Resources, University of Tehran, during summer. The insects were transferred and kept in the Laboratory at 25°C, and light; dark 12: 12h.

Sample preparation and enzyme assays

Sample preparation was done as described by Allahyari et al. (2010) with slight modifications. Briefly, Adult insect were placed on ice (about 5 min) for immobilization and dissected under light microscope. Insect gut was removed and homogenized in pre-cooled homogenizer (Teflon pestle) in distilled water. Samples then were put in the 1.5 ml centrifuge tubes and centrifuged at 15000 rpm for 15 min at 4 °C. Supernatant was separated and kept at -20 °C for subsequent analysis as an enzyme source.

α-Amylase assay

α-Amylase activity was assayed using the dinitrosalicylic acid (DNS) procedure (Bernfeld, 1955), using 1 % soluble starch (Merck, Darmstadt, Germany) as the substrate as described by Bandai et al. (2009). Ten microliter of the enzyme in addition to 10 microliter substrate (soluble starch) and 80 microliter universal buffer (0.02 M) containing succinate, glycine and 2-morpholinoethanesulfonic acid (pH 7) were incubated in 30 min at 35 °C. After addition of DNS (100µ) and heating in the boiling water for 10 min, the reaction was stopped. Then, absorbance was read at 540 nm. As a blank distilled water was used, instead of enzyme. All assays were repeated at least two times.

Effect of PH and temperature on enzyme activity
The effects of temperature and pH on α-amylase activity were examined. Optimal pH was determined using universal buffer with pH set at 3, 4, 5, 6, 7, 8, 9 and 10. The effect of temperature on α-amylase activity was determined by incubating the reaction mixture at 20, 30, 35, 40, 45, 50, 60 and 70 °C for 30 min followed by measurement of activity as described before.

Electrophoresis
The amylase present in crude homogenates of gut extract was subjected to SDS–polyacrylamide gel electro-phoresis (PAGE) as described by Laemmli (1970) and Campos et al. (1989) and Mehrabadi et al. (2010). SDS–PAGE was performed in a 10 % (w/v) separating gel and a 5 % stacking gel, both with 0.05 % SDS. The electrode buffer was prepared based on the method of Laemmli (1970), but SDS was not used. The sample buffer contained 25 % stacking buffer (0.5 mol/ L Tris–HCl [pH 6.8]), 20 % glycerol, 2 % SDS, 0.005 % (w/v) bromophenol blue, but no mercaptoethanol, and it was not heated. Electrophoresis was conducted at 4°C at 120 V until the blue dye reached the bottom of the slab gel. To prepare gels for α–amylase assay, the gel was rinsed with water and washed by shaking gently with 1 % (v/v) Triton X-100 in phosphate buffer [pH 7] containing 2 mmol/lCaCl2 and 10 mmol/l NaCl for 1.5 h. Staining the gel with 0.05% KI and 0.05% I2 solution visualized α-amylase activity as light bands in dark background.

Protein determination
Protein determination was done based on Bradford (1976) and bovine serum albumin (Bio-Rad, München, Germany) used as a standard.

RESULTS

α-Amylase activity
In this study using starch as a substrate it was determined that α-amylase activity was present in the Italian Locust, *Calliptamus italicus*. The activity of the enzyme was 0.022 U/ml.

Effect of PH and temperature on enzyme activity
The α-amylases activity in the locust was determined to be present over a wide range of pH. Activity was seen at pH 2.0 to 12.0. However, the enzyme activity was the highest at pH 8.0 (Fig. 2). The enzyme activity was decreased sharply after pH 9.0 but steadily increase in enzyme activity was seen from pH 3.0 to 9.0. Thus it could be said that the enzyme is more active in the acidic region than in alkaline region.

The effect of temperature on the enzyme activity was determined showing that the enzyme was active from 20°C to more than 70°C (Fig. 3). Optimum temperature was determined to be 40°C. Sharp decrease in enzyme activity was seen at low temperature e.g. temperature lower than 30°C. It seems that the enzyme was more active at high temperatures than low ones. As seen in the figure (Fig. 3) the enzyme was active on the wide range of the temperatures from 30 to 60°C. Even at 60°C more than 50% of the enzyme activity was present. However, at 70°C only about 30% of the enzyme activity was left and more than 70% of the enzyme activity was lost. At 30°C also something about 40% of the enzyme activity was present and 60% of the enzyme activity was lost.

Electrophoresis

Native gel electrophoresis analysis showed that a mixture of different iso-enzyme was present in the gut homogenate (Fig. 4). The analysis was shown that 9.0 amylase bands were seen in the gel thus the insect use an array of different alpha amylase for digestion of their food in the midgut.

DISCUSSION

The Italian locust *Calliptamus italicus* L. (Orthoptera: Acrididae) is a polyphagous species that feed on a variety of plant species from grasses and cereals crop to cotton and fruit plants including grape. It also creates damage on pastures and hay lands thus it considered a serious pest in many places depends on type of plants growing. The current study showed that the same trend was seen in the α-amylase activity of the insect feeding on the different plant species. The α-amylase was active on a wide range of pH values from acidic to alkaline conditions. It has been reported that pH of the Orthopteran insect is acidic to neutral (Romoser & Stopholano, 1998). However, in the current study was shown that the enzyme had optimum activity at pH 8.0. It has been reported that optimal pH correspond to the pH prevailing in the midgut of the insect which amylase has been isolated (Terra et al., 1996). High pH gut has been reported for the Lepidoptera thus optimal pH for the α-amylase activity in these insects has been shown to be high (> 7.0).There are reports that pH values of 9.0 for *Chilo suppressalis* (Lepidoptera: Crambidae), 9.2 for *A. mylitta*, 12.0 for *Acherontia atropos* (Lepidoptera: Sphingidae), 10.8 for Lasiocampa quercus (Lepidoptera: Lasiocampidae), 11.3 for *Manduca sexta* (Lepidoptera: Sphingidae) and 10.8 *Lichnoptera felina* (Lepidoptera: Noctuidae) (Dow 1984; Zibaee et al,2009). It has been reported that high values of gut pH in insects is adaptation to feed on plant materials rich in tannins (Chapman, 1998), which at lower pH values bind to proteins thus decreasing the digestion efficiency (Dow, 1986). In the current study it was found that α-amylase was active at pH 8.0. So these discrepancies in the enzyme activity among different insect species could be attributed to the different feeding habits and feeding sources. Because of living on a starch-rich diet, many insects depend on the effectiveness of their amylases for survival. In insects α-amylases are synthesized and secreted by midgut epithelial cells, along with other digestive enzymes (Baker, 1983; Terra & Ferreira, 2005). Amylases have been investigated in Coleoptera, Hymenoptera, Diptera, Lepidoptera and Heteroptera by several researchers (Santos & Terra, 1986; Baker, 1991; Terra et al., 1988; Ferreira & Terra, 1989; Schumaker et al., 1993; Ferreira et al., 1994; Mehrabadi et al., 2009).

Optimum temperature for α-amylase activity was shown to be at 40.0^{oC} which can be attributed to the environment that the insect live and feed. Interestingly, Gel electrophoresis showed that a mixture of different α-amylase enzyme was present in the insect gut. A mixture of different α-amylase isoenzymes has been reported for other insects such as *Sitophilus oryzae*, *Tribolium castaneum*, *Anthonomus grandis*, *C. maculates*, *R. dominica*, *S. granarium*, *E. intergriceps* (Terra et al., 1988; Chen et al., 1992; Oliveira-Neto et al., 2003; Kazzazi et al., 2005; Mehrabadi et al., 2011). Presence of different α-amylase isoenzymes could be related to importance of this enzyme in the insect food digestion.

In conclusion it should be mentioned that because the Italian locust feed on different plant species, it needs different α-amylases for the digestion of some carbohydrate the insect encounter during feeding. Thus, since the insect rely on

carbohydrate digestion for the growth and development it does have a mixture of isoenzymes.

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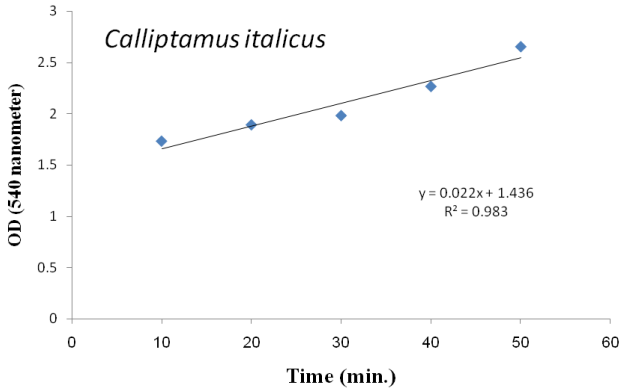

Figure 1. α-Amylase activity of the the *Calliptamus italicus*.

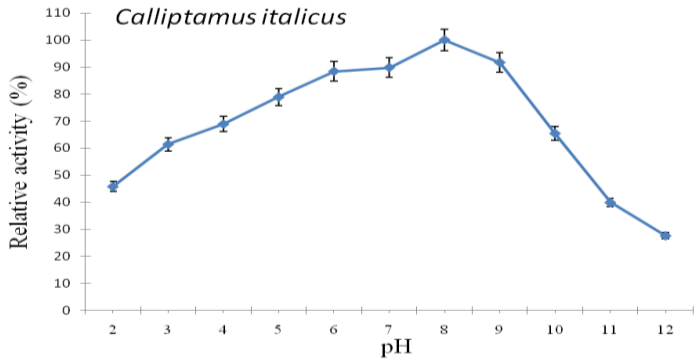

Figure 2. The effect of pH on the activity of the α-amylase activity of the *Calliptamus italicus*.

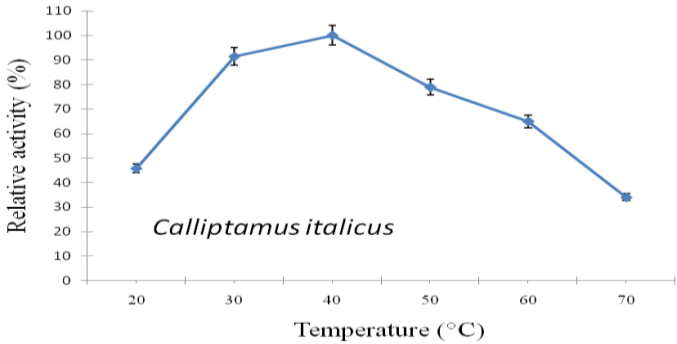

Figure 3. The effect of temperature on the α-amylase activity of the *Calliptamus italicus*.

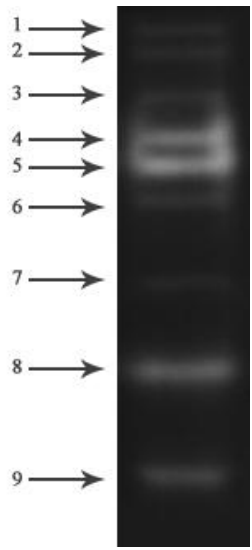

Figure 4. Native gel electrophoresis of α-amylase activity of the *Calliptamus italicus*.