SUPPLEMENT: SHORT COMMUNICATIONS

Arch. Biol. Sci., Belgrade, 56 (1-2), 1P—2P, 2004.

ISOFORMS OF LEUCYL–AMINOPEPTIDASE OF *CERAMBYX CERDO* (COLEOPTERA, CERAMBYCIDAE) LARVAE.

N. Božić[1], Z. Vujčić[2], V. Nenadović[3] and J. Ivanović[3], *Department of Chemistry, Institute of Chemistry, Technology and Metallurgy*, Studentski trg 12–16, 11000 Belgrade; *Department of Biochemistry, Faculty of Chemistry, University of Belgrade*, Studentski trg 12–16, 11000 Belgrade; *"Siniša Stanković" Institute of Biological Research, Department of Insect Physiology and Biochemistry*, 29 Novembar 142, 11000 Belgrade, Serbia and Montenegro

UDC 595.768 : 577.151.64

There are a very few literature data about *C. cerdo* proteolytic enzymes (Ivanović and Milanović 1970, Nenadović *et al.*, 1982; 1994; 1999); among a report them is concerning leucyl–aminopeptidase (LAP) (Božić *et al.*, 2001) which has been shown to be the most abundant enzyme in the midgut of *C. cerdo* larvae. It is also the most abundant enzyme in the midgut of the species *Morimus funereus*, likewise a member of the family Cerambycidae (Božić *et al.*, 2003). In the latter species, there are four isoforms, that differ in efficiency and specificity. This could be of great influence in maintenance of the high polyphagy of *M. funereus*, which attacks a number of deciduous and coniferous trees, because isoenzymes provide increased capability of the organism to adapt to different sources of food and to overcome the activities of plant proteinase inhibitors (Wagner *et al.*, 2002).

Cerambux cerdo favors all species of the genus Quercus, but can also be found on beech, ash, and walnut trees in Russia (Nenadović *et al.*,1999), thus having a much narrower host range than *M. funereus*. From that point of view, it was interesting to find out how many isoforms of leucyl–aminopeptidase are present in the midgut of *C. cerdo* larvae and clarify whether there is any connection between this enzyme and polyphagy in some members of the family Cerambycidae.

Larvae collected outdoors in December on the mountain of Fruška Gora, were used in the present work. Midguts were dissected out, weighed, and homogenized with a pre–chilled mortar and pestle in 4 vol. (g/ml) of ice–cold 50 mM Tris buffer, pH 7.5, with the addition of quartz sand. After centrifugation, the resulting supernatants were treated with an equal volume of carbon tetrachloride for lipid removal and centrifuged again (Božić *et al.*, 2003). Protein concentration was determined in the extract (Bradford, 1976).

A measured volume of midgut extract of *C. cerdo* larvae was loaded onto a Sephadex G–100 column (1,6 × 60 cm, Pharmacia, Uppsala, Sweden) equilibrated with 0.15 M NaCl in 20 mM acetate buffer, pH 6.0, and calibrated with molecular weight markers. Fractions of 2 ml were collected and A_{280} values monitored. Fractions containing LAP activity were pooled, and protein concentration and LAP activity were determined. The results are summarized in Fig. 1. Since enzyme activities were detected in the fraction responding to column void volume, the estimated molecular mass of partially purified LAP was over 100 kDa.

The activity of leucyl–aminopeptidase was determined using specific the chromogenic substrate leucine–*p*–nitroanilide (Lee and Anstee, 1995). Reaction mixtures contained 5 µl of crude midgut extracts and 20 µl of the fraction in 0.5 ml of 50 mM Tris–HCl buffer with pH 8.0, which has been shown to be the pH optimum for LAP in this species (Božić *et al.*, 2001), and 1 mM of substrate dissolved in N,N–dimetilformamide. The reaction run for 5 min at 30°C. After that, enzymatic reactions were terminated by adding 0.1 ml 30% acetic acid. The concentration of the resulting p–nitroaniline was estimated by measuring absorbance at 410 nm (Erlanger *et al.*, 1961).

Native slab 10 % polyacrylamide gel electrophoresis (Davis, 1964) was used for detection of LAP isoforms. Activity of LAP was detected by zymogram analysis based on the formation of azo–color (Božić *et al.*, 2003). The semipreparative gel contained 100 µl of crude midgut extract (2,5 U/ml) (lane B) and 300 µl of partially purified LAP (0.8 U/ml) (lane A) (Fig. 2). Two bands of LAP activity in the crude extract as well as in the partially purified fraction were visible in the zymogram resolved by native pag.

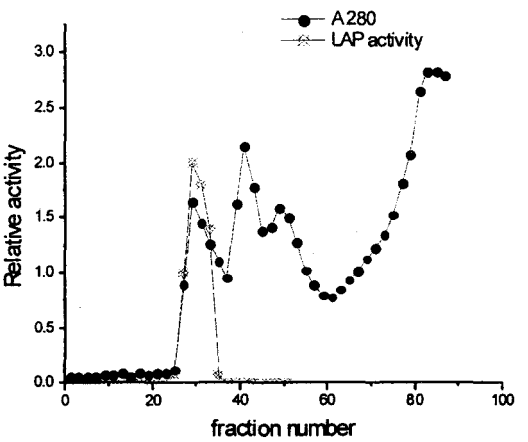

Figure 1.

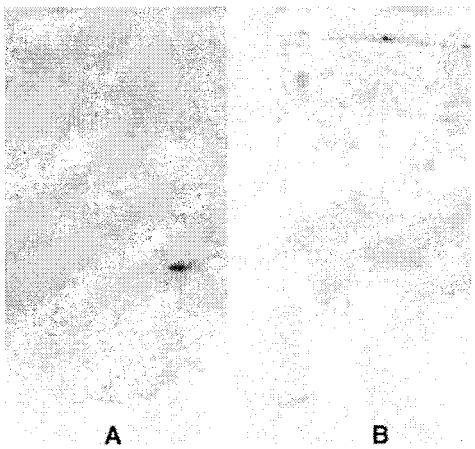

Figure 2.

Leucyl–aminopeptidase in the midgut of *C. cerdo* larvae exists in two forms. In view of the possible role of isoenzymes in polyphagy of *M. funereus* larvae and the wider host range of the species *M. funereus*, these results were expected. They enable us to assume that LAP is a potential marker for cerambycid beetles, not only through its existence, but also through the presence of different forms of it.

Acknowledgments — This work was supported by the Ministry of Science, Technology, and Development of Serbia under projects nos. 1615 and 1755.

References: Božić, N., Vujčić, Z., Janković–Tomanić, M., Ivanović, J. and Nenadović, V. (2001). *Acta Entomologica serbica* **6** (1/2), 143–146. – Božić, N., Vujčić, Z., Nenadović, V. and Ivanović, J. (2003). *Comp. Biochem. Physiol.* **134B**, 231–241. – Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254. – Davis, B. J. (1964). *Ann. NY Acad. Sci.* **121**, 404–427. – Erlanger, B. F., Kokowsky, N. and Cohen, W. (1961). *Arch. Biochem. Biophys.* **95**, 271–178. – Ivanović, J. and Milanović, M. (1970). Zbornik Za Prirodne Nauke, **39/70**, 171–175 (In Serbian). – Lee, M. J. and Anstee, J. H. (1995). *Insect. Biochem. Mol. Biol.* **25**, 63–71. – Nenadović, V., Janković–Hladni, M., Ivanović, J., Stanić, V. and Marović, R. (1982). *Acta Entomologica Yugoslavica.* **18** (1/2), 91–96. – Nenadović, V., Janković–Hladni, M., Prolić, Z. and Ivanović, J. (1994). In: Zaštita Bilja Danas I Sutra (Eds: Šestović, M., Nešković, N. and Perić, I.) 303–314 (In Serbian), Društvo Za Zaštitu Bilja Srbije. – Nenadović, V., Prolić, Z., Lazarević, J., Al Arid, L. and Ivanović, J. (1999). *Acta Veterinaria*, **49** (2–3), 105–116. – Wagner, W., Mohrlen, F. and Schnetter, W. (2002). Insect. Biochem. Mol. Biol. **32**, 801–812.