

Housefly larvae aminopeptidase separated by gel chromatography and PAGE

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Abstract

Statement of the Problem: Housefly larvae are used in alternative therapy for better wound healing. It is accepted an idea that endopeptidases and exopeptidases are involved in these processes. In many insects predominant peptidolytic activity belongs to serine proteinases of two different subclasses, trypsin-like and elastase-like. Aminopeptidases and carboxypeptidases play also an important role as described in literature. **Methodology & Theoretical Orientation:** After delipidation by chloroform supernatant fraction of larval stage II was loaded on column of Sephadex G-200 (2.5 x 90 cm) equilibrated in 100 mM Tris/HCl buffer of pH 8.0. Elution was performed by 100 mM Tris/HCl buffer with a flow rate 12 ml.h⁻¹. Aliquot of each fraction was estimated on enzymatic activity with L-leucine p-nitroanilide (L-Leu-p-NA; aminopeptidase), N_α-benzoyl-DL-arginine p-nitroanilide hydrochloride (DL-BAPA; trypsin-like) and N-succinyl-alanyl-alanyl-alanine p-nitroanilide (N-Suc-Ala₃-p-NA; elastase-like). Column was calibrated by blue dextran. Separation of aminopeptidases of three larval stages was performed in 4% polyacrylamide gel at 20 mA for 1 hour at 4 °C. Aminopeptidases on zymogram were detected by diazotization of liberated p-nitroaniline with sodium nitrite and visualized with 1-naphthylamine. **Findings:** Larval peptidolytic enzymes were separated by gel chromatography into several peaks with different enzyme activities and molecular weight. Principal enzymes with catalytic activity to L-Leu-p-NA were eluted with, or immediately after elution volume of blue dextran. Estimated molecular weight for enzymes of aminopeptidase nature was approximately 200 kDa. Using leucine-p-nitroanilide as substrate two leucine aminopeptidase activities were found in all three stages of housefly development. **Conclusion & Significance:** Using gel chromatography it was possible partially purify leucine aminopeptidase from housefly larvae. Electrophoretogram of aminopeptidases showed two fractions probably reflecting two isoforms of the enzyme.

Image

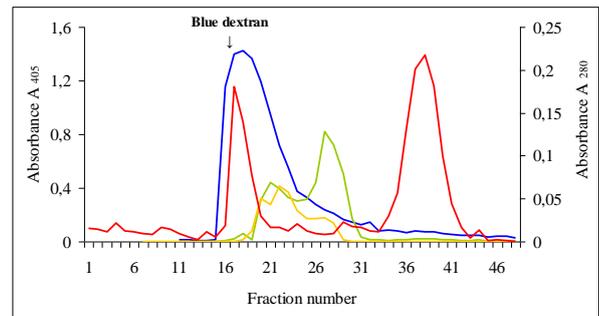


Figure 1: Gel chromatography of delipidated crude extract of larvae of housefly *Musca domestica* on Sephadex G-200 column. Protein concentration was measured at 280 nm (red). The enzyme activities of each fraction were estimated by reading of reaction product p-nitroaniline at 405 nm released of chromogenic substrates L-Leu-p-NA (blue), DL-BAPA (green), N-Suc-Ala₃-p-NA (yellow).



Figure 2: Zymogram of leucine aminopeptidases detected in three larval stages of housefly development.

Recent Publications

1. Blahovec J, Kostecka Z, Kocisova A (2006) Peptidolytic enzymes in different larval stadium of housefly *Musca domestica*. *Veterinary Medicine Czech* 51: 139-144.
2. Božić N, Vujčić Z, Nenadović V, Ivanović J (2003) Partial purification and characterization of midgut leucyl aminopeptidase of *Morimus funereus* (Coleoptera: Cerambycidae) larvae. *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology* 134: 231-241.
3. Božić N, Vujčić Z. (2005) Detection and quantification of leucyl aminopeptidase after native electrophoresis using leucine-p-nitroanilide. *Electrophoresis* 26: 2476-2480.
4. Valencia JW, de Sá MF, Jiménez AV (2014) Activity of leucine aminopeptidase of *Telchin licus licus*: an important insect pest of sugarcane. *Protein and Peptide Letters* 21:535-541.



Biography

Zuzana Kostecka has her experience in isolation of insulin-like growth factors and their binding proteins from ruminant amniotic fluids including their influence on proliferative activity of different target cells. She is interested in enzymology mainly peptidases of insect nature. Results of her research are applied in the education of students at the University of Veterinary Medicine and Pharmacy in Kosice in the field of biochemistry.

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