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Housefly larvae aminopeptidase separated by gel chromatography and PAGE

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Statement of the Problem: Housefly larvae are used in alternative therapy for better wound healing. It has been proved 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 also play 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 (2.5x90 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 to partially purify leucine aminopeptidase from housefly larvae. Electrophoretogram of aminopeptidases showed two fractions probably reflecting two isoforms of the enzyme.

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 from insects. 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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