INSECT DIGESTION: MOLECULAR, CELLULAR, PHYSIOLOGICAL AND EVOLUTIONARY STUDIES

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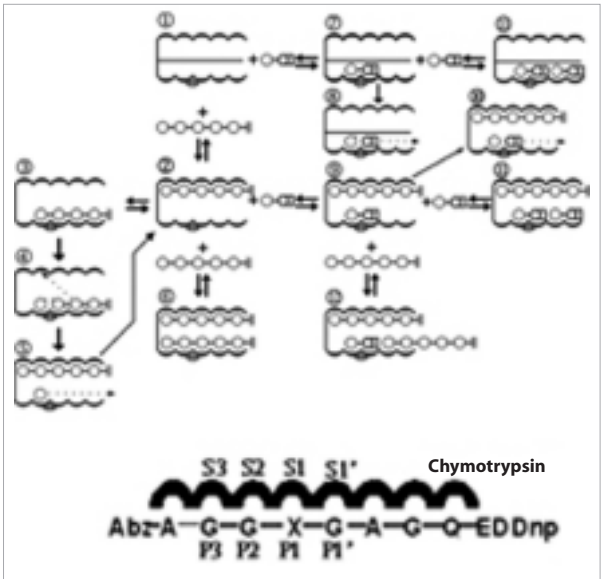


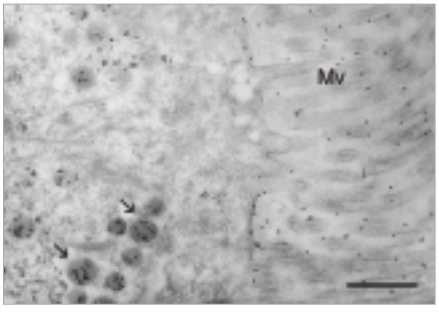
P. americana	0.6	0.1	Y	0.3
T. molitor	1.0	1.7	Y	0.5
D. saccharatis	1.8	0.2	Y	-1.5

Top: model for the action of a digestive enzyme from Abracris flavolineata. *Biochim.Biophys. Acta* ***1774****: 1079-1091 (2007). Bottom: insect chymotrypsin primary specificity (P1) and hydrophobicity (the figures) of their subsites. Insect Biochem. Molec. Biol.* ***38****: 626-633 (2008).*

Insects destroy about 10% of the word biomass each year and carry diseases to plants and animals, including man. However, the use of chemical pesticides to control insects causes environmental damages. The information that insect midgut is a large and relatively unprotected surface requires midgut studies to search for new targets of insect control. The insect midgut physiology needs a sophisticated understanding at the molecular level. It requires knowledge on digestive enzymes at the molecular structural level, as well as other proteins associated with midgut function like transporters, receptors, pumps and proteins participating in the enzyme secretory machinery. This project deals with these subjects in 4 main research lines: (a) digestive enzymology; (b) molecular midgut physiology; (c) midgut secretory enzyme machinery, and (d) evolution of digestive systems. Among our main goals is the study of structure and kinetics of digestive enzymes that led insects to overcome plant chemical defenses. The following enzymes will be studied within model insects: trypsin; chymotrypsin; cathepsins L and D; amino peptidase; trehalase; β-glucosidase; β-1,3-glucanase; α-mannosidase, and α-amylases. The development of midgut molecular physiology models of *D. peruvianus, T. molitor, M. domestica* and *D. saccharalis* will be based on several steps: (a) random sequencing of midgut cDNA libraries; (b) antibody for protein studies; (c) immunocytolocalization of recombinant proteins; (d) proposition of physiological roles for proteins, and (e) protein silencing by RNAi. Studies of insect digestive enzyme secretory mechanisms may result in valuable contributions to the physiology of digestion in insects and to cell biology. Screening midgut cDNA expression libraries with antibodies raised against proteins of microvilli cytoskeleton (*S. frugiperda*), cell apex (*T. molitor*) and microvillar membrane (both insects) will lead to the identification of positive clones. These will support secretory mechanism hypotheses following procedures including those described for the study of molecular midgut physiology.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A study of the organization of digestion in the neglected major coleopteran group Dermestidae was performed to improve details on the evolution of digestive systems hypothesis. The major amino peptidase from the aphid *Acyrtosiphon pisum* was shown to be a lectin receptor such as a target for new control strategies. Chymotrypsins were purified from insects of 3 different orders and were compared regarding their substrate specificities with internally quenched fluorescent oligopeptides. They differ characteristically and the obtained data support aspects of a chymotrypsin catalysis mechanism. A glucanase purified from the grasshopper *Abracris flavolineata* and kinetically analyzed, indicated that the processivity results from consecutive transferences of substrate between accessory and active sites and that substrate inhibition occurs by the use of these sites. The cDNA coding for *Spodoptera frugiperda* midgut trehalase was cloned and expressed. Site mutagenesis of the recombinant trehalase identified the active site proton donor and nucleophile as D322 and E520, respectively. This is the first trehalase to have its active site groups identified by site mutagenesis. A recombinant cathepsinL-like (CAL) digestive enzyme from *Tenebrio molitor* was crystallized, submitted to X-ray diffraction and its 3D structure is being resolved.



Electron transmission immunocytochemical localization of PMAP in T. molitor *middle midgut. Arrows point to secretory vesicles. Bar= 0.5 µm.*

Antibodies raised against midgut microvillar proteins from *T. molitor* and *S. frugiperda* were used for screening cDNA expression libraries. The positive clones were sequenced, assembled and searched for similarities in databases. One of the predicted proteins from *T. molitor* was cloned, expressed and characterized. The obtained data support the assumption that this protein is comprised of domains A and B arranged in AB repeats. The processed form is AB and is putatively involved in peritrophic membrane formation. A predicted protein from *S. frugiperda* is annexin, and ongoing research suggests it is part of the digestive enzyme secretory machinery.

Glucose transport by *Dysdercus peruvianus* midgut cells were shown, *in vivo*, to depend on a facilitative transporter and a K+ -symporter. A cDNA coding for the facilitative transporter (GLUT) was sequenced and is being characterized.

MAIN PUBLICATIONS

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