

Identification of Novel Odorant Binding Proteins in the Beetle *Tenebrio molitor*

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Insects must detect and interpret a variety of chemical signals in order to sense the presence of food, danger and mates. These signals are typically hydrophobic organic molecules that are poorly soluble in aqueous body fluids. Odorant-binding proteins (OBPs) are used to transport these chemical signals to their site of action. The beetle, *Tenebrio molitor*, which is a domesticated pest of stored grain products, produces a large family of 12-14 kDa hemolymph proteins (THPs) similar to OBPs. The first OBP structure to be solved was that of a 12 kDa beetle isoform (THP12a). It has a hexahelical fold with an internal hydrophobic ligand-binding pocket. The goal of this work was to determine THP isoform diversity in the hemolymph of the beetle both in terms of sequence and structure.

To examine the sequence variation in the THP isoforms, the hemolymph was fractionated by gel exclusion and reversed-phase HPLC (Figure 1). Selected peaks were reduced and the Cys residues were alkylated, protein molecular weights were determined by ESI-MS and some of the fraction was digested with trypsin. The sequences of tryptic peptides from each fraction were identified by ESI MS/MS. The challenge was to find sequence stretches that were unique to each isoform. To minimize problems with subsequent cloning it was preferable that these sequence stretches did not contain L/I, R or S.

Stretches of unique sequence tags were found for 7 different isoforms. Approximately 70% sequence coverage was obtained for each isoform. These sequence tags were then used to design degenerate oligonucleotide primers for cDNA clone isolation. Full-length sequences were derived for 9 additional clones with sequence identities from 33-99% (Figure 2). One of the derived sequences, THP12b, differed from THP12a by only two amino acids, which had been suggested by the MS spectrum of the intact protein as well as MS/MS sequencing. The 3-D structures of the isoforms have been modeled based on THP12a and have been found to vary in the shape of the binding pocket suggesting that divergent isoforms carry different compounds throughout the body of the beetle. Since hexahelical OBPs are unique to insects they make attractive targets for the rational design of environmentally friendly insecticides.

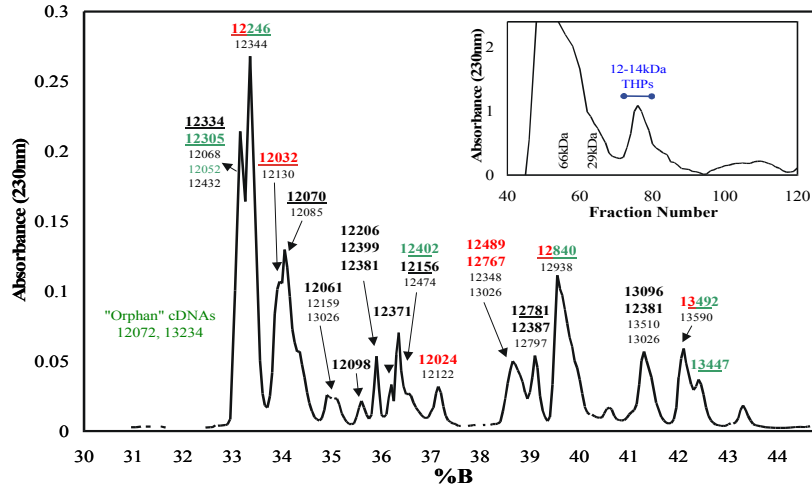


Figure 1. Gel exclusion and HPLC elution profile of THP isoforms present in larval hemolymph. The indicated peak from an S100 gel-exclusion column (inset) was fractionated by reversed-phase HPLC on a C18 analytical column (B=0.05% TFA, 80% acetonitrile). The observed masses are shown with the major components (>50% of the height of the largest peak by ESI-MS) in larger font in bold. Isoforms that were partially sequenced by tandem mass spectrometry are indicated partially or fully in red. Masses consistent with cDNA sequences are in green.

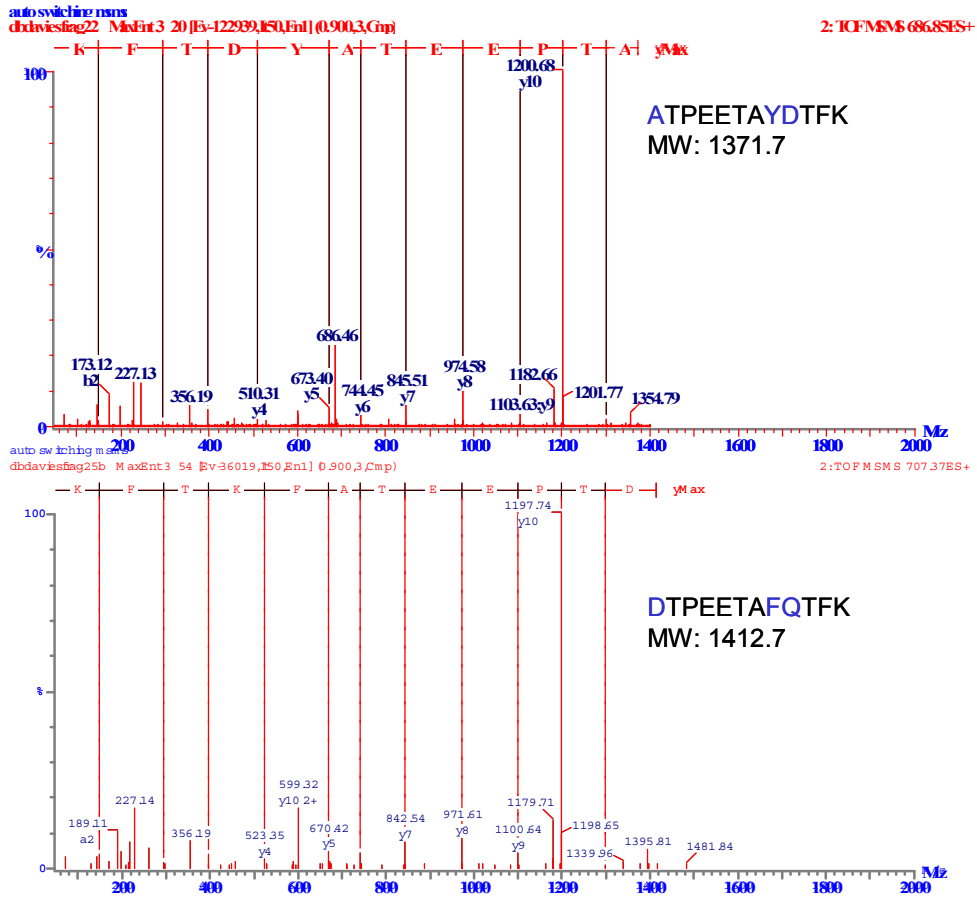


Figure 2. Sequencing of homologous tryptic fragments from proteins of the 12840 Da isoform THP13a (top), and the 13492 Da isoform THP13d (bottom). Amino acid changes are in blue.