

# Esterases from Drosophila mojavensis

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SUMMARY

The esterase-4/esterase-5 system in Drosophila mojavensis is a good model for studying especially the first stages of evolutionary divergence of a protein. Firstly, the variation of these esterases is large. Secondly, esterase-4 and esterase-5 are the products of a duplicated gene. Finally, variants of esterase-4 with a different substrate preference were detected.

Therefore, these esterases were made the basis of the study described in this thesis. The ultimate goal of the investigation is the comparison of the primary structures of the esterases and their variants in order to obtain information about cause and mechanism of variation.

Chapter 1 is a review of esterases from Drosophila, especially Drosophila mojavensis and of esterases and their classification problems in general. Purification and characterization of esterase-4 is described in chapter 2. Since only very small amounts of protein may be obtained with the method used, a new, more rapid isolation procedure by immunoaffinity chromatography was developed for both esterase-4 and esterase-5 (chapter 3). Comparison of the N-terminal amino acid sequences showed that the esterases have a common origin, since they are 82 % identical. However, the amounts isolated still were too small to determine the complete primary structures by classical protein sequencing techniques. In chapter 4, the determination of the kinetic parameters of two allozymes of esterase-4 is described. Large differences in catalytic efficiency and substrate preference were found, which are rare for variants of an enzyme. Immunologically cross-reacting esterases from closely related species were detected by double-immunodiffusion and immunoaffinity chromatography with antisera raised against esterase-4 and esterase-5 (chapter 5). Substrate preference for 1- and 2-naphthyl esters turned out to be a poor criterion for the relationship between esterases from different species. Finally, a start of attempts to obtain the primary structures of the esterases via their cDNAs is described in chapter 6. An oligonucleotide probe was synthesized on the basis of the available N-terminal sequence. This probe was used for hybridization with mRNAs present in the enriched polyadenylated RNA-fraction from Drosophila mojavensis larvae. From the enriched polyadenylated larval RNA, cDNA was synthesized.

The results described in this thesis show that esterases-4 and -5 from *Drosophila mojavensis* are indeed a perfect system for the study of molecular evolution and give the expectation that the primary structures may be determined via cDNA sequencing.