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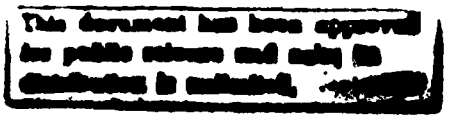

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

At least 8 proteases have been detected in the larval midgut of Tribolium castaneum by electrophoresis on polyacrylamide gels that contain gelatin. Most of the proteolytic activity of Tribolium stems from SH-proteases. The isolation and characterization of locust caecal trypsins and a chymotrypsin are reported.

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THIRD INTERIM REPORT

PROTEASES OF STORED PRODUCT INSECTS AND THEIR INHIBITION BY SPECIFIC PROTEASE INHIBITORS FROM SOYBEANS AND WHEAT GRAIN

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PREFACE

The ongoing research performed during the period to which this third interim report relates (November 1987 - June 1988) centers on the comparison of the insect digestive enzymes to respective mammalian digestive enzymes, characterizing differences in their kinetic properties and inhibition by naturally occuring and synthetic protease inhibitors. Specifically, these studies deal with a general characterization of Tribolium castaneum proteases, with a detailed characterization of Locusta migratoria caecal trypsins and with initial studies of Locusta migratoria caecal chymotrypsin.

EXPERIMENTAL RESULTS

(1) Tribolium castaneum larval midgut proteases

A variety of proteases have been detected in the larval midgut of Tribolium. The trypsin- and chymotrypsin-like activities in the larval midgut enzyme solution (LMES) have been mentioned in our previous report. The enzymatic profile of LMES can be seen by polyaczylamide gel electrophoresis (PAGE) into which either casein or gelatin were included. Differential staining of the gels indicated at least 8 distinct proteases. Using E64, a specific inhibitor of sulfhydryl proteases, which does not inhibit trypsin or chymotrypsin, it has been clearly demonstrated that most of the proteolytic activity of LMES is due to the presence of SH- proteases. The inhibition of three-SH proteases by E64 has also been visualized on PAGE-gelatin plates. These proteases are now being isolated by ion-exchange HPLC, as will be reported in the final (second annual) report.

(2) Locust proteases

2.a. Locust trypsins

Two trypsin-like enzymes were isolated from the digestive tract of Locusta migratoria. Primary purification was carried out on a diethylaminoetyhl (DEAE)-cellulose column, from which the two trypsins emerged in the anionic fraction. Further purification was achieved by affinity chromatography on a p-aminobenzamidine (PABA)-Sepharose column, which also separated between the two trypsins ($TLE_{Arr.1.}$ and $TLE_{Arr.2.}$), or by HPLC on an anion exchange column. The purity and homogeneity of the trypsins were demonstrated by electrophoresis on cellulose acetate strips and in

polyacrylamide gels, with and without SDS. The molecular weights of $TLE_{Aff.1}$ and $TLE_{Aff.2}$, as determined by SDS-PAGE, were-17000 and-24000 respectively. The amino acid compositions of the locust trypsins were similar to those of trypsins from the digestive systems of other insects, which are characterized by the lack or low content of half cystines. The isoelectric points were 3.2 for $TLE_{Aff.1}$ and 3.5 for $TLE_{Aff.2}$. Since most of the locust trypsin comprised of $TLE_{Aff.2}$, the latter served as the main object of this study. $TLE_{Aff.2}$ was unstable at low pHs, differing in this respect from mammalian trypsins. The optimum activity was at pH 8.5-9.0. The Km and k_{cat} values, were similar to those for bovine trypsin. Activation by substrate, a phenomenon known for bovine trypsin, was also observed for $TLE_{Aff.2}$. The locust trypsin was fully inhibited by the proteinaceous trypsin inhibitors Bowman-Birk (BBI) and Kunitz (STI) from soybeans, CI from chickpeas, chicken ovomucoid and turkey ovomucoid. It was inactivated by phenylemthysulfonyl fluoride (PMSF) and tosyl-L-lysine chloromethyl ketone (TLCK), indicating the involvement of serine and histidine in the active site.

2.b. Locust chymotrypsin

A chymotrypsin-like enzyme (CTLE) was isolated from the digestive tract of Locusta migratoria by ion-exchange chromatography on DEAE cellulose followed by affinity chromatography on phenylbutylamine (PBA)-Sepharose. The purity and homogeneity of CTLE have been shown by SDS-PAGE and on cellulose acetate strips. The enzyme has a molecular weight of ≈ 24000, determined by SDS-PAGE and on a Sephadex G-75 calibrated column. It has an isoelectric point of 10.1 and contains no S-S bonds. The optimal pH for enzyme activity and stability was in the range of 8.5-9.0. The enzyme was fully inhibited by BBI from soybeans and CI from chickpeas, by chicken ovomucoid and turkey overmucoid, as well as by the Kunitz (STI) soybean trypsin inhibitor that hardly inhibits bovine chymotrypsin.

SIGNIFICANT FINDINGS

1. In contrast to the digestive proteinases of Tenebrio, and the locust which, comprise mainly of serine proteases with trypsin- and chymotrypsin-like activities, Tribolium castaneum digestive proteases are predominantly sulhydryl enzymes.

2. The lack of disulphide bridges in the proteases of the Locusta migratoria possibly confers conformational flexibility upon these enzymes in situ, which may protect them against proteolysis.