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Proteinases of female *Aedes aegypti* (L.)¹

Preliminary note

R. A. YEATES

After a blood meal proteolytic activity is induced in the gut of the adult female mosquito (Gooding, 1966; Briegel and Lea, 1975). In *Aedes aegypti* this activity includes trypsin-like enzymes, with activity against the esters of basic amino acids. These enzymes have recently been purified to near homogeneity (Kunz, in press). The present paper includes an estimate of the total proteolytic activity made up by the trypsin-like enzymes. A major new proteolytic activity is also identified.

Female mosquitoes 25 h after a blood meal were homogenized in 0.025 M HEPES buffer, pH 7.7. After centrifugation the proteolytic activity of the supernatant was determined against casein, using a slight modification of the Kunitz method (Laskowski, 1955).

The caseinolytic activity could be subdivided according to its sensitivity to the proteolytic inhibitor from lima beans. 100 μ g inhibitor per mosquito inhibited only 40–50% of the activity and increasing the inhibitor concentration did not increase this inhibition. The portion of the activity which is sensitive to the inhibitor must include the trypsin-like enzymes, as these are at least 95% inhibited under these conditions. It must also include another inhibitor-sensitive component, a serine proteinase, described by Kunz (in press) as forming a “front band” after polyacrylamide gel electrophoresis. It can be estimated that the trypsin-like enzymes make up about three quarters of the lima-bean inhibitor sensitive component, and hence (30–40%) of the total proteolytic activity. This contrasts with the figure of “at least 75%” obtained by Briegel and Lea (1975) using a different and less typical protein, namely hide powder, as substrate.

Gooding (1966) has reported that the proteolytic activity of *Aedes aegypti* is inhibited by the metal chelator EDTA. Under our conditions (35–52%) of the caseinolytic activity was inhibited by 25 mM EDTA in 3 h. Experiments with

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mixed inhibitors show that only low levels (0–11%) of the caseinolytic activity were sensitive to both EDTA and lima-bean inhibitor. It follows that there is a major proteolytic activity present in *Aedes aegypti* which is not trypsin-like, but is sensitive to EDTA and insensitive to lima-bean inhibitor. Further work on the properties and purification of the enzyme(s) involved is in progress.

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- 4 Laskowski M.: Determination of activity by the casein digestion method (Kunitz). In: *Methods in enzymology* (ed. by S. P. Colowick and N. O. Kaplan) 2, p. 33–34. Academic Press, New York/London 1955.