

**PARTIAL CHARACTERIZATION OF TWO DIGESTIVE CYSTEINE  
PROTEINASES FROM THE MIDGUTS OF THE COTTON STAINER BUG  
*DYSDERCUS PERUVIANUS***

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Most major insect groups use serine proteinases as their principal digestive proteinases. In contrast, Hemiptera depend on cysteine and/or aspartic proteinases to digest their diet. We have isolated and purified two cysteine proteinases from the cotton-seed sucker Hemiptera *D. peruvianus*. Those cysteine proteinases were named Cys 1 and Cys 2. Their molecular weights were 32 and 45 kDa (SDS-PAGE) respectively. Cys 1 p*H*o 6.3 and Cys 2 p*H*o 6.3, have the same thermal inactivation profile, following apparent first-order kinetics with a half-life of 90 min at 40°C. Cys 1 and Cys 2 are inhibited by E-64 with a  $K_D$  of 17.3 nM (Cys 1) and 7.11 nM (Cys2). The kinetical parameter of Cys 1 and Cys 2 on the two substrates are ( $K_m$ ,  $\mu$ M): Cys 1 on ZFR-MCA (6.9) and on ZRR-MCA (9.1) and Cys 2 on ZFR-MCA (5.3) and on ZRR-MCA (6.8). Attempts of N-terminal sequencing of these enzymes were unsuccessful, which led us to try a random sequencing of a normalized expression midgut cDNA library. Four sequences were identified as cathepsins-L, being 3 contigs with 869, 818 and 812 pb and one singlet with 711 pb. The correlation of the purified enzymes with those sequences is in progress.

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