

## Kinetic and Structural Characterization of a Digestive Lysozyme from *Musca domestica*

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Lysozymes “c” have been implied in the digestion of bacteria in insects. These lysozymes share structural and mechanistic characteristics with hen egg white lysozyme (HEWL). However, to perform their digestive function, insect lysozymes have some specific characteristics, such as a pH optimum more acidic than those of defense lysozymes.

Here, we report kinetic and structural data of a digestive lysozyme from *Musca domestica* (Lys1 – AF3445890) expressed as recombinant protein in *Pichia pastoris*. The pH effect on catalytic activity of Lys1 showed to be dependent on two ionizable groups, D50 and E32, presenting  $pK_e$ s of 3.09 and 6.4 respectively. These values are lower than those determined for HEWL ( $pK_e$ s of 3.85 and 6.61). As these differences could be correlated with specific properties of the microenvironment of the catalytic residues of Lys1 and HEWL, the three-dimensional structure of the Lys1 (2FBD) was determined using HEWL as search model.

The structural comparison showed that the microenvironment of E32 is less hydrophobic than that of HEWL (replacement of V and A by S and T, respectively). Moreover, in Lys1 a polar residue (N) replaced a negatively charged one (D) in the vicinity of D50. Site-directed mutagenesis may contribute to reveal the importance of these modifications to the pH optimum determination in digestive lysozymes.

**Supported by FAPESP, CNPq and LNLS.**