Molecular Basis of Enzyme Properties

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Digestive enzymes from insects are good experimental models to investigate the basis of enzymatic properties. ***Substrate specificity:*** The substrate preference of β-glycosidases may largely vary depending on small alterations in the substrate structure. In order to study the basis of this characteristic, the substrate specificity of the β-glycosidase from *Spodoptera frugiperda* larvae (Sfβgly) was investigated using site-directed mutagenesis and enzyme kinetics. It was showed that Sfβgly preference for fucosides, glucosides and galactosides depends on non-covalent interactions between hydroxyl groups of the substrate glycone and residue E451 from Sfβgly active site. Differently, several residues modulate the Sfβgly preference for the substrate aglycone. E190 and E194 favor the binding of alkyl moieties, whereas K201 and M453 interact with glucose units of cellodextrins. In summary, these experiments revealed an interactions network that controls the substrate specificity of Sfβgly. ***pH effect on the catalytic activity:*** Digestive lysozymes exhibit an acidic pH optimum. The structural comparison between digestive lysozymes from *Musca domestica* larvae (MdL1 and MdL2) and lysozyme from hen egg white (Hewl) revealed changes in the residues forming the microenvironment of the catalytic residues of MdL1 and MdL2. In order to verify the participation of these modifications in the determination of the pH optimum, three residues (V106, A107 and D48), which are found in Hewl, were introduced in MdL2. Indeed, these simultaneous replacements, but not the individual ones, increased the pH optimum of MdL2 and made it identical to that of Hewl. In summary, the acidic pH optimum of digestive lysozymes is determined by the environment of their catalytic residues.

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