DECIPHERING THE CATALYTIC MECHANISM OF GLUTAMIC PEPTIDASES

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The recently formed glutamic peptidase family (Family G1 in MEROPS, also known as Eqolisins) is a distinct group of acid peptidases with structure and mechanistic features distinct from the canonical peptidases families. These enzymes have a unique β-sandwich structure composed of two 7-stranded antiparalel β-sheets and a catalytic mechanism based on a glutamate, a glutamine and a nucleoliphilic water molecule. In addition to this unique machinery for the hydrolysis of peptide bound, Eqolisin is only found in filamentous fungi and its distribution among pathogenic species represents a putative target for the development of anti-fungi drugs against invasive fungal infections in plants and humans, particularly in HIV immunocompromised populations. In this work we aim to establish the catalytic role of Glu in the water activation through a detailed study of pH-profile dependence of Eqolisin hydrolytic reaction against the fluorescence resonance energy transfer (FRET) peptide MCA-KLFSSKQ-EDDnp, where the MCA (7-amino-4 methyl coumarin) is the fluorescent group and glutaminyl-[*N*-(2,4-dinitrophenyl)-ethylenediamine] (Q-EDDnp) is the fluorescence quencher. In addition, proton inventory, i.e., the kinetic studies of solvent isotope effects (SIE) in a mixture of H_2O and D_2O were obtained. Our data clearly points to a carboxyl group being deprotonated in the ascendant acid limb of pH profile, supporting a mechanism in which the carboxylate of Glu136 would act as a general base accepting the proton from the catalytic water (at pH 4.0) and an iso-mechanism where Glu136 carboxyl proton is transferred to an aceptor residue as the substrate bounds to enzyme bellow this pH.