ENZYMATIC AND STRUCTURAL CHARACTERIZATION OF OLIGOPEPTIDASE A COMPARED WITH HOMOLOGUES ENZYMES

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Proteolysis acts controlling the protein balance keeping the integrity of the cells. In the present study, we characterized a bacterial oligopeptidase (OpdA) of the M3A subfamily that presents properties similar to mammalian thimet-oligopeptidase (TOP) and neurolysin, two Zn-dependent metallo-hydrolases. OpdA, TOP and neurolysin present close related substrate specificity and they are inhibited by the same compounds. It was also described that TOP and neurolysin have a large similarity regarding their structure: their active sites are in the bottom of a deep channel, explaining their preference for small peptides. However, nothing is known about the OpdA structure. Therefore, our work's objective was the OpdA kinetical and structural characterization, in order to better demonstrate similarities among the subfamily's members. We determined the kinetic parameters for the hydrolysis by OpdA of the bradykinin fluorescent derivatives Abz-RPPGFSPFRQ-EDDnp (K_m=0,28µM; k_{cat}=0,35s^{-1}; k_{cat}/K_m=1250$mM^{-1}s^{-1}$) and Abz-GFSPFRQ-EDDnp (K_m=0,34µM; k_{cat}=0,16s^{-1}; k_{cat}/K_m=470$mM^{-1}s^{-1}$); moreover, in contrast to TOP and neurolysin that cleave these compounds at the Pro-Phe bound, OpdA hydrolyze them at the Phe-Ser. We now head to the crystallization and modeling of OpdA, to provide data to better compare these three enzymes and develop specific inhibitors based on their structural differences. Supported by CNPq and FAPESP.