Poster E-8

Dynamics of catalysis and inhibition in oligopeptidases: Natural peptide structures as promising antihypertensive agents.

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Short Abstract: Molecular modeling, combined with docking and molecular dynamics experiments was used to study the dynamics of catalysis/inhibition in “druggable” human Oligopeptidases. The dynamics of ligand recognition and structural determinants for selective inhibition of these enzymes lead us to propose more selective oligopeptidase inhibitors with improved stability and pharmacological profile.

Long Abstract:

Oligopeptidases are wonderful biomolecular machines that catalyze the hydrolysis of small peptides and contain in their sequence the evolutionary conserved HExxH(x)23E motif to coordinate de Zn2+ ion of the catalytic site. Oligopeptidases are important enzymes in regulating intracellular and extracellular concentration of bioactive peptides and it has been an attractive target for drug design due to the critical role in cardiovascular, renal and other disease. Our "in silico" study was focused in: i) evolutionary conservation of residues surrounding the active site; ii) dynamics of substrate recognition and catalytic/inhibition mechanism and iii) structural determinants for selective inhibition of these enzymes.

We used X-ray data of prokaryotic and mammalian M3A intracellular endopeptidases, human type I membrane-anchored M2 endopeptidases and molecular modeling for other "druggable" targets as human somatic Angiotensin I-converting enzyme (hsACE), combined with docking and molecular dynamics experiments to study the dynamics of catalysis/inhibition of these enzymes. Natural substrates, commercial inhibitors and natural inhibitors from snake venom were used to form complexes and reproduce biological data obtained in animal, enzymatic and MS experiments. Substrates or inhibitors were docked using the Autodock 3.0 and in energy minimization, equilibration and production dynamics using GROMACS. Interactions in final structures were evaluated in STAR Sting package.

In spite of low sequence identity, evolutionary conserved and important to catalysis secondary structure elements were detected. Although the topology as do the sizes of individual elements differs, the overall structural similarity and catalytical specificity of these enzymes imply in a common origin. Molecular dynamic trajectories indicate conformational changes in the enzyme structure from substrate-accessible (open) conformation, to

substrate-bound (closed) conformation, as described experimentally for human ACE2 and E. coli Dcp enzymes.

The major enzyme-selective contacts were detected in S2-S5 positions whereas contacts in S2'-S1 positions were almost conserved. Residues surrounding the oligopeptidases active site (within the 5 Å diameter sphere) are evolutionary conserved and form thigh and mostly unspecific contacts with small commercial inhibitors. Our results indicate the importance of the protonation of conserved His I residue for catalytic process, in agreement whit partially inactive TOP mutants obtained in our laboratory. Natural peptides with carboxy-terminal Pro-Pro motif (BPPs) were tested as selective nM inhibitors. The "natural" mechanism of inhibition was related to the proline residue in P1', and hydrophobic residues in P1 position. The development of middle-size carboxy-Pro-Pro peptidomimetic structures will lead us to more selective oligopeptidase inhibitors with stability and improved pharmacological profile.

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