ABSTRACT

The herein presented Ph.D. dissertation describes kinetic and structural characterization of human glutamate carboxypeptidases II and III (GCPII and GCPIII) using a complete panel of their natural substrates. These enzymes hydrolyze C-terminal glutamate from their substrates. They share 67 % sequence identity and also similar enzymatic activities.

This thesis quantitatively compares human GCPII and GCPIII in terms of their ability to hydrolyze the substrates N-acetyl-L-aspartyl-L-glutamate (NAAG), folyl-poly-γ-L-glutamic acids ($FolGlu_n$) and β-citryl-L-glutamate (BCG). We demonstrated that GCPIII hydrolyzes its substrates in a metal-dependent manner, that BCG is a specific substrate of GCPIII, and that NAAG and $FolGlu_n$ are specific substrates of GCPII. We also provide indirect biochemical evidence that GCPIII might feature a heterometallic active-site cluster. Additionally, we characterized the relevance of a surface exosite of GCPII, the arene-binding site (ABS), for the hydrolysis of $FolGlu_n$ substrates using mutagenesis and enzyme kinetics and showed that polymorphic His475Tyr variant of GCPII hydrolyzes $FolGlu_n$ substrates with the same kinetic parameters as the wild-type enzyme.

Furthermore, this thesis focuses on structural aspects of the substrate specificities of GCPII and GCPIII: we present the X-ray structures of inactive mutant of GCPII, Glu424Ala, in complex with its substrates $FolGlu_{1-3}$ and BCG. The $FolGlu_{1-3}$ complexes show how ABS residues of GCPII - Arg463, Arg511 and Trp 541 - participate in binding the aromatic pteridine ring of these substrates. These findings are complemented by high-level quantum mechanics/molecular mechanics (QM/MM) calculations which reveal how BCG probably binds to the active site of GCPIII and how a calcium-zinc heterometallic active-site cluster of GCPIII might look like.

Finally, we also quantified the kinetics of the N-acetyl-L-aspartyl-L-glutamyl-L-glutamate (NAAG2)-hydrolyzing activity of GCPII and GCPIII. Further, we quantified the tissue distribution of GCPII and GCPIII (in human tissues) both at the mRNA and the protein level, showing highest expression of GCPIII in testes and discussing its possible role as an iron chelator. The thesis is rounded off with structural characterizatin of lipophilic inhibitors of GCPII and the discovery of a moderately specific inhibitor of GCPIII.