ABSTRACT

Zinc-binding proteins represent approximately one tenth of the proteome and a good portion of them are zinc-dependent hydrolases. This thesis focuses on biochemical and structural characterization of glutamate carboxypeptidase II (GCPII) and histone deacetylase 6 (HDAC6), two members of the zinc-dependent metallohydrolase superfamily. We describe here their interactions with natural substrates and inhibitors.

GCPII is a homodimeric membrane protease catalyzing hydrolytic cleavage of glutamate from the neurotransmitter N-acetylaspartylglutamate (NAAG) and dietary folates in the central and peripheral nervous systems and small intestine, respectively. This enzyme is associated with several neurological disorders and also presents an ideal target for imaging and treatment of prostate cancer. GCPII inhibitors typically consist of a zinc-binding group (ZBG) linked to an S1' docking moiety (a glutamate moiety or its isostere). As such, these compounds are highly hydrophilic molecules therefore unable to cross the blood-brain barrier and this hampers targeting GCPII to the central nervous system. Different approaches are adopted to alter the S1' docking moiety of the existing inhibitors. As a part of this thesis, we present different strategies relying on replacement of the canonical P1' glutamate residue with unbranched non-natural amino acids and glutamate bioisosteres. We also study the effect of introduction of an aminohexanoate linker on affinity and biological properties of phosphoramidate-based inhibitors. Analysis of crystal structures of GCPII in the complex with these novel inhibitors identified unprecedented plasticity of the S1' site that enables GCPII to accommodate bulky residues. We have succeeded in identifying compounds with increased hydrophobicity. These molecules, although not strong inhibitors, represent promising scaffolds for further rational design of new inhibitors.

Acetylation of Lys40 of α-tubulin protects the microtubules from mechanical ageing and plays role in cell motility, axonal branching, and growth and maintenance of neuronal processes. HDAC6 is the major tubulin deacetylase and it is emerging as a possible treatment target in cancer and neurodegenerative diseases. A better understanding of its interaction with the tubulin substrate is therefore needed. We show here that HDAC6 deacetylates the tubulin dimers at a rate 1500-fold higher than the microtubules. Our data indicates that amino acids beyond the P_1 and P_{-1} within the Lys40 loop contribute minimally to the substrate recognition and that efficient deacetylation requires complex longitudinal and lateral interactions with tubulin dimer.