Abstract

Glutamate carboxypeptidase II (GCPII) is a zinc-dependent carboxypeptidase with high expression levels in prostate carcinoma. As the enzyme represents a validated target for cancer therapy and imaging, the development of new GCPII-specific ligands is still a focus of an active academic and industrial research. However, existing assays to screen inhibitor libraries and determine inhibitor efficacy are suboptimal at best.

This thesis is aimed at the development of small internally quenched probes that could be used for continuous measurement of the GCPII enzymatic activity. These probes are derived from natural GCPII substrates and consist of a fluorophore/quencher pair connected by a GCPII-hydrolysable linker. I first characterized biophysical properties of the probes and then determined kinetic parameters of their hydrolysis by GCPII. The optimized activity assay was then used to determine inhibition constants of several GCPII-specific inhibitors. Finally, complexes between the inactive enzyme and several probes were co-crystallized and one of the complexes refined and analyzed. Our data show that the probes are involved in non-covalent interactions with the same amino acid residues of the enzyme's active site as natural substrates. The developed assay could be optimized for high-throughput screening of small-compound libraries in search of potential GCPII inhibitors.

Key words: glutamate carboxypeptidase II, internally quenched fluorescent probes, enzyme kinetics; X-ray crystallography