ABSTRACT

Dipeptidyl-peptidase I (DPPI, known also as cathepsin C, EC 3.4.14.1) is a lysosomal cysteine peptidase from the family of papain-like cysteine peptidases. DPPI is dfferent from other members of the family in that it is a tetramer in its active form, while others are active as monomers. Each subunit is composed of a catalytic and an exclusion domain that are non-covalently linked. The exclusion domain sterically interferes with substrate binding, making DPPI an exopeptidase. Biological roles of DPPI include non-specific protein degradation as well as activation of effector serine peptidases of the immune system. Excessive activity of the latter is involved in inflammatory diseases therefore inhibition of DPPI is a strategy for the treatment of these diseases.

The aim of this thesis was to identify and characterize modifiers that affect DPPI activity by binding outside of the active site. For this purpose we prepared recombinant human DPPI in a mammalian expression system (HEK293T cells) and for the first time also in soluble form in a bacterial expression system (*E. coli*). As expected, the former was a tetramer in its active form ($DPPI_{tet}$), whereas the latter was a monomer ($DPPI_{mono}$). Both enzymes had similar functional properties with the exception of the turnover number k_{cat} for the substrate H-Gly-Phe-AMC and the rate constant for the binding of the irreversible inhibitor E-64. These results show that formation of the tetrameric structure is not necessary for activity of DPPI. Since the oligomeric structure of DPPI and the exclusion domain are unique among cysteine cathepsins, we also produced recombinant DPPI without its exclusion domain (DPPIΔEX) in *E. coli*. We successfully activated DPPIΔEX and demonstrated that it acts as an endopeptidase, not an exopeptidase and has substrate specificity comparable to other monomeric cathepsin endopeptidases. The catalytic domain of DPPI has thus preserved its endopeptidase activity.

Using molecular docking and experimental testing we identified and kinetically characterized 11 modifiers of DPPI activity that acted via kinetic mechanisms consistent with binding outside of the active site. Two compounds showed different binding affinities for both forms of DPPI. S-[(2-guanidino-4-thiazoyl)methyl] isothiourea, acted as a linear mixed inhibitor with predominantly uncompetitive character and showed greater affinity for $DPPI_{mono}$. In contrast, Su-His-OMe, a hyperbolic competitive inhibitor, showed greater affinity for $DPPI_{tet}$. According to computational docking results, Su-His-OMe binds to a site, homologous to a known allosteric site of cathepsin K. We experimentally confirmed this prediction using point mutants of $DPPI_{mono}$. 2-[(3-nitrophenyl)carbamoyl]benzoic acid on the other hand, had similar affinity for $DPPI_{mono}$ and $DPPI_{tet}$, but acted via different modification mechanisms. With the identification and characterization of these modifiers we have shown that DPPI can be regulated by the binding of effectors outside of the active site and that its tetrameric structure can have a role in enzyme activity regulation.