The Crystal Structure of the (Zn/Zn)bLAP/Zofenoprilat Complex

Vincenzo Alterio[a], Mario Cappiello[b], Pietro Amodeo[c], Andrea Scaloni[d], Antonella Del Corso[b], Carlo Pedone[a], Umberto Mura[b], Giuseppina De Simone[a], [a]*IBB-CNR, Naples, Italy.* [b]*University of Pisa, Pisa, Italy.* [c]*ICB-CNR, Naples, Italy.* [d]*ISPAAM-CNR, Naples, Italy.* E-mail: alterio@chemistry.unina.it

Bovine leucine aminopeptidase (bLAP) is an exopeptidase that cleaves N-terminal hydrophobic residues from polypeptide substrates. It is a hexameric enzyme made up of six identical monomers. Each subunit contains two Zn^{2+} in the active site, which are fundamental for catalytic activity. They may be replaced by other divalent cations with different exchange kinetics. The readily exchangeable site (site 1) can be occupied by Zn^{2+}, Mn^{2+}, Mg^{2+} or Co^{2+}, while the tight binding site (site 2) can be occupied by Zn^{2+} or Co^{2+}. We recently reported that introduction of Mn^{2+} into site 1 generates a novel activity of bLAP toward Cys-Gly, which in contrast is not hydrolysed by the (Zn/Zn) enzyme. To clarify the influence of the metal present in site 1 on enzyme interaction with sulphur-containing derivatives, we have undertaken functional and structural studies on (Zn/Zn) and (Zn/Mn)bLAP forms. Here we report the kinetic analysis of various sulphur-containing derivatives with both enzyme forms and the crystal structure of (Zn/Zn)bLAP in complex with Zofenoprilat. This peptide-mimetic derivative containing a sulphydryl moiety was found to be also a potent inhibitor of (Zn/Zn)bLAP. This combined approach provided insights on interaction of bLAP with sulphydryl-containing compounds, showing that metal exchange in site 1 modulates binding to these molecules that, depending on metal nature, may result as enzyme substrates or inhibitors.

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