Mode of Metal Ligation Governs Inhibition of Carboxypeptidase A

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Carboxypeptidase is a Zn-dependent protease that specifically recognises and hydrolyses peptides with a hydrophobic side chain at the C-terminal residue. According to hydrolysis mechanisms proposed in the literature, catalysis requires a water molecule to be close to the Zn ion so as to be activated as a nucleophile. Among small molecules that resemble the slowly hydrolysed Gly-Tyr peptide, which have been previously designed as inhibitors and characterised structurally, a variant with the terminal amino acid in a D-configuration has been the most effective. Our molecular dynamics simulations of carboxypeptidase complexed with different variants of those inhibitor ligands as well as variants of the Gly-Tyr peptide show that the strength of the inhibitory effect is not related to the binding strength of the ligand. Our data rather support an earlier notion that the inhibition is, at least partially, due to blocking a coordination site at the Zn ion by the ligand coordinating the metal ion in a bidentate fashion.

[1] J. A. Amador Balderas, F. Beierlein, A. H. C. Horn, S. Volkenandt, L. Völcker, N. Mokhtari, J. C. E. Ndongue, P. Imhof, *Int. J. Mol. Sci.* **2024**, *25*, 13725. (DOI: 10.3390/ijms252413725)