

Insight to zinc-metalloproteases: QM/MM study of Carboxypeptidase A structure and mechanism

Jon. I. Mujika and Adrian. J. Mulholland

Centre for Computational Chemistry, School of Chemistry, University of Bristol, Bristol BS8 1TS

Carboxypeptidase (CPA) is a zinc-containing metalloprotease found in the digestive system. CPA catalyses the hydrolysis of the C-terminal amino acid from polypeptide substrates, exhibiting a preference toward hydrophobic residues, especially those containing aromatic side chains. Although this enzyme has been extensively studied, the catalytic mechanism is not entirely understood and more than one possible reaction pathway has been proposed. Historically, substrate activation in the reaction has been attributed to a precatalytic interaction between the metal and the peptide bond.¹ However, some experimental studies² proposed that the zinc only activates the nucleophilic zinc-bound water molecule.

We have performed QM/MM calculations using the CHARMM suite of programs in order to study the system, where the QM part has been treated with the SCCDFTB QM method³. Zinc proteins provide a challenge for QM/MM modelling⁴. We have tested the treatment of the zinc site (e.g. the metal coordination) by comparison with higher level (B3LYP) QM/MM calculations. Potential energy surfaces and potentials of mean force have been calculated. The reaction pathway was recalculated with high level QM/MM calculations, to test the validity of SCCDFTB/CHARMM to describe for this important class of enzyme. We present an overall picture of the reaction, highlighting the catalytic role of the zinc ion together with the most important amino acids involved, especially Arg127.

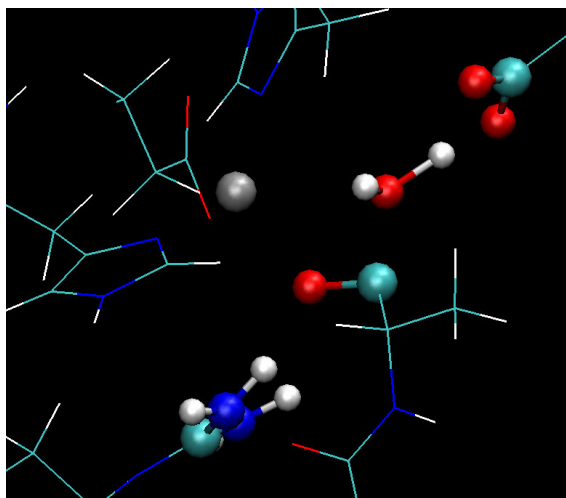


Figure 1 Transition state of the reaction catalyzed by CPA

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- (4) Zurek, J.; Bowman, A. L.; Sokalski, W. A.; Mulholland, A. J. *Struct. Chem.* **2004**, *15*, 405-414.