

## Enantioselectivity of alpha-chymotrypsin towards 4,5-disubstituted-gamma-lactams: a new perspective from molecular simulations

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In this study, we have applied an ab initio quantum mechanics (QM) and molecular dynamics/free energy (MD-FE) calculations based approach to investigate the enantioselectivity of alpha-chymotrypsin towards ester bond cleavage in 4,5-disubstituted-gamma-lactamic esters. The gamma-lactam nucleus (pyrrolidin-2-one) characterizes many compounds possessing biological and pharmaceutical activities. Among the compounds containing the gamma-lactam moiety, lactacystin plays a major role as a potent 20S proteasome peptidase inhibitor, and constitutes a synthetic challenge for researchers owing to the presence of four contiguous stereocenters. Further examples are represented by pilolactam, recently patented by Garst et al. as a muscarinic active principle, and Rolipram, an antidepressant and phosphodiesterase inhibitor synthesized by two different research groups, and currently manufactured by Schering Plough.

Among the plethora of substances featuring the lactam ring moiety as a structural component, 4-carboxy-gamma-lactams are interesting compounds since they can be considered as aza analogues of paraconic acids. Accordingly, two years ago some of us reported the optical resolution of a series of methyl esters of 1-alkyl-5-oxopyrrolidine-3-carboxylic acids by chemo-enzymatic hydrolysis of the ester function. Among the suitable enzymes available on the market, alpha-CT turned out to be the choice for the enantiomeric resolution of these lactams with high enantiomeric excess. The specificity of the enzyme and the high enantioselectivity observed experimentally were successively fully rationalized by means of molecular mechanics/dynamics simulations on the corresponding enzyme-substrate complexes. Thus, we decided to proceed with the synthesis of enantiomerically pure 4-carboxy-5-n-pentyl-pyrrolidin-2-ones, as promising candidates for biological and toxicological activity. Following this work, recently we reported our first results concerning the chemo-enzymatic synthesis of methyl 2-pentyl-5-oxo-pyrrolidine-3-carboxylates (and their acids) of type 1, in pure enantiomeric form using alpha-CT.

Given this, we have calculated the activation free energies for the acylation step of ester bond cleavage (i.e., from the Michaelis-Menten complex MMC to the tetrahedral intermediate TET) in alpha-CT-(±)-cis- ((±)-1a) and alpha-CT-(±)-trans ((±)-1b) methyl esters of 1-alkyl-5-oxopyrrolidine-3-carboxylic acid complexes. Comparison of the ester bond hydrolysis of similar reactive-site sequences in alpha-CT-substrate complexes had allowed us to investigate factors responsible for the observed enantioselectivity of the enzyme. Molecular dynamics simulations of 500 ps were

also carried out for the alpha-CT-(±)-1a and alpha-CT-(±)-1b tetrahedral intermediates (TET-(±)-1a and TET-(±)-1b), and for the alpha-CT-(±)-1a and alpha-CT-(±)-1b acyl-enzyme intermediates (AEC-(±)-1a and AEC-(±)-1b). Calculations for the TET-1 intermediates were performed to check how the catalytic groups are located into the active site for the hydrolysis of the ester bond. Acyl-enzyme calculations were carried out with the purpose of seeing how the newly formed termini in the substrate and the acyl group are located within the enzyme active site for the possible reformation of the cleaved ester bond.