DIPEPTIDYL PEPTIDASE III INHIBITION TESTS BY METAL DICATIONS



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Dipeptidyl peptidase (DPP) III is a zinc-dependent exopeptidase which motifs, "HEXXGH" and "EEXR(K)AE(D)" are responsible for the binding of metal ions to the active site of the enzyme. The peptidase activity of human dipeptidyl peptidase III (hDPP III) is inhibited by the excess of zinc ions. The aim of this study is to determine the effects of different concentrations of metal dications: Zn²⁺, Mn²⁺, Co²⁺ and Cu²⁺ on the activity of hDPP III, and identify the inhibitory binding site of the metal. The existence of a metal inhibitory binding site was hypothesized based on the structural similarity of the hDPP III and the thermolysin active sites and the observed decrease in hDPP III activity at higher zinc concentrations.¹ In the thermolysin X-ray structure (PDB: 1LND) the second metal ion is bound in the immediate vicinity of a catalytically active one.

> Influence of the concentration of metal ions on the hDPP III activity.

Future experiments

The 'stop flow' method monitors the decrease in the enzymatic activity of hDPP III caused by an excess of zinc ions.

The peptidase activity of hDPP III is inhibited by an excess of metal dications.

HR-ICP-MS (high resolution mass spectroscopy with inductively coupled plasma) \rightarrow the metal concentration in the holoenzyme will be determined, *i.e.* the number of metal ions per protein molecule.

Binding of another metal ion to 'metal inhibitory binding site' located in the substrate binding site of hDPP III and its influence on the structure and dynamics of the enzyme was identified using molecular dynamics in combination with calculation of the binding free energies and quantum mechanical - molecular mechanical calculations.

E508-OE2

E451 E316 Dummy atom model structure obtained after $1 \ \mu s$ of MD simulation

Coordination of zinc ions during 500 ns of MD simulations of the hDPP III – IVYPW complex. The results of two independent runs are given; top – results of run1 and bottom results of run2.

Literatura:

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