Effect of Large-Scale Motions on PlmII-Inhibitor Binding Modes: An Analysis by Essential Dynamics Techniques.

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Large-scale movements have been suggested to play a role in aspartyl protease activity. Previous structural studies of 3D PlmII structures, indicated that this enzyme have a great structural flexibility in the flap, L1, L2, L3 and L4 regions. The large structural flexibility of PlmII active site cavity allows the binding of different inhibitor scaffolds to this enzyme. However, is not clear the effect of large-scale motions over the different binding modes described for PlmII-Inhibitor complexes. Here, we performed Molecular Dynamics simulations of PlmII in complexes with specific (IH4) and non-specific (PepstatinA, EH58, R36 and R37) inhibitors during 40 ns. We chose these structures because it represents the three different ligand binding modes observed for this enzyme. All MD simulations showed that flap and L loops are the most flexible regions of the protein. To get a better understanding of the protein motion, we computed essential modes of the simulations from backbone covariance matrix. As result, the first 10 eigenvalues of each system contained the 90 % of the fluctuations. Then, we checked the statistical significance of the MD conformational sampling through the measure of the cosine content in the principal modes. The valid vibrations of the protein (cosine content around 0.2) involved principally motions of flap, L4 and L3 regions. These conformational changes result in open/closure movements of the two lobes of PlmII around the pseudo-symmetry axis, and therefore, provoke a variation of the molecular volume of the enzyme cavity. In addition, the movement of L3 promoted that hydrophobic residues F241, L242, P243 and F244 formed part of the S3 subsite and interacted differentially with residues in distal position of the inhibitors studied, which explains recent experimental findings. Finally, our analysis suggested that the L3 and L4 loops play a flap-like role for the PlmII function. While, the anti-parallels β-sheets that cross-link the two lobes of the enzyme acted as a hinge between the two domains. These results shed light on the role of flexible regions of the enzyme over the different binding modes described for PlmII-Inhibitor complexes, and they should be useful in structure-based design of novel, selective inhibitors that may serve as antimalarial drugs.

Key words: plasmpesin II, molecular dynamics, protein flexibility, essential dynamics