Analysis of crystallographic structures of aspartic proteases allows to conclude that nonbonded interactions of various enzymes of this family with respective specific substrates produce in every case practically identical unstable electronic systems which include catalytic groups of the active center, a cleaved peptide bond and a water molecule. The idea of a similar mutual orientation of these components in all nonbonded complexes of aspartic proteases [1] can reliably be verified by an a priori semi-empirical computation of the enzyme-substrate interactions for two representatives of this group which differ essentially in their amino acid sequences and three-dimensional structures. As the objects of theoretical investigations of catalytic mechanism of aspartic proteases, we have chosen human immunodeficiency virus protease (HIV-PR) and fungal protease rhizopuspepsin. Because of the restricted volume of the publication, here we only present the results for rhizopuspepsin and its interaction with the substrate Ac-Pro¹⁻Phe²⁻His³⁻Lys⁴⁻Phe⁵⁻Val⁶⁻OMe and the substrate-like inhibitor Ac-Pro¹⁻Phe²⁻His³⁻Phe⁴⁻(Ψ)⁻CH₂⁻Phe⁵⁻Val⁶⁻OMe which forms a stable complex, investigated by X-ray diffraction [2]. The results for the HIV-PR are to be published in a separate communication. A quantitative study of the rhizopuspepsin catalytic act is based on the theory and computation method described recently [3]. According to this scheme, the conformational properties of free molecules of the substrate, inhibitor and of the enzyme active center were analyzed first, and then a formation of the enzyme-inhibitor and enzyme-substrate complex was modeled.

1. CONFORMATIONAL PROPERTIES OF THE ASP³⁵ AND ASP²¹⁸ SIDE CHAIN

Conformational properties of the two catalytically active aspartic residues were studied using technique described in [4]. Figure 1 shows alignment of the $X_1 - X_2$ conformational maps of Asp³⁵ and Asp²¹⁸ in the potential field of rhizopuspepsin active site (623...
atoms of 48 amino acid residues according to the selected model) and $X_1$–$X_2$ plot of the N-acetyl-L-aspartic acid methyl amide.

The maps are plotted for the experimental $\phi$–$\psi$ values of the native enzyme [5]. It is noteworthy that both Asp$^{35}$ and Asp$^{218}$ side chains have almost identical conformational freedom in terms of their $X_1$ and $X_2$ dihedral angles. The energetically allowed region for both residues occupies on the plot a relatively small area with calculated minimum corresponding to the experimental $X_1$, $X_2$ crystallographic values; this minimum also lies within allowed region for a free aspartic amino acid residue. This fact indicates that aspartate side chains in the rhizopuspepsin active site structural environment do not undergo any steric tension, yet have substantially restricted conformational freedom. A special analysis of Asp$^{35}$ and Asp$^{218}$ nearest neighborhood demonstrates that their mutual orientation and conformational possibilities are directly determined by the next three residues in the sequence: Thr, Gly, Ser (Thr) which are conservative in all enzymes of this family. All types of nonbonded interactions: Van der Waals, electrostatic, torsional and hydrogen bonding contribute to the active Asp$^{35}(218)$ conformational stability with dominating contribution made by the dispersion component.

2. THE INHIBITOR AND SUBSTRATE BINDING MODES

In calculations of the enzyme–inhibitor complex we took into account 623 atomic coordinates of 48 residues of the active center and a nitrogen atom of the inhibitor Pro$^1$ pirrolydine ring. This nitrogen atom was rigidly fixed in its position, but Pro$^1$ residue was allowed to adopt any orientation about it. All side chain orientations were also fixed in the model except those which undergo a conformational change upon inhibitor binding. Evaluation of conformational properties of the Pro$^1$ residue revealed its single energetically favorable orientation in the enzyme potential field. The next step of the binding pro-

Figure 1. A cross-section of the $X_1$–$X_2$ potential energy surfaces of Asp$^{35}$ (thin line), Asp$^{218}$ (bold line) and of methyl amide of N-acetyl-L-Asp.