The proteolytic reaction of papain; ONIOM-type hybrid QM/MM calculations and Poisson-Boltzmann electrostatic correction

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Owing to the numerous experimental and theoretical works have been carried out on the papain (Fig. 1.) during the last two decades, a semi-quantitative model for the cysteine protease reactions could be derived. Nevertheless, some details, especially the exact sequence of bond breakage and formation and the relative energies of the stationary points on the potential energy surfaces have to be revealed.

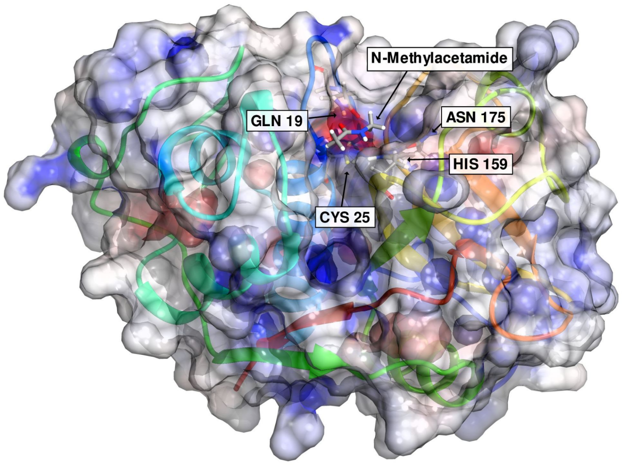

Figure 1. The structure of papain (ribbons), N-Methylacetamide and the catalytic triad (licorice). Visualized with Chimera (http://www.cgl.ucsf.edu/chimera/), the ESP surface was calculated with DelPhi (http://compbio.clemson.edu/delphi.php).

Our aim was to determine a feasible reaction path for the protease reaction of papain. ONIOM-type hybrid QM/MM calculations with electronic embedding approximation [1, 2] were used to map the potential energy surfaces and to find the local minima and first order saddle points on its. The existences of these specific points were proven by frequency analysis. MP2 method and modern density functional theories using standard basis sets with and without diffuse functions were applied throughout the study. Only the vicinity of catalytic site and the catalytic site itself were allowed to move during optimization. Poisson-Boltzmann (PB) model was used to calculate the electrostatic part of solvent effect on energy differences.

Besides the “exact” (at the levels of theories we used) stationary point geometries, energies and relative energies the most important results we obtained the instability of the “classical” tetrahedral intermediate state. The finding that the PB reaction field theory without any specific hydrogen bond is sufficient to favor the zwitterionic catalytic center [3] over the neutral one can be emphasized as well.

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