Application of X-Ray, SAXS and essential dynamics simulations to study conformational transitions of oligopeptidase B

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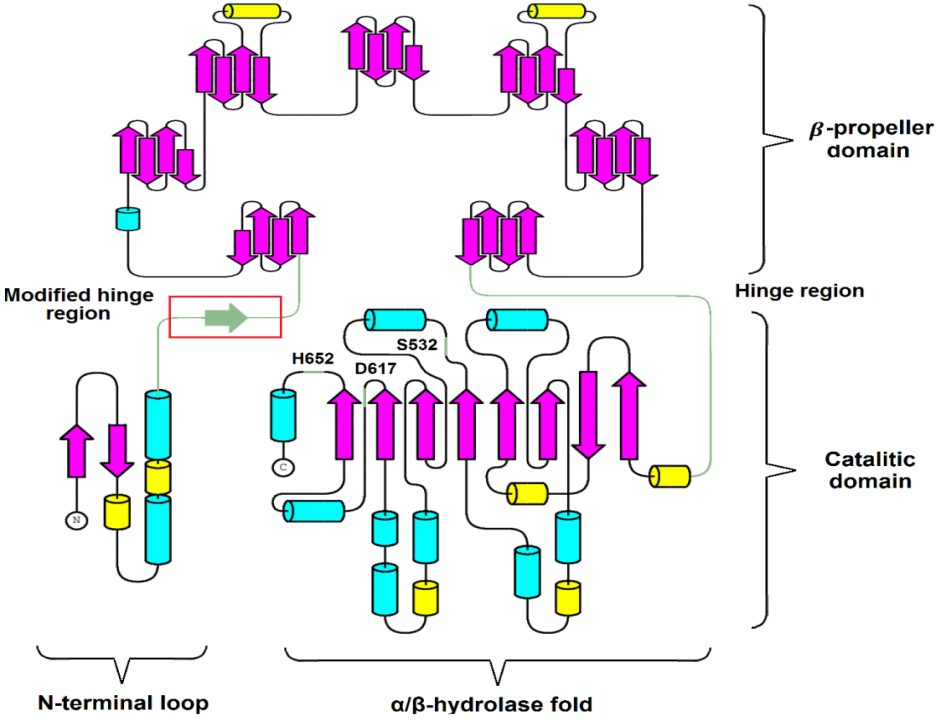
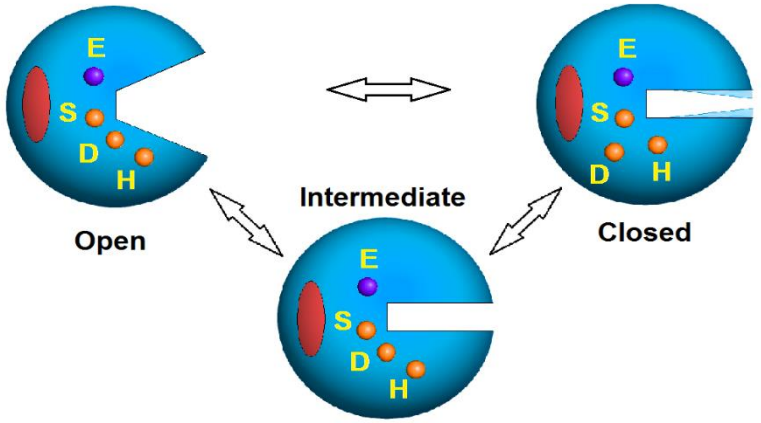
Motivation and Aim:



The schematic representation of a typical structure of POP demonstrates a domain swap in the catalytic domain. The topology was prepared using the Oligopeptidases B from bacteria Serratia proteamaculans (SpOPB) crystal structure.

Previously, X-Ray diffraction (XRD) analysis showed that OPB from bacteria *Serratia proteamaculans* (SpOPB) crystallized in the presence of polyamine spermine in the intermediate conformation; according to SAXS, in solution SpOPB adopted the open state, while spermine induced its transition to the intermediate state.

Oligopeptidases B (OPBs) are trypsin-like serine peptidases from the family of prolyl oligopeptidases. OPBs are found only in bacteria and parasitic protozoa and represent pathogenesis factors of the corresponding infections and putative pharmacological targets. According to the X-Ray study, these two-domain enzymes exist in 3 conformations: closed, open, and intermediate. In first case, the domains and residues of the catalytic triad (S, D, and H) are brought together; in second, they are separated; while in third case, the domains closure preceded the assembly of the catalytic triad. Transitions between these states regulates catalytic activation and consequently provide an avenue for inhibiting the enzymatic activity.



The schematic representation of the three conformations observed in the POP crystal structures.

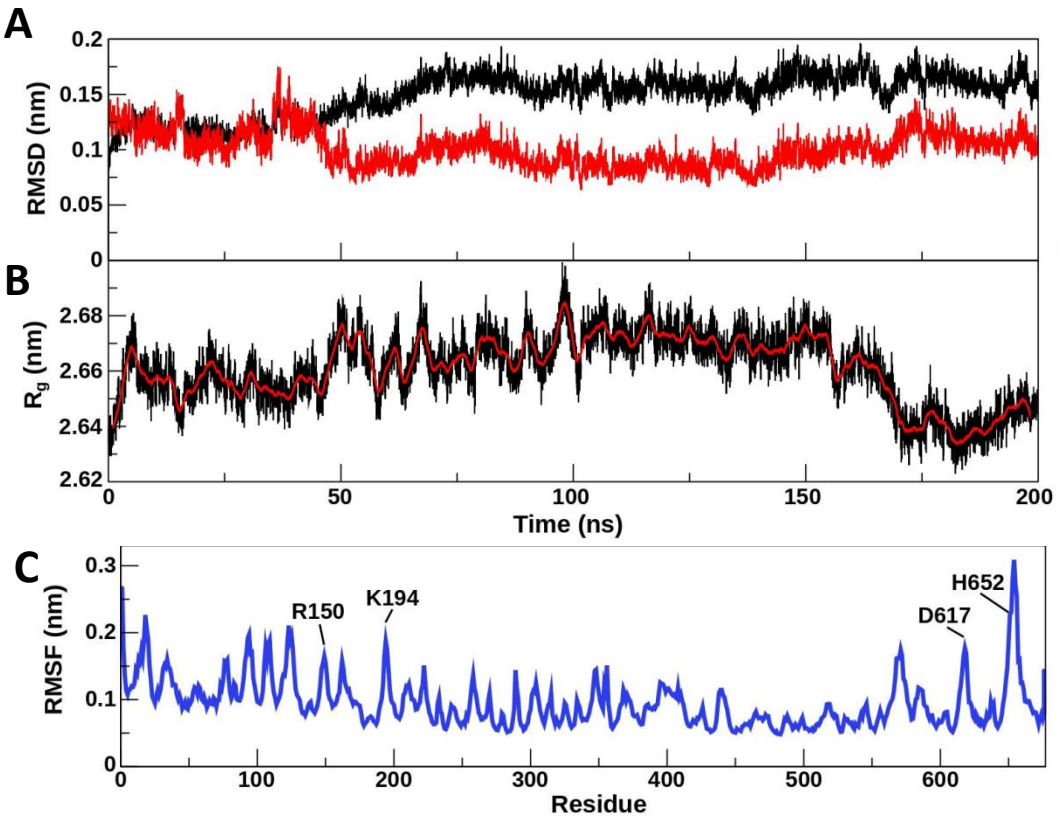
The aim of the study was the artificial transfer of the protein from the intermediate (crystal-associated) state to the open (solution-associated) state using Essential Dynamics/Molecular Dynamics (ED/MD) simulation.

Materials and methods

To elucidate the conformational dynamics of SpOPB, we applied Essential Dynamics/Molecular Dynamics (ED/MD), which combines classical MD simulation with Essential Dynamics Samling (EDS), which used to guide MD simulations.

In brief, when a definition of the collective fluctuations with largest amplitude is obtained from an initial MD simulation, EDS is used to manipulate the position of a protein along collective coordinates (eigenvectors) stimulating the system to explore new regions along these collective coordinates.

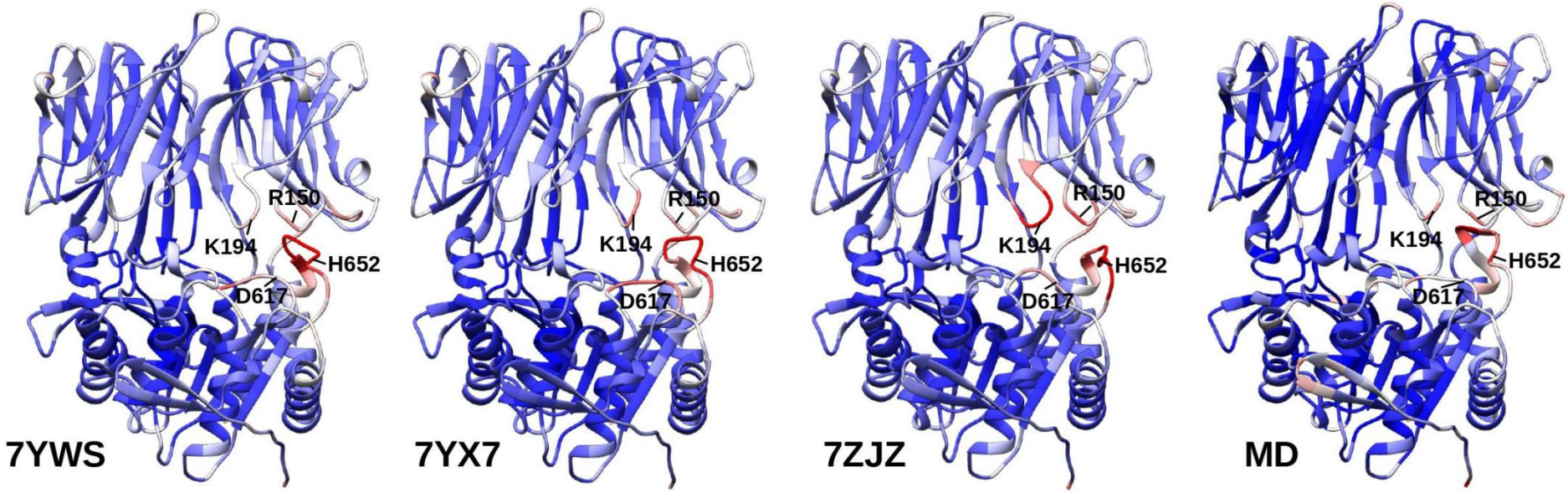
The results of this computational analysis were compared with those obtained by experimental X-Ray-based methods: XRD analysis and SAXS.



(A) The standard deviation of the main chain atoms on the MD trajectory (the black line is the RMSD relative to the initial structure, and the red line is the RMSD relative to the average structure).
(B) The change in the radius of gyration (Rg) (the red line is the average of 100 frames).
(C) Per residue RMSF for the entire MD trajectory and the mobilities of the key residues are correlated with the enhanced B-factors from the crystal structures.

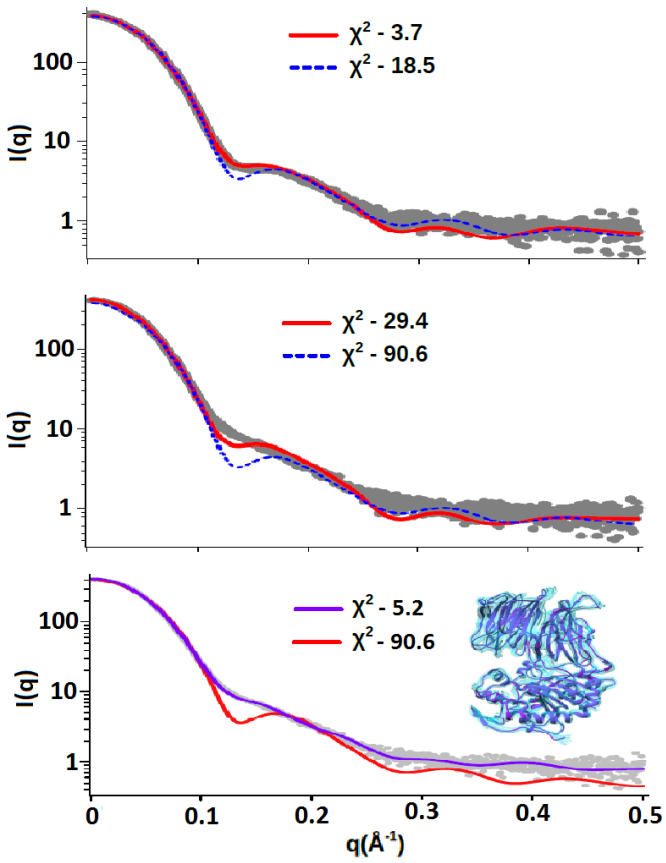
Results

The first crystal structure of catalytically deficient SpOPB (SpOPBS532A) with the intact hinge sequence was obtained (PDB ID 7ZJZ) as well as new structures of the enzymes with modified hinge regions (PDB ID 7YWS, 7YX7). Similarly to SpOPBs with modified hinges, SpOPBS532A was crystallized in the intermediate conformation. Despite the overall similarity of the crystal structures, an important difference was found in the arrangement of the catalytic S532, which could be the reason for the activity loss of the modified enzyme. The relationship between local fluctuations and conformational transitions of SpOPB was studied by ED/MD using the SpOPBS532A crystal structure (PDB ID 7ZJZ) as a starting point. As a result of the EDS simulation, the enzyme with the intact hinge was transferred from the intermediate to open state.



The crystal structures of SpOPBmod and SpOPBS532A, colored by the B-factors, and the averaged structure from the MD simulation, colored by RMSF. Red means the maximum values, blue means the minimum values.

Conclusion



Superposition of the experimental and theoretical SAXS curves. In the top and medium graphs, the curves for averaged MD structures (red) and the crystal structure 7ZJZ (blue) were fitted to the experimental SAXS curve (gray dots) obtained in presence (top) and absence (medium) of spermine. At the bottom, experimental SAXS profile for spermine-free SpOPB was aligned with the theoretical curves for the best EDS structure (violet) and the crystal structure (red).

Summarizing, we can state that during the EDS simulation, which started from the intermediate conformation, the open conformation of the protein was reached and this open conformation correspond to the conformation observed in solution with a high degree of probability.

Thus, the approach, which allowed simulation of the conformational transition, can be applicable to different systems for both study of the fundamental mechanisms of protein activity and structure-based drug design.

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