Unraveling Na^+ dependent thrombin activation with large-scale QM/MM simulations

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Thrombin is the key protease in the blood-coagulation cascade. With more than 500 unique records in the Protein Data Bank (PDB) for human thrombin only - it is one of the most studied enzymes to date. Yet there are several puzzles related to its functioning to be solved. The most intriguing one is the sodium-dependent activation which was discovered in 1980.

Na+-free thrombin has lower activity towards fibrinogen and other coagulation cascade substrates in comparison with the Na+-bound form. Sodium cation binding increases thrombin activity (altering kcat, Km or both in a substrate-dependent manner) up to 10-fold. Hence apo-form of the protein is often referred to as “slow” form, while the holo-form is called “fast”. Under physiologic conditions there is an equilibrium between three states:

$$E^* \square E \square E{:}Na^+$$

where E* is a low-activity form incapable of Na^+ binding, E and $E{:}Na^+$ (Na^+-stabilized form of E) have higher activity.

It is believed that the E form has a definite structure, while E* is represented by an ensemble of conformations. As a consequence of the ensemble heterogeneity, structural studies of the E* form proved to be more challenging than studies of the E:Na+ form. Most of the documented structures of “slow” thrombin contain mutations in proximity of Na+-binding site and crystal packing interactions that might affect conformations of the active site and Na+-binding site.

In this work, we performed large-scale hybrid QM/MM simulations with an unprecedentedly large QM part of 61 amino acid residues and 29 water molecules producing 459 atoms as well as classical MM simulations of thrombin-substrate complexes. We identified several previously undescribed or unattributed structural features of the E* form related to the configuration of the catalytic Ser195. The occurrence of the newly discovered states was confirmed with an analysis of human thrombin crystal structures from the PDB.

Our results reveal detailed picture of the E* form ensemble. We also for the first time demonstrate gradual effects of K+ and Na+ cations on the equilibria shift. Finally, our results clarify some controversies in the interpretation of existing crystal structures of “slow” and “fast” thrombin structures.

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