Structural basis of prothrombin recognition by a Type-I anti-prothrombin antiphospholipid antibody revealed by cryo-EM

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Background. Anti-prothrombin (anti-PT) antibodies are a type of antiphospholipid antibodies frequently found in antiphospholipid syndrome (APS) patients. Our previous studies have shown that prothrombin adopts closed and open forms and that Type-I antibodies prefer the open form. However, the structural basis of prothrombin recognition remains unknown.

Aims. To solve cryo-EM structure of a Type-I antibody bound to prothrombin and define its hemostatic profile.

Method. The mouse monoclonal Type-I antibody POmAb (Prothrombin Open monoclonal Antibody) was developed by immunization experiments and produced recombinantly. The mechanism of prothrombin binding was elucidated using surface plasmon resonance (SPR) and single-molecule FRET (smFRET). The structure of the complex was solved by cryo-electron microscopy (cryo-EM). Activated partial thromboplastin time (aPTT) and diluted Russell Viper Venom time (dRVV) were measured in human plasma.

Results. SPR and smFRET studies showed that POmAb binds kringle-1 of prothrombin with high affinity, forcing prothrombin to remain open. The cryo-EM structure of the complex was solved at a resolution of 3.2Å revealing an extended binding interface centered around the region R90-Y93 of kringle-1. Structural comparison between the complex and the closed form of prothrombin documents that the antibody clashes against the serine protease domain in the closed form, explaining why POmAb selectively binds to the open form. In human plasma, POmAb prolonged the aPTT and dRVV times, but the effect was modest and not entirely corrected by adding excess phospholipids, implying a weak LA effect.

Conclusions. Cryo-EM documents, for the first time, the structure of an anti-prothrombin antiphospholipid antibody bound to prothrombin. By forcing prothrombin to remain open, the Type-I antibody POmAb prolongs the clotting time with a new mechanism of action. These findings provide novel insights into autoantibody binding mechanisms and advance our understanding of prothrombin structure and function.

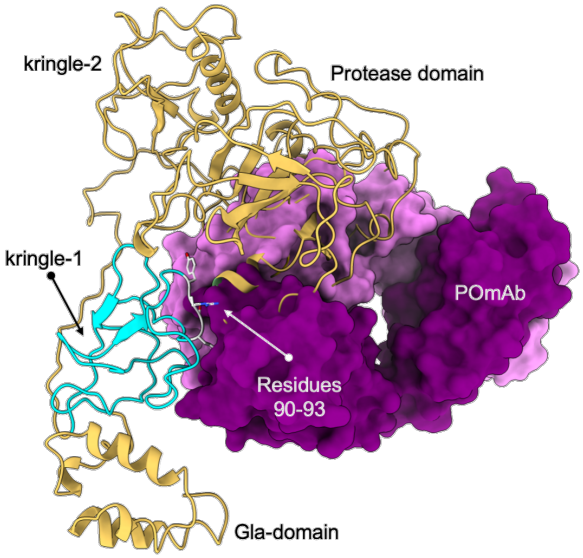

Figure 1. Cryo-EM structure of POmAb bound to prothrombin overlayed with the closed form of prothrombin (yellow) showing the protease domain clashing against the antibody. The binding of the protease domain and POmAb to residues R90-Y93 of kringle-1 is mutually exclusive.