Physico-chemical characterization of the interaction between human prothrombin and anti-thrombin aptamers

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Despite significant advances in the prevention and treatment of thrombosis, this disease is still one of the leading causes of death worldwide[1]. In this context, prothrombin pro-exosite I represents a potentially important new target for anticoagulant drug design, because it guides the prothrombin-prothrombinase interaction[2]. Interestingly, literature data demonstrated that anti-thrombin oligonucleotide aptamers, as the G-quadruplex TBA and other new generation duplex/quadruplex aptamers (RE31, NU172), binds prothrombin at the pro-exosite I and attenuates prothrombin activation by prothrombinase[3,4]. These aptamers, which are able to inhibit both activity and generation of thrombin, represent effective dual targeting therapy agents that could provide a decrease of therapeutic doses and of bleeding rates.

The structural features of the interaction between thrombin and oligonucleotide aptamers are well documented in literature[5,6]. On the contrary, up to date, a detailed structural characterization of the recognition mechanism between anticoagulant aptamers and the pro-exosite I of prothrombin is still lacking.

We performed a complete comparative thermodynamic analysis of the binding of TBA, RE31, and NU172 aptamers to thrombin and to its zymogen prothrombin by means of Isothermal Titration Calorimetry (ITC). The results clearly indicate the ability of the examined aptamers to interact with pro-exosite I with an affinity similar to that shown for exosite I, laying the foundations for an in-depth structural characterization. Furthermore, Circular Dichroism (CD) studies revealed the ability of prothrombin to act as molecular chaperone, inducing the aptamer folding.

Crystallization trials and Small-Angle Neutron Scattering (SANS) experiments of different prothrombin-aptamer complexes are in progress.

References

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