Molecular dynamics simulations shed light on the cooperative mechanisms generated by the simultaneous binding of aptamers at the two exosites of thrombin

Romualdo Troisi*, Nicole Balasco and Filomena Sica

Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Napoli, Italy
* romualdo.troisi@unina.it

Human α-thrombin is a trypsin-like serine protease endowed with the unique ability to convert soluble fibrinogen in insoluble fibrin clot. In addition to the active site, this enzyme owns two electropositive regions, exosite I and II, located at opposite sides of its globular shape [1]. The narrow substrate specificity of thrombin and its ability to change function are regulated by the exosite binding to different cofactors and modulators [2]. A special class of thrombin exosite synthetic ligands is represented by G-quadruplex anticoagulant aptamers, which are short single stranded DNA or RNA oligonucleotides that bind their targets with very high affinity and specificity [3]. The minimal 15mer DNA aptamer, named TBA, is the first and the most studied anti-thrombin aptamer. Based on several X-ray studies, this aptamer was established to bind the fibrinogen-binding site of thrombin (exosite I) by a pincer-like recognition mechanism involving the two TT loops [4]. The addition of a duplex motif to the G-quadruplex module has produced a new generation of aptamers with higher affinity against thrombin compared to TBA [5]. Among them, an aptamer, named HD22_27mer, recognizes exosite II with both quadruplex and duplex domains [6].

In the last years, great attention has been paid to the study of the effects of the simultaneous binding of two ligands on the two exosites of thrombin. Biochemical studies have suggested that thrombin is an allosterically modulated enzyme: an interplay between its two exosites as well as between the exosites and the active site has been highlighted [7]. Furthermore, the crystallographic structures of two thrombin ternary complexes, in which exosite II is bound to HD22_27mer and exosite I interacts with TBA-like aptamers, were solved and gave structural information on the effects of the simultaneous binding of two aptamers to thrombin exosites [8].

Here we present the results of an extensive molecular dynamics study performed on free thrombin and on its binary and ternary complexes with TBA and HD22_27mer in the absence of the PPACK inhibitor that is covalently bound to the protein active site in all the crystallographic α-thrombin models. This study revealed that, in the absence of any influence of the crystal packing, an inter-exosite cross-talk and an active site-exosite communication may occur in these systems. Details will be discussed at the Meeting.

[1] E. Di Cera, *J Thromb Haemost.*, **5**, (2007), 196-202.
[2] S. Krishnaswamy, *J Thromb Haemost.*, **3**, (2005), 54-67.
[3] A.D. Keefe, S. Pai, A. Ellington, *Nat Rev Drug Discov.*, **9**, (2010), 537-550.
[4] I. Russo Krauss, A. Merlino, A. Randazzo, E. Novellino, L. Mazzarella, F. Sica, *Nucleic Acids Res.*, **40**, (2012), 8119-8128.
[5] I. Russo Krauss, V. Napolitano, L. Petraccone, R. Troisi, V. Spiridonova, C.A. Mattia, F. Sica, *Int J Biol Macromol.*, **107**, (2018), 1697-1705.
[6] I. Russo Krauss, A. Pica, A. Merlino, L. Mazzarella, F. Sica, *Acta Cryst. D*, **69**, (2013), 2403-2411.
[7] N.S. Petrera, A.R. Stafford, B.A. Leslie, C.A. Kretz, J.C. Fredenburgh, J.I. Weitz, *J Biol Chem.*, **284**, (2009), 25620-25629.
[8] A. Pica, I. Russo Krauss, V. Parente, H. Tateishi-Karimata, S. Nagatoishi, K. Tsumoto, N. Sugimoto, F. Sica, *Nucleic Acids Res.*, **45**, (2017), 461-469.