The Early Phase of the Conformational Transition at Vertebrate Transglutaminases; Can We See it From Molecular Dynamics Simulations?

D. Mucs[1], A. Fekete[1], L. Muszbek[1,2] and I. Komáromi[1,2],

[1]Clinical Research Center, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary and [2]Haemostasis, Thrombosis and Vascular Biology Research Group of the Hungarian Academy of Sciences, Debrecen, Hungary

The main physiological role of transglutaminases is to form inter- or intramolecular ε(γ-glutamyl)-lysyl crosslinks. The atomic resolution 3D structure for the "open" (assumed to be "active") conformation, fixed with substrate analog inhibitor [1], is available only for human TG2. The difference between the "closed" ("inactive") and "open" X-ray structures is surprisingly large. Nevertheless, only the relative orientations of the constituting domains were changed while the domains kept their substructures.

Because of the structural and functional similarity of the "inactive" ("closed" conformation) vertebrate transglutaminases, significant similarity between their "open" conformations can be assumed as well. It was the basis of the theoretical model we proposed [2] for the "open" conformation of FXIII-A_2*.

While the whole conformational transition can be considered as „rare events" on the time scale can be reached even on nowadays high-end supercomputers, simulations can provide us valuable data on the early phase of conformational changes. Therefore our aim was to extract information from molecular dynamics trajectory on how these large Ca^{2+}-dependent conformational changes begin. Our aim was also to gain information on the binding mode of Ca^{2+} and to reveal their role in triggering the large scale conformational transition.

1μs NPT molecular dynamics simulations using explicit solvent molecules and periodic boundary condition have been carried out on the „closed" form of human TG2 in the presence and in the absence of Ca^{2+} ions. The long range electrostatic interactions were calculated by means of the particle mesh Ewald protocol implemented in the GROMACS molecular dynamics package. OPLS-AA/L force field was used for the protein while for the solvent the TIP3P water model was applied. The low frequency modes were extracted from the covariance matrices which were constructed from the trajectories of dynamic simulations. They represented motions along which the conformational changes are assumed to occur. The most probable Ca^{2+} binding sites were proposed from simulation and they are in a good accordance with those ones which were obtained from systematic mutational experiments [3]. In addition to the primary Ca^{2+} binding sites were found experimentally and which were found characteristic ones even from simulations, other, less characteristic Ca^{2+} binding sites were obtained as well.

Acknowledgments: This work was supported by Hungarian Scientific Research Funds (OTKA K-106294, NIIF-1057, TÁMOP-4.2.2.A-11/1/KONV-2012-0045 and TAMOP-4.2.2.C-11/1/KONV-2012-0010 grants.

References:

1 Pinkas DM, Strop P, Brunger AT, Khosla C. Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol.* 2007; **5**: e327.

2 Komaromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional aspects. *J Thromb Haemost.* 2011; **9**: 9-20.

3 Kiraly R, Csosz E, Kurtan T, Antus S, Szigeti K, Simon-Vecsei Z, Korponay-Szabo IR, Keresztessy Z, Fesus L. Functional significance of five noncanonical Ca2+-binding sites of human transglutaminase 2 characterized by site-directed mutagenesis. *FEBS J.* 2009; **276**: 7083-96.