Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling

Chikungunya virus (CHIKV, genus Alphavirus, family Togaviridae) has a positive sense RNA genome with length approximately 12 kb. It codes for four nonstructural (ns) proteins designated as nsP1, nsP2, nsP3 and nsP4 and for five or six structural proteins. Ns-proteins are involved in replication of virus RNA, in addition they also have functions unrelated to RNA replication. Out of all the nsPs, nsP2 plays a pivotal role towards regulation of CHIKV RNA replication. The protein was known to have NTPase, RTPase and protease activity and its N-terminal region was presumed to have helicase activity. Out of the enzymatic activities of nsP2 the helicase related functionalities were most insufficiently studied. Further, it was not known why two different and seemingly disconnected functional entities such as protease and helicase are present on a single polypeptide and what could be the importance of N terminal most part of nsP2 on the helicase activity.

The bioinformatical, biochemical and biophysical approaches were employed to characterize the helicase related activities and to reveal the apparent minimal requirements for these activities. The bioinformatics platform suggests that the 3D-structure of the first 470 aa of nsP2 resembles the fold pattern of ToMV helicase which is a superfamily 1 of helicase. In particular, this fragment was predicted to consist from three domains. From these the extreme N terminal domain appears to be disordered while the other two domains possess RecA-like fold which is commonly found in NTPases.

The biochemical analysis, carried out with purified full length and manipulated versions of nsP2, revealed that the C-terminal part of nsP2, which was known to have protease activity, is also essential for RNA helicase activity. Thus, the presence of protease region in nsP2 is clearly not accidental and these different functional domains are co-evolved to accomplish more significant tasks. The use of biophysical method (CD spectroscopy) confirmed that secondary structures of wt and manipulated versions of nsP2 are comparable; this indicates that functional defects detected in various enzymatic activities did not result from misfolding of mutant proteins. This also applies to forms of nsP2 which were engineered to contain mutations associated with noncytotoxic (NCT) phenotype of CHIKV replicons. It was found, all analyzed nsP2 enzymatic activities (protease, NTPase and helicase activities) were invariably affected by the NCT related mutations. In general, however, there was no significant correlation observed between extent of enzymatic defect(s) of nsP2 and phenotype of corresponding replicon. Thus, the development of NCT phenotype is apparently more complicated and could involve a number of underneath viral replication related functionalities. Finally, a number of ns-proteins from different alphaviruses were expressed, purified to raise polyclonal sera. These represent tools for detection of viral proteins using different immunological, such as western blot and immunofluorescence, methods. Similarly, the standardized enzymatic assays of nsP2 represent platform for screening and analysis of potential inhibitors of CHIKV infection. Taken together, these works elevated general understanding of nsP2 from a biochemical prospective and provided useful tools for studies aiming to understand molecular biology of alphaviruses.