A mechanism by which P250L mutation impairs flavivirus-NS1 dimerization: structural insights towards vaccine development

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Abstract

Flavivirus threats such as dengue, West Nile and Zika are major health issues in tropical and subtropical countries. The development of an effective anti-flavivirus strategy would represent a cornerstone in the global quality of health. Based on the NS1 antigen, this work supports novel strategies for rational virus attenuation, opening up new possibilities for anti-flavivirus measures.

Introduction

The flaviviruses comprise etiologic agents such as dengue, West Nile and Zika. These viruses are transmitted to humans by arthropods and are considered as major health issues due to their epidemiological relevance in tropical and subtropical areas worldwide. NS1 is a conserved-flavivirus glycoprotein that can be found as dimmers (Figure 1 left) in vesicular compartments within the host cell, associated to cell membranes or secreted to the extracellular space as lipid-coupled hexamers[1]. A single amino acid substitution (P250L) (Figure 1 right) is capable of preventing the dimerization of this protein resulting in lower virulence and slower virus replication[2,3]. Among the flavivirus proteins, NS1 is the only one which its precise biological function is yet unknown. Nevertheless, reports have shown the participation of NS1 in many important aspects, such as flavivirus replication, immune modulation and cell metabolism, what makes this protein an interesting target for anti-flavivirus measures.

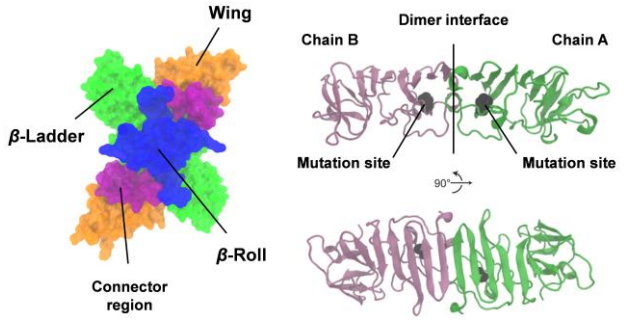

Figure 1. (left) Three-dimensional illustration of the NS1 dimer and its domains. (right) Cartoon representation of the β-ladder domain showing the dimer interface between the two monomers. Mutation sites are shown in black.

Results and Discussion

In this work, based on molecular dynamics simulations and using the NS1 β-ladder$_{179-349}$ monomer as a core model, we found that P250L mutation was able to produce long-range alterations resulting in several conformational changes. The main effect observed was a consistent conformational alteration of the β1 strand present in the β-ladder domain. As a result, the dimer interface was mainly affected and a set of critical monomer-monomer interactions, which contributes to the stabilization of the NS1-dimeric form, were impaired by this process. More specifically, the complementary hydrogen bonds between residues A186 and I188 (four bonds in total) were affected leading to the destabilization of the dimer interface. We suggested a mechanism by which a highly orchestrated sequence of events propagates the local perturbations around the mutation site towards the dimer interface. This mechanism was supported by additional simulations of other mutant models and is also probably valid for other flaviviruses due to the high degree of conservation among the residues involved. Moreover, appart from the P250L, W232A was also identified as a potential mutation to inhibit the flavivirus-NS1 dimer formation.

Conclusion

According to our *in silico* studies, P250L and W232A mutations can potentially alter the conformation of flavivirus-NS1 β-ladder monomer to inhibit the formation of the NS1 dimers. The integrity of the dimeric NS1 appear to be important in flavivirus biology since the inhibition of NS1-dimer formation implicates in loss of virulence and slow virus replication. This work supports new mutation-based strategies that can be applied for rational production of live-attenuated vaccines, highlighting a step forward in the development of novel counter measures in the field.

Aknowledgements

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