

MODELING THE FLAVIVIRUS ENVELOPE. PREDICTION OF FUNCTIONAL RESIDUES BY THE ANALYSIS OF E-GLYCOPROTEIN SEQUENCE CONSERVATION PATTERNS

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Introduction

The glycoprotein E is the major protein of the proteolipid envelope of flaviviruses. It mediates the interaction with cellular receptors and the acid catalyzed membrane fusion, constituting an important factor determining tropism, host range, virulence and protective immunity. Recently, the X-ray crystal structure of a soluble fragment of protein E from tick born encephalitis virus (TBE) has been solved, showing that the protein forms head-to-tail homodimers which apparently lay parallel to the viral membrane (1). Biochemical evidences suggest that these dimers are organized in a yet undefined network-like structure. The functional sites of protein E have not been mapped neither, though some speculations have been made concerning the location of receptor binding sites and the fusion peptide. Here we report the modeling of a symmetrical lattice structure that could be adopted by the protein E dimers in the surface of the virions and which is consistent with the experimental data. Furthermore, we have analyzed the sequence conservation patterns of flavivirus E proteins, suggesting the location of likely functional residues.

Materials and Methods

Modeling of the viral envelope and homology modeling of dengue virus E protein was achieved using the program WHATIF (2). Sequences of flavivirus E proteins were obtained from SWISSPROT, Genbank and EMBL databases. Secondary structure predictions were made with PHID (3). Prediction of functional residues were carried out by Sequence Space Analysis (4).

Results and Discussion

We have built a model of the viral envelope consisting of an icosahedral T=3 lattice of protein E monomers. Modeling was accomplished according to the following assumptions: virus diameter is 500 Å, the C₂ symmetry axis of protein E crystal dimers coincide with the icosahedral pseudo- and real C₂ symmetry axis, bumps between protein atoms are forbidden and the distance between the C-terminus (residue 395 of TBE) of each subunit and the icosahedral

pseudo C₃ symmetry axis is minimal. The modeled structure is shown in Figure 1. The resulting particle is formed by 90 dimers. The space around the pseudo-C₃ symmetry axis could be occupied by the C-terminal portions of the ectodomain which links the crystallized fragment with the membrane anchor. This segment is predicted to be constituted of two amphipathic helices separated by a highly conserved loop and it could play an important role in the pH induced oligomeric transition from dimers to trimers, which seems to be necessary for the fusogenic activity. The conservation patterns present in flavivirus E proteins should reflect the evolutionary constraints imposed by the biological function. Flavivirus conserved residues are mostly buried or exposed into the inner surface. Three major clusters were apparent: the tip of domain II, likely to be important for fusion activity; the putative loop at the C-terminal fragment of the ectodomain, whose probable role was discussed above; and an ionic cluster located in the interface between domain I and III, which we predict to participate in the low pH triggered oligomeric transition, stabilizing indirectly the dimers at neutral pH and destabilizing it at acidic pH. The outer surface of the protein is highly variable among flavivirus complexes and types. Further analysis of the dengue virus complex showed a conserved residue cluster located at the upper lateral surface of domain III, pointing toward the C₅ and C₃ symmetry axis of the modeled virions. We believe it could be a receptor binding site, involved in the virus interaction with a receptor from human and/or mosquito cells.

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4. Casari, G., Sander, C. and Valencia, A. (1995) Nature Struct. Biol., 2, 171-178.

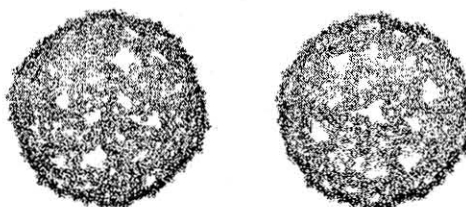


Figure 1. Stereo view of the modeled icosahedral envelope of TBE.