Hepatitis C virus envelope glycoprotein E2: critical analysis of two proposed models

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Motivation

Hepatitis C virus (HCV) represents the major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma worldwide, affecting 1-3% of the world population. Currently, interferon base therapy is effective in only 50-60% of treated patients. The HCV envelope glycoprotein E2 is largely responsible for virus antigenicity and it is involved in important viral processes including virus attachment, cell-entry and therefore it is a prime candidate for a vaccine. No crystallographic structure is available but it would represent a significant step forward in the fight against this virus. Two models obtained by fold recognition, have been proposed by Tramontano's group in 2001, and by Niccolai's group in 2006. The value of a good predicted structural model of HCV E2 is high when it is used for the definition of its biological function. Consequently, we considered that it is important to verify at a high-resolution level the difference between the two proposed models of E2 and to define each model's sustainability. We have analysed each E2 model's capacity to satisfy a number of experimental data regarding some of the major function effectors of this protein, including the glycosylation sites, the CD81 cell co-receptor and well defined epitopes generating neutralizing antibodies.

Methods

The three dimensional structure of the two models predicted by Yanik et al.(Protein Model Database ID_PM0074602) and by Spiga et al4 (PDB_2AGR) were retrieved on the basis of the literature information. In this work both models were analyzed and compared in terms of secondary structure content established using DSSP, hydropathy profile calculated according to Kyle and Doolittle plots, surface accessible area (ASA) using ASAView. The local quality was evaluated with QMEAN. By aligning several sequences belonging to the major HCV genotypes the conservation of each amino acid position was defined using Consurf. Amino acids at different conservation levels were then mapped on the tertiary structure of each model. Protein Structure Comparison, alignment and RMSD were obtained using the Combinatorial Extension (CE) method and structures visualized using PyMOL. The two models were further compared on their glycosylation sites distribution, the

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localization of single amino acid residues, segments involved in the interaction with the CD81 co-receptor and, the position of neutralizing antibody epitopes.

Results

As expected, the models have several common structural features. Their superimposition emphasizes the good structural alignment between them and a structural identity of 90%. However Tramontano's model contains a higher number of structured elements. On the other hand, the local quality is better for the Niccolai's model having overall a lower estimated energy profile along the sequence. According to experimental data in Niccolai's model, all the 18 cysteins present in the protein are involved in disulphide bridges while in Tramontano's model only 8 are. The hydropathy profile analysis versus accessibility surface area (ASA) for both monomeric and dimeric forms, shows that Tramontano's model confirm the head-to-tail homodimer conformation proposed for this protein, while in Niccolai's model, one chain is rotated 180°. Conserved amino acid residues are better distributed in Tramontano's model than in Niccolai's structure model, interestingly, in the first case both hypervariable regions I and II, are located in the same face of the dimer where, also, the majority of the conserved residues are localized within a small region in the center forming a central ring. Finally mapping functional sites show that in both models several glycosylation sites are totally or partially buried. This is in disagreement with their biological function of being involved in CD81 binding and thus playing a direct role in viral cell-entry and in neutralizing antibody formation. Due to the different position of the chains between the two models, CD81 binding sites and epitopes for neutralizing antibodies are localized differently. In Tramontano's model they are in one face, while in Niccolai's model they are in both, moreover several amino acids involved in this sites are buried or partially buried. On the basis of our results, we can conclude that both models of the HCV E2 protein present some limitations that make them only partially reliable. Tramontano's model seems to be the most consistent with the functions of E2 as supported by evidence collected so far. However, also this model presents some weak points, making the development of a new more reliable model necessary.

Availability

http://www.unipd.it/

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