Structural investigation of gp36-MPER in membrane mimicking systems

Abstract

FIV is a lentivirus that resembles the Human Immunodeficiency Virus (HIV). The lentiviral *envelope* glycoproteins (gp41 in HIV and gp36 in FIV) mediate virus entry by interacting with specific receptors present at the cell surface. Increasing evidences suggest a common structural framework for these glycoproteins, corresponding to similar roles in virus cell fusion. During the virus entry a conformational transition occurs in the glycoproteins to form a stable six-helical bundle. In this conformational arrangement, the correct assembly with the membrane of a tryptophan (Trp) rich region named membrane proximal external region (MPER) leads to the fusion of virus *envelope* and host cell membrane. The design of molecules inhibiting the correct positioning of MPER on the membrane is currently a strategy for the design of virus entry inhibitors.

C8, a Trp-rich fragment of gp36-MPER, was identified as an antiviral compound inhibiting the entry of FIV into the host cell. C8 showed remarkable membrane binding property, inducing alteration of the phospholipid bilayer and membrane fusion. In this context, part of my PhD thesis is focused on the study of C8/lipid membrane interaction employing a multiscale approach based on spectroscopic experiments, molecular dynamics simulations and confocal microscopy imaging. Our results show that the peptide is active on zwitterionic lipid environment in which it induces a

structural reorganization of the phospholipid bilayer that finally leads to the formation of membrane tubules.

The presence and the position of Trp residues in C8 are important for antiviral activity: the C8 derivative C6a, obtained by truncating the N-terminal ^{770}Trp-Glu^{771} residues, exhibits conserved antiviral activity, while the C8 derivative C6b, derived from the truncation of the C-terminal ^{776}Trp-Ile^{777}, is nearly inactive. To elucidate the structural factors that induce the different activity profiles of C6a and C6b, in spite of their similarity, in the second part of my PhD thesis I performed the study of structural behaviour of the two peptides in membrane mimicking environments using an analytical approach very similar to that employed for C8 peptide. I analysed C6a and C6b using CD and NMR spectroscopy, and confocal microscopy imaging. These data provide evidence that common antiviral activity profiles correspond to similar membrane binding properties: actually C6a, similarly to C8, has the ability to destabilize membrane vesicles, producing complex network of membrane tubes.

Eventually, I analyzed structural behavior of the full gp36-CHR-MPER in a set of membrane models characterized by increased complexity: micelles, multilamellar vesicles and cellular vesicles. Analyses with paramagnetic probes and confocal microscopy indicate that gp36-CHR-MPER and its derivatives are active on each of the mentioned bio-membrane systems, thus indicating that their impact on the size and shape is not a biophysical artefact, but an effect of biological relevance.