Solid, Liquid and Semi-liquid State NMR Studies on the Structure of HIV-Rev Protein Free and Bound to its Target RNA

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The human immunodeficiency virus Rev protein is required for export of partially spliced and unspliced viral mRNA from the nuclei of infected cells, and ultimately for viral replication. Rev is highly prone to aggregation, in both the absence and the presence of the Rev responsive element (RRE) RNA to which it binds. As a result, the full molecular structures of Rev and Rev/RRE complexes are not known.

Transmission electron microscopy and atomic force microscopy show that the morphology of pure Rev preparations and of Rev complexes with the 45-base RNA sequence of the high-affinity stem-loop IIB segment of the RRE are different. While Rev alone forms highly ordered filaments, Rev/RNA coassemblies are less ordered and not filamentous. Nonetheless, two-dimensional (2D) solid state ^{13}C-^{13}C NMR spectra of Rev filaments and Rev/RNA coassemblies, in which all Ile, Val, and Ala residues are uniformly ^{13}C-labeled, are nearly indistinguishable, indicating that the protein conformation is essentially the same in the two types of supramolecular assemblies [1]. Analysis of crosspeak patterns in the 2D spectra supports a previously developed helix-loop-helix structural model for the N-terminal half of Rev and shows that this model applies to both Rev filaments and Rev/RNA coassemblies.

Structural data on the flexible zones of both Rev filaments and Rev/RNA coassemblies have been obtained by HR-MAS, resulting in high quality NMR spectra on the viscous suspensions of these supramolecular species. Standard triple resonance spectra under MAS on uniformly ^{13}C and ^{15}N enriched Rev filaments and Rev/RNA coassemblies indicate a similar behaviour at the molecular level of the Rev protein in the filaments and in the Rev/RNA coassemblies: the C-terminal 25 residues are flexible and disordered while the N-terminal 90 residues, invisible in these spectra, form the rigid core of the fibers and assemblies.

In 6M urea the structure of Rev can be analysed in solution showing little conformational preferences in the polypeptide chain under these denaturing conditions.

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[2] Blanco F.J, Hess, S., Pannell, L.K., Rizzo, N.W. and Tycko, R. (2001) J Mol Biol, 313, 845-859.