Structural characterization of filamentous bacteriophage viruses by magic-angle spinning solid-state NMR spectroscopy

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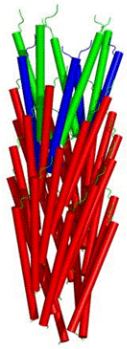
Summary



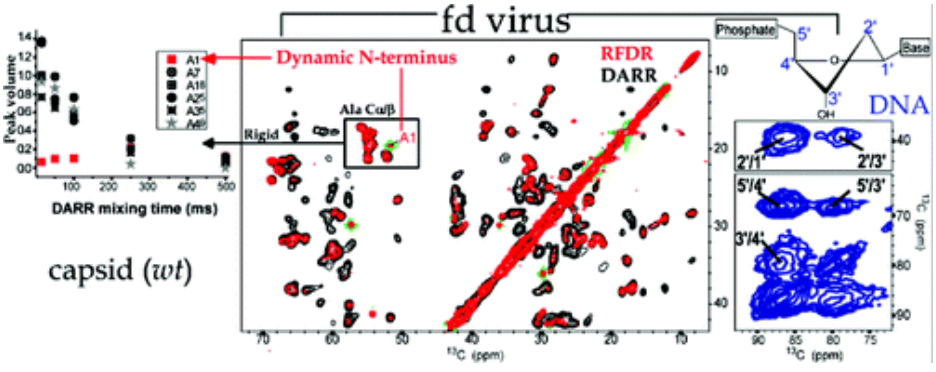
Filamentous bacteriophages comprise a family of viruses that share a similar virion structure and life cycle. The virion is composed of a circular ssDNA wrapped by thousands of similar copies of a major coat protein and several different minor coat proteins in both ends. Due to the mass (tens of MegaDaltons) and dimensions (~7 nm in diameter, 600 – 2000 nm in length) of filamentous phages, structural models have been obtained primarily from fiber diffraction, and from static solid state NMR of aligned concentrated solutions. Yet, these models are incomplete, and vary even within individual virions. Also, some key questions regarding DNA structure, subunit-DNA interactions, microscopic polymorphism, and more, remain unclear or are in debate. Magic-angle spinning solid state NMR (MAS SSNMR) has been shown to be a complementary and successful method for studying the structure and dynamics of biological macromolecules, and is particularly suitable for non-crystalline / non-soluble systems . Examples are membrane proteins, protein aggregates and fibrils, viruses and more. Also, sample preparation for MAS SSNMR is more flexible, and measurements can be performed in conditions, which are either biologically relevant, or important for their biophysical characterization.

In this project we used magic-angle spinning solid-state NMR to study the class-I bacteriophages belonging to the Ff family (fd, M13) and the class-II phage Pf1. We obtained the complete chemical shift assignment of the three phages, analyzed their intact capsid and DNA structure, examined similarities and differences, and investigated their structural behavior under different capsid and DNA perturbants and under different temperatures. We progressed significantly towards full structure calculation of M13, which is currently underway.

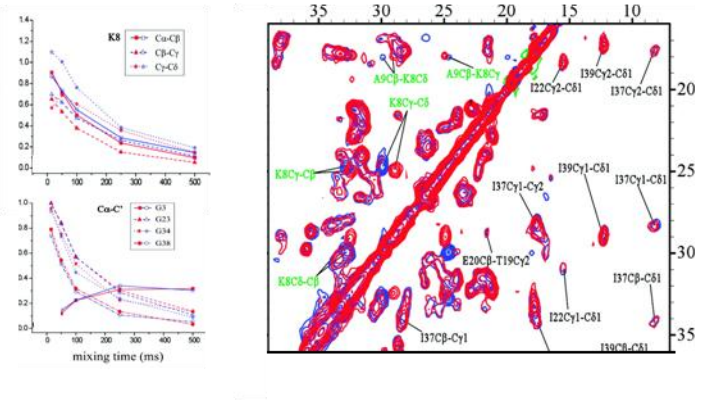
Results

Sample preparation and purification: Routine high-yield preparation, purification and biophysical characterization of M13 and fd viruses are now common in our lab and regular yields range 30-50 mg / L of culture. We managed to prepare both fully and 'check-board' enriched samples using either $^{13}C_6$-D-glucose, 1,3-^{13}C glycerol and 2-^{13}C glycerol, all combined with complete ^{15}N enrichment. All samples retain their biological infectivity prior and after NMR experiments.

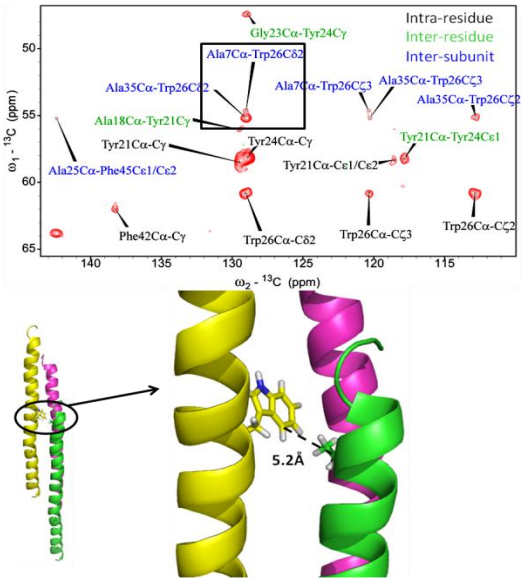
NMR experiments for assignment and conformation studies: We coded, tested and applied multi-dimensional MAS NMR experiments to assign and study the various phage systems. In particular, we employed ^{13}C-^{13}C homonuclear correlation experiments, ^{15}N-^{13}C 2D and 3D heteronuclear correlation experiments and 2D double-quantum/single-quantum experiments. Additional experiments involve through-bond transfer in the solid-state. These experiments provided us with sufficient resolution to fully assign the viruses' capsid protein. The chemical shifts for the capsid of fd have been deposited in the BMRB (Accession #17728). The Figure on the right summarizes the results of the fd study: The coat protein has a single unique structure. It is mostly helical (residues 6-47), its first 5 residues have a loop-like structure and are very mobile, and the DNA has a C2′-endo/C3′-exo sugar pucker. Our results indicated that the structure of the wild-type phage is very homogeneous despite the indication of all other methods (fiber diffraction, cryoEM, static solid-state NMR) that a Y21M mutation is required to obtain a homogeneous sample.

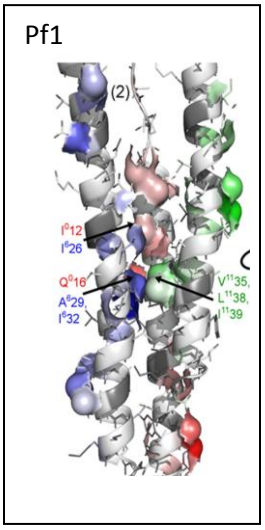


Comparison of M13 and fd phages: M13 and fd differ in a single amino acid in every capsid: the uncharged asparagine-12 in the coat protein of M13 is replaced with a negatively charged aspartate in fd. NMR data reveals that the two virions have a very conserved and stable structure, manifested in negligibly small chemical shift differences and similar dynamic properties for nearly all resonances. It explains the high fidelity of these viruses, which have a very stable and preserved structure. The principal difference between the two phages involves residues in the vicinity of residue 12. We suggested that the elimination of the single charge at position 12 throughout the entire capsid affects the electrostatic and hydrogen-bonding interaction network governing inter- and intraresidue contacts, mainly by the rearrangement of the positively charged lysine residue at position 8. The figure on the left shows the spectral overlay of the two phages (M13 in blue, fd in red). Identical resonances of most atoms including backbone and sidechains are apparent but shift deviations in Lys8 alongside differences in chain dynamics are apparent (left top).



Capsid interactions and virus structure: We used two different strategies to probe the interactions between the subunits. In Pf1, we utilized the structural phase transition in order to detect the residues involved and derived information on the hydrophobic interface. In this interface, reorganization of sidechain contacts drives the phase transition. In M13, using sparsely labeled samples, we directly probed the capsid contacts and determined the phase of the individual units with respect to the DNA axis. The NMR signals describing inter-subunit interactions were pulled out of thousands of other data points using specialized software for the automated detection of such contacts. The hydrophobic pockets describing the capsid contacts in Pf1 and examples of inter-subunit interactions from M13 are shown on the right.





Additional studies: We designed and collected data from experiments aimed to probe protein-DNA interactions, studied the dependence of spectral features of fd virus upon the addition of perturbants such as SDS and Ag, both of which affect the liquid crystalline properties of the phage, and compared results of circular-dichroism and NMR to study the relation between microscopic molecular changes and the macroscopic liquid crystals formed by the virus.

Conclusions

Our studies on several filamentous bacteriophages belonging to two different symmetry groups have demonstrated that MAS SSNMR spectroscopy is an excellent tool to study protein complexes, regardless of their shape and molecular weight. Analysis of our data enabled us to deduce the secondary structure of the coat protein, the polymorphic state of the viruses, to evaluate capsid interactions and gain information on their orientation with respect to the DNA. We obtained new knowledge on the DNA structural organization, some of which sheds new light on prior assumptions, and determined that the phages are very homogeneous in structure. Since bacteriophage viruses are widely used in molecular biology, phage display, nanotechnology and more, accurate information on their structure will have significant impact on all these fields of study.