Dynamics and rigidity in the intact fd(y21m) bacteriophage probed by automated analysis of ssNMR experimental data

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Filamentous bacteriophages are viruses that infect bacteria. Their ssDNA genome is packaged during assembly by a capsid made of thousands of identical copies of a helical coat protein, which are arranged as layered pentamers related by an approximate two-fold screw axis. While the atomic-resolution structures of several such phages have been revealed, in a site-specific manner less is known on capsid dynamics, which is related to their function, stability and interactions with the genome. Protein dynamics on various time scales can be assessed by measuring relaxation times and anisotropic interactions such as dipolar couplings, quadrupolar couplings, and chemical shift anisotropy (CSA). The magnitude of an average amide CSA interaction in proteins is approximately 6-7 kHz on a 14.1 T magnet (~110 ppm), hence, it reports on amplitudes of motions that are faster than this value. In this study, we used CSA recoupling experiments under magic-angle spinning NMR in order to probe the dynamics of the coat protein of the fd bacteriophage bearing a tyrosine-to-methionine mutation in position 21 (y21m). In order to analyze the results efficiently and in a reliable way, we developed a software, which is able to assign spectral cross peaks on the basis of experimental data, the preliminary assignment table and the known tertiary or quaternary structure, and to output the CSA line shapes (or any other data of choice) site-specifically. We show, based on fitting those automatically-generated CSA lineshapes, that residues located in the helical and in the C-terminal part (residues 6-50) have CSA values of 6.2 kHz indicating relatively high rigidity, and that residues in the N-terminus possess lower CSA values of 1.4-5 kHz indicating increased motional amplitudes. The rigid helix is indicative of the tight hydrophobic packing of the phage. The results from CSA measurements agree with qualitative analysis of build-up curves based on the homonuclear dipolar interaction, also provided by the automation tool. Our automated analysis technique is general and can be applied to study protein structure and dynamics, with data resulting from experiments that probe different interactions such as relaxation rates, dipolar order parameters and more.