STRUCTURE AND DYNAMICS OF FD COAT PROTEIN

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The major coat protein of the filamentous bacteriophage fd undergoes substantial changes in structure and dynamics during viral assembly. The fd phage particle is a flexible rod consisting of several thousand copies of the major coat protein wrapped around an extended single-stranded circle of DNA. Prior to its assembly into the virus, the coat protein is stored as an integral membrane protein. The comparison of the properties of the coat protein in the membrane environment and in the virus may give insight into the relationship between structure and function during the viral life cycle, in particular in the assembly process.

Solid state nuclear magnetic resonance (NMR) studies of biosynthetically labelled ^{15}N and ^{2}H fd coat protein provide a method for describing the structure and dynamics of the protein. The ^{15}N chemical shift and the ^{2}H quadrupolar interactions are anisotropic and are represented by second rank tensors. The powder pattern lineshapes of these interactions are determined by the principal elements of the diagonalized tensors. Large amplitude motions, which occur frequently compared with the breadth of these patterns, result in an averaging of the powder lineshape. For the ^{15}N chemical shift interaction, the time scale is 10^4/s. For the ^{2}H quadrupolar interaction the time scale is 10^6/s. The resulting lineshapes can be modeled by taking into account the rates, amplitudes, and axes of the motions (1). Rapid isotropic reorientation of a site results in a single-line resonance at the frequency defined by the average of the trace of the diagonalized tensors.

The structure of the coat protein can be determined from the orientation dependence of the spin interactions in immobile sites. The net diamagnetic susceptibility of the virus particle results in alignment of the virus filament parallel to the magnetic field (2). The ^{15}N chemical shift anisotropy results in a single resonance frequency for each labelled protein site in the oriented virus. This resonance frequency depends on the orientation of the principal axis system of the chemical shielding tensor in the laboratory frame. A second spin interaction, the ^{15}N–^{1}H dipolar interaction, results in the splitting of each amide nitrogen resonance into a doublet. The frequency difference in the splitting of this doublet is a measure of the orientation of the ^{15}N–^{1}H bond vector relative to the filament axis (3). By appropriate double labelling of the coat protein sequence, the orientation of the ^{13}C carbonyl–^{15}N amide bond vector with respect to the laboratory frame can be determined. The results from two of these interactions fully describes the orientation of a peptide unit (4).

The chemical shift spectra for the uniformly ^{15}N-labelled fd coat protein in the virus-bound and membrane-bound form are shown in Fig. 1 *A* and *B*. These spectra exhibit a full static chemical shift powder pattern in both membrane- and virus-bound coat protein, indicating that a major portion of the protein backbone is immobile in each environment. They both contain a relatively narrow isotropic resonance superimposed on the powder pattern, indicating some protein sites are mobile on the 10^4 Hz timescale. Significantly, the magnitude of the isotropic resonance component is greater in the spectrum of the membrane-bound form than for the virus. The membrane

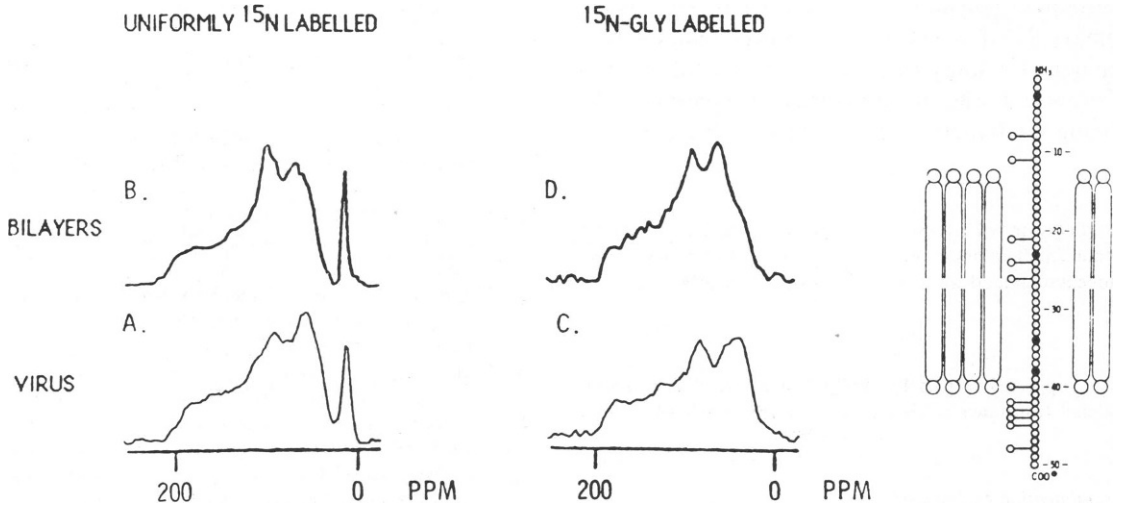

FIGURE 1 Solid-state ^{15}N spectra of fd coat protein. (*A*) Uniformly ^{15}N-labelled coat protein in dimyristoyl phosphatidyl choline (DMPC) bilayers. (*B*) Uniformly ^{15}N-labelled coat protein in the intact virus. (*C*) ^{15}N glycine-labelled fd coat protein in DMPC bilayers. (*D*) ^{15}N glycine-labelled fd coat protein in the intact virus.

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bound coat protein contains 9–13 residues that are motionally averaged. By contrast, the intact virus protein contains a maximum of four rapidly reorienting residues. Specific residue labelling can determine which sites are reorienting in each environment. Fig. 1 *C* and *D* clearly demonstrate that ^{15}N-labelled glycine in position 3 is motionally averaged in both the virus-bound and membrane-bound form of the coat protein.

The measurement of the ^{15}N–^{1}H dipolar interaction is accomplished using two dimensional separated local field spectroscopy (5). Fig. 2 *A* is the chemical shift powder pattern for the ^{15}N alanine labelled fd virus. Nine of the 10 alanine residues in the protein contribute to the static pattern. The tenth alanine is at the amino terminus and gives a narrow resonance line charecteristic of a free amino group. The oriented spectrum of ^{15}N-labelled alanine is shown in Fig. 2 *B*. The two-dimensional spectrum of Fig. 2 *C* indicates several resolved resonances for the alanine sites in the protein backbone.

The dynamics and orientation of the alanine side chains can be determined from the ^{2}H quadrupole interaction. Fig. 2 *D* and *E* indicate the relative number of mobile alanine side chains in the coat protein in membrane and virus environments. The motionally averaged central intensity is larger in the membrane-bound coat protein than in the virus. This indicates that more of the alanines are mobile in the membrane-bound form of the coat protein than in the virus. The orientation of the CD_3 net bond vectors can be determined from the splittings of the quadrupole interaction in the oriented virus. Fig. 2 *F* indicates several resolved orientations of the alanine side chains as well as an isotropic resonance which most likely corresponds to the mobile amino terminal alanine.

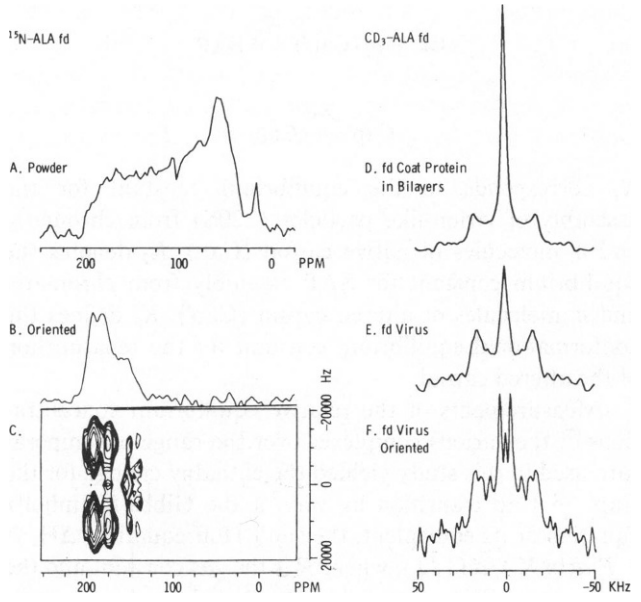

FIGURE 2 Solid-state ^{15}N and ^{2}H-NMR spectra of fd coat protein labelled in the 10 alanine residues. (*A*) ^{15}N chemical shift powder pattern in the intact virus. (*B*) ^{15}N chemical-shift spectra of oriented ^{15}N alanine fd. (*C*) Two-dimensional ^{15}N–^{1}H dipolar/chemical shift spectrum of oriented fd virus. (*D*) ^{2}H NMR spectrum of CD_3 alanine fd coat protein in DMPC bilayers. (*E*) ^{2}H NMR spectrum of CD_3 alanine fd coat protein in intact virus. (*F*) ^{2}H NMR spectrum of oriented CD_3 alanine fd virus.

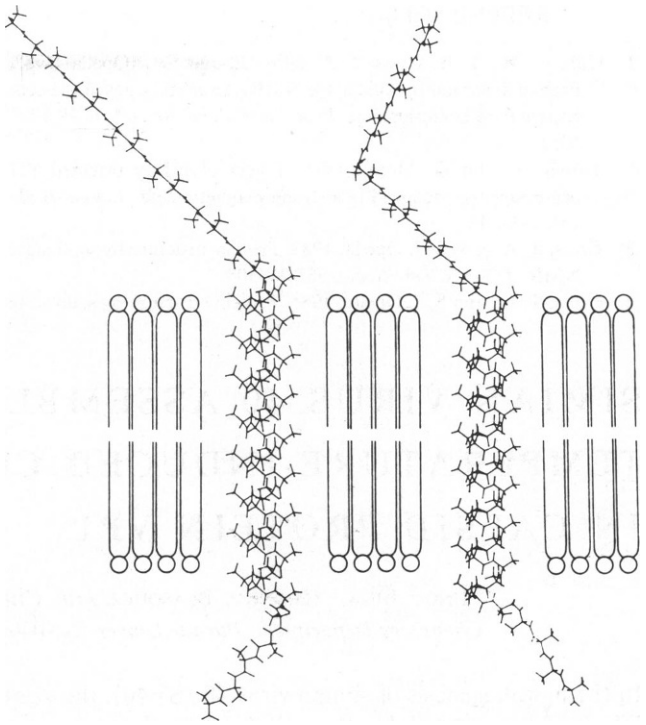

FIGURE 3 Schematic represention of the backbone properties of the fd coat protein in bilayers.

The fd coat protein shows striking differences in dynamics in the membrane-bound and virus-bound forms. The coat protein inserted in the membrane demonstrates rapid reorientation of ~10 residues in the amino terminal domain and three residues in the carboxyl terminus. The schematic representation of the protein in Fig. 3 summarizes these data. The virus-bound form of the fd coat protein exhibits rapid reorientation for only the first few residues at the amino terminal domain, with the carboxyl terminus immobile in the assembled virus particle. The interpretation of NMR results on the structure of the coat protein in the virus show that residues 28 through 32 form a helix with the helix axis directed 20° away from the filament axis; residues 33 through 39 are in a helix tilted by 7° and residues 40 through 48 are also in a helix at an angle of 14° with respect to the filament axis. This preliminary description of the protein structure is generally consistent with the overall picture of a gently slewed α-helix found from diffraction studies (6, 7).

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SIMIAN VIRUS 40 ASSEMBLY, STUDIED BY TEMPERATURE-INDUCED CONFORMATIONAL CHANGES IN CAPSID PROTEIN VP1

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In the morphogenesis of simian virus 40 (SV40), the viral DNA is first folded by the cellular core histones into nucleosomes to form the viral chromatin or minichromosome (1). Virion assembly requires the interaction of the capsid proteins VP1, VP2, and VP3 with SV40 chromatin to form a shelled particle with the minichromosome inside (reviewed in references 2 and 3).

In previous studies we combined a genetic approach with biochemical and electron microscopic studies to examine the shell assembly pathway. This involved characterization of nucleoprotein complexes formed in cells infected with conditional lethal mutants of the major capsid protein VP1 (2, 3). These mutants are temperature-sensitive in assembly and have been classified as tsB, tsC, and TsBC by complementation analysis (4, 5). Our studies of tsB mutants have revealed that at the nonpermissive temperature (40°C) the block in virion assembly results in accumulation of semiassembled particles that consist of chromatin attached to a partially assembled shell composed of VP1, VP2, and VP3 (6). We do not yet know the exact relationship between these semiassembled particles formed at 40°C and the actual intermediates that have been hypothesized (7–9) to form transiently during virion assembly. To measure the extent of temperature-induced structural pertubation in VP1 of tsB mutants, we estimated an approximate value for the enthalpy of VP1 renaturation. Conversion of altered VP1 to its native form was followed by determining the types and amounts of nucleoprotein complexes assembled at equilibrium in tsB265-infected cells incubated at various temperatures. The sedimentation profiles of these [^{3}H] thymidine-labeled complexes are shown in Fig. 1. At nonpermissive temperature (40°C), the altered VP1 yielded primarily the semiassembled particles (100–160S), as we have shown previously (2, 3, 6). At slightly lower temperatures, we detected an equilibrium mixture of chromatin (Ch) at 75S, semiassembled particles (SAP), and 220S-sedimenting particles (P). Finally, at permissive temperature (33°C), the profiles revealed predominantly two major classes of complexes: chromatin at 75S and virions at 220S. The data could be analyzed by assuming the following hypothetical assembly pathway:

$$\mathrm{Ch} + n_i\,(\mathrm{Cap}) \overset{K_1}{\rightleftharpoons} \mathrm{P}$$

$$\mathrm{Ch} + n_j\,(\mathrm{Cap}') \overset{K_2}{\rightleftharpoons} \mathrm{SAP}$$

$$\mathrm{Cap}' \overset{K_3}{\rightleftharpoons} \mathrm{Cap}.$$

K_1 corresponds to the equilibrium constant for the assembly of virion-like particles (220S) from chromatin and n_i molecules of native capsid (Cap). K_2 denotes the equilibrium constant for SAP assembly from chromatin and n_j molecules of altered capsid (Cap'). K_3 defines the conformational equilibrium constant for the renaturation of the altered capsid.

Measurements of the relative equilibrium concentrations of the various complexes over the range of temperature used in this study yielded the enthalpy change for the Cap' → Cap transition by way of the Gibbs-Helmholtz equation or its equivalent, the van't Hoff equation: $\Delta H_v = -R\,d(\ln K_3)/d\,(1/T)$, where R is the gas constant and the temperature T is expressed in °K. By solving for Cap and Cap' from the K_1 and K_2 equilibrium expressions

$$[\mathrm{Cap}] = ([\mathrm{P}]/[\mathrm{Ch}])^{1/n_i} \cdot 1/(K_1)^{1/n_i}$$

and

$$[\mathrm{Cap}'] = ([\mathrm{SAP}]/[\mathrm{Ch}])^{1/n_j} \cdot 1/(K_2)^{1/n_j},$$

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