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Direct Comparison of the Membrane Bound and Structural Forms of the Coat Protein of the Filamentous Bacteriophage fd

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Abstract

The coat protein of the filamentous bacteriophage fd exists in a membrane bound form as well as in the structural form of the virus during its lifecycle. The changes in the dynamics of the coat protein that accompany the infection of *E. coli* and the assembly of new virus particles are described with NMR experiments. Solid state NMR is used to describe the dynamics of isotopically labelled peptide backbone and amino acid sidechain sites. Motional averaging of powder pattern lineshapes gives qualitative information about the amplitudes and rates of motions.

Introduction

The filamentous bacteriophages are large nucleoprotein complexes (1). fd consists of 90% by weight coat protein and 10% DNA. fd infects *E. coli* and reproduces in a non-lytic infection. At infection the DNA enters the bacterial cell cytoplasm and the coat protein becomes associated with the inner cell membrane. Many copies of viral DNA and coat protein are synthesized within the infected cells (2). These new coat protein molecules are stored along with the salvaged ones from the infecting virus particles in the cell membrane. New virus particles are assembled at the cell membrane from this total pool of new and old coat proteins and DNA. In the assembly process the coat protein changes from its membrane bound form to the form it adopts as the major structural element of the virus particles (3). The changes in the coat protein that occur during infection and assembly processes must be reversible. The changes in the dynamics of the coat protein are the subject of this paper. This is an initial account of the findings derived from comparisons of the solid state NMR studies of the coat protein in the virus (4) and in reconstituted membrane bilayers (5).

Each coat protein molecule of fd has 50 residues. The amino acid sequence shows the protein to have a central hydrophobic midsection of about 20 residues, sur-

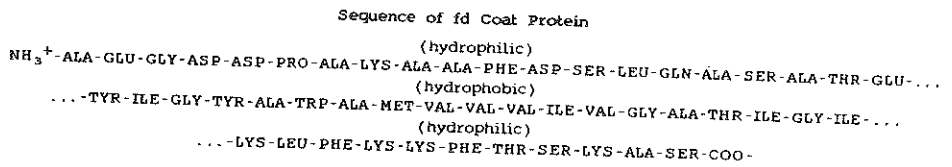

Figure 1. Amino acid sequence of fd coat protein (6a,b).

rounded by relatively hydrophilic C- and N- terminal regions. The sequence of the protein is given in Figure 1 (6a,b).

The two major techniques employed in these studies are biosynthetic isotopic labelling of the coat protein in the virus and the analysis of powder pattern lineshapes from the labelled sites obtained in solid state NMR experiments. Since *E. coli* can be grown on chemically defined media when infected with fd, labels can be readily incorporated into specific sites of amino acids in the viral proteins. Since the major coat protein of fd is small there are only one or several of each amino acid type, enabling a high degree of selectivity through labelling of one amino acid at a time.

Solid state NMR methods, rather than solution NMR methods, are essential for these studies, since neither the virus nor the coat protein-membrane complex reorient rapidly in solution. The lineshape analysis of powder patterns provides a direct way to describe molecular dynamics in molecules that are not undergoing overall rapid reorientation. These methods have been used successfully to describe the dynamics of several proteins, including bacteriorhodopsin in membrane bilayers (7-9), collagen in fibrils (10), and the coat proteins of the filamentous bacteriophages (4). Figure 2 shows a collection of calculated lineshapes for powder patterns with and without motional averaging from chemical shift and quadrupole interactions, specifically for ^{15}N in peptide linkages and ^{2}H in sidechains (11). The shape and breadth of the rigid lattice powder patterns are determined by the fundamental properties of the nuclear spin interactions. Sites that do not have large amplitude motions that are rapid compared to the frequency breadth of the spin interaction have powder patterns that are very similar to those of the rigid lattice. Large amplitude, rapid motions have substantial effects in averaging the rigid lattice patterns. Given the rigid lattice lineshapes and specific models for motion, then motionally averaged lineshapes can be calculated using standard procedures. Lineshapes can be calculated for other nuclei, spin interactions, and types of motion. Peptide backbone and sidechain sites have been shown to have large amplitude, rapid motions in some protein sites and to be immobile in others by these methods.

Materials and Methods

Sample Preparation

Isotopically labelled fd was grown and purified as described previously(4). The isotopically labelled amino acids were obtained from commercial sources with the

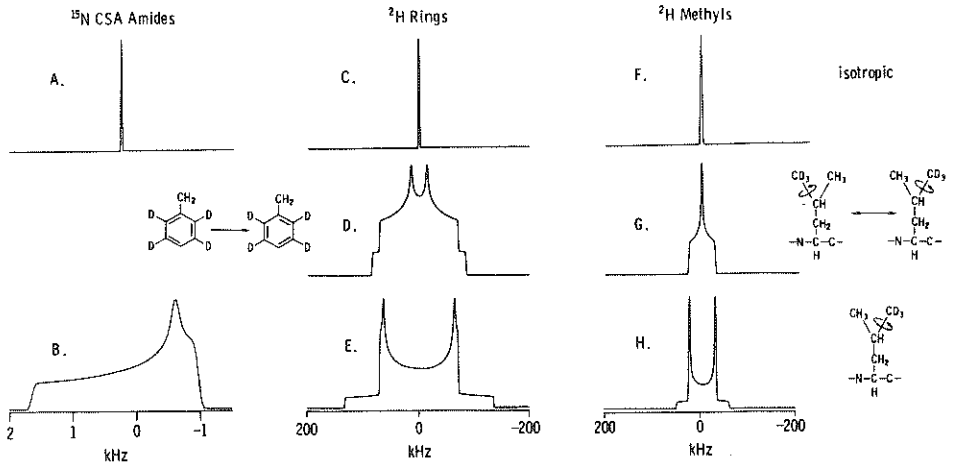

Figure 2. Calculated lineshapes based on static values from model compounds. (A) ^{15}N chemical shift anisotropy of amide group averaged by isotropic motion. (B) ^{15}N chemical shift anisotropy of static amide group. (C) ^{2}H quadrupole averaged by isotropic motion. (D) ^{2}H quadrupole averaged by two-fold ring flips. (E) ^{2}H quadrupole of static C-D bond. (F) ^{2}H quadrupole averaged by isotropic motion. (G) ^{2}H quadrupole averaged by two-site tetrahedral jump plus three-fold methyl reorientation. (H) ^{2}H quadrupole averaged by three-fold methyl reorientation.

exception of d_4-Thr which was synthesized using published procedures (12). The spectra of the intact virus were obtained from solutions with a concentration of 200 mg/ml in 40 mM borate buffer at pH 8. The bilayer samples were prepared by sonicating the virus in the presence of phospholipids followed by lyophilization and rehydration (13). The membrane samples contained about 100 mg protein and slightly greater amount of lipid in excess water.

Spectroscopy

The NMR experiments were performed on home-built spectrometers with 3.5T and 5.7T fields and a modified JEOL GX-400WB spectrometer with a 9.4T field. The ^{2}H NMR spectra were obtained using the quadrupole echo pulse sequence (14) using 2-4 μsec pulses and 20-50 μsec interpulse delays. The ^{15}N NMR spectra were obtained using the spin-lock version of cross-polarization (15) with a 1 msec mixing interval and proton decoupling during data acquisition.

Results

The experimental results described in this section are in the form of powder pattern lineshapes. Direct comparisons are made in the Figures between the membrane bound form of the coat protein in phospholipid bilayers and the structural form of the same protein in the virus. Specific models for motion are examined by comparing the experimental lineshapes to the calculated motionally averaged lineshapes in Figure 2 of the Introduction.

Backbone Sites

^{15}N NMR is an effective way of studying backbone dynamics of proteins even when all nitrogen sites are uniformly labelled. Nearly all of the nitrogens in proteins are in the peptide groups of the backbone. The ^{15}N chemical shift anisotropy is a relatively small interaction at the magnetic fields used in these experiments, resulting in a timescale for motional averaging of 1-10 kHz. A static powder pattern is observed for a labelled site when all orientations are present and large amplitude, rapid motions are absent. Large amplitude motions that occur more frequently than the breadth of the powder pattern result in an averaging of the lineshape. The lineshapes for the two extreme cases of a rigidly held and an isotropically reorienting amide group are shown in Figure 2. Labelling of individual amino acids adds greatly to the specificity of these experiments by allowing the lineshapes for one or a few sites to be examined.

Figure 3 compares the ^{15}N chemical shift spectra for the coat protein in membrane bilayers (top row) to the coat protein in its structural form in the virus (bottom row). Spectra from protein samples that are uniformly labelled with ^{15}N in all sites, labelled only in the four glycine residues, and labelled only in the two leucine residues are presented in Figure 3. These spectra are distinctive in showing a heterogeneity of protein backbone dynamics in both the membrane bound and structural forms of the protein. This is seen most directly in the spectra of the

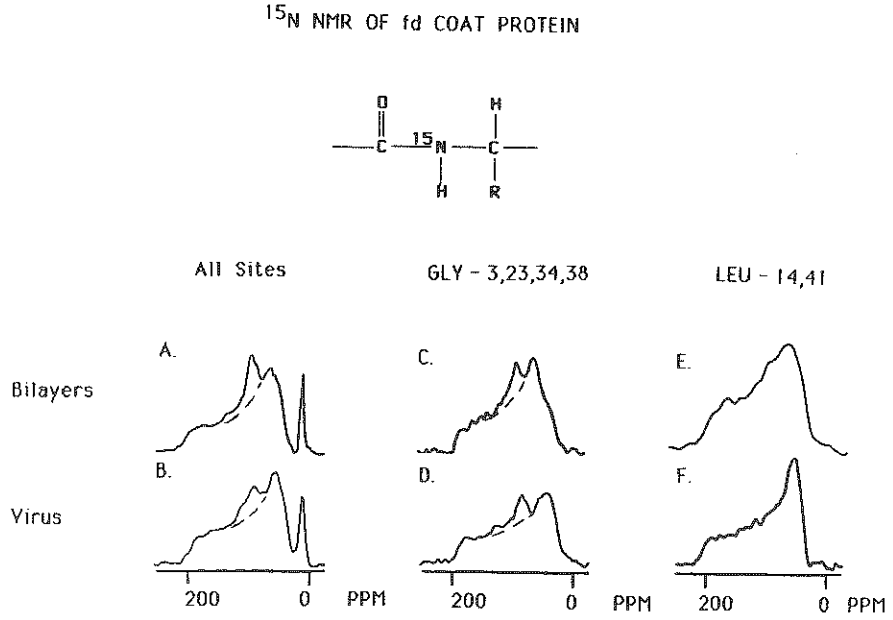

Figure 3. ^{15}N NMR spectra of labelled fd coat protein. (A) All sites uniformly labelled in the coat protein in the bilayers. (B) All sites uniformly labelled in the coat protein in the virus. (C) Glycines labelled in the coat protein in bilayers. (D) Glycines labelled in the coat protein in the virus. (E) Leucines labelled in the coat protein in bilayers. (F) Leucines labelled in the coat protein in the virus.

uniformly labelled and of the glycine labelled protein samples which have a narrow resonance near the isotropic frequency superimposed on the powder pattern lineshapes. Many of the amino acids in the protein have been individually labelled in order to identify which are mobile and which are immobile in both the membrane bound and structural forms. For example, Gly-3 is mobile while Gly-23, 34, 38 are all immobile in both forms of the protein based on the spectra in Figures 3c and 3d and the assignment of the single isotropic resonance to Gly-3 through proteolytic cleavage experiments. Leu-14 and 41 are immobile in both forms of the protein as seen in Figures 3e and 3f.

The sidechain of alanine is a methyl group bonded to the alpha carbon; therefore, the dynamics of the methyl group directly reflect on those of the backbone for these residues. The ^2H NMR spectrum of a CD_3 group is a narrowed powder pattern due to the reorientation of the methyl group about its C_α-C_β bond axis as shown in Figure 2h. This occurs whether the backbone is mobile or immobile at the residue. Additional motions can only occur in the methyl sidechain as a result of backbone motions. The spectra in Figure 4 show that the ten alanine residues in the coat protein show heterogeneous dynamics in both the membrane and in the virus, since a narrow central resonance is superimposed on the powder pattern from a reorienting methyl group. There is an increase in the magnitude of the isotropic

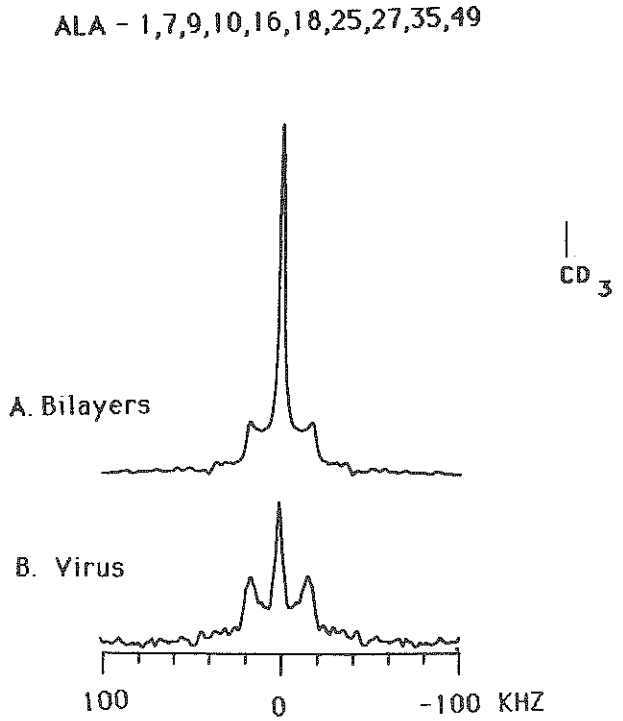

Figure 4. ^2H NMR spectra of d_3-Ala labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

resonance in the membrane bound form over the structural form of the coat protein. This indicates that a larger percentage of the backbone sites are mobile in the membrane bound form of the protein.

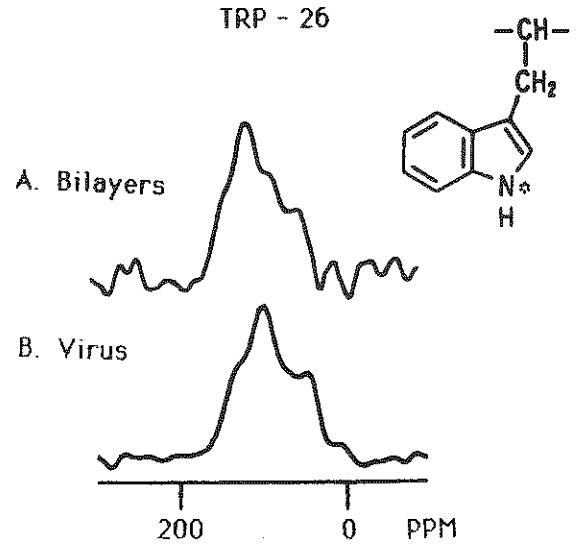

Figure 5. ^{15}N NMR spectra of ^{15}N-Trp labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

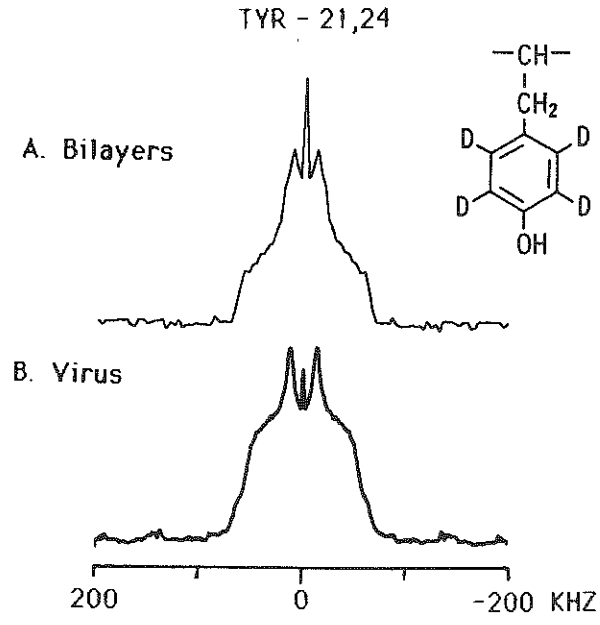

Figure 6. ^{2}H NMR spectra in d_4-Tyr labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

Aromatic Sidechains

fd coat protein has five aromatic residues. The single Trp (26) residue and the two Tyr (21,24) residues are in the hydrophobic midsection of the protein, while the three Phe (11,42,45) residues are in the terminal regions. Figure 5 compares the ^{15}N chemical shift anisotropy powder patterns for the Trp sidechain in the membrane bound and structural forms of the coat protein. In both cases the sidechain is immobile, since the powder patterns are not averaged compared to the rigid lattice model of polycrystalline tryptophan. Many of the initial results on protein dynamics by solid state NMR were obtained on aromatic sites of proteins, showing some Tyr and Phe residues to undergo two fold jumps about their C_β-C_γ bond axis (4,7,8). Figure 6 contains the ^2H NMR spectra from d_4-Tyr labelled coat protein samples. These experimental powder pattern lineshapes show that both of the tyrosine sidechains are undergoing rapid two-fold jumps in both forms of the protein by comparison with the lineshapes calculated for two-fold jump motions of this sidechain in Figure 2d. Phenylalanine sidechains have been generally found to behave like tyrosine sidechains in proteins, either being immobile or undergoing two-fold jumps. The ^2H NMR spectra in Figure 7 show the phenylalanine sidechains in the coat protein in membrane bilayers to have a great deal of additional motion as compared to the same sites in the structural form of the coat protein and other proteins.

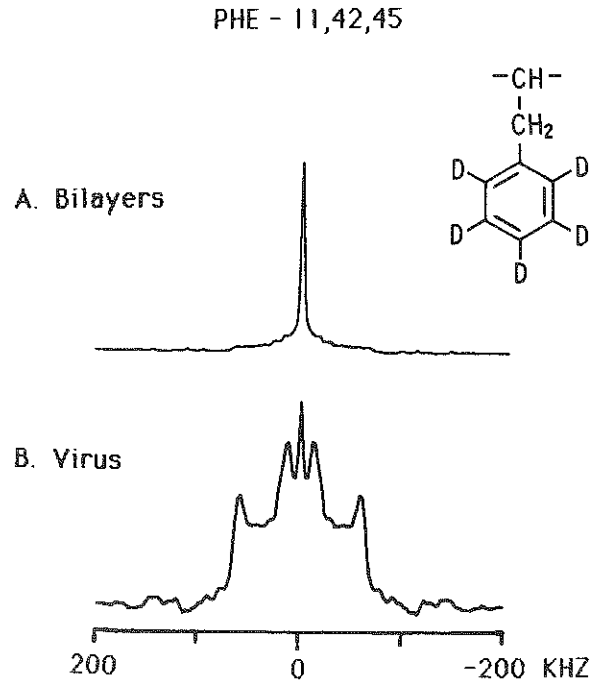

Figure 7. ^2H NMR spectra of d_5-Phe labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

Aliphatic Sidechains

The alanine sidechains discussed above were used to monitor the backbone sites of those residues. The CD_3 labelled methyl sites of the leucine residues are capable of additional modes of motion, even with immobile backbone sites as observed from the ^{15}N labels in Figure 3. Leucine sidechains have been shown to have a two-site hop motion superimposed on the methyl rotation in several systems (16,17); the calculated lineshape for this motion is in Figure 2g. Figure 8 shows that this is the case for both Leu-14 and -41 in the membrane bound and structural forms of the coat protein.

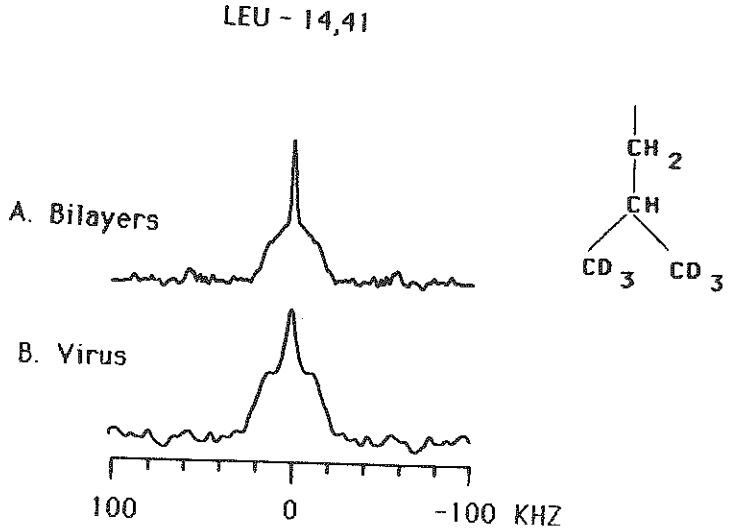

Figure 8. ^2H NMR spectra of d_{10}-Leu labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

The methyl group in threonine also provides an interesting monitor of protein dynamics (17). The threonine sidechain can clearly undergo additional modes of motion, even when the backbone at that residue is immobile. The methyl rotation powder lineshape is additionally averaged by a flip about the C_α-C_β bond. The membrane bound coat protein demonstrates that all three threonine residues undergo methyl rotation as well as the C_α-C_β jump motion. In the virus, there appears to be only one threonine sidechain which exhibits both types of motion. The remaining two threonine sidechains appear to have only the methyl rotation. The data in Figure 9 for the labelled coat protein in the virus and in the membrane bound form show differences for the two forms. These well defined lineshapes also serve to demonstrate the absence of large amplitude backbone motions for the threonine residues in both forms of the protein.

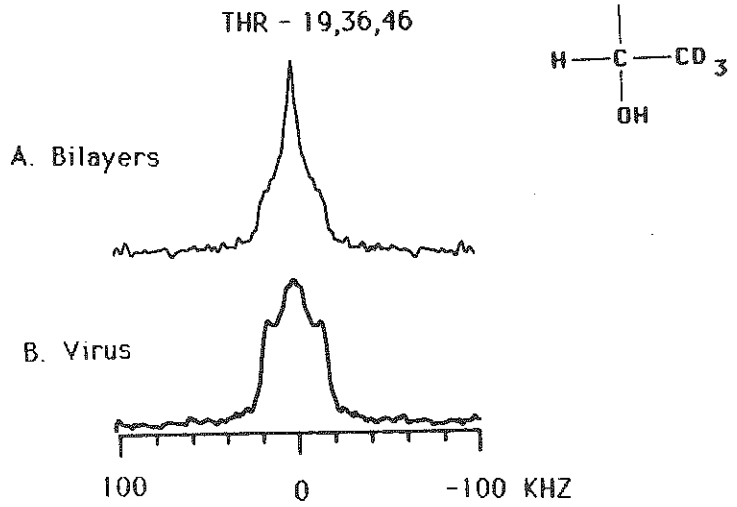

Figure 9. ^2H NMR spectra of d_4-Thr labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

Methionine sidechains are capable of many motions (18). The ^2H NMR spectra in Figure 10 are for CD_3 labelled methionine in both forms of the coat protein. This is a particularly favorable case, because there is only a single Met residue in the protein. Methyl rotation combined with one flip motion gives a calculated spectrum that is similar to the experimental lineshape. This interpretation indicates that the protein backbone is immobile at Met-28 in both forms of the coat protein.

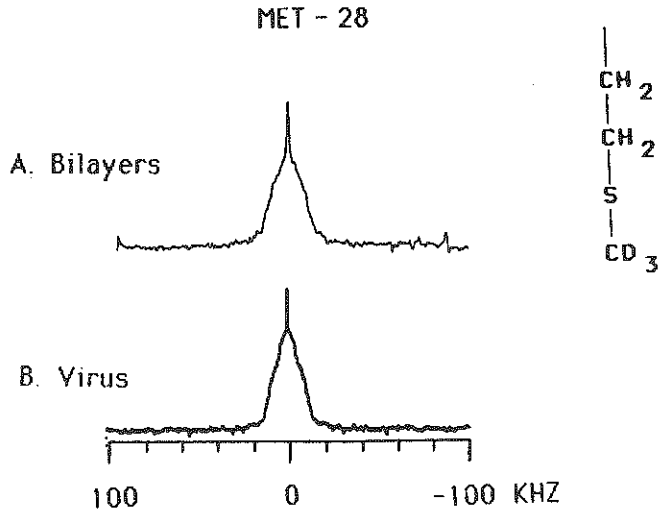

Figure 10. ^2H NMR spectra of d_3-Met labelled fd coat protein. (A) Coat protein in bilayers. (B) Coat protein in the virus.

Discussion

The major coat protein of fd goes from the structural form of the virus to the membrane bound form upon infection of the bacterial cell. The reverse occurs in the assembly process. A number of experiments indicate that the protein changes substantially in going between these two forms; for example circular dichroism (CD) spectra have been interpreted to show that the protein has about 90% alpha helix in the virus and about 50% alpha helix in the membrane (3). This suggests that substantial rearrangements of the protein occur. This contrasts to the conformational changes in allosteric proteins which typically have barely observable changes in secondary structure or even coat proteins of other viruses which apparently change very little during assembly.

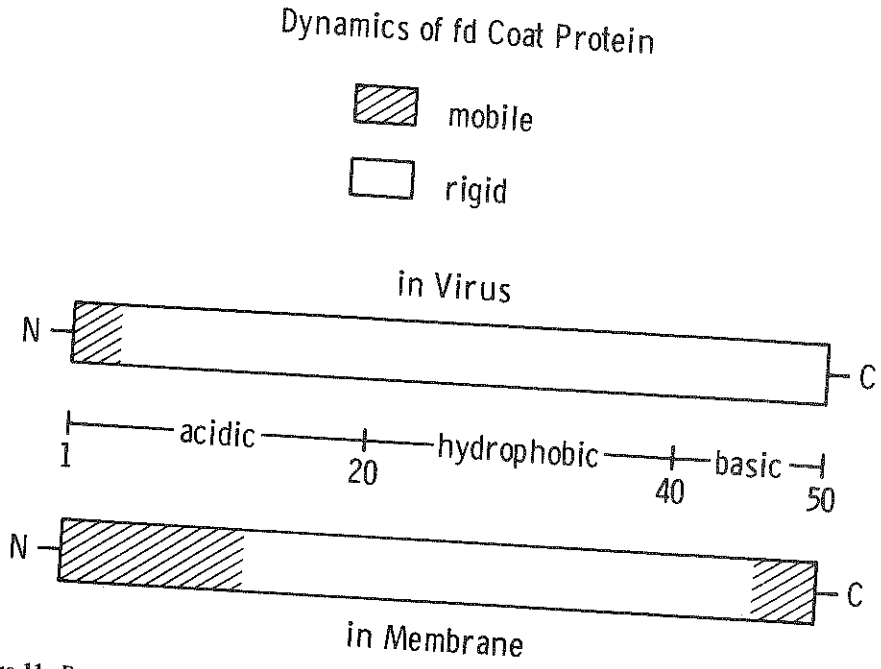

Figure 11. Representation of the distribution in backbone dynamics in fd coat protein.

The results presented here indicate that the changes in the coat protein that accompany infection and assembly must be explained in terms of dynamics as well as structure. Table I qualitatively summarizes the dynamics of the protein sites discussed in this paper. A clear trend emerges for increased mobility in the membrane bound form of the protein. Approximately 20-30% of the protein backbone is isotropically reorienting in the membrane bound form, in contrast to only 5-10% in the virus. The change of about 10 residues in the amino terminal region from immobile to isotropic motion on the kHz timescale is the largest influence of assembly on the protein. Changes in sidechain dynamics are also observed. Figure

Table I
Coat Protein Dynamics

Residue Type	Labeled Position	Structural Form Coat Protein	Membrane Bound Coat Protein
All sites	^{15}N amide backbone	4 mobile 46 static	~12 mobile ~38 static
GLY 3, 23, 34, 38	^{15}N amide backbone	1 mobile 3 static	1 mobile 3 static
LEU 14, 41	^{15}N amide backbone	2 static	2 static
	^{2}H methyl sidechain	2 methyl rotation with tetrahedral flip	2 methyl rotation with tetrahedral flip
ALA 1, 7, 9, 10, 16, 18, 25, 27, 35, 49	^{2}H methyl sidechain	1 mobile 9 static	~4-6 mobile ~4-6 static
TRP 26	^{15}N aromatic sidechain	1 static	1 static
TYR 21, 24	^{2}H aromatic sidechain	25°C 2-180° ring flip 40°C 2-180° ring flip	25°C 2-180° ring flip 40°C 2-180° ring flip with additional motion
PHE 11, 42, 45	^{2}H aromatic sidechain	3-180° ring flip	2-180° ring flip 1-180° ring flip with additional motion
THR 19, 36, 46	^{2}H methyl sidechain	2-methyl rotation 1-methyl rotation with tetrahedral flip	3-methyl rotation with tetrahedral flip
MET 28	^{2}H methyl sidechain	1-methyl rotation with twofold flip	1-methyl rotation with twofold flip

11 illustrates the distribution of the mobile and rigid regions of the backbone of the coat protein in the virus and membrane forms. If the protein spans the membrane bilayers with the C-terminal region outside the cell, then the mobile N-terminal region on the inside of the cell may be involved with displacing the gene 5 protein and wrapping about the DNA during the assembly process (19).

Acknowledgments

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