Dynamics of fd Coat Protein in the Bacteriophage†

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ABSTRACT: The dynamics of the coat protein in fd bacteriophage are described with solid-state ^{15}N and ^{2}H NMR experiments. The virus particles and the coat protein subunits are immobile on the time scales of the ^{15}N chemical shift anisotropy (10^3 Hz) and ^{2}H quadrupole (10^6 Hz) interactions. Previously we have shown that the Trp-26 side chain is immobile, that the two Tyr and three Phe side chains undergo only rapid twofold jump motions about their C_β–C_γ bond axis [Gall, C. M., Cross, T. A., DiVerdi, J. A., & Opella, S. J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 101–105], and that most of the backbone peptide linkages are highly constrained but do undergo rapid small amplitude motions [Cross, T. A., & Opella, S. J. (1982) *J. Mol. Biol. 159*, 543–549] in the coat protein subunits in the virus particles. In this paper, we demonstrate that the four N-terminal residues of the coat protein subunits are highly mobile, since both backbone and side-chain sites of these residues undergo large amplitude motions that are rapid on the time scales of the solid-state NMR experiments. In addition, the dynamics of the methyl-containing aliphatic residues Ala, Leu, Val, Thr, and Met are analyzed. Large amplitude jump motions are observed in nearly all of these side chains even though, with the exception of the N-terminal residue Ala-1, their backbone peptide linkages are highly constrained. The established information about the dynamics of the structural form of fd coat protein in the virus particle is summarized qualitatively; backbone sites are found to be either immobile or highly mobile, while side-chain sites are found either to be immobile or to undergo apparently well-defined high-frequency jump motions.

The filamentous bacteriophage fd consists of approximately 2700 copies of a small (50 amino acid) coat protein arranged to form a cylinder that encases the DNA, which is extended lengthwise within the particle (Marvin & Hohn, 1969; Makowski, 1984). The coat protein is the major structural element of the virus, contributing about 90% of the mass of the 1.6×10^6 dalton particle. The virus particle contains no membrane components; however, the coat protein exists as a membrane-bound protein during the viral life cycle after infection and prior to assembly. The coat protein is nearly completely helical in the virus. The structure of the coat protein in the virus is being determined by solid-state NMR (Cross & Opella, 1985; Opella et al., 1987) and X-ray fiber diffraction (Makowski & Caspar, 1981; Banner et al., 1981) methods.

Solid-state NMR provides a way of characterizing the dynamics of proteins that have limited or no overall motions by virtue of their being in the crystalline solid state or part of supramolecular structures in solution [for reviews see Torchia (1984) and Opella (1986)]. The dynamics of the coat protein in the filamentous bacteriophages (Gall et al., 1981, 1982; Cross & Opella, 1982; Valentine et al., 1985; Colnago et al., 1986) and in membrane bilayers (Frey et al., 1983; Bogusky et al., 1985; Colnago et al., 1986; Leo et al., 1987) have been described by solid-state NMR experiments. Other protein systems characterized by solid-state NMR include collagen (Torchia, 1982, 1984; Batchelder et al., 1982), bacteriorhodopsin (Kinsey et al., 1981a,b; Oldfield et al., 1982; Keniry et al., 1984a,b; Smith & Oldfield, 1984; Rice et al., 1981; Schramm et al., 1981), and myoglobin (Keniry et al., 1983).

Since these protein molecules are immobilized by their environments, motions within residues or protein segments can be readily identified. Solid-state NMR studies of molecular dynamics rely on the changes in powder pattern line shapes due to motional averaging to describe the amplitudes and directions of the motions, as well as the lower limits of the frequencies of the motions. Relaxation parameters can be analyzed in combination with the line shapes to determine the actual frequencies of the motions. These studies typically require labeling the protein with stable isotopes and measuring the various spectroscopic parameters, especially powder pattern line shapes. The chemical shift anisotropy of ^{15}N-labeled amide backbone sites and the ^{2}H nuclear quadrupole interaction of methyl groups (CD_3) in aliphatic side chains are utilized in the studies presented here.

Large amplitude motions in the polypeptide backbone are detected through the motional averaging of the ^{15}N chemical shift powder pattern at labeled amide sites. Sites that are immobile on the 10^3-Hz time scale of the chemical shift interaction give rigid lattice powder patterns. Sites that undergo rapid, effectively isotropic reorientation on this time scale give narrow resonances at the isotropic, or average, chemical shift frequency. Motions intermediate in amplitude or frequency result in partially averaged chemical shift powder patterns. Small amplitude motions ($<\pm 10°$) have little effect on the rigid lattice powder pattern line shapes (Frey et al., 1983). Relaxation measurements have indicated the presence of rapid motions with relatively small amplitudes in protein backbone sites that yield powder pattern line shapes very similar in shape and breadth to those from rigid crystalline model peptides (Cross & Opella, 1982). Therefore, we rely on the absence of large amplitude motions to characterize a site as "immobile", even though all sites analyzed to date show evidence of small amplitude motions. The experimental results for the fd coat protein backbone are interpreted qualitatively in terms of the sites being either immobile or highly mobile. Calculated powder pattern line shapes for ^{15}N chemical shift anisotropy in amide backbone sites are shown in Figure 1.

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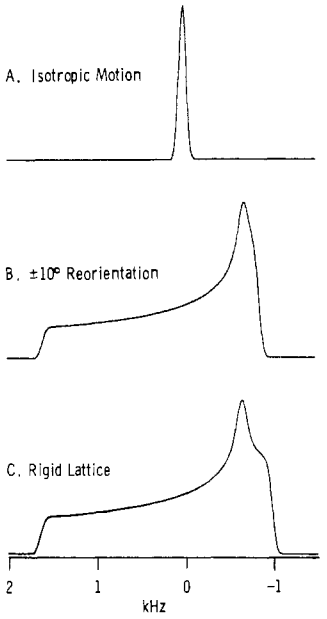

FIGURE 1: Calculated line shapes for ^{15}N chemical shift anisotropy of amide nitrogen sites in peptide linkages: (A) narrow resonance resulting from isotropic motional averaging; (B) powder pattern calculated where ±10° of librational motion is averaging the rigid lattice powder pattern (Frey et al., 1983); (C) rigid lattice powder pattern calculated for principal values typical of those observed in polycrystalline peptide and fd coat protein (σ_{11} = −34 ppm, σ_{22} = −57 ppm, σ_{33} = −206 ppm) (Cross & Opella, 1985).

The effects of the motional averaging on methyl groups have been observed in ^2H NMR experiments of CD_3-labeled amino acid side chains in peptides and proteins (Jelinski et al., 1980; Batchelder et al., 1982, 1983; Kinsey et al., 1981a,b; Keniry et al., 1984a,b; Smith & Oldfield, 1984; Colnago et al., 1986; Leo et al., 1987). Figure 2 shows several calculated ^2H NMR powder patterns that are characteristic of the methyl group motions observed in proteins. At the temperatures studied, all methyl groups undergo rapid reorientation about the C_3 symmetry axis. This results in the axially symmetric powder pattern in Figure 2D, which is reduced substantially in breadth (with a major quadrupolar splitting of about 40 KHz) compared to that from a C–D bond in a rigid lattice (with a major quadrupolar spliting of about 135 kHz). The side chains of many aliphatic amino acids are capable of undergoing jump motions about tetrahedral or near-tetrahedral carbon sites. The CD_3 deuterium powder pattern line shapes resulting from two-site jump motions, in addition to the C_3 axis methyl group reorientation, can be calculated by standard methods.

The calculation of the motionally averaged powder pattern line shapes can be readily visualized by considering a two-site "fast-exchange" process consisting of jumps between two equally populated positions (Soda & Chiba, 1969), as shown in Figure 2. The difference in orientations of the two positions is described by the angle θ as calculated with eq 1. The

$$\cos\theta = \cos^2\phi + \sin^2\phi\cos\psi \quad (1)$$

motionally averaged effective principal elements V_{ii}^M, which are represented as the discontinuities in the powder patterns, can be calculated from the rigid lattice values V_{ii} of the ^2H quadrupolar interaction with eq 2–4.

$$V_{xx}^M = V_{xx}\cos^2(\theta/2) + V_{zz}\sin^2(\theta/2) \quad (2)$$

$$V_{yy}^M = V_{yy} \quad (3)$$

$$V_{zz}^M = V_{xx}\sin^2(\theta/2) + V_{zz}\cos^2(\theta/2) \quad (4)$$

According to the definitions of angles shown in Figure 2, jump motions about tetrahedral centers have ϕ = 70.5° and

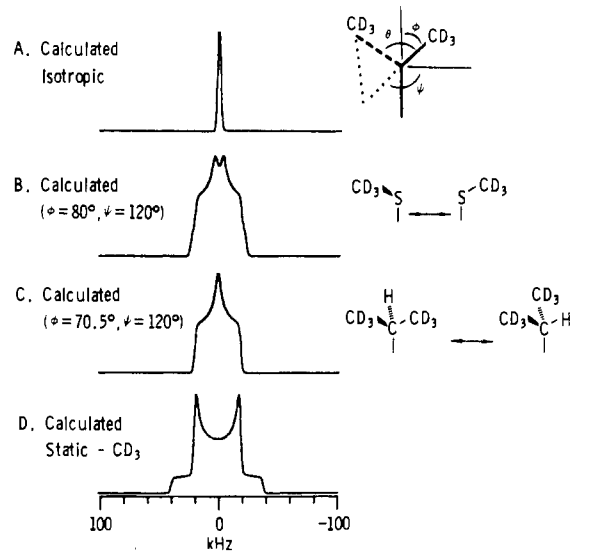

FIGURE 2: Calculated line shapes for ^2H quadrupole interaction of CD_3 groups. All spectra have 3 kHz of Gaussian line broadening: (A) narrow resonance resulting from isotropic motional averaging; (B) calculated for twofold jumps between angles ϕ = 80° and ψ = 120° as shown starting with the spectrum in (D); (C) calculated for twofold jumps between angles ϕ = 70.5° and ψ = 120° as shown starting with the spectrum in (D); (D) calculated for CD_3 groups undergoing only rapid reorientation about the C_3 axis. The major splitting is ±20 kHz.

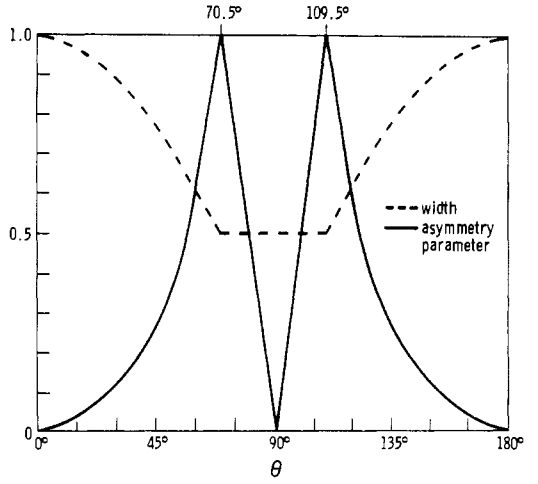

FIGURE 3: Plots of the asymmetry parameter (η) and breadth of the motionally averaged powder pattern as a function of the angle of reorientation. The width is relative to the rigid lattice powder pattern or, in the case of methyl groups, relative to the powder pattern resulting from threefold reorientation.

ψ = 120°, resulting in θ = 109.5°. For methyl groups, this yields a powder pattern line shape with discontinuities given by $V_{xx}^M = \pm 20$ kHz, $V_{zz}^M = \pm 0$ kHz, and $V_{yy}^M = \pm 20$ kHz, as shown in the calculated spectrum in Figure 2C. This spectrum has an asymmetry parameter η = 1, where $\eta = V_{xx} - V_{yy}/V_{zz}$). The geometry of carbon sites in aliphatic side chains in proteins has been found to deviate from tetrahedral due to intra- and interresidue constraints (Janin et al., 1978; Gelin & Karplus, 1979). Twofold jump motions about carbon sites that differ even slightly from tetrahedral result in substantially different line shapes in the ^2H NMR spectra. Figure 3 relates the asymmetry parameter η and the breadth of the motionally averaged powder pattern to the jump angle θ for two-site jump motions where the rigid lattice powder pattern has η = 0, as for CD_3 groups. Figure 3 demonstrates that relatively small deviations from tetrahedral geometry can have a large influence on the line shapes of the motionally averaged

powder patterns. For example, the methyl group in methionine is attached to a sulfur atom with a linkage that differs slightly in geometry from tetrahedral (Gurskaya, 1968). The motionally averaged powder pattern resulting from two-site jumps appropriate for the methionine methyl group ($\phi = 80°$, $\psi = 120°$, and $\theta = 117°$) is shown in Figure 2B; it differs markedly from that for a tetrahedral two-site jump shown in Figure 2C.

Large amplitude motions that occur in all directions result in isotropic averaging of the powder pattern line shapes to single line resonances. These isotropically averaged resonances occur at the central frequency for the interaction as seen in Figure 1A for ^{15}N chemical shift anisotropy and Figure 2A for ^{2}H quadrupole interactions. Because of the limitations imposed by the side chains, the C–D groups can undergo isotropic reorientation only if the backbone of the residues undergoes isotropic reorientation.

Materials and Methods

All labeled amino acids were obtained from Merck Isotopes in DL form, except for threonine, which was synthesized by condensing [$^{2}H_4$]acetaldehyde (Merck) and bis(glycine)copper(II) (Keniry et al., 1984b). The dipeptide Boc-L-alanyl-L-proline-OBz (Ala-Pro) was synthesized by A. Rockwell and L. Gierasch at the University of Delaware. The sample used in these experiments was crystallized from ether/methanol solution.

Virus samples with individually ^{15}N- or ^{2}H-labeled amino acids were prepared by growing *Escherichia coli* on defined media containing 100 mg/L of the labeled amino acid and 500 mg/L of all of the other unlabeled amino acids (Cross & Opella, 1981). All specifically ^{15}N-labeled proteins were solubilized in sodium dodecyl sulfate (SDS) and the incorporation and absence of scrambling to other sites verified by solution NMR. [^{15}N]Pro was incorporated into the coat protein with an *E. coli* strain (NH4104) that is a proline auxotroph supplied by B. Bachmann (*E. coli* Genetics Stock Center). The incorporation of [^{15}N]Asp was facilitated by the addition to the growth media of a transaminase inhibitor DL-methionine sulfoximine (MSX) (Sigma) (Kim & Hollocher, 1982). The uniformly ^{15}N-labeled protein samples were obtained by growing the infected bacteria on a minimal mineral media where the sole source of nitrogen was $(^{15}NH_4)_2SO_4$ (Monsanto) (Cross et al., 1982). The virus was separated from the bacteria by centrifugation, precipitated with poly(ethylene glycol), and purified by centrifugation on KBr density gradients. The virus was then exhaustively dialyzed against a buffer of 10 mM tris(hydroxymethyl)aminomethane (Tris) at pH 8.4. The 1–2-mL samples for the NMR experiments typically had a virus concentration of 70–150 mg/mL, obtained by centrifugation of a solution at 40000 rpm for 7 h. All samples contained 0.02% NaN_3. For the ^{2}H NMR experiments, the virus samples were resuspended in the same Tris buffer made from deuterium-depleted water (Sigma) and reconcentrated by centrifugation.

The ^{2}H NMR spectra were obtained on a home-built spectrometer with a 5.8-T magnet at 38.4 MHz. The spectra were recorded with the quadrupole-echo pulse sequence (Davis et al., 1976), with composite pulses in the sequence 135_X–90_{-X}–$(45_X$–$\tau)$–(-135_Y)–90_{-Y}–$(45_Y$–$\tau)$–acquisition (Levitt et al., 1984). The same pulse sequence was repeated but preceded by a saturation pulse with a 1-ms delay prior to the first pulse; this resulted in a free induction decay with only coherent and inchoherent noise that was subtracted from the free induction decay with the NMR signals to minimize spectral distortion. The $\pi/2$ pulse length was 2–4 μs, and the interpulse spacing was 25–35 μs. The center of the echo was obtained by deleting the first three to six data points, as appropriate. All spectra were recorded by use of quadrature detection with a 12-bit analog to digital converter into 2K memory points with an acquisition rate of 1 μs/point. The recycle delay was 100 ms. Each spectrum required that 10^4–10^5 transients be coadded, depending on sample size. Because the relative intensities from narrow and broad spectral features are highly dependent on data processing procedures, all spectra were processed with and without zero filling, with and without symmetrization, with and without base-line correction, and with a variety of apodization functions. In general, zero filling and base-line correction had no effect on intensities, and symmetrization equalized only relatively minor intensity variations, since major distortions were effectively removed by the pulse sequence. The ^{2}H NMR spectra presented in the figures are without zero filling and with base-line correction, symmetrization, and moderate (10^3-Hz) Gaussian apodization.

The ^{15}N NMR spectra were obtained on a modified JEOL GX-400 spectrometer with a 9.4-T field using a home-built probe. The phase-alternated spin-lock version of the cross-polarization pulse sequence (Pines et al., 1973) was used in order to minimize the effects of mismatches of the Hartmann–Hahn condition (Levitt et al., 1986). Spin-temperature alternation was used to minimize artifacts (Stejskal & Schaefer, 1975). In addition, a 180° refocusing pulse, with an interpulse spacing of 100 μs, was used following cross-polarization to avoid distortions due to probe ring down. Approximately 10^4 free induction decays were coadded with a 6-s recycle delay for each spectrum. The displayed spectra were processed with 250 Hz of Gaussian apodization. The ^{15}N chemical shifts are referenced to external $(^{15}NH_4)_2SO_4$ at 0 ppm.

Results

^{15}N NMR of Backbone Sites. Bacterial proteins can be readily labeled with ^{15}N uniformly at all sites or selectively in one type of residue at a time. Since the fd coat protein is so small, there are only one or a few of most of the amino acids, and selective labeling offers favorable opportunities for spectroscopic discrimination among sites.

The experimental ^{15}N NMR spectra in Figures 4 and 5 were obtained from unoriented solutions of the virus where the coat proteins are labeled in various sites with ^{15}N. Two distinct types of line shapes are observed in these spectra: relatively narrow resonances at the isotropic frequency and broad powder patterns. Previously published ^{15}N NMR powder patterns of uniformly ^{15}N-labeled fd virus, for example, Figure 1B of Cross et al. (1983), showed only slight evidence of isotropic resonance intensity. Motionally averaged ^{15}N resonances are from sites with correspondingly reduced heteronuclear dipolar couplings, which result in the cross-polarization of the magnetization being inefficient and critically dependent on the Hartmann–Hahn match condition. In practice, spectra with reproducible ratios of isotropic and powder pattern intensities could only be obtained with the phase-alternated cross-polarization sequence of Levitt et al. (1986). Most of the backbone sites are highly constrained and undergo only small amplitude motions; these sites yield powder patterns similar to those from a rigid lattice. However, a few amide sites undergo large amplitude motions that are rapid on the 10^3-Hz time scale of the ^{15}N chemical shift interaction and yield relatively narrow resonances near the isotropic frequency. The experimental spectra can be interpreted qualitatively by direct comparison with the calculated spectra in Figure 1.

The uniformly ^{15}N-labeled virus sample gives the spectrum in Figure 4G. This spectrum has resonance intensity near the

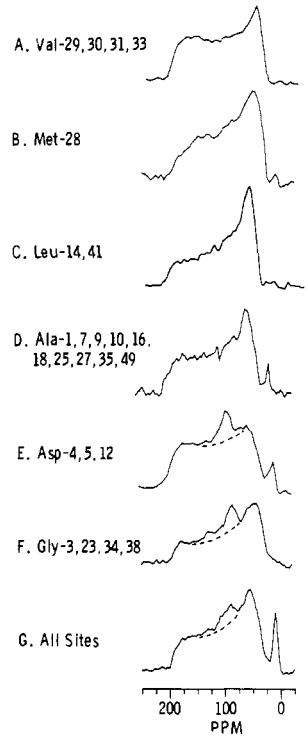

FIGURE 4: Experimental ^{15}N NMR spectra of fd virus samples at 25 °C. The samples were labeled with ^{15}N at the sites indicated.

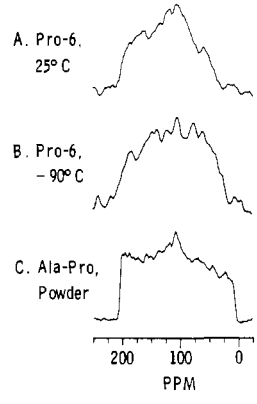

FIGURE 5: Experimental ^{15}N NMR spectra of [^{15}N]Pro-labeled samples. fd coat protein has a single Pro residue at position 6: (A) [^{15}N]Pro^6-labeled fd virus at 25 °C; (B) same as (A) except at −90 °C; (C) Ala-[^{15}N]Pro polycrystalline model peptide.

isotropic position superimposed on a powder pattern from the amide sites. The resonance near 15 ppm in this sample is from the amino groups of the lysine side chains and the N-terminal alanine residue (Cross et al., 1983). Through selective incorporation of ^{15}N-labeled amino acids, the dynamics of the peptide backbone site for individual residues can be described. The spectra of [^{15}N]Asp- (Figure 4E) and [^{15}N]Gly-labeled (Figure 4F) coat proteins have narrow motionally averaged resonance intensity from one mobile residue superimposed on the powder pattern from the other, immobile residues of each type (two of Asp and three of Gly). This estimate of intensities is reasonable since there are only three Asp residues (4, 5, and 12) and four Gly residues (3, 23, 34, and 38) in the coat protein. The ^{15}N data in Figure 4E–G clearly show the heterogeneous dynamics of coat protein backbone sites. The finding of individual Gly and Asp residues that are mobile is strongly reinforced by one- and two-dimensional spectra obtained on oriented virus samples where one Gly and one Asp resonance are observed at the isotropic chemical shift position with no heteronuclear dipolar couplings (Opella et al., 1987).

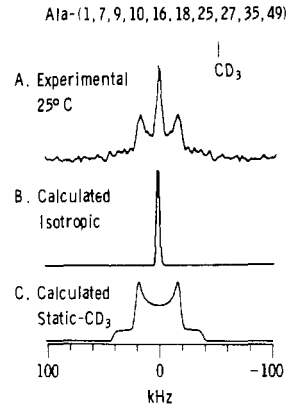

FIGURE 6: ^2H NMR spectra for CD_3 Ala labeled fd virus: (A) experimental spectrum obtained at 25 °C; (B) calculated isotropic spectrum as in Figure 2A; (C) calculated powder pattern as in Figure 2D with major splitting of ±20 kHz. Both calculated spectra have 2 kHz of Gaussian line broadening.

In these same oriented spectra, the resonances from the other labeled sites have chemical shifts and dipolar couplings appropriate for immobile N–H bonds oriented approximately parallel to the direction of the applied magnetic field. This is the orientation expected for residues immobilized in the helical protein with the helix axis parallel to the filament axis of the virus particles oriented parallel to the field (Cross & Opella, 1985; Opella et al., 1987). These results suggest that residues 3 and 4 are mobile and that residue 5 is immobile.

The spectra in Figure 4A–D show that residues 7–49 have immobile backbone sites, since no isotropic resonance intensity is observed in these spectra from virus samples that have labeled residues distributed throughout the sequence between residues 7 and 49. Additional evidence that the four valine residues (29, 30, 31, and 33) have immobile peptide linkages comes from the ^{13}C NMR powder pattern of [^{13}C]Val-labeled coat protein (Cross & Opella, 1982), which is well simulated by a broadened powder pattern corresponding to a ^{13}C chemical shift powder pattern for a rigid carbonyl carbon. The N-terminal residue is Ala-1, and its amino group gives the narrow resonance near 15 ppm in the spectrum in Figure 4D; since amino groups have very small chemical shift anisotropy, the line width of this resonance does not discriminate between a rigid or a mobile N-terminus. The imino acid proline has a different ^{15}N chemical shift tensor than the peptide linkages of the amino acids. This is apparent in the spectra in Figure 5. Proline is residue 6 of fd coat protein, and the spectrum in Figure 5A shows that this site is immobile by comparison with the breadth of the powder pattern for the virus at −90 °C (Figure 5B) and a polycrystalline model peptide (Figure 5C).

^2H NMR of Methyl Groups of Aliphatic Side Chains. The motional averaging of methyl groups in aliphatic amino acid side chains can be described by comparing calculated spectra, such as those in Figure 2, to those obtained in ^2H NMR experiments on virus samples where the coat proteins have CD_3-labeled side chains. The motions of the methyl groups of alanine residues reflect the properties of the backbone at these sites because the methyl group is directly bonded to the α-carbon. Figure 6A contains the experimental ^2H NMR spectrum of CD_3 Ala labeled fd coat protein at 25 °C. The spectrum in Figure 6A has a narrow central resonance superimposed on the CD_3 group powder pattern. The relative intensities of the narrow central component and the powder pattern component of this spectrum do not change over the temperature range 0–40 °C. This spectrum can be interpreted by comparison to the calculated spectra in Figure 6B,C. Ala-1

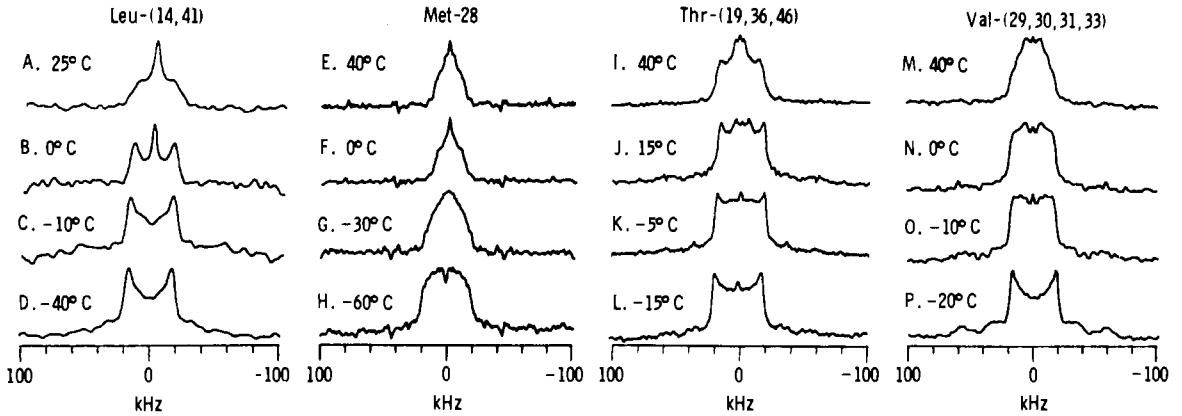

FIGURE 7: Experimental ^2H NMR spectra of specifically labeled fd virus samples at various temperatures. The labeled sites are designated above each column of spectra. The temperature of the samples are next to each spectrum.

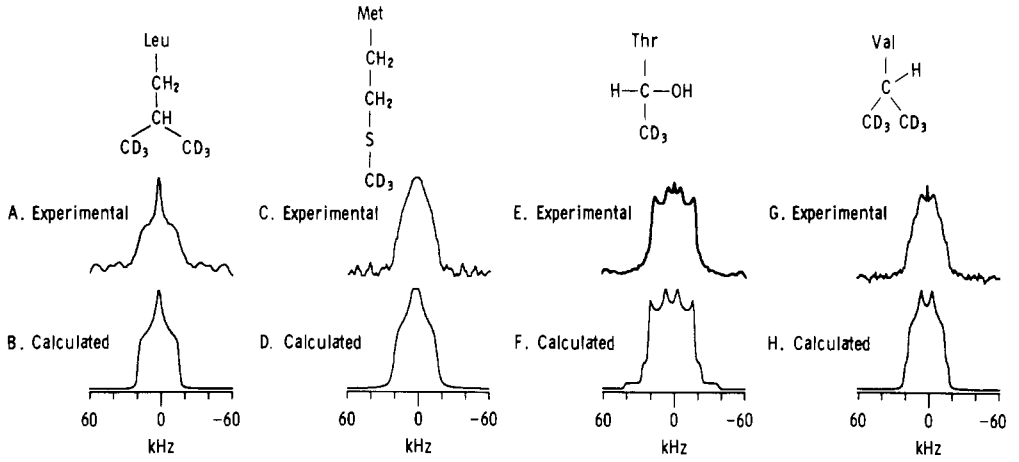

FIGURE 8: Comparisons of experimental and calculated spectra for the specifically labeled fd virus samples: (A) experimental 25 °C spectrum of Leu-labeled fd; (B) calculated spectrum with motional averaging resulting in $\eta = 1.0$ on a powder pattern with major splitting of ±18 kHz and 1 kHz of Gaussian and Lorentzian line broadening; (C) experimental −30 °C spectrum of Met-labeled fd; (D) calculated spectrum with motional averaging resulting in $\eta = 0.8$ on a powder pattern with major splitting of ±18 kHz and 2 kHz of Gaussian and Lorentzian line broadening; (E) experimental 40 °C spectrum of Thr-labeled fd; (F) calculated spectrum that is the sum of one powder pattern with $\eta = 0$ and ±20-kHz major splitting and two powder patterns with motional averaging resulting in $\eta = 0.6$ on the same ±20-kHz major splitting (2 kHz of Gaussian line broadening is added); (G) experimental 40 °C spectrum of Val-labeled fd; (H) calculated spectrum with motional averaging resulting in $\eta = 0.52$ on a powder pattern with major splitting of ±20 kHz and 2 kHz of Gaussian and 1 kHz of Lorentzian line broadening.

is the N-terminus of the coat protein, and it is mobile and yields the narrow deuterium resonance in the center of the spectrum. This is consistent with the results in Figure 4D because it is a CD_3 group on a residue with an amino rather than amide nitrogen that is mobile. This finding is reinforced by the corresponding deuterium spectrum of an oriented sample, where only one of the Ala sites gives a doublet with a very small quadrupole splitting (Valentine et al., 1985); the presence of small residual quadrupole splitting indicates that although the motional averaging of the N-terminus of the protein is substantial, it is not complete on the 10^6-Hz time scale. Additional evidence that one alanine residue is mobile is contained in the ^{13}C NMR spectrum of [$^{13}C_\alpha$]Ala-labeled coat protein obtained with magic angle sample spinning (Cross & Opella, 1982). This spectrum has one very narrow $^{13}C_\alpha$ resonance superimposed on broad resonance band from the other Ala-C_α resonances. The Ala residues are distributed throughout the protein sequence (1, 7, 9, 10, 16, 18, 25, 27, 35, and 49), and all are immobile, except for Ala-1, on the basis of the results in Figures 4D and 6A.

The interpretation of the line shapes from the other CD_3-labeled aliphatic side chains requires the consideration of additional motions, in particular jump motions about carbon sites in the side chain. fd coat protein has two leucine residues. The ^{15}N NMR powder pattern from Leu-14 and Leu-41 in Figure 4C shows that they both have highly constrained peptide linkages and are effectively immobile. Leucine side chains have been shown to undergo twofold jump motions about the tetrahedral γ-carbon site in proteins by ^2H NMR (Batchelder et al., 1982; Colnago et al., 1986). The $\eta = 0$ powder pattern for methyl threefold reorientation (Figure 2D) or the $\eta = 1$ powder pattern with twofold jumps (Figure 2C) is expected in the fast motional limits for the CD_3 groups of leucine residues. Experimental ^2H NMR spectra for CD_3 Leu labeled coat protein obtained at temperatures between −40 and 25 °C are shown in Figure 7A–D. At low temperatures, the side chains of both Leu residues appear to be immobile. Above about 10 °C, both of the Leu side chains give powder patterns with $\eta = 1$, indicative of the presence of rapid tetrahedral jump motions. The two Leu residues differ in their activation energy for the jump motion, since at 0 °C one of the side chains is immobile and the other undergoes twofold jump motions. Even at temperatures where the jump motions occur in both Leu residues, the backbone sites are immobile as seen in the ^{15}N chemical shift anisotropy powder pattern (Figure 4C) and required by the ^2H NMR powder pattern line shapes. The experimental line shape is very similar to that calculated for a methyl group undergoing only tetrahedral two-site jump motions, as shown with the comparison in Figure 8 (parts A and B).

The coat protein has a single methionine residue, Met-28. ^2H NMR spectra of this single CD_3 site as a function of

Table I: Dynamics of fd Coat Protein in the Bacteriophage

residue[a]		backbone		side chain		residue[a]		backbone		side chain	
no.	type	dynamics[b]	figure	dynamics[c]	figure	no.	type	dynamics[b]	figure	dynamics[c]	figure
1	Ala	M	6A[d]	M	6A[d]	26	Trp	R	*k*	R	*k*
2	Glu					27	Ala	R	4D, 6A	R	6A
3	Gly	M	4F[e]			28	Met	R	4	J	10A
4	Asp	M	4E[f]			29	Val	R	4A	J	14A
5	Asp	R	4E[f]			30	Val	R	4A	J	14A
6	Pro	R	5A			31	Val	R	4A	J	14A
7	Ala	R	4D, 6A	R	6A	32	Ile	R	*j*		
8	Lys	R	*g*			33	Val	R	4A	J	14A
9	Ala	R	4D, 6A	R	6A	34	Gly	R	4F[e]		
10	Ala	R	4D, 6A	R	6A	35	Ala	R	4D, 6A	R	6A
11	Phe	R	*h*	J	*h*	36	Thr	R	11	(R or J)	11
12	Asp	R	4E[f]			37	Ile	R	*j*		
13	Ser					38	Gly	R	4F[e]		
14	Leu	R	4C	J	7A	39	Ile	R	*j*		
15	Gln					40	Lys	R	*g*		
16	Ala	R	4D, 6A	R	6A	41	Leu	R	4C	J	7A
17	Ser					42	Phe	R	*d*	J	*h*
18	Ala	R	4D, 6A	R	6A	43	Lys	R	*g*		
19	Thr	R	11	(R or J)	11	44	Lys	R	*g*		
20	Glu					45	Phe	R	*h*	J	*h*
21	Tyr	R	*i*	J	*i*	46	Thr	R	11	(R or J)	11
22	Ile	R	*j*			47	Ser				
23	Gly	R	4F[e]			48	Lys	R	*g*		
24	Tyr	R	*i*	J	*i*	49	Ala	R	4D, 6A	R	6A
25	Ala	R	4D, 6A	R	6A	50	Ser				

[a] Asbeck et al., 1969; Nakashima & Konigsberg, 1974. [b] Backbone dynamics are represented as M (mobile) for sites that undergo effectively isotropic motion on the 10^3-Hz time scale of the ^{15}N amide chemical shift anisotropy and R (rigid) for sites that are immobile on the 10^3-Hz time scale. Immobile sites labeled as R undergo small amplitude (<10°) reorientations that are not taken into account in this scheme [Cross and Opella (1982) and Frey et al. (1983)]. [c] Side-chain dynamics are represented as M (mobile) for side chains that undergo effectively isotropic motion on the 10^6-Hz time scale of the ^2H quadrupole interaction, J (jump) for side chains where there are motions that yield nonaxially symmetric ^2H line shapes indicative of jump motions characterized by long residence times and short transit times that occur with a frequency greater than 10^6 Hz, and R (rigid) for side chains that are immobile on this time scale. Threefold methyl group reorientation occurs in all cases, and a methyl group with only this motion would be called rigid. [d] Figure 2F of Valentine et al. (1985) contains the ^2H NMR spectrum of CD_3 Ala labeled fd obtained on an oriented sample. This spectrum consists of overlapping doublets, one of which has a very small splitting and probably corresponds to the central narrow resonance in Figure 6A and indicates nearly complete motional averaging on the 10^6-Hz times scale. [e] A two-dimensional ^{15}N chemical shift/^1H–^{15}N dipolar spectrum of an oriented [^{15}N]Gly-labeled sample shows one distinct isotropic site and three unresolved rigid sites with N–H bonds approximately parallel to the filament axis (unpublished results). [f] A two-dimensional ^{15}N chemical shift/^1H–^{15}N dipolar spectrum of an oriented [^{15}N]Asp-labeled sample shows one distinct isotropic site and two sites with chemical shifts at positions appropriate for rigid N–H bonds approximately parallel to the filament axis; one of these sites has a dipolar splitting near maximal as expected (attributed to Asp-12), and the other site has an unusual "smeared" dipolar spectrum suggestive of partial motional averaging (attributed to Asp-5) on the 10^5-Hz time scale of this interaction. There is no evidence of motional averaging on the 10^3-Hz time scale of the ^{15}N chemical shift interaction for two of the Asp sites (unpublished results). [g] Figure 6C in Cross and Opella (1985) of an oriented [^{15}N]Lys-labeled sample shows that all five Lys residues are immobile with their N–H bonds approximately parallel to the filament axis. [h] Figure 7 of Gall et al. (1981) shows the three Phe rings to undergo only two-site 180° ring flips, and Figures 7 and 8 of Cross and Opella (1985) show all three amide linkages to be immobile with their N–H bonds approximately parallel to the filament axis. [i] The ^{15}N chemical shift anisotropy powder pattern for [^{15}N]Tyr-labeled coat proteins indicates that the backbone sites for both Tyr residues are immobile (unpublished results). In addition, Figures 5C and 6B,C of Gall et al. (1982) show that both Tyr rings undergo twofold 180° ring flips; the observed line shapes are consistent only with a rigid peptide backbone at these sites. [j] A two-dimensional ^{15}N chemical shift/^1H–^{15}N dipolar spectrum of an oriented [^{15}N]Ile-labeled sample shows that all of the Ile residues are immobile with N–H bonds approximately parallel to the filament axis (unpublished results). [k] Figures 3A, 4B,D, and kA of Gall et al. (1985) show the Trp-26 side chain to be immobile. These results also indicate that the Trp backbone is also immobile.

temperature are in Figure 7E–H. This side chain has some motions that seem to have very low activation energies and are not completely stopped at temperatures as low as −60 °C (Figure 7H). The spectra in Figure 8 (parts C and D) compare the calculated powder pattern for a twofold jump about the C–S bond in methionine to the experimental spectrum obtained at −30 °C. The experimental and calculated spectra are very similar, suggesting that the line-shape averaging results from the jump motion alone at this temperature. At higher temperatures, some additional motional averaging is observed, since the line shape is narrowed somewhat compared to that in Figure 7G. The peptide backbone at this residue is immobile as seen in the ^{15}N chemical shift anisotropy powder pattern in Figure 4B.

The coat protein has three threonine residues (19, 36, and 46). The experimental spectrum in Figure 7L shows that at −15 °C all three of the Thr side chains are immobile. At higher temperatures, overlapping motionally averaged powder patterns are observed. Figure 8 (parts E and F) shows the close correspondence between the experimental spectrum obtained at 40 °C and that calculated for two immobile Thr side chains and one undergoing two-site jumps about a carbon site that has close to tetrahedral geometry.

The four valine residues (29, 30, 31, and 33) present in the coat protein yield the $\eta = 0$ powder pattern at −20 °C that is characteristic of immobile side chains in Figure 7P. At higher temperatures, additional modes of motion are present. The analysis of the temperature dependence of the Val powder pattern is complicated by the overlap of the powder patterns from four residues. However, it is clear that large amplitude motions are present in all four of the Val side chains at temperatures above 0 °C. The comparison of experimental and calculated spectra in Figure 8 (parts G and H) is consistent with all four Val residues undergoing side-chain jump motions.

Discussion

The dynamics of fd coat protein in the virus particle are summarized in Table I. Over 90% of the peptide backbone sites have small amplitude librational motions on the 10^9-Hz time scale but no large amplitude motions with frequencies

that are rapid compared to 10^3 Hz. These sites are categorized as immobile on the basis of the observation of static powder patterns for ^{15}N chemical shift anisotropy. The four N-terminal residues are highly mobile, since their backbone and side chain sites yield resonances that exhibit extensive motional averaging on the 10^3- and 10^6-Hz time scales. There is an abrupt change in backbone dynamics between residue Asp-4 and residue Asp-5.

The finding of a mobile N-terminus suggests that these residues are exposed to the solvent and are not strongly interacting with either DNA or protein subunits. This interpretation is consistent with discussions of virus structure based on the sequence of amino acids in the coat protein where the basic C-terminal region is placed near the DNA to neutralize the phosphate groups and the acidic N-terminal region is exposed to solvent (Makowski, 1984).

The side chains of those residues where the backbone site is immobile either have two-site jump motions or are immobile. In the case of the aromatic residues Phe and Tyr, the jump motions are twofold 180° flips, and Trp-26 is completely immobile (Gall et al., 1982). The aliphatic residues undergo jump motional averaging about tetrahedral or near-tetrahedral side-chain sites.

fd coat protein has Leu residues at positions 14 and 41. Both have immobile backbone sites. Their side chains undergo tetrahedral twofold jump motions with somewhat different activation energies. They present a particularly clear-cut case of jump motional averaging in a protein.

Although there are many possible motions for the methyl group of a Met residue, only a single type of twofold jump motion is observed at −30 °C. Some additional motions are seen at higher temperatures; nonetheless, only limited motional averaging is observed.

All three of the threonine residues have immobile peptide linkages. The comparison between calculated and experimental spectra of the CD_3 Thr side chains suggests that at temperatures above 25 °C one of the Thr side chains undergoes motional averaging and two are immobile. The four valine backbone sites are immobile, and at 40 °C all four valine side chains appear to undergo motional averaging.

The analysis and interpretation of protein dynamics, especially for the large amplitude high-frequency motions described here, are at a very early stage. While the four N-terminal residues are mobile, as defined by their backbone sites undergoing effectively isotropic averaging on the 10^3-Hz time scale, the rest of the peptide backbone sites are immobile, as defined by the absence of large amplitude motions. Most of the side chains of the residues with immobile backbone sites have large amplitude motions that occur more frequently than 10^6 Hz at temperatures above 25 °C. These side-chain motions are well-defined in terms of amplitude and directions, as indicated by the characteristic powder pattern line shapes with $\eta \neq 0$. Unrestricted diffusional motions about one or several axes would yield axially symmetric ($\eta = 0$) line shapes and in the limit of motions in many directions would yield a single isotropic line.

In general, conclusions based on spectral simulations are not unique. There are typically several models that can yield a particular calculated line shape. In the case of the two-site jump motions of phenylalanine and tyrosine rings (Gall et al., 1981) and of leucine methyl groups (Batchelder et al., 1982), and highly characteristic patterns and the close fit to the experimental spectra give confidence in the results. The interpretation of Met, Thr, Val, and other aliphatic side-chain data is based on reasonable extensions of the Leu model; however, the calculated spectra could be obtained from other models of motion. "Intermediate" exchange rates, unequal population distributions, diffusional motions of limited amplitude, and alterations of quadrupole coupling constants are not considered in this analysis, primarily because of the difficulties imposed by working with overlapping resonances from several sites. More detailed analysis, including rates of motions, are in progress on proteins with only one of a particular type of residue to obviate problems of resolution.

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REFERENCES

Asbeck, F., Beyreuther, K., Kohler, H., Von Wettstein, G., & Braunitzer, G. (1969) *Hoppe-Seyler's Z. Physiol. Chem. 350*, 1047–1066.

Banner, D. W., Nave, C., & Marvin, D. A. (1981) *Nature (London) 289*, 862–863.

Batchelder, L. S., Sullivan, C. P., Jelinski, L. W., & Torchia, D. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 386–389.

Batchelder, L. S., Niu, C. H., & Torchia, D. A. (1983) *J. Am. Chem. Soc. 105*, 2228–2231.

Bogusky, M. J., Leo, G. C., & Opella, S. J. (1985) in *Magnetic Resonance in Biology and Medicine* (Govil, G., Khetrapal, C. L., & Saran, A., Eds.) pp 375–383, Tata McGraw-Hill, New Delhi.

Colnago, L. A., Leo, G. C. Valentine, K. G., & Opella, S. J. (1986) in *Biomolecular Stereodynamics, Proceedings of the Fourth SUNYA Conversation in the Discipline Biomolecular Stereodynamics* (Sarma, R. H., & Sarma, M. H., Eds.) Vol. III, pp 147–158, Adenine, Guilderland, NY.

Cross, T. A., & Opella, S. J. (1981) *Biochemistry 20*, 290–297.

Cross, T. A., & Opella, S. J. (1982) *J. Mol. Biol. 159*, 543–549.

Cross, T. A., & Opella, S. J. (1985) *J. Mol. Biol. 182*, 367–381.

Cross, T. A., DiVerdi, J. A., & Opella, S. J. (1982) *J. Am. Chem. Soc. 104*, 1759–1761.

Cross, T. A., Tsang, P., & Opella, S. J. (1983) *Biochemistry 22*, 721–726.

Davis, J. H., Jeffrey, K. R., Bloom, M., Valie, M. I., & Higgs, T. P. (1976) *Chem. Phys. Lett. 42*, 390–394.

Frey, M. H., Hexem, J. G., Leo, G. C., Tsang, P., Opella, S. J., Rockwell, A. L., & Gierasch, L. M. (1983) in *Peptides: Structure and Function, Proceedings of the Eighth American Peptide Symposium* (Hruby, V. J., & Rich, D. H., Eds.) pp 763–771, Pierce Chemical Co., Rockford, IL.

Gall, C. M., DiVerdi, J. A., & Opella, S. J. (1981) *J. Am. Chem. Soc. 103*, 5039–5043.

Gall, C. M., Cross, T. A., DiVerdi, J. A., & Opella, S. J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 101–105.

Gelin, B. R., & Karplus, M. (1979) *Biochemistry 18*, 1256–1268.

Gurskaya, G. V. (1968) *The Molecular Structure of Amino Acids*, p 76, Consultants Bureau, New York.

Janin, J., Wodak, S., Levitt, M., & Maigret, B. (1978) *J. Mol. Biol. 125*, 357–386.

Jelinski, L. W., Sullivan, C. E., & Torchia, D. A. (1980) *Nature (London) 284*, 531–534.

Keniry, M. A., Rothgeb, T. M., Smith, R. L., Gutowsky, H. S., & Oldfield, E. (1983) *Biochemistry 22*, 1917–1926.

Keniry, M. A., Gutowsky, H. S., & Oldfield, E. (1984a) *Nature (London) 307*, 383-386.
Keniry, M. A., Kintanar, A., Smith, R. L., Gutowsky, H. S., & Oldfield, E. (1984b) *Biochemistry 23*, 288-298.
Kim, C. H., & Hollocher, T. C. (1982) *J. Bacteriol. 151*, 358-364.
Kinsey, R. A., Kintanar, A., & Oldfield, E. (1981a) *J. Biol. Chem. 256*, 9028-9036.
Kinsey, R. A., Kintanar, A., Tsai, M.-D., Smith, R. L., James, N., & Oldfield, E. (1981a) *J. Biol. Chem. 256*, 4146-4149.
Leo, G. C., Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry* (following paper in this issue).
Levitt, M., Suter, D., & Ernst, R. R. (1984) *J. Chem. Phys. 80*, 3064-3068.
Levitt, M., Suter, D., & Ernst, R. R. (1986) *J. Chem. Phys. 84*, 4243-4255.
Makowski, L. (1984) in *Biological Macromolecules and Assemblies* (McPherson, A., Ed.) pp 203-253, Wiley, New York.
Makowski, L., & Caspar, D. L. D. (1981) *J. Mol. Biol. 145*, 611-617.
Marvin, D. A., & Hohn, B. (1969) *Bacteriol. Rev. 33*, 172-209.
Nakashima, Y., & Konigsberg, W. (1974) *J. Mol. Biol. 88*, 598-600.
Oldfield, E., Kinsey, R. A., & Kintanar, A. (1982) *Methods Enzymol. 88*, 210-323.
Opella, S. J. (1986) *Methods Enzymol. 131*, 327-361.
Opella, S. J., Stewart, P. L., & Valentine, K. G. (1987) *Q. Rev. Biophys.* (in press).
Pines, A., Gibby, M., & Waugh, J. S. (1973) *J. Chem. Phys. 59*, 569-590.
Rice, D. M., Blume, A., Herzfeld, J., Wittebort, R. J., Huang, T. H., Das Gupta, S. K., & Griffin, R. G. (1981) in *Biomolecular Stereodynamics, Proceedings of the Second SUNYA Conversation in the Discipline Biomolecular Stereodynamics* (Sarma, R., Ed.) pp 255-270, Adenine, Guilderland, NY.
Schramm, S., Kinsey, R. A., Kintanar, A., Rothgeb, T. M., & Oldfield, E. (1981) in *Biomolecular Stereodynamics, Proceedings of the Second SUNYA Conversation in the Discipline Biomolecular Stereodynamics* (Sarma, R., Ed.) pp 271-286, Adenine, Guilderland, NY.
Smith, R. L., & Oldfield, E. (1984) *Science (Washington, D.C.) 225*, 280-288.
Soda, G., & Chiba, T. (1969) *J. Chem. Phys. 50*, 439-455.
Stejskal, E. O., & Schaefer, J. (1975) *J. Magn. Reson. 18*, 560-566.
Torchia, D. A. (1982) *Methods Enzymol. 82*, 174-186.
Torchia, D. A. (1984) *Annu. Rev. Biophys. Bioeng. 13*, 125-144.
Valentine, K. G., Schneider, D. M., Leo, G. C., Colnago, L. A., & Opella, S. J. (1985) *Biophys. J. 49*, 36-38.

Dynamics of fd Coat Protein in Lipid Bilayers†

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ABSTRACT: The dynamics of backbone and side-chain sites of the membrane-bound form of fd coat protein are described with solid-state ^2H and ^{15}N NMR experiments. The samples were isotopically labeled coat protein in phospholipid bilayers in excess water. The protein itself is immobile and does not undergo rapid rotation within the bilayer. Like the structural form of the protein, the membrane-bound form has four mobile residues at the N-terminus. The membrane-bound form differs from the structural form in having several mobile residues at the C-terminus. Many of the side chains of residues with immobile backbone sites undergo large amplitude jump motions. The dynamics are generally similar in both the structural and membrane-bound forms of the protein.

The major coat protein of the filamentous bacteriophages is an interesting and popular system for study because it has roles as both a membrane-bound and a structural protein during the viral life cycle (Marvin & Hohn, 1969; Makowski, 1984). We are studying the coat protein in both its membrane-bound (Cross & Opella, 1979, 1980, 1981; Frey et al., 1983; Bogusky et al., 1985a,b, 1987; Valentine et al., 1985; Colnago et al., 1986) and its structural (Opella et al., 1979, 1980; Cross et al., 1981, 1982, 1983; Cross & Opella, 1982, 1983, 1985; Gall et al., 1981, 1982; Colnago et al., 1986, 1987) forms by NMR spectroscopy. The overall goal of these investigations is to develop an understanding of the relationship between the two forms of the protein and their roles in the infection and assembly processes of the viral life cycle.

The coat protein adopts its membrane-bound conformation in the presence of a variety of lipids and detergents (Nozaki et al., 1976). Samples of the protein in detergent micelles and in sonicated phospholipid vesicles are well suited for high-resolution solution NMR studies as pursued by us (Cross & Opella, 1979, 1980, 1981; Bogusky et al., 1985a,b, 1987; Schiksuis et al., 1987) and others (Hagen et al., 1978, 1979a,b; Dettman et al., 1982, 1984; Henry et al., 1985, 1987; Wilson & Dahlquist, 1985). Samples of the protein in phospholipid bilayers are well suited for solid-state NMR studies as described here and in earlier publications (Frey et al., 1983;

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