excellent typing of the manuscript.

Registry No. ATP, 56-65-5; insulin, 9004-10-8; protein kinase, 9026-43-1.

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Comparison of Protein and Deoxyribonucleic Acid Backbone Structures in fd and Pf1 Bacteriophages†

T. A. Cross, P. Tsang, and S. J. Opella*

ABSTRACT: The conformations of the protein and nucleic acid backbones in the filamentous viruses fd and Pf1 are characterized by one- and two-dimensional solid-state NMR experiments on oriented virus solutions. Striking differences are observed between fd and Pf1 in both their protein and DNA structures. The coat proteins of fd and Pf1 are almost entirely α helical and in both viruses most of the helix is oriented parallel to the filament axis. fd coat protein is one stretch of α helix that is slightly slued about the filament axis. In Pf1 coat protein two distinct sections of α helix are present, the smaller of which is tilted with respect to the filament axis by about 20°. The DNA backbone structure of fd is completely disordered. By contrast, the DNA backbone of Pf1 is uniformly oriented such that all of the phosphodiester groups have the O–P–O plane of the nonesterified oxygens approximately perpendicular to the filament axis.

The filamentous bacteriophages are nucleoprotein complexes of apparently simple design (Marvin & Hohn, 1969). Approximately ten of these viruses have been isolated. They are all filaments with a single-stranded circle of DNA surrounded by several thousand copies of a small protein. The coat protein subunits are arranged symmetrically in a helical array. Two classes of viral structures have been identified on the basis of X-ray diffraction patterns from oriented fibers of the viruses. The class I viruses (Marvin et al., 1974a) such as fd, M13, and If1 have distinctly different symmetry than the class II viruses (Marvin et al., 1974b) Pf1 and Xf.

This paper presents structural data for the class I virus fd and the class II virus Pf1 that directly address some of the most important questions concerning differences between these viruses (Day & Wiseman, 1978; Marvin et al., 1974a,b). The 1:1 ratio of nucleotides to coat protein subunits for Pf1 suggests specific protein–nucleic acid interactions and limits the possible DNA conformations. By contrast, this ratio is 2.3:1 for fd, which not only is much larger than that for Pf1 but also is significantly nonintegral, implying that the protein–nucleic acid interactions are nonspecific. There is general agreement that the coat proteins of both fd and Pf1 have a very high per-

† From the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received November 16, 1982.* This research is being supported by a grant from the National Institutes of Health (GM-24266). S.J.O. is a fellow of the A. P. Sloan Foundation (1980–1982).

0006-2960/83/0422-0721$01.50/0

centage of α helix. There is, however, considerable debate about the precise arrangement of the α helix of the proteins in the viral filaments (Marvin, 1978; Makowski & Caspar, 1978; Makowski et al., 1980; Nave et al., 1981).

Two possibilities have been proposed to explain the details of the X-ray diffraction patterns for Pf1. In one model the coat protein has two separate α-helical segments, where one segment is parallel to the filament axis and the other is tilted by 25° relative to this axis (Makowski & Caspar, 1978; Makowski et al., 1980). An alternative model for Pf1 coat protein has a single long α helix slued about the viral filament axis (Marvin et al., 1974a; Marvin & Wachtel, 1975; Marvin, 1978). This second, simpler model has also been proposed for fd (Marvin et al., 1974b; Banner et al., 1981).

In all models for the coat proteins in these viruses, nearly all of the α helix is extended approximately parallel to the filament axis. This results in the peptide carbonyl groups with their substantial local diamagnetic susceptibility being aligned parallel to each other and the filament axis. The resulting large net diamagnetic anisotropy and the liquid-crystalline character of the viral solutions combine to align the viral particles parallel to the applied magnetic field (Torbet & Maret, 1979, 1981).

A number of aspects of the filamentous bacteriophage systems have been studied by NMR[1] spectroscopy, including the dynamics of the coat protein and DNA in the intact viruses (Gall et al., 1981, 1982; DiVerdi & Opella, 1981; Cross & Opella, 1982). The structures of the protein and DNA components are being determined by the NMR methods described here. This paper emphasizes comparative aspects of both the protein and nucleic acid backbone structures at fairly low resolution because of the overlap of many similar amide ^{15}N and phosphodiester ^{31}P resonances in the spectra. However, these methods are capable of determining protein structure at atomic resolution using single site labels (Cross & Opella, 1983).

Materials and Methods

Both labeled and unlabeled fd viruses have been grown under conditions described in detail previously (Cross & Opella, 1981). Unlabeled Pf1 virus was obtained from cultures of *Pseudomonas aeruginosa* on LB-rich media infected at early log phase (OD_{550} of 0.5–1.0) with a multiplicity of 50. The growths continue for 5–8 h after infection with vigorous oxygenation. Uniformly ^{15}N-labeled Pf1 was obtained by growing infected *P. aeruginosa* on minimal mineral media (M10) with $(^{15}NH_4)_2SO_4$ as the sole nitrogen source; these cells were infected at an OD_{550} of 0.25–0.40.

Purification of the viruses was carried out as described elsewhere (Cross & Opella, 1981) with several modifications. Instead of extensive dialysis against distilled water following KBr step gradients, fd was first dialyzed against 0.5 M NaCl, 1 mM EDTA, and 10 mM Tris (pH 8.0) at room temperature and then against 10 mM Tris (pH 8.0) at 4 °C. fd samples at concentrations between 10 and 50 mg/mL obtained by suspending centrifuge pellets (VTi50 rotor at 33 000–40 000 rpm for 3 h) orient almost instantaneously in the magnetic fields used (3.5 and 5.9 T) in the NMR spectrometers. Pf1 has been purified similarly and orients in the magnetic field in the concentration range of 7–30 mg/mL. The orientation of the virus samples was checked by observing oriented NMR spectra and optical birefringence of samples immediately after their removal from the magnet.

[1] Abbreviations: NMR, nuclear magnetic resonance; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

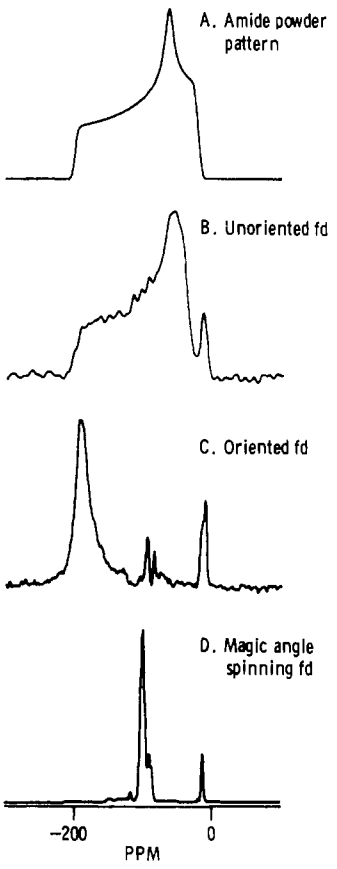

FIGURE 1: ^{15}N NMR spectra of uniformly ^{15}N-labeled fd at 25.3 MHz: (A) calculated powder pattern based on the spectrum of acetyl-[^{15}N]glycine (σ_{33} = −192, σ_{22} = −51, and σ_{11} = −10 ppm); (B) unoriented fd solution, 150 mg/mL, pH 6; (C) magnetic field oriented fd solution, 45 mg/mL, pH 8; (D) magic angle sample spinning of fd gel at 15.2 MHz, ~200 mg/mL, pH 6.

NMR spectra were obtained on home-built double-resonance spectrometers. ^{31}P spectra were recorded at 60.9 MHz in a 3.5 T magnet and ^{15}N spectra at 25.2 MHz in a 5.9 T magnet. ^{31}P chemical shifts are relative to H_3PO_4 and ^{15}N chemical shifts relative to $^{15}NH_3NO_3$. All spectra were obtained by cross polarization with a 1.0–2.0 mT ^1H decoupling field applied during data acquisition (Pines et al., 1973). The sample temperature was maintained at 25 °C. Spin temperature alternation and phase cycling were used to avoid experimental artifacts. A flip-back ^1H pulse at the end of the cycle allowed the use of shorter recycle delays. All ^{15}N spectra were recorded following an echo (τ = 200 μs) to eliminate spectral distortions due to probe ringing. The two-dimensional ^{15}N–^1H dipolar spectra were recorded by using separated local field spectroscopy (Waugh, 1976) with the 4-pulse WAHUHA cycle (Waugh et al., 1968) applied to suppress ^1H–^1H couplings during the first variable time interval.

Results

Solid-state NMR spectroscopy is well suited for studies of filamentous bacteriophages because their length (≥1000 nm) and mass (>10^7 daltons) make them immobile on the NMR time scales (10^3–10^7 Hz) (Opella et al., 1980), although the coat proteins do have well-defined rapid internal fluctuations (Gall et al., 1982). The angular dependence of the spin interactions is directly manifested in solid-state NMR spectra. The data presented here are from ^{15}N and ^{31}P chemical shift anisotropy and ^{15}N–^1H dipolar interactions.

The one-dimensional chemical shift spectra were acquired in the presence of ^1H decoupling, so the resonance line shapes

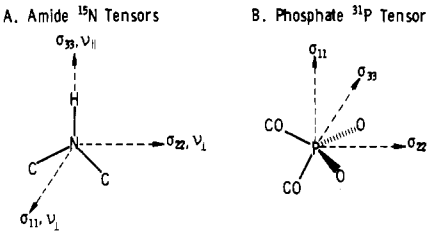

FIGURE 2: Orientation of the spin interaction tensors drawn schematically in the molecular frame for (A) ^{15}N in a peptide linkage and (B) ^{31}P in the DNA backbone. σ_{ii} are the principal elements of the chemical shift tensor, and $\nu_{\parallel}$ and $\nu_{\perp}$ are the components of the N–H dipolar interaction.

are representative of only the chemical shift interaction. The ^{15}N NMR spectra of fd in Figure 1 are dominated by the resonance band from the many similar peptide amide backbone sites. The amino groups from the five lysines and the N-terminal alanine contribute to the narrow peak near 10 ppm. Intensity from DNA sites is lost in the base line at this level of presentation because the phages are ≥90% by weight protein (Cross et al., 1982). The similar breadth of the powder patterns derived from polycrystalline *N*-acetylglycine in Figure 1A and the observed spectrum of unoriented fd in Figure 1B demonstrate the rigid nature of the protein backbone (Cross & Opella, 1982, 1983).

Structural parameters are determined from chemical shift and the associated heteronuclear dipolar spectra of oriented samples. The chemical shift and dipolar interactions have the angular dependence of second rank tensors. Both the ^{15}N amide and ^{31}P phosphodiester chemical shift tensors shown in their relevant molecular frames in Figure 2 are nonaxially symmetric, which means that the principal values are of unequal magnitude ($\sigma_{11} \neq \sigma_{22} \neq \sigma_{33}$). This is the reason that ^{15}N and ^{31}P powder pattern spectra from unoriented samples in Figures 1 and 4 have three distinguishable discontinuities, although the ^{15}N amide tensor is nearly axially symmetric with $\sigma_{11} \approx \sigma_{22}$. These patterns result because all angles are present in the samples; the magnitudes of the principal values can be determined directly from the frequencies of the spectral discontinuities.

The assignment of the principal axes of the ^{15}N chemical shift tensor and the N–H dipolar interaction (Figure 2A) in the molecular frame of a peptide linkage is based on a single crystal study of a dipeptide (R. Griffin, personal communication) and molecular symmetry. The orientation of the ^{31}P chemical shift tensor (Figure 2B) in the molecular frame of the DNA backbone is based on that found for the model compound barium diethyl phosphate (Herzfeld et al., 1978) and symmetry as used in previous studies of DNA (Nall et al., 1981). The orientation of a tensor with respect to a coordinate system such as that imposed by the applied magnetic field is fully described with two direction cosines. It is not possible to uniquely determine the angles relating a chemical shift tensor to a coordinate system solely on the basis of the chemical shift position of a resonance from an oriented molecule when that tensor is not axially symmetric. It is necessary to either limit the interpretation of the data, as is the case for the ^{31}P chemical shift anisotropy of the phosphodiester sites, or utilize a second interaction, for example, the ^{15}N–^{1}H dipolar coupling, to complement the ^{15}N chemical shift anisotropy at the amide sites. The combined use of the chemical shift and dipolar interactions at N–H sites is particularly favorable because these tensors are approximately collinear as shown in Figure 2A. Since the dipolar interaction is axially symmetric, the orientation of the dipolar vector (the N–H bond) relative to the magnetic field is described by a single direction cosine. The combined use of the dipolar and chemical shift interactions can uniquely determine the two direction cosines necessary to completely specify the orientation of a site.

Macroscopic uniaxial orientation along the direction of the applied magnetic field of a polymeric sample composed of identical subunits results in spectra in the form of single lines for chemical shift spectra and doublets for dipolar spectra (Opella & Waugh, 1977). There are a number of remarkable features about the ^{15}N NMR spectrum of oriented fd in Figure 1C. Chief among them is the absence of an equal distribution of intensity about the isotropic chemical shifts given in Figure 1D. The observation of nearly all of the ^{15}N amide signals at −185 ppm, which is approximately equal to σ_{33} of the amide chemical shift tensor, clearly demonstrates that the sample is oriented in the magnetic field. There are also a few distinguishable narrow peaks in other positions.

Even though the ^{15}N chemical shift anisotropy for the amide group is nonaxially symmetric, the oriented spectrum in Figure 1C can be directly interpreted because of the coincidence of the viral amide resonance with σ_{33}, the downfield discontinuity of the powder pattern. This principal element of the chemical shift tensor has been assigned to the N–H bond direction parallel to the applied magnetic field. Therefore, the oriented NMR spectrum of fd shows that nearly all of the amide groups of the coat protein are arranged with their N–H bonds parallel to the applied magnetic field. The line width of the amide resonance band in Figure 1C reflects the spread in individual resonance frequencies and not aspects of powder patterns. A limited range of angles for the N–H bonds relative to the filament axis is present.

The two-dimensional chemical shift and heteronuclear dipolar spectrum in Figure 3A for oriented fd confirms the assignment of the orientation of the peptide groups derived from the chemical shift data in Figure 1 and enables interpretation of the few resolved upfield peaks in the oriented spectrum of Figure 1C. The two-dimensional contour plots directly correlate heteronuclear dipolar splittings with their respective chemical shift positions. The projection of the dipolar spectrum is on the left side of the figure. The ^{15}N chemical shift resonance band at σ_{33} has a dipolar splitting of 14 kHz, which is close to the maximum calculated splitting corresponding to the N–H bond parallel to the applied magnetic field. Both the chemical shift and dipolar interactions demonstrate that nearly all of the N–H bonds of the amide groups are parallel to the field and, hence, the filament axis of the virus. Of the two, the dipolar results are more reliable, because they do not depend on a model compound determination of the tensor and can be calculated directly. The other distinguishable nitrogen sites have very small or no dipolar splittings, due to motional averaging (Lys, Ala-1), lack of a bonded hydrogen (Pro-6), or the orientation of the N–H bond being very near the magic angle (~55°) with respect to the applied magnetic field (Trp-26) (Cross & Opella, 1983).

Figure 3 compares the one- and two-dimensional ^{15}N NMR spectra of oriented fd and Pf1 samples. The spectra for Pf1 are clearly similar to those observed for fd. However, there are important differences, especially in that there is a pronounced shoulder on the upfield side of the main amide resonance peak in the Pf1 chemical shift spectrum. The upfield shift of a substantial number of the amide sites by ~35 ppm indicates that there are two populations of backbone amide orientations in Pf1. This is confirmed in the contour plot of Figure 3B, where there is a distinct dipolar doublet with a

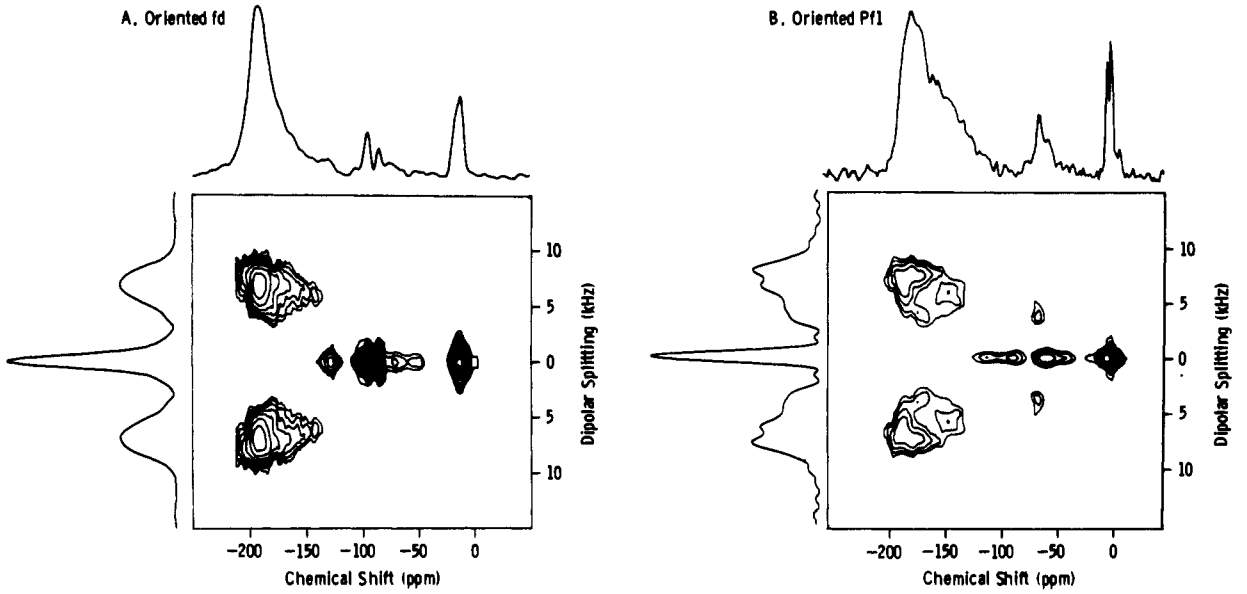

FIGURE 3: Two-dimensional ^{15}N NMR spectra at 25.3 MHz of magnetic field oriented viral solutions uniformly ^{15}N labeled: (A) fd (45 mg/mL, pH 8); (B) Pf1 (30 mg/mL, pH 8). The contour plots represent the data from two-dimensional separated local field experiments where the scale has been adjusted to account for the effect of the homonuclear decoupling scheme. The projections of the dipolar splittings are displayed on the left side of the contour plot, and the oriented chemical shift spectra are displayed above the contour plot.

reduced splitting ($\Delta\nu_d$ = 12 kHz) associated with the chemical shift position at 150 ppm; this is particularly clear in the dipolar projection on the left side. These chemical shift and dipolar spectra show that Pf1 has a group of amide N–H bonds tilted ~20° away from the majority of the amide N–H bonds, which are aligned parallel to the filament axis.

The ^{31}P NMR spectra reflect the structure of the DNA backbone in these phages. While the ^{15}N NMR data of fd and Pf1 show that the coat proteins have many similarities, there are completely opposite results for the ^{31}P chemical shift spectra of the two viruses (Figure 4). The nonaxially symmetric chemical shift powder pattern for a rigid phosphodiester group is shown in Figure 4A. The discontinuities are more clearly defined in the calculated powder pattern than in the spectrum of unoriented fd in Figure 4B, probably because some limited motions are present in the DNA backbone of the phage (DiVerdi & Opella, 1981). The oriented fd spectrum in Figure 4C is identical with that for unoriented fd in Figure 4B. This is in great contrast to the ^{15}N NMR comparison made in Figure 1, where the unoriented fd sample gives a powder pattern and the oriented fd sample gives a spectrum characteristic of aligned N–H bonds in the protein. Since the ^{15}N NMR results, as well as optical birefringence, show these same samples to be oriented by the magnetic field, the conclusion to be drawn from the ^{31}P spectrum of oriented fd is that the protein is ordered in a simple way, but the phosphodiester backbone of the DNA is not.

Pf1 differs strongly from fd with regard to the ^{31}P NMR results. Figure 4D shows that the phosphodiester linkages of DNA in Pf1 are uniformly oriented and that the chemical shift is close to the downfield discontinuity, σ_{11} of the powder pattern in Figure 4B. The phosphates of Pf1 DNA are therefore oriented so that the chemical shift tensor axis of σ_{11} is approximately parallel to the filament axis and the applied magnetic field.

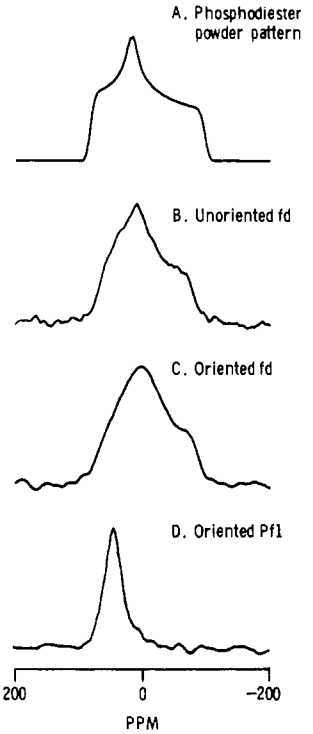

FIGURE 4: ^{31}P NMR spectra of the filamentous viruses fd and Pf1 at 60.9 MHz recorded with the same conditions as given in Figure 3: (A) calculated powder pattern based on the spectrum of unoriented frozen fd (σ_{11} = 81, σ_{22} = 15, and σ_{33} = −100 ppm); (B) unoriented fd spectrum; (C) magnetic field oriented fd spectrum; (D) magnetic field oriented Pf1 spectrum.

Discussion

The ^{15}N NMR data from fd show that nearly all of the N–H bonds of the amide groups are approximately parallel with the applied magnetic field and, hence, the filament axis of the virus. These N–H bonds in the peptide linkages of an α helix are aligned approximately parallel with the helix axis. Therefore, the NMR results for fd indicate that most of the coat protein's α helix is aligned to within about 10° of the filament axis, which is consistent with the model for class I viruses where the coat protein is a single stretch of α helix slued slightly about the filament axis (Marvin et al., 1974a; Banner et al., 1981). Even in a model α helix such as that shown in Figure 5A the N–H bonds are not aligned perfectly parallel with the helix axis, but vary by about ±10°. This range of

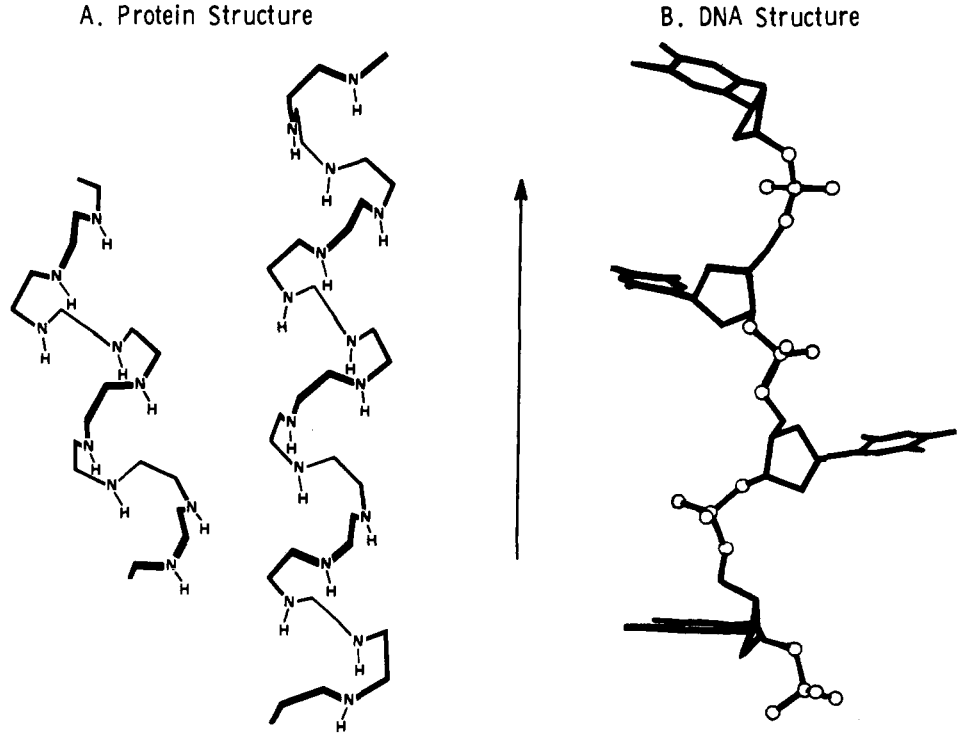

FIGURE 5: Models for the protein and DNA structures in Pf1 on approximately the same scale. The coat protein is almost entirely α helix mostly oriented parallel to the filament axis as indicated by the arrow, with a small portion tilted with respect to this axis by approximately 20°. A possible structure for a single strand of the DNA in Pf1 is shown with the phosphates oriented according to the NMR results reported and the backbone conformation extended as is expected from studies of others (Day et al., 1979).

angles accounts for the breadth of the amide resonances in the oriented spectra of fd and Pf1.

The comparison of ^{15}N NMR data for fd and Pf1 show that their coat proteins are different, since Pf1 coat protein has two distinct sections of α helix, one of which is tilted with respect to the filament axis by 20°. These NMR results disagree with the interpretations of X-ray diffraction data that indicated that Pf1 coat protein was one lone stretch of α helix (Marvin et al., 1974b; Marvin & Wachtel, 1975; Marvin, 1978) like fd coat protein (Marvin et al., 1974a). These NMR results are in general agreement with the model for Pf1, which has two segments of α helix in the Pf1 coat protein (Makowski & Caspar, 1978; Makowski et al., 1980). The model of Makowski and Caspar suggests that Pf1 coat protein contains a bend region between Ile-21 and Tyr-24, separating the two α-helical segments of approximately equal size. It is apparent from the relative intensities in Figure 3 that the tilted portion of helix is substantially smaller than the portion parallel to the filament axis; therefore, the NMR data suggest that the bend in the protein should be somewhat closer to one end of the peptide chain. The NMR results and the model of Makowski & Caspar (1978) (Makowski et al., 1980) based on X-ray diffraction agree that one of the α-helical segments in Pf1 coat protein is tilted by 20–25° from parallel to the filament axis.

The observation of diffraction from DNA in fd (Banner et al., 1981) and the ^{31}P NMR data showing that the DNA backbone in fd is disordered are difficult to reconcile. It is possible that the bases, but not the phosphodiester linkages of fd DNA, are ordered. The ^{31}P NMR spectrum of oriented Pf1 clearly shows that the DNA of this virus is highly ordered with the phosphates oriented such that the plane formed by the phosphorus bonds to the nonesterified oxygens is approximately perpendicular to the axis of the filament. On the basis of this feature and some geometrical considerations proposed for the DNA in Pf1 (Day et al., 1979), a plausible structure for one of the strands of the DNA is shown in Figure 5. The limitations imposed by the orientation of the phosphates are useful as a constraint for modeling the DNA in Pf1, although they are not sufficient to completely specify the structure of the DNA in the virion.

Because all of the protein and nucleic acid backbone sites contribute to the resonance bands in the spectra analyzed here, the view of the viral structures is necessarily of relatively low resolution. However, the general NMR approach utilized is capable of atomic resolution and precision of 1–2° in orientation when individual resolved resonances are studied (Cross & Opella, 1983). The studies of these viruses labeled at specific sites are in progress.

Acknowledgments

We thank Drs. D. A. Marvin and D. W. Banner of the European Molecular Biology Laboratory, Heidelberg, for showing us how to orient fd in a magnetic field; in particular, one of us (T.A.C.) is grateful for their hospitality during a visit to that laboratory. We also thank Dr. H. Berman for her help in using the computer graphics facilities at the Institute for Cancer Research.

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Articles

Assembly and Breakdown of Mammalian Protein Synthesis Initiation Complexes: Regulation by Guanine Nucleotides and by Phosphorylation of Initiation Factor eIF-2†

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ABSTRACT: Eukaryotic cell polypeptide chain initiation factor eIF-2 forms ternary complexes with GTP and initiator Met-tRNA$_f$. These complexes can be destabilized in vitro by the addition of salt-washed 40S ribosomal subunits. Our evidence suggests that this destabilization is mediated by GDP generated by premature hydrolysis of the GTP molecule present in the ternary complex. With complexes formed by using a partially purified preparation of eIF-2 from Ehrlich ascites tumor cells, it is possible to reverse the 40S subunit induced inhibition by creating conditions which eliminate free GDP from the system. This reversal probably occurs due to exchange of GTP for the GDP bound to the initiation factor, in a reaction catalyzed by another factor present in the eIF-2 preparation. However, if the eIF-2 has previously been phosphorylated by the reticulocyte heme-controlled repressor, the 40S subunit induced inhibition cannot be reversed by elimination of free GDP. The instability of initiation complexes containing eIF-2, together with the impairment of guanine nucleotide exchange after phosphorylation of eIF-2 [Clemens, M. J., Pain, V. M., Wong, S.-T., & Henshaw, E. C. (1982) *Nature (London) 296*, 93-95], may be an important aspect of the mechanism of the inhibition of translation by the heme-controlled repressor.

Polypeptide chain initiation in eukaryotic cells involves a complicated series of events [reviewed in Thomas et al. (1982) and Clemens & Pain (1980)] in which initiator Met-tRNA$_f$ is first bound in a ternary complex with initiation factor eIF-2[1] and GTP; this complex then binds to a native 40S ribosomal subunit. In the presence of several other initiation factors, mRNA associates with the 40S complex, and finally a 60S subunit joins, with concomitant GTP hydrolysis (Trachsel & Staehelin, 1978; Merrick, 1979; Peterson et al., 1979), to give a functional 80S initiation complex. The factor eIF-2, as well as having a crucial role in this pathway, is subject to regulation by phosphorylation catalyzed by specific protein kinases such

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[1] Abbreviations: eIF, eukaryotic initiation factor; HCR, heme-controlled repressor; PEP, phosphoenolpyruvate; PK, pyruvate kinase (EC 2.7.1.40); HPLC, high-performance liquid chromatography; DEAE, diethylaminoethyl; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

0006-2960/83/0422-0726$01.50/0