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STRUCTURAL PROPERTIES OF fd COAT PROTEIN IN SODIUM DODECYL SULFATE MICELLES

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SUMMARY

Natural abundance ^{13}C and high field ^{1}H NMR spectroscopy are used to characterize the major coat protein of the filamentous bacteriophage fd in sodium dodecyl sulfate micelles. Chemical shift dispersion of protein resonances, slow and differential exchange rates of amide protons, and relaxation parameters of the alpha carbons of the protein indicate that the detergent solubilized coat protein has a stable native conformation. The structure of the coat protein in micelles differs from that found for typical globular proteins in solution in that parts of the peptide backbone exhibit rapid segmental motion.

INTRODUCTION

Physical properties of proteins in the presence of detergents are of interest for both membrane bound and water soluble proteins. Detergent-protein complexes may be good models for membrane-protein complexes, since enzymatic and other biological activities of membrane proteins often are retained when detergents are substituted for the natural membrane lipids (1,2). In addition, detergents often have profound effects on the structure of water soluble globular proteins which allow the study of protein stabilizing forces (3).

The major coat protein of the filamentous bacteriophage fd is stored within the bacterial cell membrane during the infectious cycle prior to the assembly of the virus particle at the membrane (4,5). This coat protein is similar to other intrinsic membrane proteins in having a long 19 amino acid stretch of hydrophobic residues in the center of the 50 amino acid sequence (6) and in being completely insoluble in water (7). Circular dichroism and other physical techniques show that the coat protein in membranes and a variety of detergents exists as a dimer and has a structure with about 50% α helix (8,9). The coat protein in the virion has a quite different structure with about 90% α helix (10,11).

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Recently, NMR spectroscopy has been used to study several peptides in lipid environments (12-16). Sykes and coworkers have employed ^{19}F NMR to describe the tyrosines of filamentous phage coat protein in which fluorotyrosines were incorporated biosynthetically (17-20). These studies differ from the majority of NMR studies of membranes by monitoring the peptides rather than their influence on surrounding lipids. Most molecules immersed in membrane bilayers, such as peptides or steroids, do not give high resolution NMR spectra because the ordered membrane environment does not allow sufficient motional averaging to eliminate the severe broadening influences of dipole-dipole couplings. The cited papers (12-20) as well as the present work utilize a strategy of increasing the effective rotational reorientation by reducing the size of aggregates through the use of vesicle or micelle samples. An alternative approach is to retain large bilayer systems and use double resonance NMR procedures to narrow the resonances (21).

The NMR results presented here are from aqueous solutions of fd coat protein in excess sodium dodecyl sulfate (SDS). Individual micelles consist of a protein dimer and about 60 molecules of SDS (22) for a total particle weight of 28,000 daltons.

MATERIALS AND METHODS

fd virus was obtained in gram quantities from 10L growths of infected Escherichia coli (K3300). The bacterial cultures were grown to late log phase prior to phage infection. After several hours of postinfection growth the bacteria were removed; the phage remained in the supernatant which was made 2% in polyethylene glycol and 0.5M in NaCl to precipitate the phage (23). The virus was pelleted with low speed centrifugation. Final purification was accomplished with CsCl step gradient ultracentrifugation and dialysis against distilled water.

The major coat protein was isolated from fd after disrupting the virus structure by adding 2g of SDS per gram of phage, saturating the solution with chloroform, and heating at 50°C for 30 minutes. The coat protein was purified by chromatography on Sephacryl S-200 superfine in 10mM SDS, 40mM borate, 8% glycerol, pH 9.0 buffer. After concentration and washing with 10mM borate the protein in SDS was lyophilized for storage. Individual NMR samples were prepared by adding H_2O or ^2H_2O to the lyophilized material. Final protein concentration was typically 7mM.

All ^1H NMR spectra were obtained at 360 MHz in 5mm tubes with a sample volume of about 0.5mL. The coat protein spectra were obtained with rapid scan correlation spectroscopy to avoid interference from the large aliphatic detergent resonances. ^1H chemical shifts were referenced to DSS. The ^{13}C NMR experiments were performed on a Nicolet NT-150 spectrometer at 37.7 MHz with broad band proton decoupling at 150.0 MHz in 20mm tubes with a 10mL sample volume. The ^{13}C spectra of the coat protein were obtained by signal averaging 10^3 free induction decays and "block averaging" the Fourier transformed spectra to reduce dynamic range problems from the detergent resonances. The inversion-recovery pulse sequence (24) was used to measure T_1s and gated-decoupling (25,26) for determination of nuclear Overhauser enhancements. All ^{13}C chemical shifts were referenced to external TMS.

RESULTS AND DISCUSSION

The precise isotropic chemical shift observed for a nucleus of a molecule in solution reflects the influence of a large number of environmental factors in addition to the electronic configuration of the chemical group. The folding of a globular protein results in groups from distant parts of the peptide chain being close enough to affect their chemical shifts. The presence of structure induced chemical shift dispersion is indicative of the protein having tertiary structure, since denaturation causes a loss of these shifts (27). For oligomeric proteins, the protein-protein interactions can influence chemical shifts. The binding of lipids to a protein in a membrane also can be a source of chemical shift changes.

Both the ^{13}C and ^{1}H NMR spectra of the coat protein in SDS display considerable structure induced chemical shift dispersion (20). Figure 1 contains ^{1}H NMR spectra of the aromatic region of the coat protein; in particular, spectrum 1E shows well resolved resonances from carbon bound protons. fd coat protein has tyrosines at positions 21 and 24 (6) which are in the central hydrophobic region, therefore they most likely have very similar interactions with lipids or other protein molecules, yet all four types of Tyr ring protons are distinguishable. The Tyr $C_{3,5}$ proton doublets at 6.8ppm and 6.9ppm are particularly well resolved. There is some dispersion among the 3 sets of phenylalanine ring protons near 7.3ppm. These shifts, as well as those in other spectral regions and in the ^{13}C spectra combined with the constrained protein structure demonstrate that the coat protein in micelles does not exist as a random coil, but rather has significant amounts of specific folding.

Exchange rates of nitrogen bound protons reflect their solvent accessibility (27). Figure 1A shows that in $^{1}H_2O$ solution at pH 8.4 and 25°C there is intensity from approximately 50 protons in the amide proton resonance region between 7.5 and 10ppm. This large number of resonances accounts for most of the 48 amide protons of the coat protein backbone, since some of the intensity in this region is probably due to exchangeable sidechain protons. Spectrum 1A is strong evidence for most

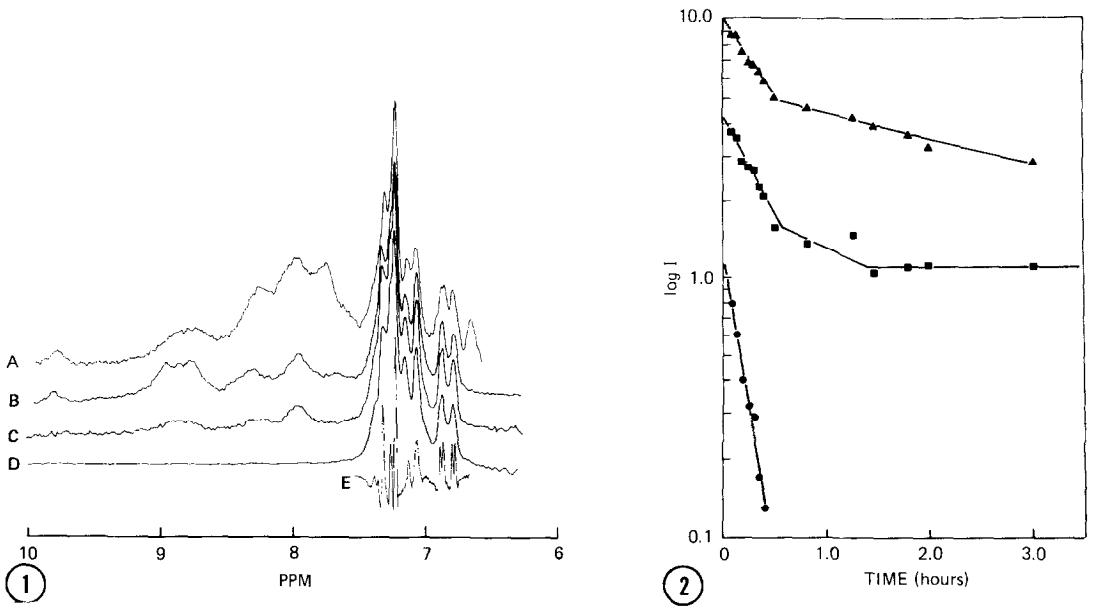


Figure 1. ^{1}H NMR spectra of fd coat protein in SDS micelles. Samples were 3mM in protein. A) in H_2O at 30°C (64 scans), B) in $^{2}H_2O$ immediately after dissolving lyophilized powder at 30°C (64 scans), C) sample B after 6.5 hrs at 30°C (64 scans), D) sample C at 30°C (256 scans) after changing pH to 10 and heating at 45°C for 30 min, E) data of D with resolution enhanced by multiplying the cross correlated data with a trapezoidal function.

Figure 2. Log of the number of protons versus time for the coat protein in SDS and $^{2}H_2O$ at pH 7.4 at 30°C. Circles represent 9.7-10.0ppm, triangles represent 8.6-9.3ppm, and squares 8.2-8.6ppm.

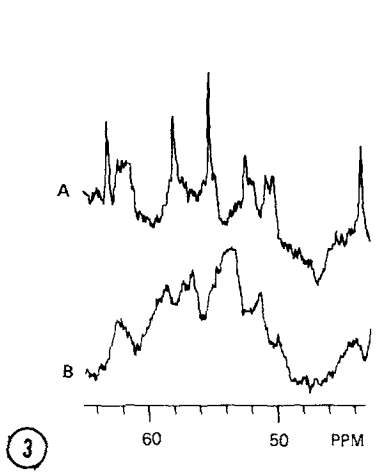
of the polypeptide chain having a defined conformation, because peptide amide protons of random coil polypeptides exchange too rapidly with water under these conditions to give rise to downfield resonances (28). Approximately 20 proton resonances are observed at 25°C in the 7.5 to 10ppm region just after adding $^{2}H_2O$ to protein-detergent lyophilized from $^{1}H_2O$. The intensities of a number of resonances can be followed as a function of time; some of these data are shown in Figure 2 where large differences in rates of exchange are visible for various protons The rates of exchange and their sensitvity to pH and temperature are comparable to those of previously studied globular proteins in solution, such as lysozyme or trypsin inhibitor (27). A similar lack of solvent accessibility of amide protons has been observed for the peptide melittin in micelles (16).

Protein dynamics can be described with ^{13}C NMR relaxation studies. In small globular proteins in solution the α carbons are tightly constrained by protein

folding and do not have internal motions, thus the rotational reorientation rates of the C_α resonances reflect the overall tumbling of the protein (29). In general, only limited resolution exists among the α carbon resonances because there are so many of this type with similar chemical shifts (50-70ppm) and broad linewidths in a protein. The value of α carbons comes from their being located uniformly in the peptide backbone with each carbon nucleus relaxed by well-characterized dipole-dipole interactions with a single proton a fixed distance away.

Figure 3 compares the α carbon resonance region for lysozyme and the fd coat protein in SDS micelles. Spectrum 3B is a typical C_α pattern at moderate field strengths for a native protein; denatured lysozyme gives a collection of narrow resonances in this region. The coat protein α carbons in Figure 3A show a distinctly different appearance from a globular protein, with several sharp lines superimposed on a background of overlapping broad lines. In the context of α carbon relaxation properties of proteins, the existence of narrow resonances means that α carbons have rapid local motions; in a denatured protein all α carbons are undergoing rapid reorientation.

We have carried out extensive relaxation measurements of T_1, NOE, and linewidth of the coat protein to more completely characterize the dynamics. Representative data on a C_α T_1 determination by inversion-recovery are shown in Figure 4. Time delays, τ, between 180° and 90° rf pulses that are not much greater or less than T_1 result in partially relaxed spectra (30) that are shown in Figure 4 and illustrate the range of T_1s of the α carbons. Clearly, there are large differences in T_1 among C_α resonances of the coat protein in SDS which is unlike the very homogeneous relaxation behavior observed for globular proteins in solution. Most of the coat protein α carbon resonances are broad and their intensity is nulled at τ = 60msec and fully relaxed at τ = 250msec. Nuclear Overhauser enhancement measurements show that the broad C_α intensity has minimal NOE (1.1) while the sharp lines have an NOE value >2. Since the narrow resonances have larger NOE than the broad resonances, their relative intensity contribution to the continuously decoupled spectra of Figures 3 and 4 is exaggerated. From integrations of ^{13}C



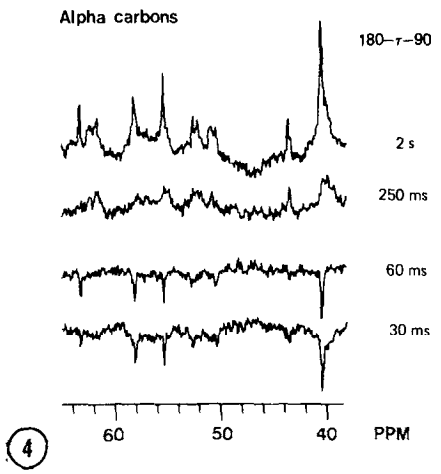


Figure 3. ^{13}C NMR spectra of α carbon resonance regions. A) fd coat protein (7mM) in SDS. 81 blocks of 1024 free induction decays obtained with 2 sec recycle delay B) lysozyme (14mM) in H_2O. 3600 free induction decays with 2 sec recycle delay.

Figure 4. Inversion-recovery T_1 measurement of α carbons of fd coat protein (7mM) in SDS. Each spectrum is from 28 blocks of 1024 free induction decays with 2 sec recycle delay.

spectra without NOE it appears that about 8 of the carbons give narrow lines. The three ^{13}C relaxation parameters are consistent in showing that most of the coat protein peptide backbone is constrained with a rotational correlation time appropriate for the entire micelle (broad lines, short T_1, minimal NOE) and that a small part experiences rapid segmental motion (narrow lines, long T_1, large NOE).

The ^{1}H chemical shift, amide proton exchange rate, and ^{13}C relaxation data show that the fd coat protein in SDS micelles has a folded native conformation over most of its length. While some of the chemical shifts and reduced rates of exchange of amide protons could be attributed to the burying of a peptide in a micelle, the differential rates of N-H exchange must be due to more subtle structural features of protein folding or protein-protein interactions of the dimer. These results are compatible with the protein having a rigid hydrophobic mid-section that spins the micelle with the hydrophilic terminal regions exposed to solvent and susceptible to proteolysis (31). The amino aicds near the ends of the polypeptide could then be the ones with rapid motion and fast amide proton exchange.

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