Location of M13 Coat Protein in Sodium Dodecyl Sulfate Micelles As Determined by NMR

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ABSTRACT: The major coat protein (gVIIIp) of bacteriophage M13 solubilized in sodium dodecyl sulfate (SDS) detergent micelles was used as a model system to study this protein in the lipid-bound form. In order to probe the position of gVIIIp relative to the SDS micelles, stearate was added, spin-labeled at the 5- or 16-position with a doxyl group containing a stable nitroxide radical. The average position of the spin-labels in the micelles was derived from the line broadening of the resonances in the ^{13}C spectrum of SDS. Subsequently, we derived a model of the relative position of gVIIIp in the SDS micelle from the effect of the spin-labels on the gVIIIp resonances, monitored via ^{1}H–^{15}N HSQC and TOCSY experiments. The results are consistent with the structure of gVIIIp having two helical strands. One strand is a long hydrophobic helix that spans the micelle, and the other is a shorter amphipathic helix on the surface of the micelle. These results are in good agreement with the structure of gVIIIp in membranes proposed by McDonnell et al. on the basis of solid state NMR data [McDonnell, P. A., Shon, K., Kim, Y., & Opella, S. J. (1993) *J. Mol.Biol. 233*, 447–463]. This study indicates that high-resolution NMR on this membrane protein, solubilized in detergent micelles, is a very suitable technique for mimicking these proteins in their natural environment. Furthermore, the data indicate that the structure of the micelle near the C-terminus of the major coat protein is distorted. This is probably due to the interaction of the positively charged lysine side chains with the sulfate head groups of the detergent molecules.

The major coat protein, gene VIII protein (gVIIIp),[1] encoded by the filamentous *Escherichia coli* phage M13, is a versatile model system for studying fundamental processes such as protein–membrane interactions and phage-assembly (Wickner, 1988).

During the phage lifecycle, the coat protein occurs in various forms. It is both a membrane protein and a DNA binding protein (Wickner, 1975; Zimmerman et al., 1982; Rasched & Oberer, 1986). It is encoded by the viral gene VIII as a procoat protein, which has an additional sequence of 23 residues at the N-terminus. Upon insertion in the inner cell membrane, this leader sequence is removed by a bacterial peptidase. Assembly of progeny virions takes place at the plasma membrane where the ssDNA binding protein encoded by gene V is removed and replaced by gVIIIp and a few minor coat proteins. Concomitant with phage extrusion, the protein is packed in the virus particle. Several observations suggest that the structure of the coat protein in the membrane differs significantly from that in the virus particle (Glucksman et al., 1992; McDonnell et al., 1993).

The structures of the coat proteins in virus particles have been well characterized for both class I (fd, M13, IKe) and class II (Pf1, Xf) filamentous bacteriophages. They have been determined by X-ray crystallography (fd, M13) (Banner et al., 1981; Glucksman et al., 1992; Makowski, 1992), solid state NMR (fd) (Cross & Opella, 1985), and neutron diffraction (Pf1) (Stark et al., 1988). Although the major coat proteins of the two classes of phages do not have much sequence homology, their structures in the virus particle are very similar. They contain more than 90% α-helix; the central portion consists of a long stretch of hydrophobic residues which allows for a close packing of the coat protein molecules in a helical arrangement around the viral DNA_f. Flanking the hydrophobic core are a short acidic N-terminus, which probably is in contact with the solvent, and a basic C-terminus. The latter most probably interacts with the negatively charged DNA phosphate backbone.

The membrane-bound form of the coat protein has been studied extensively, which has led to the formulation of several different structural models. The coat protein dissolved in detergent micelles and bilayers has been described as an arrangement of dimers with a large fraction of α-helix and β-sheet (Nozaki et al., 1978; Datema et al., 1987), but also as an almost completely α-helical monomer (Shon et al., 1991; McDonnell et al., 1993). A form in which the protein mainly adopts a β-sheet structure has been found too, but this seems to be of no biological relevance (Datema et al., 1987; Spruyt & Hemminga, 1991). Hemminga et al. (1992) showed that the mentioned differences are brought about by the isolation and purification procedures used for the preparation of the samples. The detergent/gVIIIp ratio in the NMR samples and the ratio of acrylamide/gVIIIp in gel electrophoresis influence the state of the coat protein as well (McDonnell et al., 1993).

Multidimensional high-resolution NMR methods, which have been very successful in the elucidation of the structures of small globular proteins in solution (Wüthrich, 1986), unfortunately, are less suited for studying membrane-bound proteins. In a system where the protein molecules are associated with a membrane, e.g., of a lipid vesicle, the tumbling of the molecules is slow, resulting in broad resonances. Therefore, one is forced to turn to model systems, and use

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[1] Abbreviations: gVIIIp, gene VIII protein (major coat protein); NMR, nuclear magnetic resonance; SDS, sodium dodecyl sulfate; HSQC, heteronuclear single quantum shift correlation; TOCSY, total correlation spectroscopy; NOESY, nuclear Overhauser enhancement spectroscopy; ppm, parts per million; TPPI, time proportional phase incrementation; TSP, (trimethylsilyl)propionic-d_4 acid; ESR, electron spin resonance.

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detergent micelles as membrane mimetics. GVIIIp is a small protein (50 residues, molecular mass = 5.2 kDa) that, when complexed with small micelles, e.g., sodium dodecyl sulfate (SDS), rotates rapidly enough to give reasonably well-resolved NMR spectra. This sytem has been studied for many years, but until recently, the NMR experiments were mostly restricted to measurements of the dynamics and solvent exchange rates of amide protons (Cross & Opella, 1980; O'Neil & Sykes, 1988; Henry et al., 1987; Henry & Sykes, 1990).

The amide protons and ^{15}N resonances of M13 gVIIIp have been assigned using two-dimensional NMR techniques (Henry & Sykes, 1992), followed by the identification of the $^{13}C_\alpha$, ^{13}CO, $^1H_\alpha$, and $^1H_\beta$ resonances by 3D NMR (Van de Ven et al., 1993). In the latter study, the secondary structure of M13 gVIIIp was found to consist of two α-helices, comprised of residues 6–20 and 24–50, connected by a "hinge" region around residue 22. Using a combination of two-dimensional high-resolution NMR and solid state NMR, the membrane-bound form of the coat protein of filamentous phage Pf1 has been studied. The results suggest a long hydrophobic helix crossing the membrane, and a shorter amphipathic helix which lies in the plane of the bilayer (Shon et al., 1991). Nearly identical results were obtained in a parallel study on the fd major coat protein in the membrane-bound form by McDonnell et al. (1993).

In the present study, we report on the location of gVIIIp, which is in the monomeric form (Van de Ven et al., 1993), in SDS micelles. To this end, two different NMR relaxation probes were used, 5-doxylstearate and 16-doxylstearate, each containing a nitroxide radical as a spin-label. First the position of the spin-labels relative to the detergent molecules was established by ESR and NMR. Next, the location of the major coat protein in the micelle was determined by comparing ^1H–^{15}N HSQC and TOCSY spectra recorded in the absence and presence of the spin-labels. We conclude that the structure of gVIIIp in SDS micelles is similar to that in the lipid membrane found by McDonnell et al. (1993), which consists of two α-helices. One amphipathic helix lies on the surface of the SDS micelle and another, hydrophobic, helix runs through the micelle. Our findings indicate that the surface of the micelle is distorted around the C-terminus of the protein, which is probably due to the interaction between the highly positively charged DNA binding region of gVIIIp and the sulfate head groups of the detergent molecules.

MATERIALS AND METHODS

Isolation of gVIIIp. The filamentous M13 phages were propagated on *Escherichia coli* K38 grown in minimal medium as described previously (Konings, 1980). $^{15}NH_4Cl$ (Isotech) was used as the sole nitrogen source to obtain uniformly ^{15}N-labeled M13 phages. The isolation and purification of the phages were performed as described earlier (Konings, 1980; Spruyt et al., 1989). Separation of gVIIIp from the phage particles was carried out by phenol extraction (Knippers & Hoffman-Berling, 1966; Konings et al., 1970). This procedure is different from the one described by Henry et al. (1986), in which the protein is extracted directly from the phages with SDS, followed by column chromatography. The NMR spectra indicate that the same results are obtained for both procedures (Van de Ven et al., 1993). No attempt was made to remove the minor coat proteins.

NMR Methods. The concentration of gVIIIp in the samples was 1.5–2.0 mM per 500 mM $[^2H_{25}]$SDS (MSD Isotopes). The spectra were acquired at 37–39 °C and pH 4.80 (pH meter reading). Two detergent-like spin-labeled compounds were used, 5-doxylstearate and 16-doxylstearate (Aldrich), which were solubilized in $[^2H_4]$methanol (Merck) to a final concentration of 0.1 M. The spin-labels differ in the location of the nitroxide radical relative to the alkyl chain of the stearate. HSQC spectra of samples with various amounts of the 16-doxylstearate spin-label added (1.6 and 3.2 M) were recorded on a Bruker AMX600 spectrometer, using the pulse sequence described by Bodenhausen (1980). The spectra were recorded with 256 increments in the t_1 dimension and 2K real points in the t_2 dimension. The sweep width was 37.4 ppm in the nitrogen dimension and 12.0 ppm in the proton dimension. All other experiments were carried out on a Bruker AM400 spectrometer. Other HSQC experiments, with the 5-doxyl-stearate spin-label, were performed as described by Messerle et al. (1989). The NOESY spectra were acquired using weak continuous irradiation during the relaxation delay of 1.0 s and a semiselective jump–return observation pulse pair (Plateau & Guéron, 1982) to suppress the water resonance. The NOESY mixing time was 500 ms. The TOCSY experiments were carried out in D_2O solution as described by Bax and Davis (1985) with a mixing time of 35 ms. In both the NOESY and the TOCSY experiments, the sweep width was 12.5 ppm in both dimensions, and the acquisition times were 51.2 and 205 ms in the t_1 and t_2 dimensions, respectively. All spectra were recorded using the TPPI method (Marion & Wüthrich, 1983). The processing of the time domain data involved window multiplication using a shifted sine bell. Spectra were calibrated relative to (trimethylsilyl)propionic-d_4 acid (TSP) as described by Ikura et al. (1991).

The ESR experiments were performed on a Bruker ESP300 X-band continuous wave spectrometer. The temperature was 40 °C for all experiments. The data were obtained as described in the literature (Brown et al., 1981).

RESULTS AND DISCUSSION

General Remarks. Three-dimensional high-resolution NMR measurements on the coat protein of bacteriophage M13 in SDS micelles have led to a structural model for this protein (Van de Ven et al., 1993). Both the NOE data and the chemical shift deviations from random coil values of $^{13}C_\alpha$ and $^1H_\alpha$ showed that the protein adopts a structure with two α-helices when bound to SDS micelles. One helix comprises residues Y24–F45, possibly extending to residue 50, while a second, amphipathic, helix comprises residues P6–E20. The two helices are connected by a flexible hinge region. Line width measurements showed that the first, hydrophobic, helix is rigid (^{15}N line widths = 15–20 Hz), whereas the second helix is more mobile, with ^{15}N line widths of 5–10 Hz. An abrupt change in line width was observed in the region near residue 22, which is in the region connecting the two helices.

To obtain information about the interaction of the major coat protein with the SDS molecules in the micelle, we compared NOESY spectra of gVIIIp recorded with and without protonated detergent. Figure 1 displays the "NH" regions of two NOESY spectra of the M13 coat protein in 500 mM SDS, with 98% ^2H SDS (A) and 80% ^2H/20% ^1H SDS (B). The spectra are similar except for the differences arising from the different protonation levels of SDS. The presence of 20% protonated SDS has two consequences for the cross-peaks in the spectrum. First, some peaks are not present, or are less intense in Figure 1B (e.g., 34NH-α and 26NHring-α), due to the increased line widths of the peaks in the protonated sample. Second, some small peaks appear in the "NOESY ladders" of residues 24, 26, 33, and 34, all at 1.27 ppm. SDS protons attached to carbons 3–11 resonate

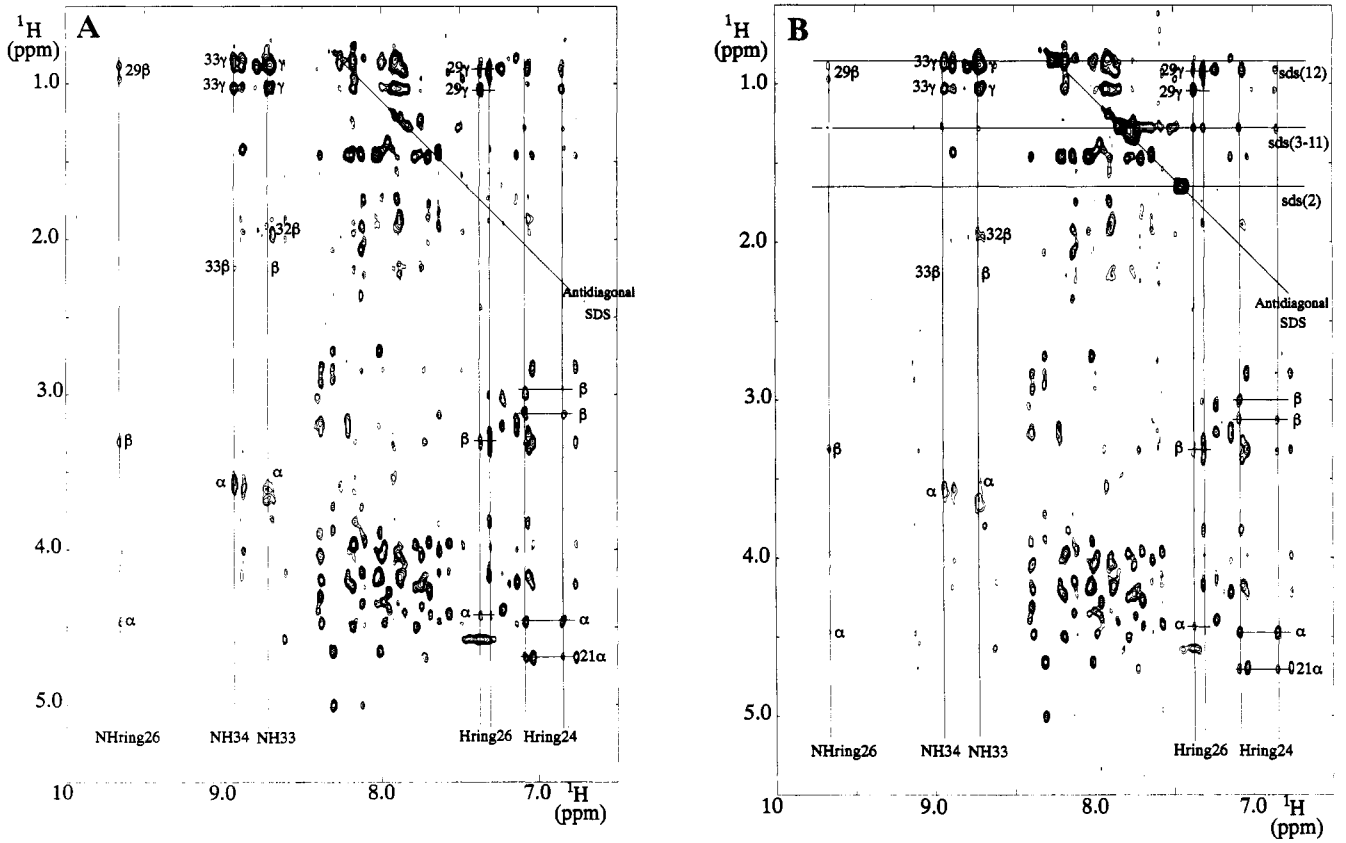

FIGURE 1: "NH" region of a 400 MHz NOESY spectrum of 2 mM gVIIIp/500 mM [^{2}H]SDS with 2% ^{1}H SDS (A) and 20% ^{1}H SDS (B), recorded at 39 °C. The spectra were recorded with a mixing time of 500 ms. The NOESY ladders which show magnetization transfer to the SDS resonances are indicated with lines, as is the antidiagonal of the SDS spectrum. In the second spectrum (B), the SDS resonances of the protons on carbons 2, 3–11, and 12 are denoted with lines as well.

at this chemical shift value. The cross-peaks between these SDS protons and the aforementioned residues show that parts of the coat protein are in contact with the SDS molecules. Although a larger part of the coat protein is expected to be in contact with SDS, no more NOE contacts are seen in the spectrum. These peaks are probably too small to be observed due to the increased line width. The other three peaks in the SDS proton spectrum resonate at 4.0, 1.65, and 0.85 ppm, which are assigned to the protons on carbons 1, 2, and 12, respectively. No NOE contacts to the gVIIIp residues are visible at these resonance positions (see Figure 1B). This is probably due to the fact that the ^{1}H SDS concentration (100 mM) is too low to produce any detectable NOESY peaks from the SDS protons at a single carbon site. The small peaks at 1.27 ppm are due to the combined effect of at least 18 SDS protons resonating at this chemical shift. Using higher concentrations of protonated SDS is not feasible because it results in additional line broadening and the large SDS peaks cause strong base-line distortions. Note also that both figures show an antidiagonal which is most likely due to the fact that the relaxation delay was short compared to the T_1 relaxation time of SDS. The NOESY experiment indicates that at least part of the gVIIIp protein is located within the SDS micelle, but it is difficult to draw more specific conclusions from these data alone.

Location of the Spin-Labels in the Micelles. A more detailed description of the position of the coat protein in the SDS environment is obtained by using spin-labels. This method of probing the relative orientation of micelles and proteins bound to them has been applied before to glucagon and melittin bound to dodecylphosphocholine (Brown et al., 1981, 1982). We used two different spin-labeled stearates, which resemble the detergent molecules. In one spin-label probe, the radical is located near the head group of the stearate, while in the other spin-label probe it is situated at the end of the alkyl chain. In this way, we can probe positions throughout the micelle.

The use of spin-labels also enables us to gain additional information about the SDS/gVIIIp system from ESR measurements. The results obtained for gVIIIp in SDS are in perfect agreement with earlier results of other proteins in dodecylphosphocholine (Brown et al., 1981, 1982). We determined the SDS/gVIIIp stoichiometry by measuring the line width as a function of SDS concentration as described by Brown et al. (1981), and found that approximately 60 detergent molecules were needed to form 1 micelle (data not shown). This means that at the conditions used for the NMR experiments (2 mM gVIIIp, 3 mM spin-labeled molecule, and 500 mM SDS) less than one gVIIIp or spin-label is bound per micelle (four micelles for each gVIIIp molecule).

More precise information about the positions of the spin-labels inside the SDS/gVIIIp micelles was obtained from ^{13}C NMR measurements. Figure 2 displays the ^{13}C spectra of SDS recorded using a SDS/gVIIIp sample in the absence of spin-label (A) and in the presence of 8 mM 16-doxylstearate (B) and 10 mM 5-doxylstearate (C). Only the resonances of carbons 1, 2, 3, 10, 11, and 12 have been assigned in this spectrum (Kragh-Hansen & Riisom, 1976). Adding the two spin-labels affects the SDS resonances in two ways. Small shifts can be observed, the origin of which is unclear. Second, the line widths of the resonances are increased to various extents. The effect of 5-doxylstearate on the line widths is most profound on resonances 1, 2, and 3, while 16-doxylstearate causes line broadening of resonances of carbon atoms 11 and

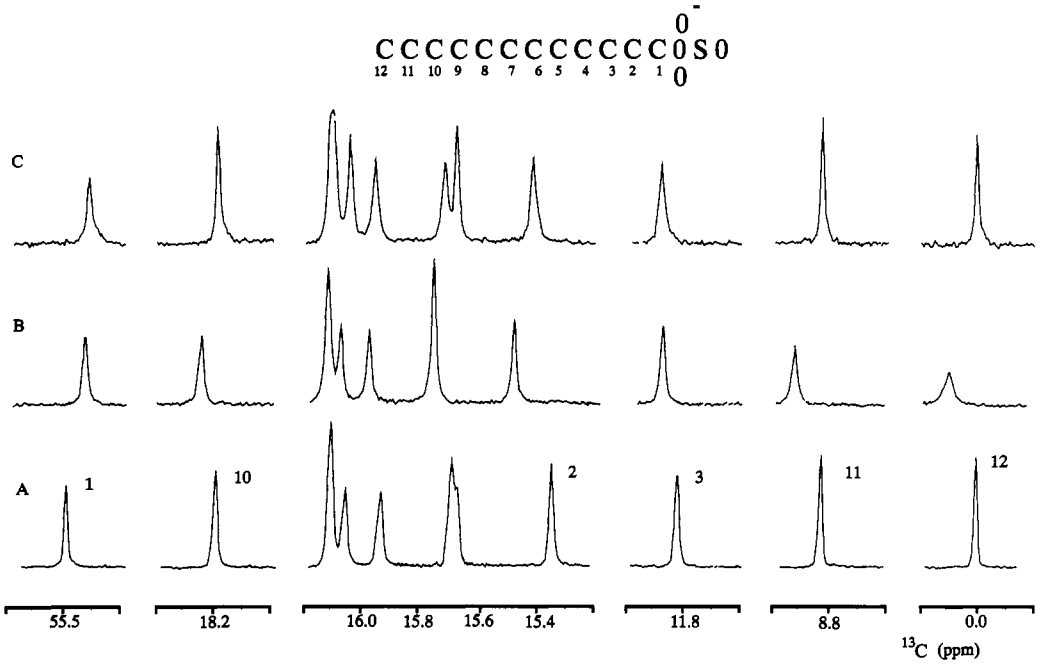

FIGURE 2: 100 MHz ^{13}C SDS spectra at 39 °C of (A) 2 mM gVIIIp in 400 mM ^{1}H SDS, (B) 1 mM gVIIIp in 300 mM ^{1}H SDS with 8 mM 16-doxylstearate, and (C) 2 mM gVIIIp in 400 mM ^{1}H SDS with 10 mM 5-doxylstearate. The resonances of carbons 1, 2, 3, 10, 11, and 12 are annotated.

12. The broadening is predominantly due to enhanced relaxation caused by the unpaired electron of the nitroxide radical. There may also be a contribution due to the dynamics of the spin-labeled stearate, i.e., broadening caused by fast chemical exchange. In any case, broadening of a resonance directly indicates its proximity relative to the average position of the spin-label. It should be noted that the micelle structure is known to be quite dynamic. The average residence time of a detergent molecule in a micelle, and by inference also that of a stearate molecule, is about 10^{-5} s (Bales & Kevan, 1992). Thus, the information obtained for the position of the nitroxide radical in the micellar interior should be regarded as a mean over all occupied locations. From this experiment, it can be concluded that the distribution of the nitroxide radical of 5-doxylstearate is highest near the sulfate group of the detergent molecules. On the other hand, for 16-doxylstearate, the spin-label is on average positioned near the end of the alkyl chains of the SDS molecules in the center of the micelle. Knowing the average position of the radicals relative to the SDS molecules, we subsequently use the effect of the spin-labels on the resonances of the coat protein to determine the location of gVIIIp in the micelles.

Positioning of gVIIIp in a Micellar Environment. Figure 3 presents the two-dimensional ^{1}H–^{15}N heteronuclear correlation spectra (HSQC spectra) of uniformly ^{15}N-labeled M13 coat protein in deuterated micelles without (Figure 3A) and with 16-doxylstearate (Figure 3B). Addition of spin-label only affects a specific subset of the resonances. The peaks that are affected most by 16-doxylstearate are annotated in Figure 3A. Addition of 5-doxylstearate also causes specific resonances to broaden significantly [data not shown (*vide infra*)]. Since every amino acid, with the exception of residues A1 and P6, gives rise to a cross-peak in this spectrum, it is very suitable for determining specific line broadening effects. The percent reduction of every cross-peak's intensity, upon addition of spin-labeled stearate, was measured and categorized into four classes. The results are given in Table 1. Residues that were not influenced by the presence of the spin-label belong to category I, while residues which showed the greatest line broadening after adding a small amount of spin-label were grouped in category IV. The effect of the spin-label on the residues in category III was intermediate, and the resonances in category II showed only a very small line broadening when the spin-label was added.

The 5-doxylstearate spin-label has a very large effect on the side-chain resonances of residue Q15, while the broadening of the backbone resonance of this residue is small. As this residue is positioned in the amphipathic part in the coat protein (residues 6–20) (McDonnell et al., 1993), we wanted to gain some more information about the influence of 5-doxylstearate on the side chains of this section of the protein. For this purpose, we recorded a TOCSY spectrum in D_2O and determined the line broadening effect on cross-peaks belonging to the aliphatic spin systems upon addition of 5-doxylstearate. Where multiple connectivities were observed per residue, the average decrease (only measured for cross-peaks that did not overlap) in the peak intensities belonging to one amino acid was taken. The results from this experiment are also collected in Table 1. In both the two-dimensional HSQC spectrum and the TOCSY spectrum, some minor shifts were observed (<0.03 ppm in the proton dimension) which did not have any correlation with the observed broadenings.

From Table 1, it can be concluded that 5-doxylstearate causes a "background" broadening of the resonances of almost every residue of the coat protein except for the N-terminal region. Additionally, large effects are observed for residues 26–27, 38–39, and 22 and the side chain of 15. On the other hand, addition of a small amount of 16-doxylstearate (1.6 mM) only affects the resonances of residues 34, 35, and 38. Somewhat higher concentrations (3.2 mM) also influence those of the surrounding amino acids. Given that 5-doxylstearate is located near the surface of the micelle and 16-doxylstearate in the center, a consistent and clear-cut interpretation of the results obtained with the spin-label experiments is possible for residues 1–35.

We interpret the results in terms of the following model: The SDS molecules are aggregated in the form of micelles, the surfaces of which are probed by 5-doxylstearate and the

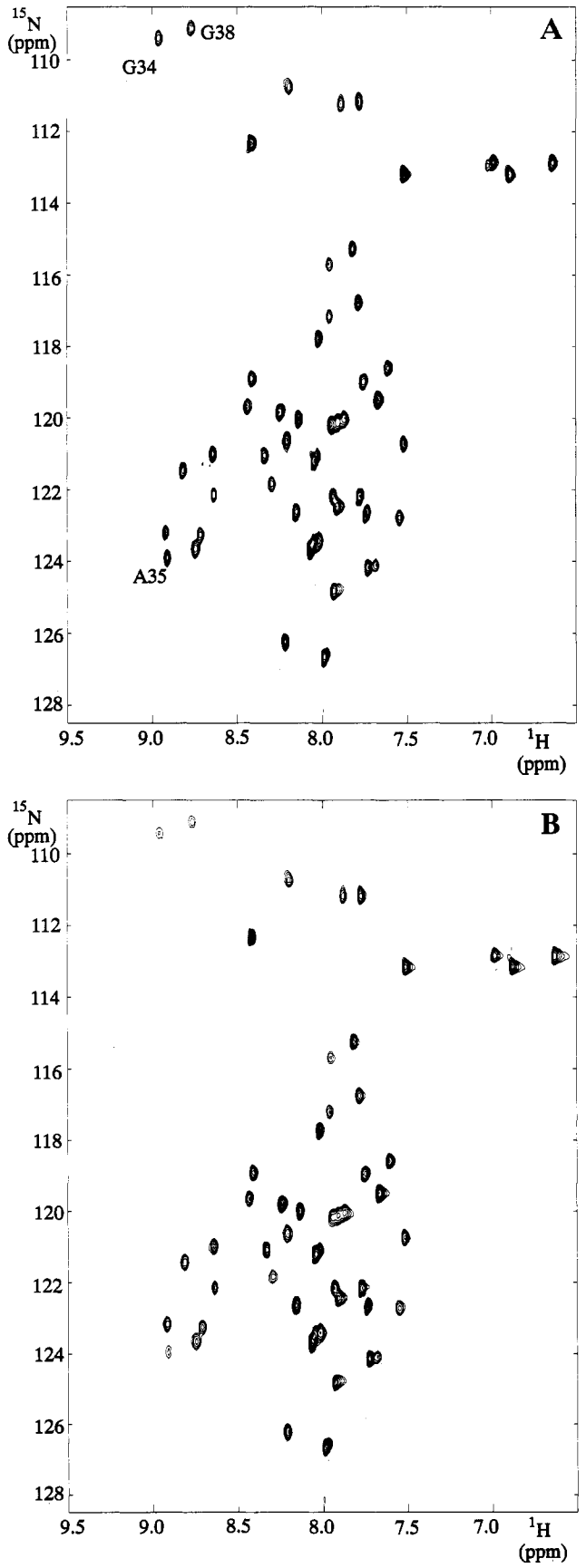

FIGURE 3: 600 MHz ^{1}H–^{15}N HSQC spectrum of gVIIIp in the absence of spin-label (A) and in the presence of 1.6 mM 16-doxylstearate (B) at 39 °C. In (A), the ^{1}H–^{15}N cross-peaks affected by this spin-label are marked by the corresponding residue number.

centers by the 16-doxylstearate spin-labels. We first consider the amphipathic helix, extending from residue P6 to residue

Table 1: Effect of Spin-Labels on the Residues of the Major Coat Protein[a]

	3.0 mM 5-doxylstearate				3.2 mM 16-doxylstearate			
residue	I	II	III	IV	I	II	III	IV
A1								
E2	h,t[b]					h		
G3	h				h			
D4	h,t				h			
D5	h,t				h			
P6		t						
A7	h				h			
K8	h	t			h			
A9	h				h			
A10	h				h			
F11	h	t			h			
N12	h,t,h*				h,h*			
S13	h,t				h			
L14		h	t		h			
Q15		h	t	h*	h	h*		
A16		h			h			
S17	h,t				h			
A18	h				h			
T19	h	t			h			
E20	h	t			h			
Y21		h,t			h			
I22			t	h	h			
G23			h		h			
Y24	h	t			h			
A25		h				h		
W26			t	h	h			
A27				h,t		h		
M28		t	h		h			
V29		h,t			h			
V30		h	t			h		
V31		h				h		
I32		h,t					h	
V33		h					h	
G34		h						h
A35		h						h
T36		h	t			h		
I37		t	h				h	
G38				h				h
I39			h	t			h	
K40	h					h		
L41		h				h		
F42		h,t			h			
K43		h	t[c]		h			
K44	h,t					h		
F45		h,t			h			
T46	h	t				h		
S47	h,t				h			
K48	h,t				h			
A49	h,t				h			
S50	h				h			

[a] The classification into categories I until IV was based on the decrease in peak intensity upon addition of spin-labeled stearate as follows: I, <20%; II, 20–40%; III, 40–60%; IV, 60–80%. [b] h = data from a HSQC experiment (h* = side-chain resonance); t = data from aliphatic resonances in a TOCSY experiment (data from some residues are missing due to overlap). [c] For K43, the broadening was almost exclusively observed for cross-peaks involving $^{1}H_{\epsilon}$.

E20. It interacts predominantly with the "heads" of the SDS molecules, as witnessed by the effects of the 5-doxylstearate spin-label on the side-chain resonances of particular residues in this section of the protein. In particular, its effect is very strong on the side-chain resonances of residues 14 and 15. As this part of the protein is less rigid than the other helix (Van de Ven et al., 1993) and only the side-chain resonances of the mentioned residues are influenced, it is not likely to be covered by many SDS molecules. This idea is supported by the results of 16-doxylstearate, which is located in the center of the micelle and only affects the resonances of residues 32–39. The amphipathic helix is connected to the hydrophobic helix, which comprises residues Y24–F45, by a hinge centered around I22.

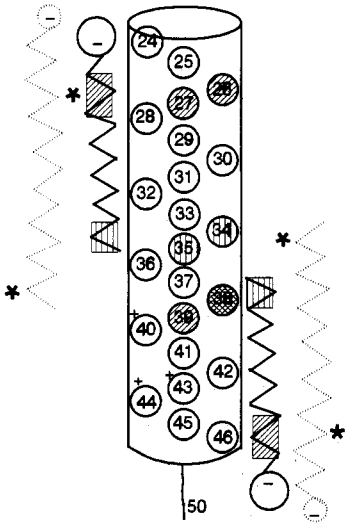

FIGURE 4: Schematic drawing of the hydrophobic α-helix comprising residues 24–46 (circles), SDS (solid line), and spin-labeled stearate (broken line). All molecules are drawn to scale. The positions of both spin-labels are indicated by asterisks (displayed here on one stearate). The largest effects on SDS are indicated by a hatched box for 5-doxylstearate and by a striped box for 16-doxylstearate, while the effects on the gVIIIp resonances of different amino acids are represented by hatched and striped circles. The resonances of residue 38 are influenced by both spin-labels, i.e., 5-doxylstearate as well as 16-doxylstearate (see text).

As both the backbone and the side-chain resonances of this residue are strongly influenced by 5-doxylstearate, it must be positioned near the surface of the micelle as well.

The hydrophobic helix is located within the micelle, since it is affected by both 5-doxylstearate and 16-doxylstearate. The ^{15}N line widths of these residues also indicate that this part of the coat protein is more rigid because of the presence of the surrounding SDS molecules. Specifically, 5-doxylstearate affects W26 and A27, and 16-doxylstearate influences G34 and A35. This is in perfect agreement with the line broadening exhibited by the ^{13}C resonances of SDS (see Figure 2). In an extended alkyl chain, the distance between carbon 2 and carbon 11 of the SDS molecule, which are affected most by 5-doxylstearate and 16-doxylstearate, respectively, is approximately 11 Å. This corresponds to a length of about eight consecutive residues in a regular α-helix, which conforms to the distance between W26/A27 and G34/A35 (*vide supra*). Hence, the conclusion is that the second α-helix runs through the micelle, with residues 26 and 27 near the surface and residues 34 and 35 in the hydrophobic center of the micelle.

If we assume the shape of the micelle containing the protein to be a regular ellipsoid, then broadening of the resonances of residues 26 and 27 by 5-doxylstearate should be accompanied by broadening of the resonances belonging to residues F42 and K43. Furthermore, no other effects from 16-doxylstearate would be expected. This is not what we observe; both spin-labels have a significant effect on the signals of G38 (5-doxylstearate also on that of I39), while the backbone resonances of residues 42 and 43 are hardly affected (N.B.: a broadening of the $^1H_\epsilon$ resonance of the side chain of K43 was observed). It is not possible to explain this result within the context of the aforementioned model with, on average, extended SDS alkyl chains. To illustrate this point, the relative lengths of the various moieties involved have been drawn to scale in Figure 4. According to this figure, the broadening effects from 16-doxylstearate on the resonances of the glycines-34 and -38 could be explained by assuming that the 16-carbon alkyl chain of the stearate penetrates the micelle beyond carbon 12 of the SDS chain, so that the 16-doxyl spin-label is off-center. In this case, residue 36 is in the center of the micelle, but its resonances would not be affected as much by the 16-doxylstearate spin-label as those of residues 34 and 38, which is what we observe (see Table 1). It is noted in passing that the relatively large effects on G34 and G38 can also be the result of the ability of the doxyl groups to more closely approach the backbone ^{15}N–^1H moieties of the glycines than those of bulkier amino acids. That the 16-doxyl spin-label is off-center has been previously suggested by Bales and Kevan (1992), who reported the effect of these stearic acid spin-labels on the photoionization of an aromatic molecule in SDS micelles. However, if this approach, with extended SDS alkyl chains, were correct, one would still expect broadening by the 5-doxylstearate spin-label of the resonances of residues 45 and 46 (see Figure 4). Instead, we observe large effects of 5-doxylstearate on residues 38 and 39.

Therefore, we propose the following model, in which the micellar environment around the C-terminal part of the second helix is distorted. The simplest explanation of the data is to assume that the presence of gVIIIp induces a disturbance of the SDS micellar surface, as depicted in Figure 5. In this way, residue G38 is simultaneously close to the surface and the core of the micelle, which explains the observed effects of both 5-doxylstearate and 16-doxylstearate on this residue. Figure 5A reflects the effects of 5-doxylstearate on the gVIIIp resonances, and Figure 5B represents the effects of 16-doxylstearate. The model features the two helices introduced above. One, less rigid, helix lies on the surface of the micelle with the side chain of residue 15 pointed toward the micelle (Figure 5A). The residues affected by the 5-doxylstearate label, such as K8, F11, L14, and Q15, are located on one side of the N-terminal α-helix, facing the micelle, while the unaffected residues such as N12, S13, and S17 are located on the opposite, solvent-exposed, side. It should be noted that in Figure 5 the positions of the residues relative to the faces of the helices are only roughly indicated. Moreover, it should be noted that this helix is flexible, i.e., its position relative to the micellar surface is not expected to be fixed; there is probably significant motion, most likely via the hinge region around residue 22.

The other helix runs through the micelle, with residues 26 and 27 at one end of the micelle and residue 38 at the other end. These residues are largely affected by 5-doxylstearate. The center of the micelle is situated near residues 34, 35, and 38 as reflected by the effect of 16-doxylstearate.

The asymmetric arrangement of the SDS around the C-terminal α-helix of gVIIIp is most likely caused by the presence of three lysines, at positions 40, 43, and 44. These lysines may "pull in" the micellar surface to produce an optimal arrangement for electrostatic interactions between the sulfate head groups of the SDS and the positively charged lysyl NH_3^+. Henry et al. (1987) also suggested a stable complex of the lysines and the negatively charged head group of the surfactant molecules based on the results of digestion on the C-terminus of gVIIIp using proteinase K. It is known from other NMR studies on gVIIIp in membrane environments that K40, K43, and K44 are near the surface of the bilayer (Sanders et al., 1991). Assuming that the head group of the stearate molecule interacts with the positive charges of the lysines, the position of the 5-doxyl group is such that it will have a large influence on residue 38. The charged amino acids in the N-terminal, amphipathic, helix do not appear to influence the arrangement of the SDS molecules. On the basis of our results, we cannot make a firm statement regarding the average shapes of the

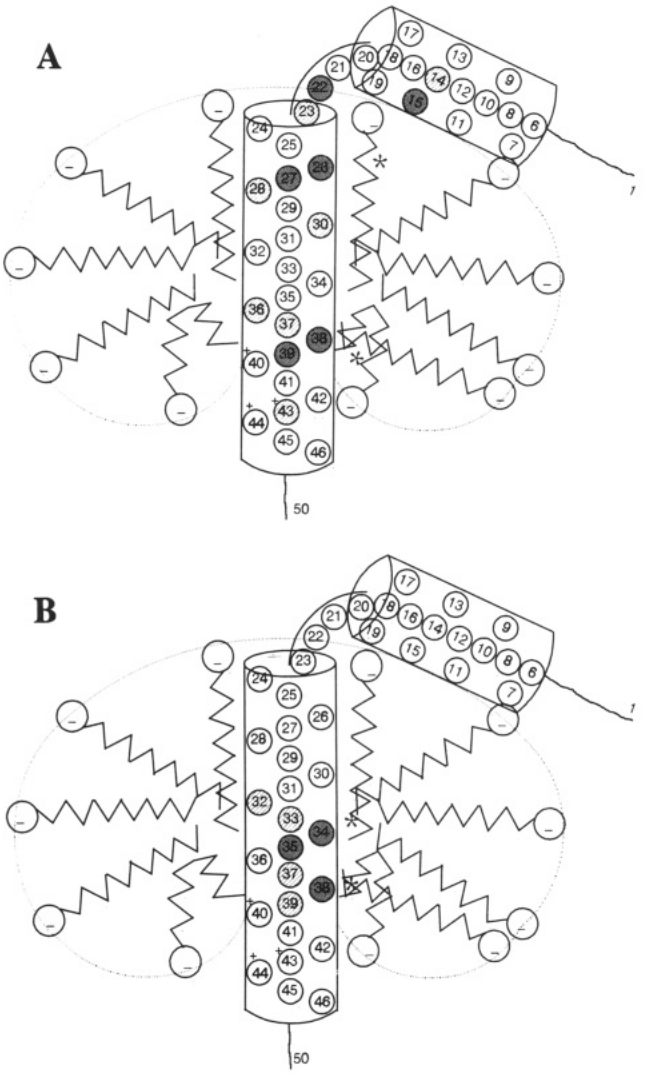

FIGURE 5: Model of the location of gVIIIp in SDS micelles. All molecules are drawn to scale. The two helices are displayed as cylinders, and the residues are represented as circles. The effects of 5-doxylstearate (A) and 16-doxylstearate (B) on the gVIIIp resonances are shown. Open circles represent residues that are not affected or weakly affected by the spin-label. Hatched circles represent residues that had a medium effect, and the darkly shaded circles indicate residues that were strongly affected. The dotted line represents the micelle surface, and some SDS molecules are included. The asterisks denote the largest effects of the spin-labels on the SDS resonances.

alkyl chains of the SDS molecules; they may well be bent in the distorted region in the vicinity of G38. The chain conformations of the SDS molecules in the micelle and the structure of micelles are still subject of controversy (Dill, 1982; Dill et al., 1984; Menger & Doll, 1984). NMR measurements of the relaxation times of the ^{13}C resonances of the SDS chain indicated that chain folding occurs and that the terminal methyl group sometimes touches the surface of the micelle (Van Bockstaele et al., 1980). ESR measurements showed that although the alkyl chain of SDS may be slightly bent, the overall conformation of the chain must be extended (Szajdzinska-Pietik et al., 1985). This may not be the case in our system in which gVIIIp is solubilized by SDS. If there were, however, many strongly curved SDS molecules in the micelle, one would expect the effects of 5-doxylstearate and 16-doxylstearate on the ^{13}C NMR spectrum of SDS to be less specific; i.e., other resonances of the alkyl chain should also be broadened. However, if such bending of SDS only occurs in the gVIIIp-containing micelles, which comprise only 25% of all micelles present, this may go unnoticed in the SDS ^{13}C NMR spectrum.

In summary, our interpretation of the results is that gVIIIp consists of an amphipathic helix lying on the surface of the micelle, connected via a short hinge region to a long hydrophobic helix that runs through the micelle. Furthermore, the positively charged C-terminal segment protrudes from the micelle into the solution. The evidence suggests that the structure of the micelle near the DNA binding region of gVIIIp, i.e., the cluster of three lysines at positions 40, 43, and 44, is perturbed. We propose that the surface of the SDS micelle is disturbed in such a way that it is pulled nearer to residue G38, as is shown in Figure 5. The results indicate that a high-resolution NMR structure of this membrane protein in detergent micelles is a very good mimetic for the structure in its natural environment. The data are highly consistent with the bihelical structure of gVIIIp in lipid bilayers proposed by McDonnell et al. (1993) on the basis of solid state NMR.

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