Assignment of ^{1}H, ^{15}N, and Backbone ^{13}C Resonances in Detergent-Solubilized M13 Coat Protein via Multinuclear Multidimensional NMR: A Model for the Coat Protein Monomer†

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ABSTRACT: The major coat protein (gVIIIp) of bacteriophage M13 complexed with SDS detergent micelles was used as a model system to study the lipid-bound conformation of the protein. Conditions were found that allowed the recording of good quality of NMR spectra. By making extensive use of three-dimensional heteronuclear (^{13}C, ^{15}N) NMR, we obtained a complete set of resonance assignments for ^{1}HN, $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{13}C^{\alpha}$, CO, and ^{15}N and partially assigned the rest of the ^{1}H spectrum. Analysis of NOE and chemical shift data reveals that gVIIIp is composed of two α-helical domains, one ranging from Pro-6 to Glu20 and the other ranging from Tyr-24 all the way to the C-terminus Ser-50. In contrast to the results reported by Henry and Sykes [Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31,* 5285–5297], at a high SDS to protein ratio the protein appears to be monomeric.

The major coat protein (also known as gene VIII protein and henceforth referred to as gVIIIp)[1] of filamentous *Escherichia coli* phage M13 has the intriguing property that during the phage life cycle it plays the role of a true coat protein as well as an intrinsic membrane protein. Upon host-cell penetration, the gVIIIp is left behind in the inner cell membrane of the host while the single-stranded viral DNA enters the cytoplasm. During the phage replication cycle a water-soluble precursor of gVIIIp is synthesized which contains an additional leader sequence of 23 residues at its N-terminus. Concomitant with insertion of this precursor in the plasma membrane, the leader sequence is cleaved off by a leader peptidase. Progeny virions are assembled at the plasma membrane whereby the ss DNA binding protein encoded by gene V is removed and replaced by gVIIIp and a few minor coat proteins (Wickner, 1975; Ohkawa & Webster, 1981; Rasched & Oberer, 1986; Model & Russel, 1988).

The structure and organization of gVIIIp in the phage particle have been well characterized by X-ray crystallography (Banner et al., 1981; Glucksman et al., 1992; Makowski, 1992), neutron diffraction (Stark et al., 1988), and solid-state NMR (Cross & Opella, 1985). The protein is almost completely α-helical; the basic C-terminus points inward, quite probably in contact with the DNA phosphate backbone, and the acidic N-terminus points outward. The hydrophobic central parts of the gVIIIp molecules contact each other and provide a close packing of the approximately 3000 units per virion in a helical arrangement.

During the last two decades the lipid-bound gVIIIp has also been extensively studied. Due to its small size of 50 residues and its relatively facile isolation in large quantities, it has become a popular model system for study of membrane assembly (Wickner, 1988) and dynamic properties of membrane-bound proteins (Henry et al., 1987a,b; Henry & Sykes, 1990a,b; O'Neil & Sykes, 1989; Leo et al., 1987; Colnago, et al., 1987; Schiksnis et al., 1987). Various models have been advanced for lipid-bound gVIIIp that differ substantially. GVIIIp in detergent micelles has been reported to be a dimer with α-helical N- and C-termini and a β-structured central part (Nozaki et al., 1978; Datema et al., 1987a,b), but also to be an almost complete α-helical monomer (Shon et al., 1991). Moreover, so-called "β-polymers" can be formed (Datema et al., 1987a,b, 1988). In recent years it has become clear how these different states are brought about via the isolation/purification procedures of the various preparations (Hemminga et al., 1992). The β-structure, and the associated aggregation, most likely does not occur *in vivo*.

Here we report on high-resolution multidimensional multinuclear NMR studies of gVIIIp in sodium dodecyl sulfate (SDS) micelles. The system of gVIIIp complexed to SDS has been studied by NMR for more than a decade [e.g., Cross and Opella (1980)]. When 2D NMR in combination with distance geometry calculations became established as an alternative to X-ray crystallography for unraveling the three-dimensional structures of small proteins (Wüthrich, 1986), it was anticipated that gVIIIp could be quite amenable to such an approach. Unfortunately, previous attempts to assign the resonances in the ^{1}H NMR spectrum of gVIIIp have met with limited success. Until now, only the assignments of the HN protons have been reported for the major coat proteins of the *Pseudomonas* filamentous phage Pf1 (Shon & Opella, 1989) and phage M13 (Henry & Sykes, 1992). It turned out that many of the *J* connectivities, especially H^{α}–NH, could not be observed in homonuclear 2D spectra such as COSY or

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[1] Abbreviations: gVIIIp, gene VIII protein (major coat protein); NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; ppm, parts per million; SDS, sodium dodecyl sulfate; TOCSY, total correlation spectroscopy, also known as HOHAHA, or homonuclear Hartmann–Hahn, spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence spectroscopy; HMQC, heteronuclear multiple quantum coherence spectroscopy; CT, constant time; 1D, 2D, and 3D, one-, two-, and three-dimensional; TSP, (trimethylsilyl)propionic-d_4 acid; TPPI, time proportional phase incrementation; HNCO, HN(CO)CA, HCACO, and HCA(CO)N, acronyms for three-dimensional heteronuclear NMR experiments connecting backbone atoms, i.e., H, N, CO, CA, and HA.

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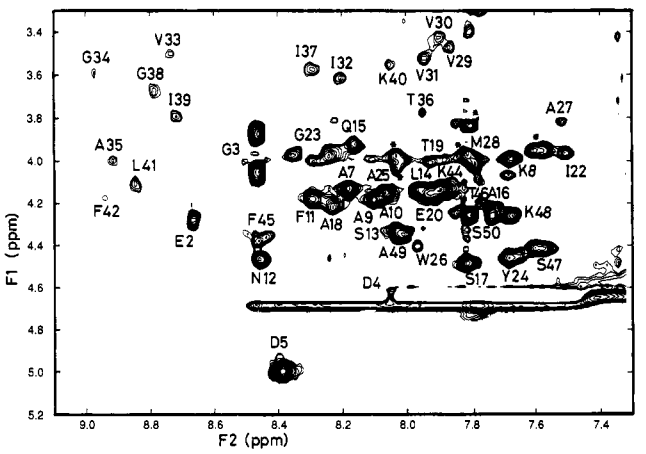

FIGURE 1: Fingerprint region of a 600-MHz "clean" TOCSY spectrum of gVIIIp, recorded in 90% H_2O/10% D_2O with a mixing time of 35 ms. The assignments of the HN–H^{α} connectivities have been indicated. Relayed cross peaks, i.e., HN–H^{β}, are marked with asterisks.

TOCSY, because of the unfavorable ratio of line width to ^3J coupling constants. However, we employed different sample conditions, i.e., a higher SDS/protein ratio, than was customary and found that we were able to obtain good ^{1}H 2D TOCSY spectra. In combination with ^{1}H–^{15}N 2D HSQC and 3D NOESY–HSQC, this allowed the almost complete assignment of the ^{15}N and ^{1}H spectra. Recently, an alternative method for obtaining NMR resonance assignments for larger proteins was put forward by Bax and co-workers (Ikura et al., 1990), making use of heteronuclear (^{13}C, ^{15}N, ^{1}H) three-dimensional NMR. We used some of these techniques, i.e., HNCO, HN(CO)CA, and HCA(CO)N experiments, and obtained the assignments of the $^{13}C\alpha$ and ^{13}CO resonances.

The interproton contacts and the $^{13}C^{\alpha}$ and $^1H^{\alpha}$ chemical shift data were interpreted, which yielded a clearcut picture of two α-helices: one ranging from residue Pro-6 to Glu-20 and one from Tyr-24 to the C-terminus Ser-50.

MATERIALS AND METHODS

Materials. E. coli cells (K38) were grown in minimal medium and infected with M13 phages using standard protocols. ^{13}C and ^{15}N labeling was achieved by using [^{13}C]glucose (fermentation grade) and/or $^{15}NH_4Cl$ as sole carbon and nitrogen source. These labeled compounds were obtained from Isotech. The phage particles were isolated and purified as described before (Konings et al., 1980; Spruijt et al., 1989). Three M13 preparations were used in this study: (i) unlabeled, (ii) uniformly labeled with ^{15}N only, and (iii) labeled with both ^{13}C and ^{15}N. Judging from the NMR spectra, the labeling percentages were greater than 90%.

Two different isolation procedures for gVIIIp were employed. The first method consisted of directly extracting the protein from the phages with SDS, followed by separation of the protein from the phage DNA via column chromatography on Sephadex G150 (Henry et al., 1986). The second method entailed extraction of the protein via phenol treatment (Knippers & Hofmann-Berling, 1966; Konings et al., 1970). No attempt was made to remove the minor coat proteins, as they constitute a negligible fraction of the preparations. Both methods yielded electrophoretically pure gVIIIp.

NMR Methods. The NMR samples typically consisted of 1.5–2.0 mM gVIIIp and 300 mM (^{2}H)SDS. The pH was adjusted to 5.1 (pH meter reading). Spectra were usually recorded at 38 °C.

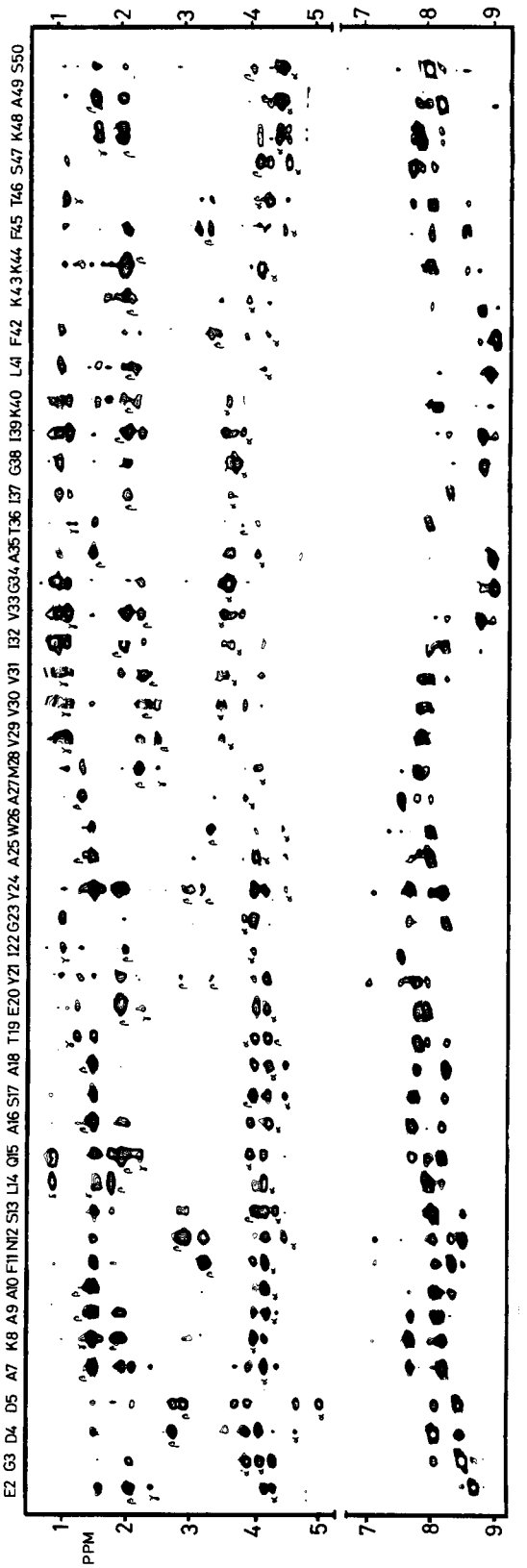

FIGURE 2: NOESY "ladders" collected from a 3D NOESY–HSQC spectrum, recorded at 600 MHz with a mixing time of 150 ms. Each ladder (in the *F1* direction) is obtained by cutting out a strip at the appropriate ^{15}N plane (*F2*) and ^{1}HN chemical shift (*F3*) of a given residue. Pro-6 is obviously absent from this figure. Overlap of both *F2* and *F3* occurred for the pairs Lys-8/Tyr-24 (note that the Tyr-24 ladder has been plotted at lower contour levels) and Val-33/Ile-39. The *intra*residual HN–H^{α}, HN–H^{β}, etc. NOEs have been marked α, β, etc.

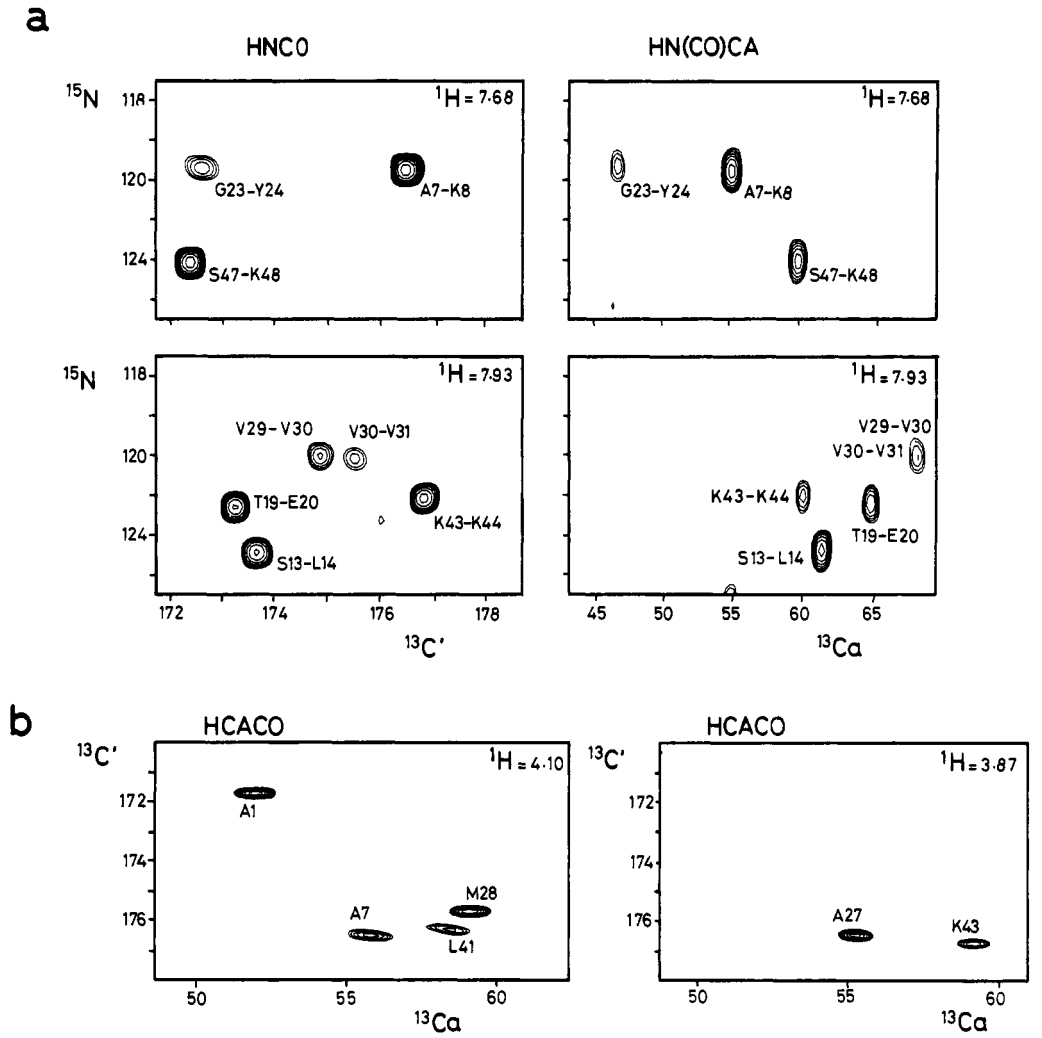

FIGURE 3: Planes, corresponding to particular ^{1}H frequencies of HNCO (a, left), HN(CO)CA (a, right), and HCACO (b). Chemical shifts are in ppm, as described under NMR Methods. The assignments of the cross peaks have been indicated.

The two-dimensional spectra recorded were obtained via total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY), and inverse-detected heteronuclear correlation spectroscopy (HSQC). The TOCSY experiments were carried out, in both H_2O and D_2O solution, as described by Griesinger et al. (1988). In H_2O solution, suppression of the solvent peak was performed as described by Bax et al. (1987). The NOESY spectra were recorded in a standard manner. The HSQC experiment was performed as described by Messerle et al. (1989); for the ^{13}C-labeled sample a constant-time HSQC spectrum was also recorded according to the method of Van de Ven and Philippens (1992). The two-dimensional spectra were recorded on three different Bruker AM spectrometers operating at ^{1}H frequencies of 400, 500, and 600 MHz.

A three-dimensional (3D) NOESY–HSQC spectrum was recorded using the ^{15}N-labeled sample on an AM600 spectrometer, as described by Messerle et al. (1989). The other 3D experiments were performed on an AM500 spectrometer. These experiments typically involved rf pulsing on different channels: ^{15}N, ^{1}H, and two regions of the ^{13}C spectrum, i.e., where the $^{13}C^{\alpha}$ and the ^{13}CO carbons resonate. Since an AM spectrometer features only two rf channels, we used home-built equipment to control the rf gating, power, and phase of the third and fourth channel as described by Kay et al. (1990). CT-HCACO and CT-HCA(CO)N (Powers, 1991) heteronuclear 3D spectra were recorded in D_2O, and CT-HNCO and CT-HN(CO)CA (Grzesiek & Bax, 1992) spectra were recorded in H_2O. Some experiments were slightly modified to employ optimized constant-time frequency labeling (Van de Ven & Philippens, 1992). Absorption phased spectra were recorded using the TPPI method (Marion & Wüthrich, 1983). Typical acquisition parameters for ^{15}N, $^{13}C\alpha$, and ^{13}CO, respectively: carrier position, 118, 57, and 175 ppm; spectral width, 1000, 3333.3, and 1000 Hz. Whenever possible, 64 real pints were sampled in the t_1 or t_2 dimension, leading to acquisition times of 32.0, 9.6, and 32.0 ms. Fewer points were taken when constant-time frequency labeling imposed an upper limit to the t_1 or t_2 acquisition time. This was 7 ms for $^{13}C\alpha$ in CT-HCACO and CT-HCA(CO)N and 18–20 ms for ^{15}N in CT-HNCO and CT-HN(CO)CA. In the t_3 (^{1}H) dimension the carrier was at 4.62 ppm on the solvent resonance, which was presaturated during the relaxation delay of 1 s. The ^{1}H spectral width was 5000 Hz, and 2048 points were taken, resulting in an acquisition time of 204.8 ms. Phase cycling was as in the papers cited above and was repeated several times for the less sensitive experiments, leading to overall measuring times ranging between 30 h for CT-HNCO and 72 h for CT-HCA(CO)N. The processing of the spectra consisted of window multiplication using a shifted sine bell and zero-filling to 128 points in f_1 and f_2. Spectra were calibrated relative to (trimethylsilyl)propionic-d_4 acid (TSP) as described by Ikura et al. (1991), whereby the 0 ppm value

Table I: ^{15}N, ^{13}C, and ^{1}H Assignments for SDS-Solubilized M13 Coat Protein, gVIIIp, pH 5.1, 38 °C[a]

residue	N	CO	CA	HN	H^{α}	other[b]
Ala-1		171.8	52.0		4.10	H^{β}, 1.51
Glu-2	122.2	174.6	57.8	8.67	4.26	H^{β}, 2.35; H^{γ}, 1.99/2.06
Gly-3	112.6	171.8	45.5	8.48	4.04	
					3.85	
Asp-4	121.4	173.4	54.8	8.05	4.63	H^{β}, 2.71
Asp-5	121.3	171.7	53.1	8.40	5.00	H^{β}, 2.84/2.89
Pro-6	139.1	176.0	65.4		4.31	H^{β}, 2.05/2.35; H^{γ}, 1.87; H^{δ}, 3.65/3.88
Ala-7	122.8	176.5	55.0	8.19	4.12	H^{β}, 1.44
Lys-8	119.6	175.9	59.1	7.67	3.97	H^{β}, 1.92/1.82; $H^{\beta,\gamma}$, 1.79/1.88/1.72/1.57; H^{ϵ}, 2.97; H3N, 7.42
Ala-9	123.7	177.8	54.9	8.11	4.17	H^{β}, 1.45
Ala-10	123.5	177.1	55.0	8.07	4.14	H^{β}, 1.46
Phe-11	119.9	175.0	61.0	8.29	4.13	H^{β}, 3.23/3.16; Hring, 7.11/7.18
Asn-12	118.8	175.3	56.3	8.45	4.45	H^{β}, 2.80/2.92; NH2, 113.1; H2N, 7.54/6.90
Ser-13	117.9	173.9	61.4	8.03	4.30	H^{β}, 3.97/4.03
Leu-14	124.8	176.2	57.6	7.95	4.12	H^{β}, 1.73/1.53; H^{δ}, 0.79
Gln-15	119.9	175.1	58.8	8.17	3.92	H^{β}, 1.91/2.14; H^{γ}, 2.22; NH2, 112.8; H2N, 6.98/6.65
Ala-16	122.4	176.8	54.1	7.73	4.20	H^{β}, 1.44
Ser-17	115.2	172.9	60.0	7.80	4.47	H^{β}, 3.97
Ala-18	126.1	176.3	55.5	8.24	4.22	H^{β}, 1.43
Thr-19	111.0	173.2	64.9	7.79	3.97	H^{γ}, 1.15/1.21
Glu-20	122.5	174.6	58.0	7.93	4.16	H^{β}, 1.87; H^{γ}, 2.13/2.21
Tyr-21	119.0	174.3	59.1	7.77	4.70	H^{β}, 3.29/2.81; Hring, 7.04/6.76
Ile-22	120.7	174.1	63.9	7.51	3.95	$H^{\gamma}M$, 0.97
Gly-23	110.5	172.6	46.8	8.24	3.97	
Tyr-24	119.4	174.8	59.8	7.68	4.45	H^{β}, 3.27/311; Hring, 7.10/6.83
Ala-25	123.4	176.6	56.1	8.03	4.15	H^{β}, 1.46
Trp-26	117.0	175.5	60.1	7.96	4.39	H^{β}, 3.35/3.25; Hring, 7.32/7.38/7.24; HNring, 9.80
Ala-27	122.9	176.5	55.8	7.48	3.81	H^{β}, 1.26
Met-28	116.7	175.3	58.9	7.78	4.05	H^{β}, 2.17; H^{γ}, 2.54/2.44
Val-29	119.9	175.0	68.1	7.86	3.45	H^{β}, 2.44; H^{γ}, 1.03/0.92
Val-30	120.0	175.5	68.2	7.90	3.41	H^{β}, 2.29; H^{γ}, 0.88/1.05
Val-31	120.3	174.9	67.9	7.98	3.51	H^{β}, 2.22; H^{γ}, 1.01/0.83
Ile-32	120.5	177.0	66.6	8.20	3.59	H^{β}, 1.94; $H^{\gamma}M$, 0.84
Val-33	123.6	175.0	68.2	8.73	3.47	H^{β}, 2.17; H^{γ}, 0.87/1.02
Gly-34	109.2	172.6	47.9	8.96	3.59	
Ala-35	123.9	176.5	55.9	8.91	3.99	H^{β}, 1.41
Thr-36	115.4	174.4	68.9	7.95	3.77	H^{β}, 4.38; H^{γ}, 1.13
Ile-37	121.8	175.7	66.1	8.29	3.59	H^{γ}, 1.94; $H^{\gamma}M$, 0.83
Gly-38	109.0	172.7	48.4	8.79	3.68	
Ile-39	123.2	175.0	66.4	8.71	3.77	H^{β}, 1.99; $H^{\gamma}M$, 1.00
Lys-40	121.0	178.2	60.2	8.04	3.54	H^{β}, 1.97; $H^{\gamma,\delta}$, 1.86/2.07
Leu-41	121.5	176.4	58.4	8.85	4.09	$H^{\beta,\gamma,\delta}$, 2.06/0.89/1.95/0.88/2.09/1.45/1.61/1.31
Phe-42	123.2	176.7	63.0	8.94	4.18	H^{β}, 3.37/3.25; Hring, 7.05/7.34
Lys-43	121.0	176.8	60.0	8.63	3.80	H^{β}, 1.84
Lys-44	122.2	176.9	59.5	7.92	3.98	H^{β}, 1.10/1.32; H^{γ}, 1.52/1.62; H^{ϵ}, 2.88; H3N, 7.38
Phe-45	119.7	175.4	61.0	8.46	4.37	H^{β}, 3.01/3.19; Hring, 7.26/7.22
Thr-46	110.6	173.6	63.7	7.89	4.14	H^{β}, 4.40; H^{γ}, 1.16
Ser-47	118.6	172.7	59.7	7.58	4.41	H^{β}, 3.95
Lys-48	124.3	174.2	57.1	7.66	4.24	H^{β}, 1.46/1.78; H^{γ}, 1.84; H^{δ}, 1.43/1.64; H^{ϵ}, 2.92; H3N, 7.33
Ala-49	126.7	174.8	52.9	8.01	4.35	H^{β}, 1.36
Ser-50	122.4			7.80	4.25	H^{β}, 3.82

[a] Assignments are for the gVIIIp monomer in ppm relative to TSP as described under NMR Methods. [b] Assignments of side-chain ^{1}H resonances were obtained from 2D TOCSY spectra; specific assignments to H^{γ}, H^{δ}, etc. are tentative.

is obtained by multiplying the ^{1}H TSP frequency by 0.25144954 for ^{13}C and by 0.10132914 for ^{15}N.

RESULTS AND DISCUSSION

Effect of Sample Preparation and Conditions. It has been shown for phage Pf1 that the secondary structure of its coat protein is preserved when it is extracted with SDS (Schiksnis et al., 1988). This result indicates that no major conformational switches are required to transfer the protein from its coat environment to an SDS micelle. We have compared 2D NMR spectra of both phenol- and SDS-extracted gVIIIp. The results were very clear-cut: there were no significant spectral differences at all. We used phenol-extracted gVIIIp for most of the 3D NMR, because these samples were somewhat easier to prepare.

We conducted our NMR studies at an SDS to protein ratio of about 150:1, which was higher than was typically reported in the literature. It was clear that the higher SDS/gVIIIp ratio improved the spectra. Adding salt or varying the pH had no significant effect. Since gVIIIp molecules pack tightly along the DNA in the virion, it is natural to expect this protein

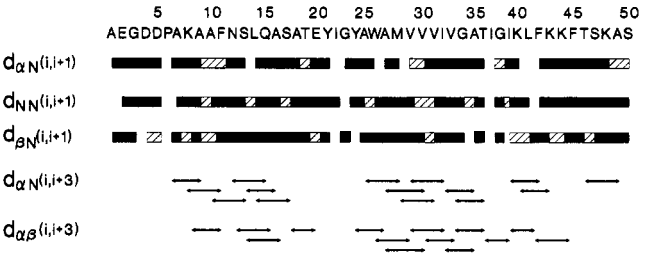

FIGURE 4: Overview of sequential and (i, i+3) NOE connectivities for gVIIIp. The relative sizes of the NOEs have not been indicated since the overall cross-peak intensities differ for different regions of the protein (cf. Figure 2). Dashed bars indicate NOEs that are ambiguous because of overlap.

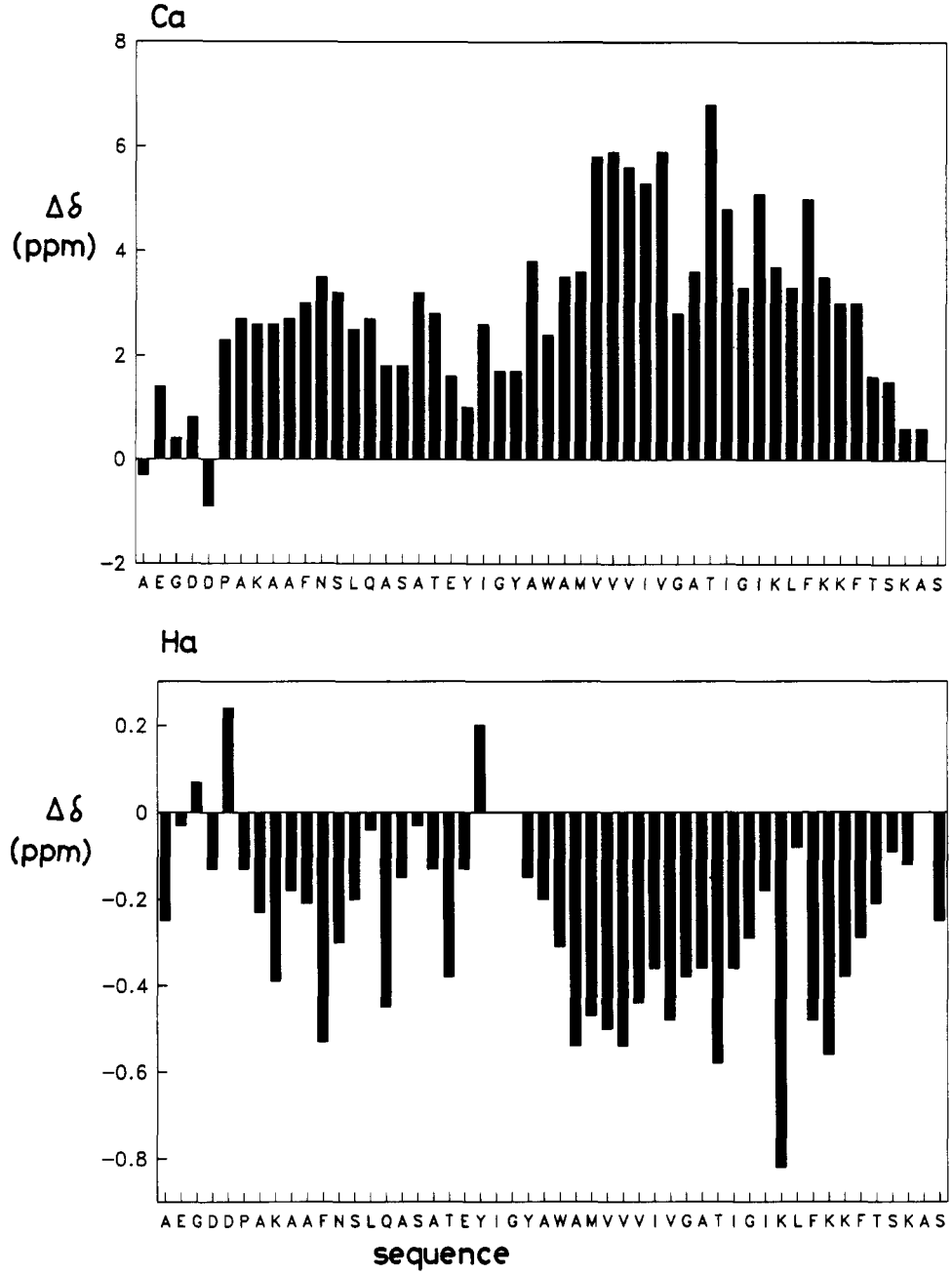

FIGURE 5: Secondary chemical shifts, defined as ppm(observed) – ppm(random coil) for the $^{13}C^{\alpha}$ and $^{1}H^{\alpha}$ resonances of gVIIIp, plotted against the amino acid sequence.

to have a tendency to aggregate. Increasing the SDS/gVIIIp ratio, or in other words the micelle/gVIIIp ratio, reduces the chance of obtaining multiple gVIIIp molecules associated with a single micelle. The most severe test for the quality of the sample was the TOCSY (H_2O) spectrum, featuring the J connectivities between NH and CαH protons. In helical proteins the ^{3}J values for this coupling are at most 4.0 Hz and may be as small as 2.0 Hz. In practice this means that when the ^{1}H line width exceeds 15 Hz, is almost impossible to obtain a sufficient signal to noise ratio in TOCSY. A typical example of a TOCSY spectrum is shown in Figure 1. Clearly, we can observe all expected J(Hα–HN) connectivities, although the ones in the 25–45 region, and especially 33–35, are significantly weaker than the others.

Toward the end of this study we learned (S. J. Opella, personal communication) that even better results can be obtained at SDS concentrations as high as 500 mM, i.e., at an SDS/protein ratio of 250:1.

Resonance Assignments. The main purpose of the study reported here was to assign the ^{1}H resonances of gVIIIp, which is a prerequisite for the elucidation of the three-dimensional structure of the protein via NMR. We employed the well-known Wagner–Wüthrich method (Wüthrich, 1986) to arrive at these assignments.

It can be gleaned from Figure 1 that there is substantial overlap of NH and H^{α} resonances. This causes ambiguities in the interpretation of 2D NOESY spectra, i.e., several NH protons may qualify for the same H^{α}–NH or NH–NH NOE. Problems associated with overlap of NH resonances were overcome almost completely by adding a third dimension: ^{15}N. We found that a single NOESY–HSQC spectrum, in combination with 2D TOCSY, was sufficient to obtain a complete "sequential walk" with resonance assignment of the NH–H^{α}–H^{β} spin systems and most of the side-chain protons. The NH NOE "ladders" of all the residues but Pro-6 of gVIIIp

are shown in Figure 2, illustrating the continuous sequential walk via H^{α}–HN and HN–HN NOEs.

An alternative method of resonance assignments (Ikura et al., 1990) is based on several 3D heteronuclear experiments, known by acronyms such as HNCO, HN(CO)CA, etc. In each of these experiments, 3D cross peaks are generated at the chemical shift positions of three different backbone ^{13}C, ^{15}N, and ^{1}H nuclei. Using this method, ambiguities arising because of accidental overlap of resonances are kept to a minimum, rendering the method very reliable and particularly suited for larger proteins. We used this method to extend our ^{1}H and ^{15}N assignments to the backbone carbons, $^{13}C^{\alpha}$ and ^{13}CO. For each ^{1}H–^{15}N pair, the chemical shifts of the ^{13}CO of the preceding residue can be read off in the HNCO spectrum, and that of $^{13}C^{\alpha}$ can be read off in the HN(CO)CA spectrum. For the bulk of the assignments this was trivial, as the ^{1}H–^{15}N cross peaks were already resolved in the 2D HSQC spectrum (cf. Figure 6). There were a few cases of partial overlap of ^{1}H–^{15}N cross peaks, e.g., Lys-8/Tyr-24 and Glu-20/Lys-44. Therefore a HCACO spectrum was recorded to allow a double-check of the ^{13}CO–$^{13}C^{\alpha}$ pairs. These examples are illustrated in Figure 3. At the $^{1}H/^{15}N$ combination of 7.68/119.5 ppm, both Lys-8 and Tyr-24 resonate; hence, two candidates are found for ^{13}CO and $^{13}C^{\alpha}$ (Figure 3a, top). The same kind of ambiguity arises because of the Glu-20/Lys-44 overlap (Figure 3a, bottom). The correct $^{13}CO/^{13}C^{\alpha}$ pairing is then read off from HCACO for Ala-7 (Figure 3b, left) and Lys-43 (Figure 3b, right). Also, the ^{13}C assignment of Asp-5 was obtained from the HCACO spectrum. Another extra control was provided by the HCA(CO)N experiment, which also allowed the ^{15}N assignment of Pro-6. For a few residues, i.e., Ala-1 and -49, Lys-8, -40, and -48, Phe-42 and -45, and Pro-6, assignments of ^{13}CO resonances of gVIIIp have been reported before (Henry et al., 1987b). Our results are in good agreement with these, and also with the previously reported ^{1}H–^{15}N assignments of Henry and Sykes (1992).

Secondary Structure. The main results of this study are presented in Figure 4 and Table I. The (i, i+3) connections, indicated in Figure 4 are a strong indication of the presence of α-helical structure. There are enough unambiguous cross peaks to conclude that the majority of the residues are involved in α-helix. This is confirmed by the fact that for most of the residues sequential HN(i)–NH(i+1) contacts were observed. It should be noted that earlier reports about the α-helical structure of Pf1 or M13 coat protein (Shon et al., 1991; Henry & Sykes, 1992) were essentially based on these contacts only; no assignments of $^{1}H^{\alpha}$ or $^{1}H^{\beta}$ resonances, required to determine (i, i+3) NOEs, have been reported before. There is an interruption in the typical helical stretch around Ile-22. The NH of this residue does not give rise to any sequential connectivities.

The patterns for residues Ala-7–Glu-20 and Thr-46–Ser-50 differ from that of the Tyr-24–Phe-45 segment. The cross peaks in the various spectra belonging to residues in the latter region are much weaker, i.e., these residues feature broader resonances indicating a longer rotation correlation time. Moreover, their $H^{\alpha}(i)$–HN(i+1) NOEs are in general weaker than the corresponding HN(i)–HN(i+1) NOEs, in contrast to the N- and C-terminal regions where the two types of sequential NOEs are of similar strength. We interpret these results as follows: there is a rigid helix in the Tyr-24–Phe-45 region with ^{15}N line widths ranging from 15 to 20 Hz. This helix may extend all the way to Ser-50, but then there is enhanced flexibility beyond Phe-45. There is another helix

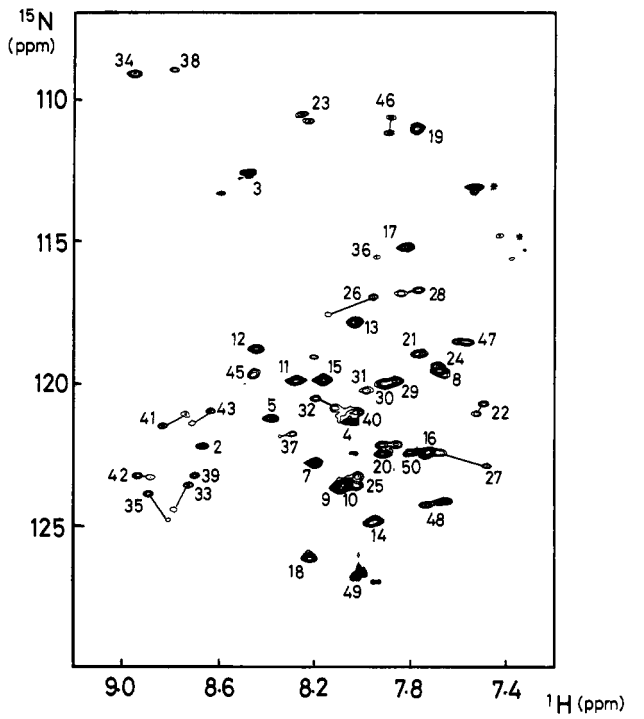

FIGURE 6: ^{1}H–^{15}N HSQC spectrum of gVIIIp. Several residues give rise to pairs of cross peaks, representing a major and a minor component. Paired cross peaks have been connected. The minor component peaks have not been assigned in the present study; their assignments were taken from Henry and Sykes (1992).

in the Pro-6–Glu 20 region which has ^{15}N line widths of 5–10 Hz. This helix appears less rigid and may be unfolded part of the time. The transition of one helix to the other, with a rather abrupt change in the line widths of the resonances, appears to be around residue Ile-22. A similar model was advanced by Opella and co-workers on the basis of solid-state NMR of the coat protein of Pf1 in oriented lipid bilayers (Shon et al., 1991).

This model of two separate α-helices is supported by the chemical shift profiles of the $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ resonances. These resonances are found at shifted positions, relative to their random-coil values, when the $^{1}H^{\alpha}$ and $^{13}C^{\alpha}$ nuclei are located in an α-helical segment of a protein. As can be seen in Figure 5, there are two separate regions of upfield-shifted $^{1}H^{\alpha}$ and downfield-shifted $^{13}C^{\alpha}$ resonances. The random-coil values, used to calculate the chemical shift differences, were taken from Wishart et al. (1992) and Spera and Bax (1991).

Asymmetric Dimer. Recently, Henry and Sykes (1990a, 1992) reported that gVIIIp in SDS micelles exists as an asymmetric dimer. They arrived at this conclusion because they observed that several residues gave rise to two distinct peaks in a ^{15}N HMQC spectrum. Since these pairs consisted of peaks of almost identical magnitude, they reasoned that it was unlikely that the doubling originated from conformational heterogeneity, but rather represented the individual monomers of asymmetric dimers. They speculated that these dimers were parallel and that the monomers were oriented relative to each other in a way similar to that in the virion.

We have made two observations that render this conclusion highly unlikely. We too have observed the doubling of cross peaks in the HSQC spectrum. However, we find that their ratio strongly depends on the amount of SDS present. At higher SDS/gVIIIp ratios we can clearly distinguish between a major and a minor component (Figure 6). Moreover, we have recorded HSQC spectra with extra long acquisition times in both the t_1 and the t_2 dimension, allowing us to estimate

the line widths of the various peaks. We consistently observe that the line widths of the resonances belonging to the minor component are about twice those of the major component. Actually, the minor component hardly ever shows up in the other spectra (cf. Figure 1). Therefore we conclude that the minor component most likely represents a higher aggregation state, while the major component is monomeric. If the minor component represents the dimeric state, then this must be symmetric. Recently, we learned that, by using a more extreme SDS/protein ratio, one can get rid of the minor component altogether (S. J. Opella, personal communication).

CONCLUSIONS

We have, for the first time, succeeded in completely assigning the $^1H^{\alpha}$, $^1H^{\beta}$, $^{13}C^{\alpha}$, and ^{13}CO resonances of gVIIIp in SDS micelles, in addition to making the assignments of the ^1H–^{15}N moieties, for which we obtained the same results as Henry and Sykes (1992). This allowed us to observe (i, i+3) NOEs, and shifts for $^1H^{\alpha}$ and $^{13}C^{\alpha}$ resonances, which point to the presence of two α-helices: one in the N-terminus and one in the hydrophobic region of the molecule. Our model is in agreement with data obtained by solid-state NMR of the functionally related protein of phage Pf1 in lipid bilayers (Shon et al., 1991). This suggests that SDS is not a poor membrane mimetic for gVIIIp. We are currently in the process of deriving a high-resolution NMR structure for this protein.

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REFERENCES

Banner, D., Nave, C., & Marvin, D. (1981) *Nature 289*, 814–816.
Bax, A., Sklenar, V., Clore, G. M., & Gronenborn, A. M. (1987) *J. Am. Chem. Soc. 109*, 6511–6513.
Bax, A., Clore, G. M., & Gronenborn, A. M. (1990) *J. Magn. Reson. 88*, 425–431.
Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry 26*, 847–854.
Cross, T. A., & Opella, S. J. (1980) *Biochem. Biophys. Res. Commun. 92*, 478–484.
Cross, T. A., & Opella, S. J. (1985) *J. Mol. Biol. 182*, 367–381.
Datema, K. P., Visser, A. J. W. G., Van Hoek, A., Wolfs, C. J. A. M., Spruijt, R. B., & Hemminga, M. A. (1987a) *Biochemistry 26*, 6145–6152.
Datema, K. P., Wolfs, C. J. A. M., Marsh, D., Watts, A., & Hemminga, M. A. (1987b) *Biochemistry 26*, 7571–7574.
Datema, K. P., Spruijt, R. B., Wolfs, C. J. A. M., & Hemminga, M. A. (1988) *Biochim. Biophys. Acta. 944*, 507–515.
Glucksman, M. J., Bhattacharjee, S., & Makowski, L. (1992) *J. Mol. Biol. 226*, 455–470.
Griesinger, C., Otting, G., Wüthrich, K., & Ernst, R. R. (1988) *J. Am. Chem. Soc. 110*, 7870–7872.
Grzesiek, S., & Bax, A. (1991) *J. Magn. Reson. 96*, 432–440.
Hemminga, M. A., Sanders, J. C., & Spruijt, R. B. (1992) in *Progress in Lipid Research* (Sprecher, H., Ed.) Vol. 31, pp 301–333, Pergamon Press, Oxford.
Henry, G. D., & Sykes, B. D. (1990a) *J. Mol. Biol. 212*, 11–14.
Henry, G. D., & Sykes, B. D. (1990b) *Biochemistry 29*, 6303–6313.
Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284–5297.
Henry, G. D., Weiner, J. H., & Sykes, B. D. (1986) *Biochemistry 25*, 590598.
Henry, G. D., Weiner, J. D., & Sykes, B. D. (1987a) *Biochemistry 26*, 3626–3634.
Henry, G. D., Weiner, J. D., & Sykes, B. D. (1987b) *Biochemistry 26*, 3619–3626.
Ikura, M., Kay, L.E., & Bax, A. (1990) *Biochemistry 29*, 4659–4667.
Ikura, M., Kay, L. E., Krinks, M., & Bax, A. (1991) *Biochemistry 30*, 5498–5504.
Kay, L. E., Ikura, M., Tschudin, R., & Bax, A. (1990) *J. Magn. Reson. 89*, 496–514.
Knippers, R., & Hofmann-Berling, H. (1966) *J. Mol. Biol. 21*, 293–304.
Konings, R. N. H. (1980) *Methods Enzymol. 65*, 795–811.
Konings, R. N. H., Ward, R., Francke, B., & Hofschneider, P. H. (1970) *Nature 226*, 604–607.
Leo, G. C., Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry 26*, 854–862.
Makowski, L. (1992) *J. Mol. Biol. 228*, 885–892.
Marion, D., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 113*, 967–974.
Messerle, B. A., Wider, G., Otting, G., Weber, C., & Wüthrich, K. (1989) *J. Magn. Reson. 85*, 608–613.
Model, P., & Russel, M. (1988) in *The Bacteriophages II* (Calender, R., Ed.) pp 375–456, Plenum, New York.
Nozaki, Y., Reynolds, J., & Tanford, C. (1978) *Biochemistry 17*, 1239–1246.
Ohkawa, I., & Webster, R. E. (1981) *J. Biol. Chem. 256*, 9951–9958.
O'Neil, J. D. J., & Sykes, B. D. (1989) *Biochemistry 28*, 6736–6745.
Powers, R., Gronenborn, A. M., Clore, G. M., & Bax, A. (1991) *J. Magn. Reson. 94*, 209–213.
Rasched, J., & Oberer, E. (1986) *Microbiol. Rev. 50*, 401–427.
Schiksnis, R. A., Bogusky, M. J., Tsang, P., & Opella, S. J. (1987) *Biochemistry 26*, 1373–1381.
Schiksnis, R. A., Bogusky, M. J., & Opella, S. J. (1988) *J. Mol. Biol. 200*, 741–743.
Shon, K. J., & Opella, S. J. (1989) *J. Magn. Reson. 82*, 193.
Shon, K. J., Kim, Y., Colnago, L. A., & Opella, S. J. (1991) *Science 252*, 1303–1305.
Spera, S., & Bax, A. (1991) *J. Am. Chem. Soc. 113*, 5490–5492.
Spruijt, R. B., Wolfs, C. J. A. M., & Hemminga, M. A. (1989) *Biochemistry 28*, 9158–9165.
Stark, W., Glucks, M. J., & Makowski, L. (1988) *J. Mol. Biol. 199*, 171.
Van de Ven, F. J. M., & Philippens, M. E. P. (1992) *J. Magn. Reson. 97*, 637–644.
Wickner, W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4749–4753.
Wickner, W. (1988) *Biochemistry 27*, 1081–1086.
Wishart, D. S., Sykes, B. D., & Richards, F. M. (1992) *Biochemistry 31*, 1647–1651.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.