Biophysical Characterization of Wild-Type and Mutant Bacteriophage IKe Major Coat Protein in the Virion and in Detergent Micelles†

Karen A. Williams‡ and Charles M. Deber*

Division of Biochemistry Research, Research Institute, Hospital for Sick Children, Toronto M5G 1X8, and Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Ontario, Canada

Received March 8, 1996; Revised Manuscript Received May 22, 1996⊗

ABSTRACT: Interactions between the filamentous bacteriophage major coat protein and its environment differ markedly between the membrane-bound assembly intermediate which spans the lipid bilayer and the phage coat protein which makes up the capsid of the virion. Nonetheless, both reflect successful strategies to sequester the hydrophobic regions of the coat protein away from the aqueous milieu. To characterize the roles of individual residues in the conformation, stability, and oligomerization of the coat protein in both the virion and in detergent micelles, wild-type IKe and M13 coat proteins, together with a library of over 40 IKe coat protein mutants, were studied using circular dichroism (CD), fluorescence, and solution nuclear magnetic resonance (NMR) spectroscopies. The largely helical conformations of coat protein in IKe wild-type and mutant virions were found to be very similar by CD, demonstrating that the overall organization of the phage can accommodate a diverse range of amino acid substitutions in the major coat protein. Intrinsic Trp fluorescence showed that the polarity of the Trp 29 environment in the virion was modulated by residues within one helical turn of this locus. Characterization of IKe phage growth and plaquing properties highlighted the importance of Pro 30 in maintaining viability. As well, the Pro 30 mutants were the only substitutions which rendered the detergent-solubilized coat protein less thermostable and additionally altered the polarity of the Trp 29 environment. The Pro 30 Gly mutant exhibited numerous ^{1}H and ^{15}N chemical shift changes between residues Ile 25 and Ala 38 in the 2D $^{1}H-^{15}N$ HSQC spectrum in myristoyllysophosphatidylglycerol (MPG) micelles, demonstrating that the effect of the substitution is propagated beyond adjacent residues. The overall results highlight the stabilizing effect of Pro in the first turn of a transmembrane helix and the importance of hydrophobicity in modulating the oligomerization and stability of coat protein both in the phage and in detergent micelles.

The filamentous bacteriophage have been extensively employed as model systems to characterize such diverse areas as DNA replication, gene expression (Russel & Model, 1988; Webster & Lopez, 1985), and membrane assembly (Wickner, 1988). Bacteriophage M13 is widely used in DNA cloning and sequencing (Sambrook et al., 1989) as well as in phage display libraries (Makowski, 1993; Cesareni, 1992; Scott & Smith, 1990). The appeal of the filamentous phage is in their simplicity: the genome encodes just 10 proteins, of which five make up the capsid. The phage major coat protein occurs both as an integral membrane protein on the bacterial inner membrane prior to phage assembly and as a structural coat protein in the virion (Figure 1). Pure phage can readily be obtained in large quantities for direct characterization of the virion or extracted with detergent for study of the membrane-bound form of the coat protein. For this reason, the filamentous phage major coat proteins have been widely used as model membrane proteins (e.g., Deber et al., 1993; Hemminga et al., 1992; Henry & Sykes, 1990) and the virus as a model macromolecular assembly (Williams et al., 1995; Marvin et al., 1994; Glucksman et al., 1992; Liu & Day, 1994).

Filamentous bacteriophage IKe has a circular single-stranded DNA genome of 6883 nucleotides which is encapsulated by a helical sheath of about 2700 copies of the major coat protein (gene 8), together with a few copies of the minor coat proteins at either end, to produce an elongated virion about 100-fold longer than it is wide (Marvin et al., 1994). Two classes of filamentous phage are distinguished on the basis of slight differences in virion symmetry (Marvin et al., 1974, 1975). IKe and M13 belong to class 1 phage since they exhibit five-fold rotational symmetry and a two-fold screw axis. IKe infects bacteria such as *Escherichia coli* by adsorbing to cell-surface N-pili which are encoded by the N-conjugative plasmid (Khatoon et al., 1972), while M13 is specific for bacteria harboring the F plasmid. IKe and M13 share 55% sequence identity, with less similarity in capsid proteins than in proteins involved with DNA replication and morphogenesis (Peeters et al., 1985). Mature IKe coat protein is 53 residues in length and exhibits 37% identity with the 50 residue M13 coat protein (Figure 2).

Coat protein is synthesized as a precursor (procoat) with an N-terminal leader peptide that is cleaved by host leader peptidase after post-translational, *sec*-independent assembly into the membrane (Wickner, 1988). Coat protein accumulates in the membrane until protein and phage DNA levels are sufficient for phage assembly; it then undergoes a change in conformation and is incorporated into the capsid

† This work was supported, in part, by a grant to C.M.D. from the Medical Research Council of Canada. K.A.W. held a Medical Research Council of Canada Studentship.

* Correspondence should be addressed to this author at the Research Institute.

‡ Present address: European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, 69012 Heidelberg, Germany.

⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1996.

S0006-2960(96)00578-8 CCC: $12.00

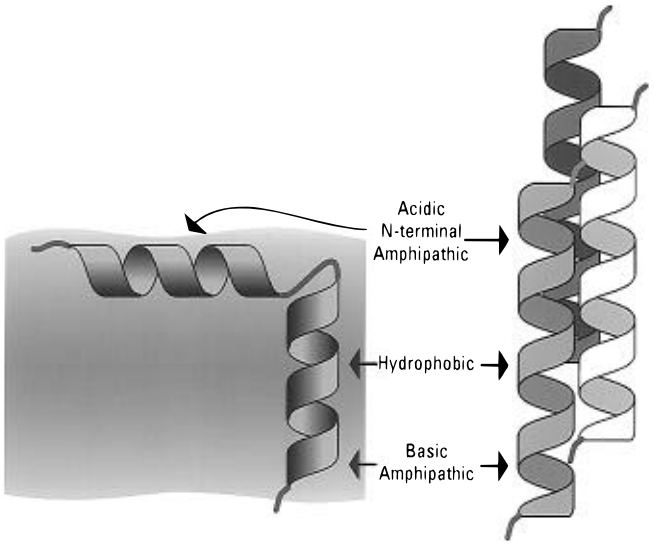

FIGURE 1: Schematic representation of the helical membrane-bound and virion forms of IKe major coat protein. Note that the ribbon model illustrates helicity but does not reflect specifically the number of turns of the helices. The conformation of the membrane-bound form is taken from solution NMR studies of coat protein solubilized in MPG micelles (Williams et al., 1996), while the almost fully helical virion form is taken from X-ray fiber diffraction data (Marvin et al., 1994). (Left: Membrane-bound) The coat protein consists of two helical regions, an N-terminal amphipathic helix (Asn 4 to Ser 26), and a membrane-spanning helix (Trp 29 to Phe 48). The N-terminal helix is situated at the membrane−aqueous interface such that the nonpolar residues contact the acyl chains of the lipid, while the polar residues are exposed to the aqueous environment. The C-terminal helix spans the bilayer with the cluster of positively charged residues (Arg 43, Lys 46, Lys 47) interacting with acidic lipid head groups. (Right: Virion form) The almost fully helical coat protein is oriented such that the polar face of the N-terminal amphipathic region is exposed to the aqueous environment, while the apolar face interacts with other (out-of-register) subunits in the capsid [see also Williams et al. (1995)]. The hydrophobic region which spans the bilayer in the membrane-bound form interacts with other subunits in the capsid, while the positively charged residues mediate interactions to DNA.

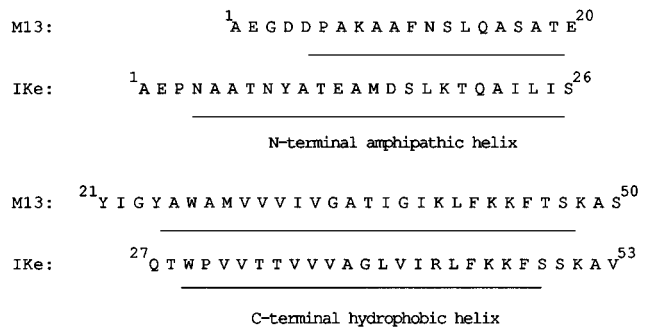

FIGURE 2: Sequences of M13 and IKe major coat proteins (Nakashima et al., 1981; Peeters et al., 1985). Residues underlined are in a helical conformation in the membrane-bound form of the coat protein, as determined by solution NMR [e.g., M13 (Henry & Sykes, 1992; van de Ven et al., 1993) and IKe (Williams et al., 1996)].

of progeny virion (Smilowitz, 1974). The sequence of major coat proteins includes an acidic N-terminal region, a hydrophobic segment which spans the bilayer in the membrane-bound form, and a highly conserved basic C-terminal region which mediates DNA binding in the virion (Nakashima et al., 1981; Asbeck et al., 1969). When the coat protein spans the bacterial inner membrane, it occurs as a type 1 single-spanning integral membrane protein, with the N-terminus situated in the periplasm and the C-terminus located in the cytoplasm. Micelle-solubilized M13 coat protein occurs as a monomer as well as in dimeric and oligomeric forms of up to ca. 20 subunits, which can be modulated by adjusting the lipid to protein ratio (Li et al., 1993; Hemminga et al., 1992; Makino et al., 1975; Nozaki et al., 1978). Detergent-solubilized coat protein undergoes an irreversible conformational transition to a high molecular weight β-sheet form over time; the transition is favored by high protein concentrations and elevated temperatures (Li et al., 1993; Hemminga et al., 1992; Sanders et al., 1991). Several features of the amino acid sequence of IKe coat protein are characteristic of membrane proteins, including (a) a stretch of hydrophobic residues which span the bilayer in an α-helical conformation together with an amphipathic surface helix, (b) interfacial Pro and Trp residues, and (c) a locus of positively charged residues in the C-terminal part of the transmembrane (TM)[1] helix (Williams et al., 1996). The conformation of phage coat proteins in micelles has been shown to be largely helical by CD (Nozaki et al., 1976, 1978; Williams & Dunker, 1977) and solution NMR (Williams et al., 1996; McDonnell et al., 1993; van de Ven et al., 1993; Henry & Sykes, 1992).

Models of phage architecture have been proposed on the basis of X-ray [e.g., fd (Marvin et al., 1994), M13 (Glucksman et al., 1992), pf1 (Liu & Day, 1994)] and neutron [e.g., Pf1 (Nambudripad et al., 1991a)] fiber diffraction of oriented phage. Current fiber diffraction methods are unable to resolve interactions among coat protein subunits at the level of individual amino acids. As a result, the models rely on a variety of biophysical [e.g., Raman (Overman et al., 1994; Aubrey & Thomas, 1991); flow linear dichroism (Clack & Gray, 1992); CD (Nozaki et al., 1976; Clack & Gray, 1989; Arnold et al., 1992); NMR (Gall et al., 1982; Cross et al., 1983; Colnago et al., 1987); Fourier Transform infrared (Azpiazu et al., 1993)] and mutational (Williams et al., 1995; Greenwood et al., 1991; Greenwood & Perham, 1989; Hunter et al., 1987) data. Major coat protein subunits are oriented some 10−20° from the parallel of the long axis of the virion and follow a right-handed helix. Coat protein occurs as a gently curving helix which slopes from a narrow inner radius to wide outer radius so that successive coat protein subunits overlap (Marvin et al., 1994; Liu & Day, 1994). The virus is solubilized in the aqueous environment by the polar face of the N-terminal domain, while the nonpolar face sequesters the hydrophobic TM domain of the preceding subunit.

The filamentous bacteriophage provide an excellent opportunity to study both macromolecular assemblies and membrane proteins, since the major coat protein occurs as an integral membrane protein, while the virion represents a macromolecular assembly. Randomized and saturation site-directed mutagenesis of residues Leu 24 to Ile 42 of IKe major coat protein previously yielded a library of over 40 single and double amino acid mutants (Williams & Deber, 1993). The only selection criterion imposed by mutagenesis was that the mutations must permit the phage to be viable.

[1] Abbreviations: MPG, myristoyllysophosphatidylglycerol; LPC, lauryllysophosphatidylcholine; β-OG, β-octyl glucoside; DOC, sodium deoxycholate; SDS, sodium dodecyl sulfate; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; HSQC, heteronuclear single-quantum correlation spectroscopy; CD, circular dichroism; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TM, transmembrane; YT, yeast tryptone; TE, tris EDTA (ethylenediaminetetraacetic acid).

While this restricted the selection of mutants obtained for subsequent characterization of the membrane-bound form, it also meant that the mutational analysis yielded a wealth of information about the nature of interactions between the major coat protein subunits in the phage, since all of the requirements of the phage, including its primary role as coat protein in the virion, must be satisfied (Williams et al., 1995). A preliminary study of the wild-type and mutant detergent-solubilized coat proteins suggested that Pro 30 played a role in maintaining the thermal stability of the protein (Williams & Deber, 1993). In the present work, the roles of individual residues in the conformation, stability, and oligomerization of both virion and membrane-bound forms of wild-type and mutant IKe and wild-type M13 coat proteins are characterized using CD, fluorescence, and solution NMR [see also Williams et al. (1995, 1996)]. This work highlights the importance of hydrophobicity in modulating the stability and oligomerization of both the membrane-bound and virion forms of the coat protein as well as the stabilizing effect of Pro in the first turn of a TM helix.

MATERIALS AND METHODS

Growth of Bacteriophage IKe. Bacteriophage IKe and *E. coli* JE2571/N3 (leu$^-$, thr$^-$, str^r, fla$^-$, pil$^-$) were gifts of Dr. D. Bradley (Memorial University, St. John's, Newfoundland) and the plasmid pCU109 (chl^r) containing the IncN transfer genes was provided by Dr. V. N. Iyer (Carleton University, Ottawa, Ontario) (Thatte et al., 1985). M13mp18 single-stranded DNA was obtained from Pharmacia (Uppsala, Sweden). *E. coli* JM101/pCU109 (supE, thi, Δ(lac-proAB)/F′[traD36, proA$^+$, proB$^+$, lacI^q, lacZΔM15]) was used as bacterial host for site-directed mutagenesis because of the high efficiency of transformation, as well as for the preparation of ^{15}N-labeled phage samples for NMR (Williams et al., 1996). Unlabeled large scale preparations were obtained using either host strain. Bacteriophage M13 was propagated using JM101, and the phage was purified and stored in the same manner as IKe. Over 40 IKe coat protein mutants were previously isolated using randomized site-directed mutagenesis of residues Leu 24 to Ile 42, in conjunction with saturation mutagenesis at Pro 30 (Williams & Deber, 1993).

Wild-type and mutant phage were prepared by infecting 100 μL of log phase host cells with 10 μL of diluted phage stock for 10 min prior to the addition of top agar and plating on 2YT plus chloramphenicol plates. Plates were kept at room temperature overnight and transferred to 37 °C for 8 h the following day. Phage were isolated from single plaques and grown in 1.5 mL of 2YT with 15 μL of overnight bacterial culture for 8 h at 37 °C, followed by the removal of bacteria by centrifugation. Phage was obtained in large scale by infecting a 1 L 2YT culture of host cells at OD_{550} = 0.3 with 10 mL of phage stock (1 mL of a 3 h bacterial culture which had been inoculated with one drop of overnight culture, together with 10 mL of 2YT and 1 mL of phage 2YT supernatant, and grown for 4−6 h) and grown for a further 4−8 h with subsequent removal of bacteria by centrifugation (JA-14 rotor, 6000 rpm for 20 min).

Purification of Bacteriophage IKe. Phage was harvested from media by the addition of 0.2 volume of 20% PEG/15% NaCl. After 1 h at room temperature, or overnight at 4 °C, phage were pelleted by centrifugation and resuspended in TE buffer (10 mM tris, 1 mM EDTA) at pH 8.0. Phage concentration was determined by absorbance at OD_{265} = 3.5 corresponding to 1 mg of IKe phage (Thomas et al., 1983). Phage was purified on a KBr step gradient prepared by layering 2 mL of 22%, 3 mL of 29%, 4 mL 33%, 4 mL 36%, and 2 mL of 44% KBr and centrifuging at 24 000 rpm for 20 h at 4 °C in a Ti-28 rotor (McDonnell et al., 1993). The phage sample was desalted using three rounds of concentration and dilution in TE buffer pH 8 using 3000 Da cutoff centriprep tubes (Amicon, Inc., Beverly, MA). Phage was stored at 10 mg/mL at 4 °C.

Isolation of Detergent-Solubilized Coat Protein. KBr purified phage was extracted with detergent by directly mixing the 10 mg/mL phage stock with detergent and adding a small volume of chloroform prior to shaking at 200 rpm at 37 °C until no trace of chloroform remained and then diluted to make the appropriate protein/detergent concentration (15−40 mM DOC for fluorescence and CD experiments; 400 mM MPG in NMR studies). With this method of detergent extraction, the DNA and minor coat proteins remain in the solution but have been shown not to interfere with the biophysical characterization of the major coat protein (Williams & Dunker, 1977; Dunker et al., 1991). Protein concentration was determined by absorption where OD_{279} = 1 corresponds to 1 mg/mL coat protein (Williams & Deber, 1993).

Circular Dichroism (CD). CD spectra were recorded on a Jasco J-720 spectropolarimeter (JASCO International Co., Tokyo, Japan). Detergent-solubilized coat protein samples were prepared just prior to use. Each curve is the average of four scans, recorded between 250 and 190 nm in a cell with path length of 0.01 cm for 1 mg/mL protein or phage samples or in a 0.1 cm cell for 0.1 mg/mL protein or phage solutions. CD spectra were collected at 5 °C intervals from 25 to 95 °C and again after return to 25 °C to assess coat protein or phage thermal stability. To monitor the transition from intact phage to detergent-solubilized coat protein (phage disassembly), spectra were recorded on a 1 mg/mL phage solution in 25 mM DOC (no chloroform) from 25 to 95 °C.

SDS−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE experiments were performed in 4−20% Tris-glycine preformed gradient gels from NOVEX (Helix technologies) with 5−10 μg of protein loaded per lane. Gels were run at 125 V for ca. 1.5 h and stained with Coomassie blue.

Intrinsic Tryptophan Fluorescence. Trp emission spectra of 30 μM coat protein in 25 mM DOC, or 15 mM phage in H_2O, were recorded from 290 to 400 nm at 25 °C in a Hitachi F-4000 fluorescence spectrometer with the excitation wavelength set at 295 nm to minimize interference from Tyr (Eftink & Ghiron, 1977). The excitation and emission bandpass were set at 5 nm, and the scan speed was set at 60 nm/min. Three independent samples were employed for each measurement.

Solution NMR of Coat Protein in MPG Micelles. Uniformly ^{15}N-labeled IKe coat protein was solubilized in 400 mM fully protonated myristoyllysophosphatidylglycerol (MPG) (Avanti Polar Lipids, Inc., Alabaster, AL) with 5 mM NaCl and 5 mM sodium borate in 95% H_2O/5% D_2O at pH 4.5 (Williams et al., 1996). NMR experiments were recorded at 45 °C on a Varian UNITY 500 MHz spectrometer equipped with a pulsed field gradient unit and triple-resonance probe with an actively shielded z-gradient. Carrier positions employed were 119.5 ppm for ^{15}N and 4.67 ppm

Table 1: Summary of Randomized Mutagenesis Data for IKe Major Coat Protein

IKe coat protein residue	total no. mutants	no. silent mutants	no. amino acid mutants	mutations (no. of occurrences)	mutations not arising[a]
Leu24	7	4	3	I(3)	P, F, V, H, R
Ile25	7	5	2	L(1), T(1)	S, F, V, N
Ser26	10	4	6	T(1), A(1), F(1), Y(3)	P, C
Gln27	13	2	11	K(2), R(2), H(4), stop codon[b] (3)	P, E, L
Thr28	9	4	5	S(2), A(2), I(1)	P, N
Trp29	0				C, S, R, G, L
Pro30[c]	12	7	5	A(5)	L, S, T, H, R
Val31	4	1	3	I(3)	L, F, A, D, G
Val32	8	2	6	I(3), L(1), A(2)	E,G
Thr33	5	3	2	S(2)	P, A, M, R
Thr34	3	0	3	S(1), A(2)	P, I, N, S
Val35	2	0	1	I(1)	L, F, A, D, G
Val36	10	4	6	I(2), L(1), A(3)	E, G
Val37	15	4	11	I(4), A(1), G(6)	L, F, D
Ala38	5	2	3	S(2), G(1)	P, T, V, D
Gly39	16	6	10	S(2), C(4), V(2), A(2)	R, D
Leu40	3	1	2	I(1), V(1)	P, F, H, R
Val41	3	1	2	A(2)	L, I, E, G
Ile42	7	6	1	V(1)	L, F, T, N, S

[a] Mutations which could have arisen from a single nucleotide change. [b] The stop codon "tag" is suppressed as Gln in *E. coli* JM101. [c] Saturation mutagenesis at Pro 30 additionally yielded seven silent, eight P30A, 12 P30G, seven P30S, and three P30C mutants.

for ^{1}H. The 2D ^{1}H−^{15}N HSQC spectra were recorded with 16 transients per t_1 point and 160 complex t_1 points (160 × 512 complex matrix) with a spectral width of 1650 Hz in F_1, the ^{15}N dimension (Kay et al., 1992; Bodenhausen & Ruben, 1980). The Pro 30 Gly 150 ms ^{15}N-NOESY experiment was recorded with eight transients per t_1 point (110 × 32 × 512) and a spectral width of 5499.8 Hz in F_1 (^{1}H) and 1500 Hz in F_2 (^{15}N) (Zhang et al., 1994). These experiments employed WALTZ-16 ^{15}N decoupling (Shaka et al., 1983) during acquisition and used pulsed field gradients for coherence transfer pathway selection (Kay et al., 1992; Muhandiram & Kay, 1994) making use of an enhanced sensitivity approach (Cavanagh et al., 1991; Palmer et al., 1991) with minimal H_2O saturation and dephasing (Kay et al., 1994). Spectra were processed using the NMRPipe software system (Delaglio, 1993) and analyzed with the peak picking program PIPP (Garrett et al., 1991). A 60° phase-shifted sine bell and single zero-fill were applied in each of the F_1 and F_2 dimensions prior to Fourier transformation. The ^{15}N-NOESY was processed with forward−backward linear prediction in F_2.

RESULTS

Bacteriophage IKe Major Coat Protein Mutants. Randomized mutagenesis of the IKe major coat protein hydrophobic segment (Leu 24 to Ile 42) together with saturation mutagenesis at Pro 30, resulted in a mutant library of over 40 viable single and double amino acid coat protein mutants (Williams & Deber, 1993). The mutant library is exhaustive only at Pro 30; it is very likely that additional mutants throughout the remainder of the hydrophobic region could be isolated with further screening. Nonetheless, the mutants which were obtained yield insights into the backbone and side-chain requirements throughout the protein (Table 1). The distribution of base changes along the two oligos (Leu 24 to Thr 33 and Thr 33 to Ile 42) is uneven, with a large number of mutations obtained at Val 37 and Gly 39, but very few at Thr 34 and Val 35. The scarcity of mutations at the later two loci may reflect poor annealing at the end of the oligo, as was observed in the mutagenesis of M13 coat protein (Li & Deber, 1991). The overall efficiency of mutagenesis was ca. 30% [see also Williams and Deber (1993)].

Polar and charged mutations are enriched among residues Leu 24 to Pro 30, while nonpolar substitutions predominate in residues Val 31 to Ile 42 of IKe coat protein. This parallels findings of M13 coat protein (Li & Deber, 1991) and confirms the accessibility of these polar residues (i.e., residues 24−30 in IKe) to the aqueous environment during the life cycle of both phage. Although many of the mutations are conservative in nature, a number of more broadly substituted positions are also observed. For example, bulky aromatics (Tyr and Phe) were successfully introduced into the protein at Ser 26, while the positively charged residue Arg was introduced at Gln 27. No negatively charged residues were obtained upon mutagenesis of IKe coat protein, a result which may, in part, reflect few opportunities for negatively charged residues in this region (Table 1). The polar side chains of mutants in the region of Ser 26 to Pro 30 may hydrogen-bond to the backbone, a situation which is favorable in the first turn of the helix (Harper & Rose, 1993). Ser 26 and Gln 27 were particularly susceptible to mutation, as was also observed for the same region in M13 coat protein (Gly 23−Tyr 24), thus suggesting the presence of few requirements at these locations.

The hydrophobic segment (Val 31−Ile 42) displayed mutations to seven residues: Ala, Gly, Ser, Cys, Ile, Val, and Leu. Mutations at Gly 38 resulted in the elimination of the only Gly residue from this protein. This demonstrates that Gly residues in IKe coat protein are not essential for mediating conformational transitions, as has been suggested for Pf1 (Liu & Day, 1994). Ala was permissible at 10 of 18 sites in the region mutagenized, while Ile occurred at 9 of 18 residues. β-Branched residues (i.e., Thr, Val, Ile) are considered as β-sheet formers (Chou & Fasman, 1978), although these residues frequently occur in TM helices (Landolt-Marticorena et al., 1993). β-Branched residues were introduced at four locations in the IKe coat protein (Leu 24 Ile, Ser 26 Thr, Gly 39 Val, and Leu 41 Ile), while there was an interchange among the β-branched residues at eight

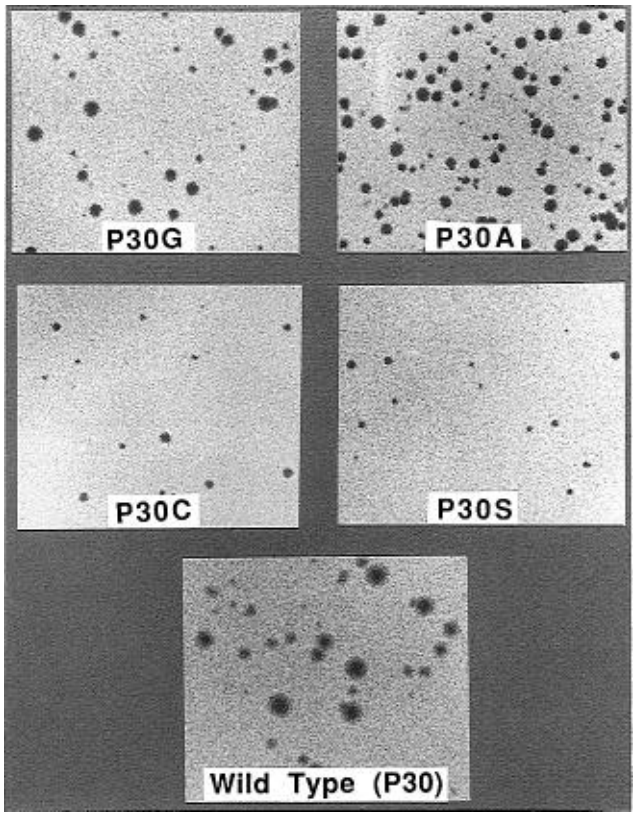

FIGURE 3: Wild-type and Pro 30 mutant plaque morphology. Phage were grown on an *E. coli* JM101/pCU109 lawn on 2YT plates supplemented with chloramphenicol at 25 °C overnight and at 37 °C for 8 h the following day. Plaque morphology is dependent upon the specific plating conditions and varies even within a single plate. The photographs are representative of the phenotypes typically observed and were taken from a single plating.

other locations (Ile 25 Thr, Thr 28 Ile, Val 31 Ile, Val 32 Ile, Val 35 Ile, Val 36 Ile, Val 37 Ile, and Ile 42 Val).

Viability of Wild Type and Mutant Bacteriophage IKe. Bacteriophage IKe grows well in rich media with yields of ca. 60 mg routinely obtained per liter of 2YT, in contrast to considerably lower quantities obtained with M13 (10−20 mg). At the same time, however, IKe fails to grow in common minimal media such as M9 or M63, whereas these media sustain the growth of bacteriophage M13. IKe plaques are much smaller than those of M13 when grown on 2YT plates at 37 °C overnight (not shown). When plates are incubated at room temperature overnight and at 37 °C for 8 h the following day, wild-type IKe plaques are large and have a characteristic halo (Figure 3), while M13 plaques are very large, opaque, and somewhat difficult to observe. Such modulation of plaque phenotype by incubation conditions likely reflects alterations in the kinetics of phage and cell growth, of both infected and neighboring cells. The growth and plaquing properties of IKe mutants largely resemble that of wild-type, with several exceptions. The Pro 30 mutants give rise to very distinctive plaques which are sharply delineated, clear in the center, and lack the diffuse halo characteristic of wild-type (Figure 3). Mutants which exhibited very small plaques (e.g., Pro 30 Ser, Pro 30 Cys, and Thr 34 Ala) were characteristically difficult to propagate in large scale.

Characterization of the Virion Form of Wild Type and Mutant IKe Coat Protein. Wild-type and mutant coat protein conformations in the virion are largely indistinguishable by

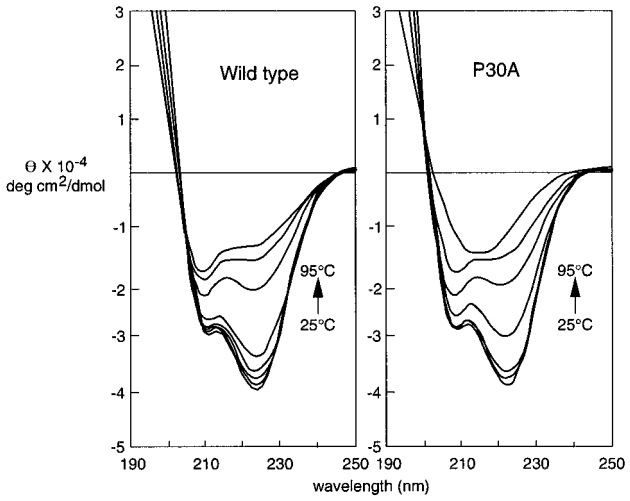

FIGURE 4: IKe wild-type and Pro 30 Ala phage disassembly mediated by 25 mM DOC upon heating 1 mg/ml phage in TE buffer by 5 °C increments from 25 to 95 °C, with spectra plotted at 10 °C intervals. The transition from phage to micelle-solubilized coat protein is observed around 70 °C. The micelle-solubilized wild-type coat protein remains partially helical upon heating to 95 °C, while the Pro 30 Ala mutant undergoes an irreversible transition to a β-sheet conformation.

CD and appear to be almost fully helical (e.g., 25 °C spectra of wild-type IKe and Pro 30 Ala in Figure 4). The spectra exhibit suppression of ellipticity at 208 nm, which is characteristic of many filamentous phages (Arnold et al., 1992).

Intrinsic tryptophan fluorescence exploits the sensitivity of Trp to the polarity of its environment; in a nonpolar environment the maximum wavelength (λ_{max}) of emission is shifted to shorter wavelengths (e.g., 330 nm), while in a polar environment λ_{max} is shifted to longer wavelengths (e.g., 350 nm). The wild-type IKe phage fluorescence emission spectrum after excitation at 295 nm is illustrated in Figure 5A. This spectrum is similar in shape to that of M13 (Johnson & Hudson, 1989), although λ_{max} is shifted to a longer wavelength in IKe (337 nm) compared to M13 (331 nm). This shift is consistent with the presence of polar residues surrounding Trp 29 in IKe (24-LISQT**W**PVV-32) vs Trp 26 in M13 (21-YIGYA**W**AMV-29). The Trp emission spectra of mutant IKe phages were recorded and the results summarized in Figure 5B. The majority of mutants were similar to wild-type, although values ranged from 330 nm for Gln 27 Leu to 339 nm for Pro 30 Ala. Several trends among the mutants were discerned. Residues immediately adjacent to Trp 29 either resembled wild-type (e.g., Thr 28 Ala) or were shifted to a slightly longer (more polar) wavelength (e.g., Pro 30 Ala, Pro 30 Gly). In contrast, mutations at residues further from Trp 29 in linear sequence, but close in space when the region through Trp 29 is in a helical conformation, shift λ_{max} to shorter wavelengths, indicative of a more nonpolar environment (e.g., single mutants Ser 26 Tyr, Ser 26 Phe, Gln 27 Leu, and double mutant Ser 26 Ala/Val 32 Ile). While some of these changes arise simply from substitution of polar amino acids with nonpolar residues, others do not (e.g., Ile 25 Leu and Val 32 Ile involve changes in volume and/or geometry but nonetheless alter the λ_{max} of Trp 29). This implies that the changes arise from alterations in coat protein packing among adjacent subunits so as to change the environment of Trp 29. Another somewhat surprising example is the STQR (Ser

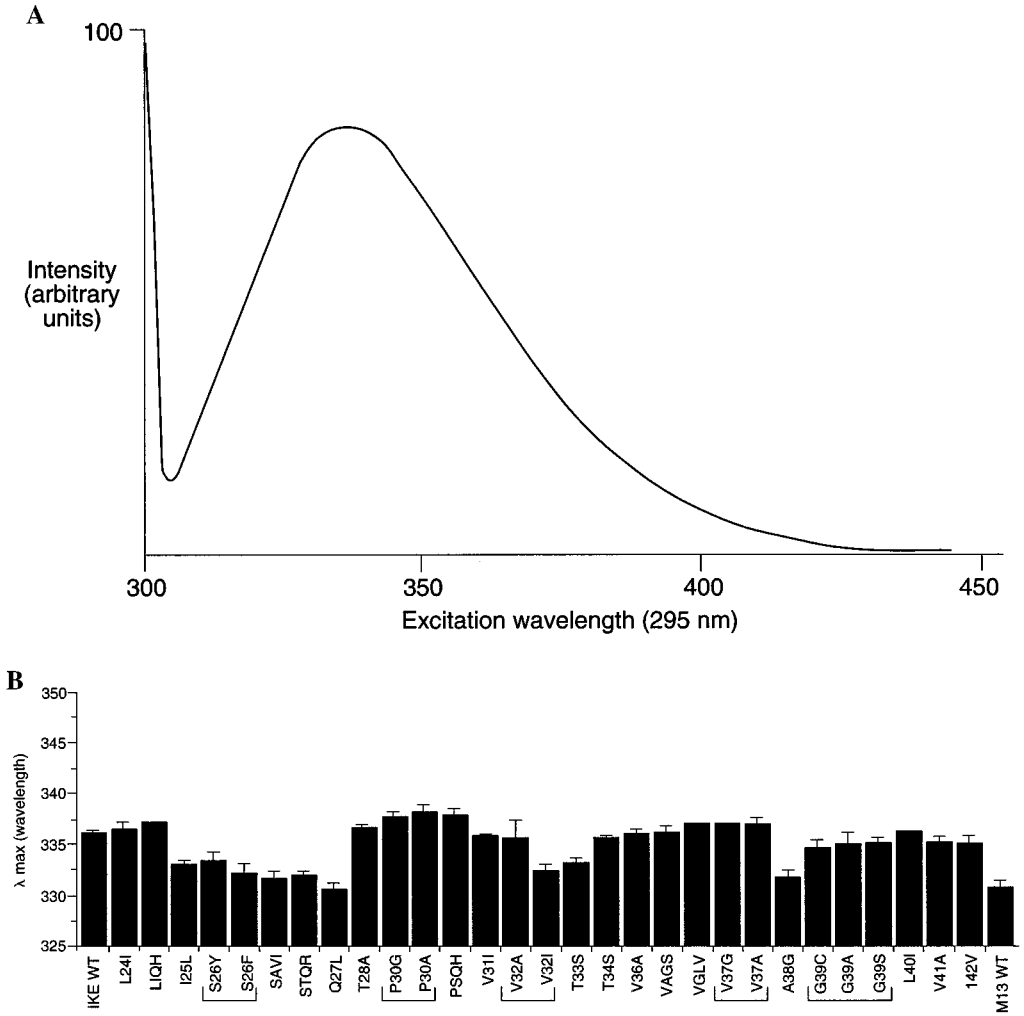

FIGURE 5: (A) Intrinsic tryptophan fluorescence emission spectra of 15 μM wild-type IKe phage in water after excitation at a wavelength of 295 nm at 25 °C. (B) Comparison of wavelength of maximum emission (λ_{max}) after Trp excitation at 295 nm of wild-type IKe (on the left), wild-type M13 (on the right), and the library of IKe TM mutant phage. IKe mutants are listed by residue number from Leu 24 to Ile 42. Residues with two or more mutations are grouped by a square bracket. Double mutants are as follows: Leu 24 Ile/Gln 27 His (LIQH); Ser 26 Ala/Val 32 Ile (SAVI); Ser 26 Thr/Gln 27 Arg (STQR); Pro 30 Ser/Gln 27 His (PSQH); Val 36 Ala/Gly 39 Ser (VAGS); Val 37 Gly/Leu 40 Val (VGLV).

26 Thr/Gln 27 Arg) double mutant which has a more nonpolar λ_{max} than wild-type, even though the mutant introduces the positively charged residue Arg. This may reflect changes in packing or indicate that Trp experiences the nonpolar portion of the Arg side chain, not the charged guanidino group. The only mutant which exerts a change in Trp environment without being within one helical turn of Trp 29 is Ala 38 Gly. The effect of this mutation is transmitted either intramolecularly to the Trp 29 in the protein or intermolecularly through alterations in coat protein packing between subunits.

Wild-Type and Mutant IKe Bacteriophage Structural Stability. To assess the integrity of coat protein interactions in the bacteriophage, the phage was slowly heated to 95 °C and the change in conformation monitored by CD (Figure 6). IKe is more stable structurally than M13 since the latter undergoes an irreversible transition to β-sheet conformation at high temperatures. Another strategy to assess the integrity of coat protein interactions is to examine the ease with which coat protein is extracted from phage DNA upon the addition of a detergent such as DOC (Figures 4 and 7). Extraction is normally carried out upon the addition of chloroform and incubation at 37 °C, but when DOC is added in the absence

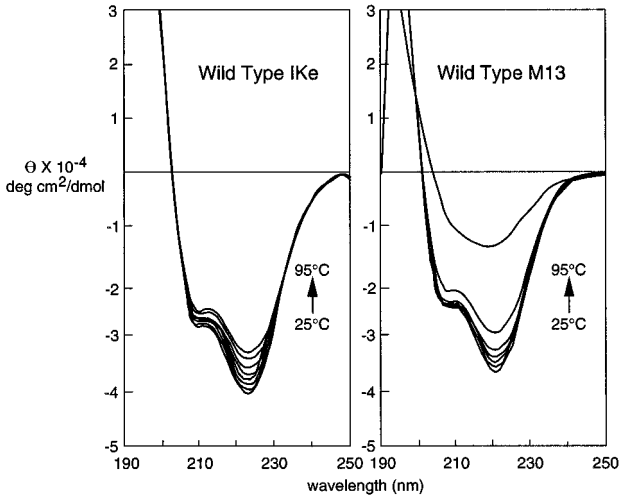

FIGURE 6: Wild-type bacteriophage IKe and M13 conformational changes upon heating detected using CD. Spectra of 0.1 mg/ml phage in TE buffer were recorded at 5 °C intervals from 25 to 95 °C with the spectra plotted at 10 °C intervals.

of chloroform, phage disassembly occurs gradually over time, with the rate enhanced by heating. The DOC-induced

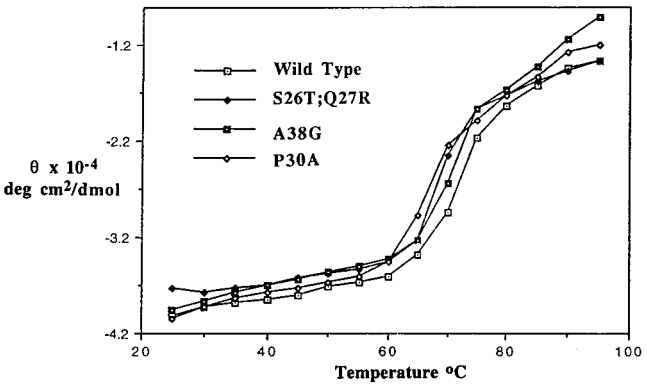

FIGURE 7: Comparison of the DOC-mediated disassembly of 1 mg/mL wild-type IKe and mutant IKe phage (single mutants Pro 30 Ala, Ala 38 Gly, and double mutant Ser 26 Thr/Gln 27 Arg) in TE buffer upon heating from 25 to 95 °C in 5 °C increments. Ellipticity at 222 nm is plotted against temperature. Each curve shown represents the result of a single experiment; duplicate runs typically yielded ellipticity values at each point within ±2%.

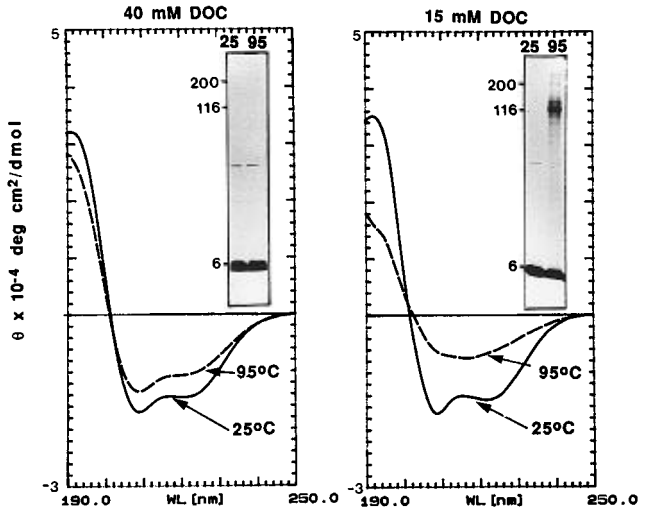

FIGURE 8: Circular dichroism spectra of 1 mg/mL wild-type IKe at 25 °C and at 95 °C in 40 mM DOC (left) and 15 mM DOC (right). Parallel samples were run on an SDS 4−20% gradient Tris-glycine gel at 25 °C.

disassembly of wild-type IKe and Pro 30 Ala is shown in Figure 4. The phage exhibit little change in conformation until around 70 °C when the coat protein becomes solubilized in DOC micelles. Disassembly is cooperative, with the midpoint of transition varying from about 65 to 75 °C for different mutants (Figure 7).

Conformation of Wild-Type and Mutant IKe Coat Protein in DOC Micelles. The conformation of IKe coat protein in DOC micelles is largely helical by CD and occurs as a 6 kDa monomeric species in SDS−PAGE (Figure 8). The CD spectra of IKe coat protein solubilized in a variety of detergents (e.g., SDS, MPG, LPC, or β-OG) and detergent concentrations (e.g., 15 and 40 mM DOC) are indistinguishable. At 40 mM DOC, the coat protein remains stable upon heating to 95 °C, and the slight reduction in helicity which occurs upon heating is fully reversible upon return to 25 °C. In contrast, at lower DOC concentrations, IKe coat protein undergoes an irreversible transition to a β-sheet conformation which exhibits a minimum at 217 nm in its CD spectrum and migrates at >100 kDa on SDS−PAGE. β-Polymerization also occurs over time in the absence of heating and is promoted by high protein concentration. Therefore, the stability and β-polymerization of IKe coat protein can be modulated by adjusting the protein-to-detergent (lipid) ratio.

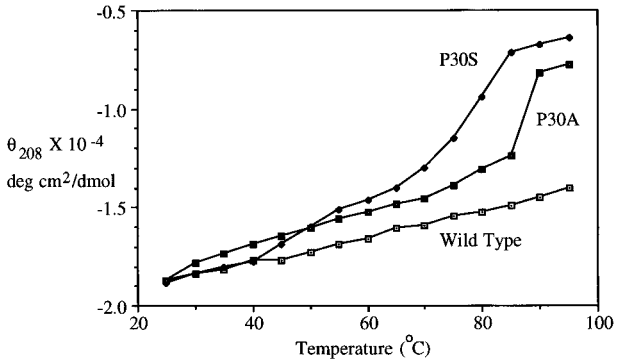

FIGURE 9: Thermal transition of 1 mg/mL wild-type, Pro 30 Ala, and Pro 30 Ser mutant IKe coat proteins in 40 mM DOC micelles at pH 9.0 obtained upon heating the sample from 25 to 95 °C. The change in ellipticity at 208 nm is plotted against temperature. Each curve shown represents the result of a single experiment; duplicate runs typically yielded ellipticity values at each point within ±2%.

The conformations of IKe coat protein mutants at 25 °C were generally similar to that of wild-type on the basis of CD (not shown). The stability of IKe mutants was also similar to that of wild-type, with the exception of the Pro 30 mutants which underwent an irreversible transition to a high molecular weight β-sheet species upon heating at a DOC concentration which prevents this transition in wild-type IKe (Williams & Deber, 1993). To examine this thermal transition in more detail, the change in ellipticity at 208 nm is plotted against temperature for wild-type and two Pro 30 mutants in Figure 9. Wild-type exhibits a gradual increase in ellipticity, which is fully reversible upon return to room temperature, while the Pro 30 mutants undergo an irreversible transition to β-sheet conformation between 80 and 90 °C, with Pro 30 Ser appearing somewhat less stable than Pro 30 Ala. Even though Pro is generally considered to be a helix destabilizing residue, these results show that the wild-type coat protein in micelles is in fact stabilized at high temperatures by the presence of Pro 30.

Another strategy to assess the impact of mutations on the conformation of the micelle-solubilized coat protein is to employ intrinsic Trp fluorescence to monitor changes in the Trp 29 environment. The Trp emission spectrum of wild-type IKe coat protein in DOC micelles exhibits a broad peak, similar in shape to that of M13 coat protein, but with the IKe λ_{max} dramatically shifted to 344 nm, in contrast to 332 nm in M13 coat protein (Figure 10). This demonstrates that Trp 29 in IKe coat protein experiences a far more polar environment than Trp 26 in M13. The Trp 29 λ_{max} of emission was similar among the IKe wild-type and mutant coat proteins in DOC micelles, with the exception of the Pro 30 mutants (Figure 10). Pro 30 Gly and Pro 30 Ala exhibit a λ_{max} (ca. 338 nm) shifted to a slightly more nonpolar environment than wild-type IKe but still more polar than Trp 26 in M13. The shift in Trp 29 fluorescence by Pro 30 mutants does not appear to reflect a change in the polarity of the sequence surrounding Trp 29 since the mutations to Ala and Gly do not dramatically alter polarity, and the other Trp 29 adjacent mutant, Thr 28 Ala, does not impact Trp fluorescence. None of the mutants which render the Trp 29 environment more hydrophobic in the virion (e.g., Gln 27 Leu, Ala 38 Gly, see above) affect the λ_{max} of coat protein solubilized in micelles.

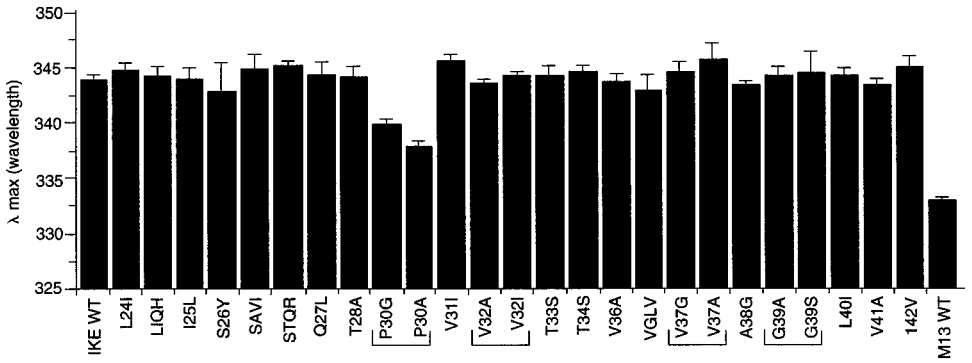

FIGURE 10: Comparison of the maximum wavelength of tryptophan fluorescence emission (λ_{max}) of 60 μM coat protein in 25 mM DOC after excitation at 295 nm at 25 °C. IKe wild-type is on the left (344 nm); M13 wild-type is on the right (332 nm). IKe mutants listed sequentially by residue number. Residues with two or more mutants are grouped with a square bracket. See the legend to Figure 4 for identification of double mutants.

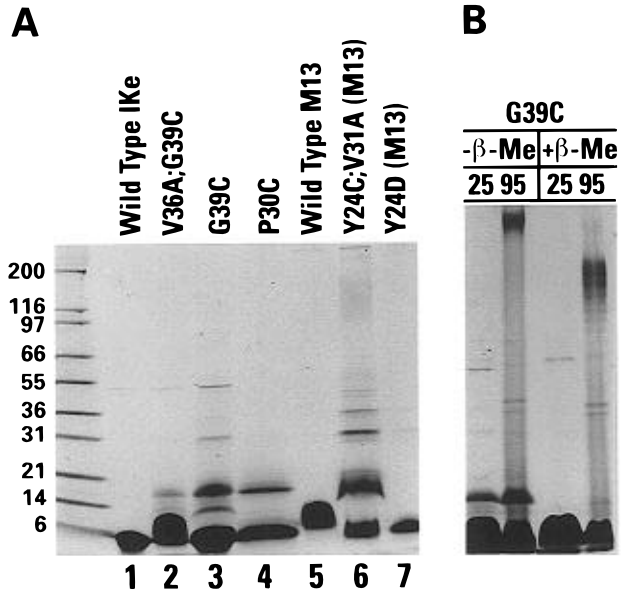

FIGURE 11: (A) Comparison of mobility of wild-type and Cys-mutant (Pro 30 Cys, Gly 39 Cys/Val 36 Ala, Gly 39 Cys) IKe coat protein, together with wild-type M13, the disulfide-bonded M13 double mutant Tyr 24 Cys/Val 31 Ala, and the monomeric M13 mutant Tyr 24 Asp. In each lane, 5−10 μg of protein was loaded in a nonreducing SDS 4−20% gradient Tris-glycine polyacrylamide gel and stained with Coomassie blue. (B) Reduction of IKe Gly 39 Cys covalent dimer with β-mercaptoethanol.

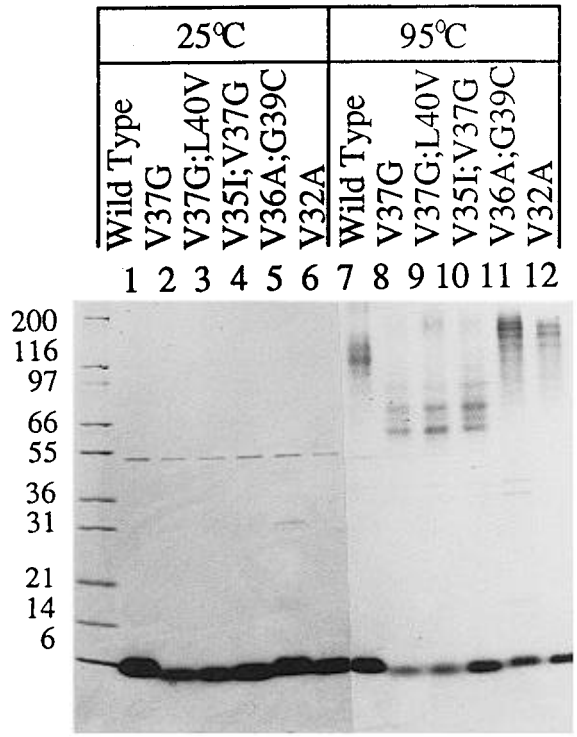

FIGURE 12: Comparison of mobility of wild-type and mutant IKe coat proteins in 15 mM DOC at 25 °C (lanes 1−6) and after heating to 95 °C (lanes 7−12). Samples of 5−10 μg of protein were loaded in a 4−20% gradient Tris-glycine polyacrylamide gel at 25 °C and stained with Coomassie blue.

Oligomerization of Wild-Type and Mutant IKe Coat Protein in DOC Micelles. The majority of IKe coat protein mutants migrate as monomeric species on SDS−PAGE with mobility similar to that of wild-type. Four mutants, Gly 39 Ala, Gly 39 Cys, Pro 30 Ala, and Val 36 Ile, migrated more slowly than wild-type in SDS−PAGE, while two mutants, Val 32 Ala and Val 41 Ala, migrated farther than wild-type (not shown). While these differences in mobility may simply reflect altered interactions of the mutant coat protein with SDS, the mutants with slower mobility may conceivably represent a combination of monomeric and dimeric species. The three Cys-containing mutants, Pro 30 Cys, Gly 39 Cys, and Val 36 Ala/Gly 39 Cys, all exhibited a small amount of covalent (disulfide) dimer together with the monomeric species (Figure 11A). The disulfide-linked dimer was susceptible to reduction with β-mercaptoethanol to produce the monomeric species (Figure 11B) [see also Khan et al. (1995)].

Wild-type and mutant IKe coat proteins produce a high molecular weight β-sheet species (ca. 120 kDa) upon heating at high temperatures (95 °C) (Figure 12). The mobility of the high molecular weight species differed for the mutants containing Val 37 Gly (including the single mutant Val 37 Gly and the double mutants Val 37 Gly/Leu 40 Val and Val 37 Gly/Val 35 Ile), since three main bands between 60 and 90 kDa were observed after heating to high temperatures. This suggests that the replacement of Val 37 with Gly promotes the formation of discrete high molecular weight complexes which differ from that of wild-type and supports the notion that this mode of oligomerization is sequence-dependent.

Comparison of IKe Mutants in MPG Micelles by Solution NMR. The ^{1}H, ^{15}N-HSQC spectra of the mutants Pro 30 Ala, Pro 30 Gly, and Val 37 Gly (Figure 13) are similar to that of the wild-type (Williams et al., 1996). The assignment

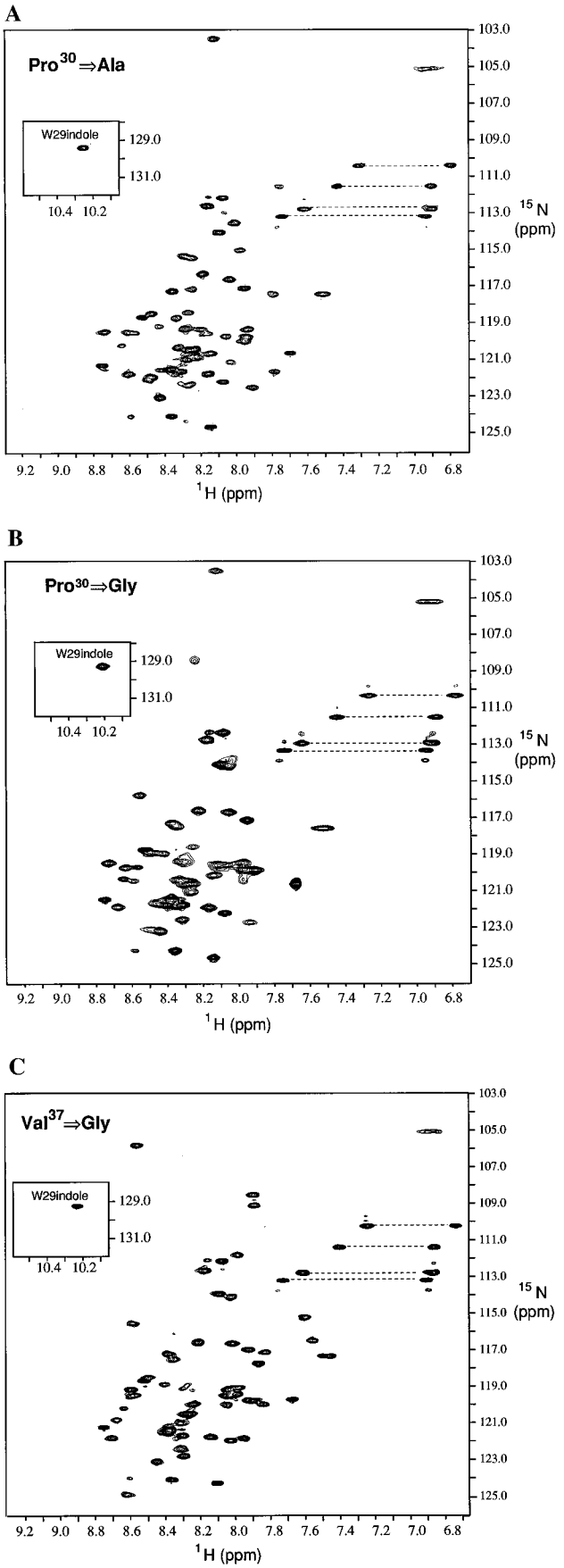

FIGURE 13: Comparison of $^1H-^{15}N$ HSQC spectra of ^{15}N-labeled mutant IKe coat protein in 400 mM fully protonated ^1H-MPG in H_2O at pH 4.5, 45 °C. Spectra are: (a) Pro 30 Ala, (b) Pro 30 Gly, and (c) Val 37 Gly.

of the Pro 30 Gly HSQC using a 3D ^{15}N-NOESY-HSQC revealed that the peaks corresponding to the amides of Ile 25, Gln 27, Thr 28, Trp 29, Val 31, Val 37, and Ala 38 were not observed, while the peaks corresponding to Ser 26 and Val 53 were shifted slightly in both ^1H and ^{15}N dimensions (Figure 13B). The occurrence of chemical shift changes in residues situated some distance away from the mutated amino acid indicates that the effect of the mutation is transmitted through the protein. The peak corresponding to the Gly 30 amide in the Pro 30 Gly mutant was easily identified at 108.5 ppm in the ^{15}N dimension and 8.2 ppm in the ^1H dimension. The two Pro 30 mutants, Pro 30 Gly and Pro 30 Ala, exhibited generally similar changes in the HSQC compared to that of wild-type and may indicate that the effect of substitution at Pro 30 by these residues was similar regardless of whether the mutant residue was Ala or Gly. The HSQC spectra of the Val 37 Gly mutant differed more dramatically from that of wild-type (Figure 13C). The Val 37 Gly single and double mutants were the only mutants which exhibited several discrete 60−90 kDa species upon heating, although the CD and fluorescence properties of these mutants were similar to wild-type. Solution NMR, however, indicates that the introduction of Gly at Val 37 results in a substantial change in the environment of amides throughout the protein. In the absence of a high-resolution structure, it is not possible to assess whether the changes in chemical shift are manifested as alterations in the structure, dynamics, or orientation of the coat protein with respect to the micelle.

DISCUSSION

The primary strategy we employed to characterize IKe coat protein in both the membrane-bound and virion forms was to establish a library of major coat protein mutants for comparison to wild-type (Williams & Deber, 1993). The region of IKe coat protein targeted for mutagenesis (Leu 24 to Ile 42) was selected to facilitate comparison to the same region in M13 (Tyr 21 to Ile 39), which had previously been subjected to randomized mutagenesis (Li & Deber, 1991). The occurrence of one or more mutations at every residue between Leu 24 and Ile 42, with the exception of Trp 29, demonstrates the ability of many different sequences to meet the structural and functional requirements of the coat protein packing in the virion, as well as throughout the phage life cycle. This is consistent with the major coat protein sequence variability observed among the filamentous phage (e.g., M13, Pf1, Pf3, and Xf), which nonetheless pack to form very similar structures (Kostrikis et al., 1995).

Wild-Type and Mutant Bacteriophage IKe. Wild-type bacteriophage IKe was found to be more stable structurally than M13 since the latter undergoes an irreversible transition to β-sheet conformation at high temperatures (Figures 4 and 6). This may reflect the difference in hydrophobicity between the IKe and M13 coat proteins: M13 coat protein is markedly more nonpolar than IKe, especially in the vicinity of the conserved Trp residue (i.e., 21-YIGYAWAMVVV −31 in M13 vs 24-LISQTWPVVTT-34 in IKe). Coat protein hydrophobicity promotes aggregation at high temperatures since the coat protein becomes more exposed to the aqueous environment. Structural stability does not correlate with phage viability, however, since bacteriophage IKe is inactivated more readily by exposure to high temperatures than M13 (Khatoon et al., 1972) and is readily digested by trypsin and pronase compared to M13 (Schwind et al., 1992; Khatoon et al., 1972). As well, IKe displays

more fastidious growth requirements than M13 since it is unable to grow in the common minimal media M9 or M63.

The Pro 30 locus appears subject to steric constraint since substitution at this position was limited to the four smallest residues (Ala, Gly, Ser, and Cys), even though all 20 amino acids were accessible using saturation mutagenesis. The Pro 30 mutants were less viable than wild-type IKe (Figure 3), although it is not clear whether this stems from the properties of the coat protein while in the bacterial inner membrane, while in the virion, or during some other part of the phage life cycle. When the mutants obtained at Pro 30 are compared with those isolated at the same position in M13 (Ala 27 Glu, Ala 27 Ser, and Ala 27 Thr), only Ala and Ser are found to occur in both proteins. It is striking that Thr was unable to replace Pro 30 in IKe since the latter residue could readily result from a single base change at the Pro codon. As well, Glu could also have arisen from saturation mutagenesis. Attempts to mutagenize Ala 27 to Pro in M13 coat protein have proven unsuccessful (Marvin et al., 1994). These results suggest that although the Pro 30/Ala 27 sites are homologous, the differences between the proteins (i.e., the residues which are not identical) are important in determining the nature of residues which can occur. This implies that the sequence differences between IKe and M13 are, in part, cooperative in nature, being dependent upon local amino acid environment.

The conformations of IKe wild-type and mutant phages were shown to be similar by CD. Intrinsic Trp fluorescence revealed that the polarity of the Trp 29 environment can be modulated by both the hydrophobicity of residues within one helical turn of Trp 29 (e.g., Ser 26 Phe) and/or subtle changes in the side chain volumes which modulate packing interactions among adjacent coat protein subunits (e.g., the shift of Trp 29 to a more nonpolar environment in the Ala 38 Gly mutant). The helical periodicity of mutations affecting Trp 29 fluorescence suggests that Pro 30 is in a helical conformation in the phage, despite the potentially helix-destabilizing properties of this residue. Trp 29 is conserved among the class 1 filamentous bacteriophage and likely serves an important role either as an interfacial residue at the membrane surface (see below) or in mediating protein−protein and/or protein−DNA interactions in the phage.

IKe coat protein residues Val 37, Ala 38, and Gly 39 predominantly gave rise to small residue substitutions (i.e., Ala, Gly, and Ser). Similarly, M13 coat protein residues Gly 34, Ala 35, and Gly 38 were also limited to small residue replacements (Li & Deber, 1991). When the entire M13 coat protein was subjected to a combination of randomized and saturation mutagenesis, a series of small (Ala 7, Ala 10, Ala 18) and hydrophobic (Phe 11 and Leu 14) residues in the N-terminal amphipathic helix either resisted mutation or gave rise to small residue substitutions (Williams et al., 1995). A model in which the small residue rich faces of both the TM and N-terminal amphipathic helices mediate oligomerization between adjacent coat protein subunits in the virion was proposed, where each subunit participates in two principal interactions—layer and axial—which are stabilized by a combination of small and/or hydrophobic amino acids (Williams et al., 1995; Marvin et al., 1994). This mode of small residue-mediated oligomerization is analogous to the TM helix oligomerization observed for M13 coat protein (Deber et al., 1993) and glycophorin A (Lemmon et al., 1994). IKe coat protein also exhibits a small and hydrophobic residue-rich face (Ala 10, Ala 13, Met 14, Leu 17, Ala 20) of the N-terminal amphipathic helix and is expected to participate in similar helix−helix interactions in the virion.

Wild-Type and Mutant IKe Coat Protein in Micelles. Detergent-solubilized IKe coat protein is markedly more thermostable than M13 coat protein as shown by the retention of helical conformation to >95 °C in 40 mM DOC (Figure 8), in contrast to the irreversible transition of M13 coat protein to a β-sheet conformation at ca. 76 °C (Li et al., 1993). As with the thermal stability of the virion, the stability of IKe coat protein in micelles is due, in part, to its polarity since this makes it less prone to aggregation when the micelle is disrupted at high temperatures. Although the formation of an aggregated species from detergent-solubilized coat protein is likely driven by protein hydrophobicity, the formation of high molecular weight β-sheet additionally depends upon the formation of intersubunit hydrogen bonds, together with the appropriate backbone dihedral angles. The enrichment of β-substituted residues, such as in the TM segment (e.g., ^{31}VVTTVVV^{37}) in IKe, favors β-sheet formation in soluble proteins (Chou & Fasman, 1978). The mechanism of β-polymerization is poorly understood. Electrostatic interactions may play a role in aligning coat proteins in a head-to-tail orientation (Spruijt & Hemminga, 1991), although it is not known whether the β-strands are parallel or anti-parallel.

The environment of IKe Trp 29 in DOC micelles is substantially more polar (λ_{max} of 344 nm) than when the coat protein is packaged in the IKe virion (λ_{max} of 336 nm), whereas in M13 the environment of Trp 26 is similar in both the membrane-bound and virion forms (λ_{max} of 332 nm) (Figures 5 and 10). Trp is enriched at the membrane−aqueous interface of membrane proteins (Schiffer et al., 1992; Landolt-Marticorena et al., 1993) and may play a role in protein translocation across the bilayer (Schiffer et al., 1992). The partitioning of Trp analogs between water and cyclohexane experimentally verified the preferential localization of Trp at the membrane interface (Wimley & White, 1992; Jacobs & White, 1989). Trp is ideally suited as an interfacial residue by virtue of the large nonpolar indole ring which favors the hydrophobic acyl chain environment and the polar indole NH which can hydrogen-bond to lipid carbonyls or other polar groups in the lipid head group region (Schiffer et al., 1992; Woolf & Roux, 1994). M13 coat protein Trp 26 has been shown to bind to lipid head groups (Johnson & Hudson, 1989) and is immobile within the bilayer (Leo et al., 1987).

Pro 30 was the only locus in IKe coat protein in which the mutant coat proteins solubilized in micelles displayed decreased thermostability (Figure 9), together with a slightly more nonpolar Trp 29 environment (Figure 10). It is striking that the replacement of Pro by Ala leads to a less thermostable protein, since Pro is generally considered to be destabilizing in the context of an α-helix. These results support the suggestion that Pro 30 protects against the transition to β-conformation in the context of a hydrophobic environment (Li et al., 1996). Solution NMR studies suggest that Pro 30 occurs at the Ncap+1 position of the IKe TM helix (Williams et al., 1996). The replacement of Pro by Ala and Gly resulted in numerous amide proton chemical shift changes between residues Ile 25 and Ala 38 (Figure 13), indicating that the effect of this substitution is propagated some distance through the protein. The position of Pro in a

helix is clearly crucial in determining the effect of this residue on helix stability; Pro can indeed be quite favorable in the first turn of a helix due, in part, to the fact that the two hydrogen bonds lost when Pro occurs in the center of an α-helix are not specifically required in the first turn of a helix (Williams & Deber, 1991). Pro is enriched in the first turn of helices in soluble proteins (Richardson & Richardson, 1988) as well as in the N-terminal region flanking the TM helices of single-spanning membrane proteins (Landolt-Marticorena et al., 1993).

IKe coat protein and all of the IKe mutants (except those containing Cys) were monomeric in detergent micelles, whereas M13 coat protein occurs as a monomer and/or dimer and higher oligomers depending upon temperature and detergent concentration (Spruijt & Hemminga, 1991). The propensity of M13 coat protein to dimerize can be eliminated by the introduction of a single polar amino acid in the TM region (Li et al., 1993), since, in the initial library of M13 TM mutants (Li & Deber, 1991), all but one polar substitution gave rise to monomeric species or to monomer with only a small amount of dimer. It is not surprising, therefore, that IKe coat protein remains monomeric since it contains a number of polar residues in the TM segment. The disulfide-linked dimers of IKe Cys mutants (Pro 30 Cys, Gly 39 Cys, and Val 36 Ala/Gly 39 Cys) migrate as compact bands corresponding to a mobility of ca. 15 kDa, somewhat larger than expected for an IKe dimer on an SDS gel. In contrast, noncovalent M13 dimers [e.g., Val 31 Ala (Deber et al., 1993)] migrate as a broad band centered at ca. 12 kDa. The marked difference in mobility between the two types of dimers may reflect a difference in the interactions of the dimer with SDS (i.e., the noncovalent dimer binding more SDS and therefore migrating further than the disulfide-linked dimer) or in the orientation between the two helices (the noncovalent dimers being antiparallel and/or parallel vs the disulfide-linked dimers being locked in a parallel configuration).

Implications for Filamentous Bacteriophage Assembly. Phage assembly is a transient process which incorporates the two stable conformations of the coat protein, the membrane-bound "assembly intermediate" and the virion form. These two forms occur in very distinct environments, the lipid bilayer and the virion, respectively. The transition between the two involves a change in both the interactions which stabilize the coat protein and the conformation of the coat protein. As a result, conformational flexibility between the two forms must be encoded in the sequence and is likely manifested in part as the conformational heterogeneity which was observed in NMR studies of wild-type IKe coat protein (Williams et al., 1996). The nature of the interactions in these two environments differs considerably; however, both reflect successful strategies to sequester the hydrophobic regions of the coat protein from the aqueous milieu. The coat protein TM domain interacts with the hydrophobic acyl chains in the membrane-bound form (Williams et al., 1996), while, in the virion form, the TM domain contacts the predominantly hydrophobic residues of adjacent coat protein subunits (Williams et al., 1995; Marvin et al., 1994). The principal difference, therefore, lies in the specificity of hydrophobic interactions: the virion form is stabilized by specific amino acid contacts, whereas the TM domain in the micelle experiences far fewer specific interactions due to the mobility of the acyl chains. Similarly, in the virion, the hydrophobic face of the N-terminal amphipathic helix interacts specifically with nonpolar residues of adjacent subunits (Williams et al., 1995; Marvin et al., 1994), while in the membrane-bound form this segment is situated at the interface of the micelle (Williams et al., 1996). The two conformational states of phage coat protein also differ in the nature of electrostatic interactions at the positively charged C-terminal side chains Arg and Lys. In the virion, these residues participate in electrostatic interactions with the negatively charged phosphates of the phage DNA, while in the membrane-bound form these residues interact with the acidic lysolipid head groups in the micelle (Williams et al., 1996). The overall interactions between IKe coat protein and lipids in the membrane-bound form, and between coat protein subunits and DNA in the virion, therefore reflect two alternative, thermodynamically favorable methods of satisfying the requirements of the coat protein.

Summary. The overall hydrophobicity of phage major coat proteins plays a central role in modulating their stability and oligomerization in both the virion and membrane-bound forms. Whereas IKe coat protein solubilized in micelles is monomeric and thermostable under a wide range of detergents, detergent concentrations, and temperatures, the more hydrophobic M13 coat protein tends to dimerize and is markedly less thermostable. These studies highlight the importance of Pro 30 in maintaining phage viability as well as in modulating the stability of the detergent-solubilized coat protein. Solution NMR studies of the wild-type IKe coat protein in MPG micelles suggest that Pro 30 occurs in the first turn of the TM helix at the Ncap+1 position (Williams et al., 1996). The CD, fluorescence, and NMR studies of the Pro 30 mutants detailed herein demonstrate that Pro is indeed favorable at this position and likely reflects the specific steric and hydrogen-bonding schemes imposed by this residue.

ACKNOWLEDGMENT

We thank Professor Lewis Kay for assistance with NMR experiments and Carolin Joensson for assistance with IKe mutant protein preparations.

REFERENCES

Arnold, G. E., Day, L. A., & Dunker, A. K. (1992) *Biochemistry 31*, 7948−7956.

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

Aubrey, K. L., & Thomas, G. J. (1991) *Biophys. J. 60*, 1337−1349.

Azpiazu, I., Gomez-Fernandez, J. C., & Chapman, D. (1993) *Biochemistry 32*, 10720−10726.

Bodenhausen, G., & Ruben, D. H. (1980) *Chem. Phys. Lett. 69*, 185−189.

Cavanagh, H., Palmer, A. G., Wright, P. E., & Rance, M. (1991) *J. Magn. Reson. 91*, 429−436.

Cesareni, G. (1992) *FEBS Lett. 307*, 66−70.

Chou, P. Y., & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol. 47*, 45−148.

Clack, B. A., & Gray, D. M. (1989) *Biopolymers 28*, 1861−1873.

Clack, B. A., & Gray, D. M. (1992) *Biopolymers 32*, 795−810.

Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry 26*, 847−854.

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

Deber, C. M., Khan, A. R., Li, Z., Joensson, C., Glibowicka, M., & Wang, J. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 11648−11652.
Delaglio, F. (1993) NMRPipe System of Software, Lab of Chemical Physics, NIDDK, NIH.
Dunker, A. K., Ensign, L. D., Arnold, G. E., & Roberts, L. M. (1991a) *FEBS Lett. 292*, 271−274.
Eftink, M. R., & Ghiron, C. A. (1977) *Biochemistry 16*, 5546−5551.
Gall, C. M., Cross, T. A., DiVerdi, J. A., & Opella, S. J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 101−105.
Garrett, D. S., Powers, R., Gronenborn, A. M., & Clore, G. M. (1991) *J. Magn. Reson. 95*, 214−220.
Glucksman, M. J., Bhattacharjee, S., & Makowski, L. (1992) *J. Mol. Biol. 226*, 455−470.
Greenwood, J., & Perham, R. (1989) *Virology 171*, 444−452.
Greenwood, J., Hunter, G. J., & Perham, R. N. (1991) *J. Mol. Biol. 217*, 223−227.
Harper, E. T., & Rose, G. D. (1993) *Biochemistry 32*, 7605−7609.
Hemminga, M. A., Sanders, J. C., & Spruijt, R. B. (1992) *Prog. Lipid Res. 31*, 301−333.
Henry, G. D., & Sykes, B. D. (1990) *Biochem. Cell Biol. 68*, 318−329.
Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284−5297.
Hunter, G. J., Rowitch, D. H., & Perham, R. N. (1987) *Nature* 327, 252−254.
Jacobs, R. E., & White, S. E. (1989)*Biochemistry 28*, 3421−3437.
Johnson, I. D., & Hudson, B. S. (1989) *Biochemistry 28*, 6392−6400.
Kay, L. E., Keifer, P., & Saarinen, T. (1992) *J. Am. Chem. Soc. 114*, 10663−10665.
Kay, L. E., Xu, G. Y., & Yamazaki, T. (1994) *J. Magn. Reson. Series A* 109, 129−133.
Khatoon, H., Iyer, R. V., & Iyer, V. N. (1972) *Virology 48*, 145−155.
Khan, A. R., Williams, K. A., Boggs, J., & Deber, C. M. (1995) *Biochemistry 34*, 12388−12397.
Kostrikis, L. G., Liu, D. J., & Day, L. A. (1994) *Biochemistry 33*, 1694−1703.
Landolt-Marticorena, C., Williams, K. A., Deber, C. M., & Reithmeier, R. A. F. (1993) *J. Mol. Biol. 229*, 602−608.
Lemmon, M. A., Treutlein, H. R., Adams, P. D., Brunger, A. T., & Engelman, D. M. (1994) *Nature Sruct. Biol. 1*, 157−163.
Leo, G. C., Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry 26*, 854−862.
Li, S.-C., Goto, N., Williams, K. A., & Deber, C. M. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 6676−6681.
Li, Z., & Deber, C. M. (1991) *Biochem. Biophys. Res. Commun. 180*, 687−693.
Li, Z., Glibowicka, M., Joensson, C., & Deber, C. M. (1993) *J. Biol. Chem. 268*, 4584−4587.
Liu, D. J., & Day, L. A. (1994) *Science 265*, 671−674.
Makino, S., Woolford, J. L., Tanford, C., & Webster, R. E. (1975) *J. Biol. Chem. 250*, 4327−4332.
Makowski, L. (1993) *Gene 128*, 5−11.
Marvin, D. A., & Wachtel, E. J. (1975) *Nature 253*, 19−23.
Marvin, D. A., Wiseman, R. L., & Watchel, E. J. (1974) *J. Mol. Biol. 82*, 121.
Marvin, D. A., Hale, R. D., Nave, C., & Citterich, M. H. (1994) *J. Mol. Biol. 235*, 260−286.
McDonnell, P. A., Shon, K., Kim, Y., & Opella, S. J. (1993) *J. Mol. Biol. 233*, 447−463.
Muhandiram, D. R., & Kay, L. E. (1994) *J. Magn. Reson., Ser. B. 103*, 203−216.
Nakashima, Y., Frangione, B., Wiseman, R. L., & Konigsberg, W. H. (1981) *J. Biol. Chem. 256*, 5792−5797.
Nambudripad, R., Stark, W., & Makowski, L. (1991) *J. Mol. Biol. 220*, 359−379.
Nozaki, Y., Chamberlain, B. K., Webster, R. E., & Tanford, C. (1976) *Nature 259*, 335−337.
Nozaki, Y., Reynolds, J. A., & Tanford, C. (1978) *Biochemistry 17*, 1239−1246.
Overman, S. A., Aubrey, K. L., Vispo, N. S., Cesareni, G., & Thomas, G. J. (1994) *Biochemistry 33*, 1037−1042.
Palmer, A. G., Cavanagh, J., Wright, P. E., & Rance, M. (1991) *J. Magn. Reson. 93*, 151.
Peeters, B. P. H., Peters, R. M., Schoenmakers, J. G. G., & Konings, R. N. H. (1985) *J. Mol. Biol. 181*, 27−39.
Russell, M., & Model, P. (1988) *The Bacteriophages* (Calendar, R., Ed.) Vol. 2, pp 375−456, Plenum Press, New York.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sanders, J. C., Poile, T. W., Spruijt, R. B., Van Nuland, N. A. J., Watts, A., & Hemminga, M. A. (1991) *Biochim. Biophys. Acta 1066*, 102−108.
Schiffer, M., Chang, C.-H., & Stevens, F. J. (1992) *Protein Eng. 3*, 213−214.
Schwind, P., Kramer, H., Kremser, A., Ramsberger, Q., & Rasched, I. (1992) *Eur. J. Biochem. 210*, 431−436.
Scott, J. K., & Smith, G. P. (1990) *Science 249*, 386−390.
Shaka, A. J., Keeler, J., Frenkiel, T., & Freeman, R. (1983) *J. Magn. Reson. 52*, 335−338.
Smilowitz, H. (1974) *J. Virol. 13*, 94−99.
Smilowitz, H., Carson, J., & Robbins, P. W. (1972) *J. Supramol. Struct. 1*, 8−18.
Spruijt, R. B., & Hemminga, M. A. (1991) *Biochemistry 30*, 11147−11154.
Thatte, V., Bradley, D. E., & Iyer, V. N. (1985) *J. Bacteriol. 163*, 1229−1236.
Thomas. G. J., Prescott, B., & Day, L. A. (1983) *J. Biol. Chem. 165*, 321−356.
van de Ven, F. J. M., van Os, J. W. M., Aelen, J. M. A., Wymenga, S. S., Remerosski, M. L., Konings, R. N. H., & Hilbers, C. W. (1993) *Biochemistry 32*, 8322−8328.
Webster, R. E., & Lopez, J. (1985) in *Virus Structure and Assembly* (Casjens, S., Ed.) pp 235−267, Jones and Bartlett Publishers, Inc., Boston.
Wickner, W. (1988) *Biochemistry 27*, 1081−1086.
Williams, K. A., & Deber, C. M. (1991) *Biochemistry 30*, 8920−8923.
Williams, K. A., & Deber, C. M. (1993) *Biochem. Biophys. Res. Commun. 196*, 1−6.
Williams, K. A., Glibowicka, M., Li, Z., Li, H., Khan, A. R.,Wang, J., Chen, Y. M.-Y., Marvin, D. A., & Deber, C. M. (1995) *J. Mol. Biol. 252*, 6−14.
Williams, K. A., Farrow, N., Deber, C. M., & Kay, L. E. (1996) *Biochemistry 35*, 5145−5157.
Williams, R. W., & Dunker, A. K. (1977) *J. Biol. Chem. 252*, 6253−6255.
Wimley, W. C., & White, S. H. (1993) *Biochemistry 32*, 6307−6312.
Woolf, T. B., & Roux, B. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 11631−11635.
Zhang, O., Kay, L. E. Olivier, J. B., & Forman-Kay, J. D. (1994) *J. Biomol. NMR 4*, 845−858.

BI960578E