Reconstitution of the M13 Major Coat Protein and Its Transmembrane Peptide Segment on a DNA Template†

Weijun Li,‡,§ Itai Suez,‖ and Francis C. Szoka, Jr.*,‡

Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry, School of Pharmacy, University of California at San Francisco, San Francisco, California 94143-0446, and Department of Chemistry, University of California at Berkeley, Berkeley, California 94720-1460

Received January 25, 2007; Revised Manuscript Received May 2, 2007

ABSTRACT: The major coat protein (pVIII) of M13 phage is of particular interest to structure biologists since it functions in two different environments: during assembly and infection, it interacts with the bacterial lipid bilayer, but in the phage particle, it exists as a protein capsid to protect a closed circular, single-stranded DNA (ssDNA) genome. We synthesized pVIII and a 32mer peptide consisting of the transmembrane and DNA binding domains of pVIII. The 32mer peptide displays typically an α-helical structure in trifluroethanol or 0.2 M octylglucoside solutions similar to pVIII. Attachment of polyethylene glycol (PEG) onto the N-terminal of 32mer increased the α-helical content and the peptide thermal stability. The peptides were reconstituted with DNA from a detergent solution into a discrete (<200 nm diameter) nanoparticle on both linear double-stranded DNA (dsDNA) and linear ssDNA, where the linear dsDNA is used to mimic the closed circular, ssDNA in M13 phage, upon removal of the detergent. The peptide/DNA particle was an irregular and not a rod-shaped aggregate when imaged by atomic force microscopy. All three peptides underwent a structural transition from α-helix to *β*-sheet within ∼1 h of DNA addition to the detergent solution. There was a further decrease in α-helical content when the detergent was removed. The presence of anionic (such as octanoic acid) or cationic (such as 1,5-diaminopentane) molecules in the detergent mixture resulted in the retention of the peptide α-helical structure. Thus the interaction between the peptide and DNA in octylglucoside is driven by electrostatic forces, and peptide−peptide interactions are responsible for the transition from α-helix to *β*-sheet conformation in pVIII and its analogues. These results suggest that the assembly process to form a rod-shaped phage is a delicate balance to maintain pVIII in an α-helical conformation that requires either an oriented bilayer to solubilize pVIII prior to interaction with the DNA or other phage proteins to nucleate pVIII in the α-helical conformation on the DNA.

The M13 phage is a rod-like particle (1 *μ*m length × 10 nm diameter) containing a closed circular ssDNA[1] genome (6407 base) which is coated by ∼ 2700 copies of the phage major coat protein, pVIII, along the long axis of the virus (*1*). Each end of the phage cylinder is capped by five copies of the products of gene III, VI or VII, IX. The 50-residual M13 pVIII has segments that interact with bilayer membrane, DNA, and adjacent pVIIII. In the mature phage particle, pVIII roughly parallels the axis of DNA genome with its C-terminal domain binding to DNA. When M13 phage infects *Escherichia coli*, the pVIII capsid diffuses into the *E. coli* cell membrane and the DNA genome is introduced into the host cytoplasm. The phage structure, life cycle, and its assembly *in vivo* have been extensively reviewed (*2*−*4*).

In spite of the important biological functions of the M13 pVIII, little is known concerning how this capsid coat protein self-assembles with DNA in a cell-free system. The first successful study on virus reconstitution *in vitro* was accomplished with an RNA virus, tobacco mosaic virus (TMV) (*5*), over four decades ago. Many subsequent attempts to reconstitute other rod-shaped viruses have encountered difficulties (*6*). For the *in vitro* reconstitution of a DNA phage, a pioneering work was published by Knippers and Hoffmann-Berling (*7*), where the pVIII of fd phage (almost identical to M13 phage except that on position 12 pVIII of phage fd has an Asp, whereas pVIII of M13 has an Asn) formed particles with single-stranded phage DNA, but not with dsDNA in this work. Urea was used to solubilize the fd phage coat proteins. Reconstitution of the particle occurred upon removal of the urea by dialysis. The DNA was observed

† This work was supported by NIH EB 003008.

* Corresponding author. Mailing address: Department of Biopharmaceutical Sciences, HSE 1145, 513 Parnassus Avenue, University of California at San Francisco, San Francisco, CA 94143-0446. Tel: 415-476-3895. Fax: 415-476-0688. E-mail: szoka@cgl.ucsf.edu.

‡ University of California at San Francisco.

§ Current address: Bayer HealthCare Pharmaceuticals, Global Biological Development, Dwight Way 800, Berkeley, CA 94701-1986.

‖ University of California at Berkeley.

[1] Abbreviations: AFM, atomic force microscopy; CD, circular dichroism; dsDNA, linear double-stranded DNA; FITC, fluorescein 5-isothiocyanate; ssDNA, linear single-stranded DNA; Tris, tris-(hydroxymethyl)aminomethane; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; MALDI, matrix-assisted laser induced mass spectrometry; MOPS, 3-(*N*-morpholino)propanesulfonic acid; OG, *n*-octyl *β*-D-glucopyranoside; PCS, photon correlation spectroscopy; PEG, poly(ethylene glycol); PEGylation, PEG-modification; P/N ratio, peptide/nucleotide molar ratio; pVIII, M13 phage major coat protein; SDS, sodium dodecyl sulfate; TFE, 2,2,2-trifluoroethanol.

10.1021/bi700165m CCC: $37.00
Published on Web 06/27/2007

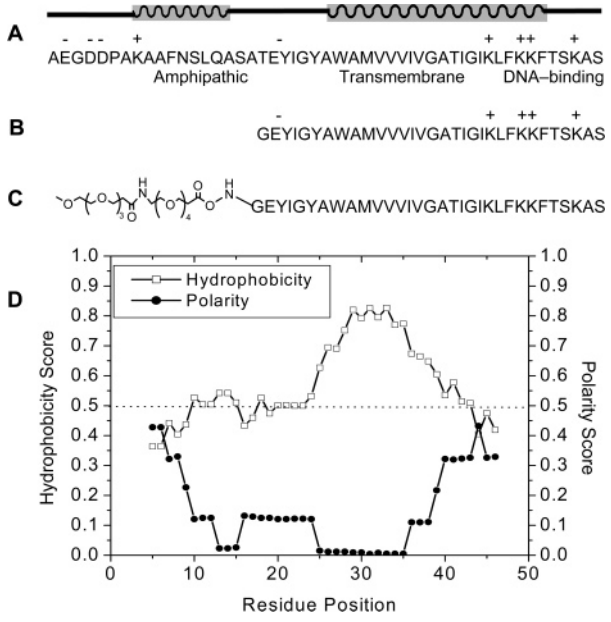

FIGURE 1: Sequence, structure, and properties of pVIII and analogue peptides: pVIII (A), 32mer (B), and PEG-32mer (C). The pVIII is roughly divided into three parts: the N-terminal amphipathic domain, the transmembrane domain, and the C-terminal DNA-binding domain, which are indicated by the cartoon scheme above the pVIII sequence (*11*). Charged amino acid residues on pVIII and 32mer at physiological conditions (pH 7.4) are indicated as + (positive) or − (negative). Calculations on the hydrophobicity and polarity of pVIII (D) are based on the methods of Kyte and Doolittle (*17*) and Zimmerman et al. (*18*), respectively. The hydrophobicity scale is normalized from 0 to 1 by setting Arg (the minimum value) as 0 and Ile (the maximum value) as 1. The polarity scale is normalized from 0 to 1 by setting Ala and Gly as 0 and Arg as 1.

by electron microscopy to be encapsidated in irregular tubular aggregates rather than as a rod-shaped phage. The particles had a very low level of infectivity ($1/10^7$ titer of mature phage). Cavalieri et al. prepared pVIII complexes with and without DNA using a sodium deoxycholate dialysis method and concluded that when the detergent was removed pVIII was not associated with DNA (*8*). More recently, Feng and colleagues employed a permeable host cell system which can postpone the assembly process of filamentous phage using the controlled accumulation of assembled components (*9*). There is an absence of structural data on the peptide when DNA interacts with pVIII in the assembly process.

The M13 pVIII protein has been extensively investigated as a model of protein−membrane interactions in detergent or bilayer environments (*10*). The sequence of pVIII can be roughly divided into three functional domains: an amphipathic α-helical domain on the N-terminal, a transmembrane α-helical domain in the middle, and a DNA binding domain on the C-terminal (Figure 1A). The solution structure of pVIII, solublized in detergent micelles, has been determined by NMR and computed by restrained molecular dynamics (*11*). By measuring the local polarity of the environment around probes, it was found that the domains of pVIII packed in various detergents, except SDS, are similar to the domain packing in phospholipid bilayers (*12*). To investigate the α-helical conformation in the micelle or bilayer environment, the peptide mimics of the M13 pVIII transmembrane segment were synthesized with lysine residues added to both the N-terminal and C-terminal to increase its solubility in aqueous buffers (*13*). The modified transmembrane segments retained an α-helical conformation similar to the corresponding pVIII, and maintained parallel α-helix in both the micelle and bilayer environments (*13*).

Although viral systems, such as M13 phage, use a multidomain peptide with a hydrophobic segment to form a capsid to protect the DNA genome, few hydrophobic peptides have been tested as DNA condensing agents for use as non-viral gene transfer vectors (*14*−*16*). In this work, our goal is to synthesize a 32mer peptide mimic embodying the M13 pVIII transmembrane and DNA binding segment (Figure 1), solubilize the peptides with DNA in a detergent environment that can permit observations of secondary structure, and then reconstitute pVIII or the peptide mimics with non-viral originated plasmid DNA by removal of the detergent. In these experiments, the linear dsDNA is used to mimic the closed circular ssDNA found in the M13 phage. The reconstitution resulted in nanoparticles that incorporated DNA into small diameter aggregates. The formation of the nanoparticles was accompanied by a secondary structural transition of the pVIII protein or peptides from a predominantly α-helical configuration into a mixed configuration that contained a substantial *β*-sheet component. These results suggest that either the bilayer organization of the bacterial cell membrane or other protein components of the phage/bacterial system are required to generate filamentous structures from the pVIII protein.

EXPERIMENTAL PROCEDURES

Peptide Synthesis, Purification, and Modification. The pVIII and the 32mer peptide corresponding to the pVIII residue 20 through 51 (with one added N-terminal glycine residue) were synthesized by Fmoc chemistry on an Applied Biosystems 431A peptide synthesizer (Foster City, CA). The standard scale (0.25 mmol substitution resin per batch) was employed to extend the peptide chain using double coupling at each residue. An Fmoc-ser(tBu)-NovaSyn TGA resin (A26467) with low substitution ratio (0.16 mmol/g) from Novabiochem (San Diego, CA) was used to extend the peptide sequence from C-terminal to N-terminal. The Fmoc protection was removed by piperidine from the N-terminal amino group of the peptide in the last step of synthesis. The peptide was cleaved from the resin with a cocktail composed of 94.0% (v/v) trifluoroacetic acid, 2.5% (v/v) pure water, 2.5% (v/v) 1,2-ethanedithiol, and 1.0% (v/v) triisopropylsilane. Cleaved peptide was precipitated with ice-cold diethyl ether. The pellets were collected by centrifugation (3000 rpm) and washed with diethyl ether four times. After drying, the peptide can be dissolved in pure 2,2,2-trifluoroethanol (TFE) with sonication under argon protection for 0.5 h. The yield of peptide synthesis calculated by dividing the weight of the cleaved peptide by the expected value based upon the resin substitution was 95% (w/w) for 32mer and 73% for pVIII. The peptide was purified on a Source reverse phase column packed with 3 mL of polystyrene-based Source 15RPC beads (Amersham Pharmacia, Piscataway, NJ). The elution gradient varied from 30% to 90% B in 50 min (phase A, pure water; B, isopropanol with 0.2% triethylamine). The purified peptides were confirmed by matrix-assisted laser induced (MALDI) mass spectrometry (Perspective Biosystems, Framingham, MA). The molecular weights determined by mass spectrum are 3450.2 (MH^+/found) and 3450.2 (MH^+/

theory) for 32mer; 5237.4 (MH^+/found) and 5237.7 (MH^+/theory) for pVIII.

After removing the N-terminal Fmoc protection group with piperidine, the N-terminal amine of the 32mer was further modified with polyethylene glycol (PEG). Briefly, 50 mg of the 32mer peptide on the resin without cleaving the side-chain protection groups was weighed into 3.3 mL of *N*-methylpyrrolidone/methylene chloride (2/1, v/v). Then 11 μL of 4-methylmorpholine and 9.2 μL of NHS-m-dPEG (Mw 580) from Quanta Biodesign (Powell, OH) were added into the mixture. The mixture was gently shaken for 3 h at room temperature. Then the resin was collected and washed with methylene chloride and methanol, respectively. The PEGylated peptide was cleaved from the resin and purified using the same procedure as the native peptide. The PEGylated peptide was identified by MALDI mass spectrometry, and no free 32mer was detected. Mass spectral data for purified PEG-32mer are 3916.0 (MH^+/ found) and 3916.6 (MH^+/theory).

Hydrophobicity and Polarity. The pVIII sequence was identified with the web-based program ProtScale (http://us.expasy.org/cgi-bin/protscale.pl) with the following parameters: the window size was set at 9, and the relative weight of the window edges compared to the window center was 100%. The hydrophobicity and polarity calculation were based on the methods of Kyte & Doolittle (*17*), and Zimmerman et al. (*18*), respectively.

DNA. In this study, the source of the DNA is linear dsDNA if no specific description is provided. Linear dsDNA was prepared by *Bam*HI restriction endonuclease cleavage of a plasmid pBG956 (~6.1 kb) from Valentis (Burlingame, CA) according to the cleavage protocol from Invitrogen (Carlsbad, CA). The linearized DNA was confirmed as a single band (~6.1 kb) with correct migration position by agarose gel electrophoresis. The DNA concentration was determined spectrophotometrically (one unit absorbance at 260 nm equal to 50 μg/mL). The nucleotide amount on DNA was calculated by assuming the average molecular mass of nucleotide base pair as 660 Da. The linear single-stranded DNA (ssDNA) was made by heating the linear dsDNA at 95 °C for 10 min, and then rapidly placing it into a water−ice bath at 0 °C. The dsDNA denaturation was confirmed by the appearance of a faster running band using agarose gel electrophoresis.

Formation of the Peptide−DNA Complex. The peptide (pVIII, 32mer, or PEG-32mer), DNA, and various specific additives were mixed in 10 mM HEPES at pH 7.0 to the final volume of 1 mL together with 200 mM octylglucoside (OG). Usually the peptide was dispersed into OG detergent first, equilibrated for 0.5−1 h, and then mixed with DNA. Brief vortexing of the sample was found to be the preferred method to form an optically clear suspension. After about 1 h, the sample was loaded into a Slide-A-Lyzer dialysis cassette (3500 Mw cutoff) from Pierce (Rockford, IL). The sample was dialyzed against 1 L of the dialysis buffer (10 mM HEPES at pH 7.0) at 4 °C for 2 days. The dialysis buffer was changed every half a day for a total of four times. Usually the volume of the peptide/DNA particle after dialysis will expand to about 1.5 mL, and this concentration change was accounted for in all experiments where the peptide concentration was critical.

Particle Size and ζ-Potential Measurements. The particle size and the ζ-potential of the peptide/DNA particle were determined by photon correlation spectrometry (PCS) on a Zeta 3000 laser light scattering spectrophotometer from Malvern Instruments (Southborough, MA). For the size determination, 0.5 mL of sample was mixed with 1.0 mL of 0.1 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) and 10 mM NaCl, pH 7.5. The resulting mixtures were briefly shaken and subjected to size determination 5 min after mixing. The measurements were repeated in triplicate. For the ζ-potential, a 20 μL sample was diluted into 2 mL of 0.1 mM MOPS and 10 mM NaCl at pH 7.5. The mixture was injected into the sample capillary slowly to avoid bubble generation. The data were processed with the Malvern software package using the automatic algorithm mode. The measurements were repeated 20 times.

Gel Retardation Assay. A solution of peptide/DNA complex corresponding to 1 μg of DNA was loaded onto a 0.8% agarose gel containing 0.5 μg/mL ethidium bromide to visualize DNA, and the Tris/EDTA/acetate buffer (pH 8.0) was the running buffer. The electrophoresis was performed at 100 V for 1.5 h. To determine if the peptide/DNA particle formed with the detergent dialysis method is different from a simple aggregate between the peptide and DNA, the peptide dialyzed using the same conditions but without DNA was directly mixed with an equivalent amount of DNA for 1 h before the sample was loaded on the electrophoresis gel and analyzed.

DNase Digestion Assay. Samples of the DNA and peptide/DNA particles containing 1 μg of DNA were brought up to a total volume of 40 μL in 10 mM HEPES buffer at pH 7.0. Ten microliters of 9 mM $MnCl_2$ and 0.5 M Tris buffer at pH 7.4 was added into the samples together with 1 μL of DNase I (50 units/mL), and then the samples were incubated for 10 min at 37 °C. After incubation, the sample tubes were placed at 4 °C and the digestion was stopped by the addition of 5 μL of 10% SDS and 5 μL of 0.5 M EDTA. The samples were analyzed by electrophoresis on 0.8% agarose as described above.

DNA Encapsidation Ratio. The peptide−DNA particle was centrifuged at ~14000 rpm for 5 min on a benchtop centrifuger (Eppendorf, Germany). The peptide/DNA particle sedimentated during centrifugation. The free dsDNA concentration (c) in the supernatant was quantitated with the PicoGreen kit (Molecular Probes, Inc., Junction City, OR). The DNA encapsidation ratio was calculated as $I(\%) = (c^\circ - c)/c^\circ \times 100$ where c° is the starting amount of DNA added to the peptide detergent suspension used to prepare the particle. The PicoGreen method is not applicable for the quantitative measurement of ssDNA concentration.

Circular Dichroism (CD). CD spectra were obtained on a Jasco-715 CD spectrometer (JASCO Corporation, Japan) in a 1 mm cell at 25 °C except where otherwise noted. Spectra were recorded under a continuous purge of 15−20 L/min of nitrogen using the Spectra Manager Program version 1.53 (JASCO Corporation, Japan) as the average of four repetitions with a data bin of 1 nm and a scan speed of 50 nm/min. The results are expressed as mean molar residue ellipticities $[\theta]$ (deg·cm^2·dmol^{-1}):

$$[\theta] = \frac{\theta}{MLn} \quad (1)$$

where θ means the ellipticity (mdeg), M is the molar concentration of peptide, L is path length of the cuvette, and n is the peptide residue number.

All spectra had the background obtained with buffer alone or free DNA solution alone, and subtracted from them, except where noted. The helical content was determined from $[\theta]_{222}$ at 222 nm according to the following relation:

$$\alpha\% = \frac{[\theta]_{222} + 2340}{(-30300)} \times 100 \qquad (2)$$

where α% is the percentage of α-helix (*19*, *20*).

For the temperature transition assay, the cuvette temperature was changed at a rate of 30 °C/h from 10 °C to 90 °C, maintained at 90 °C for 30 min until the signal was stable, and then reduced to 10 °C at the same rate. The ellipticity at 208 nm was recorded with data bins of 0.5 °C, and the spectrum was also scanned every 10 °C over the temperature range.

For the time course of the peptide−DNA interaction in detergent, the spectrum (185−320 nm) was recorded every 2 min with a fast scan mode of 100 nm/min at 25 °C just after mixing the peptide and DNA for 1 min. The ellipticities of 208 and 222 nm were used to follow the peptide secondary structural change in this period.

Tryptophan Fluorescence Spectrometry. Measurements were made on a Spex Fluorolog photon spectrometer (Edison, NJ) using a 150 W xenon light source. Peptides in solution were irradiated at 295 nm, and samples were scanned from 300 to 400 nm at 1 nm intervals. Buffers and DNA background were subtracted from each scan, and the emission spectra were recorded in triplicate.

Atomic Force Microscopy (AFM). The pVIII/DNA complex or free DNA was diluted with 10 mM ammonium acetate buffer at pH 7.0 to a final DNA concentration of 0.5 μg/mL. The M13 phage strain μE (1.5×10^{14}/mL) from our previous work (*21*) was diluted to 1.5×10^{10}/mL with 10 mM ammonium acetate buffer at pH 7.0 before imaging. Samples were spin-coated on a freshly cleaved mica surface and dried by nitrogen flow. The scanning force microscopic images were obtained using a Nanoscope IIIa system (Digital Instruments, Santa Barbara, CA) by the tapping mode in air. Representative images are selected from three independent experiments. The height of DNA and M13 phage was determined as an average value from the object. The height of pVIII/DNA particle was determined as a range from at least four spots per particle to represent the variety of morphologies observed in the sample.

Fluorescence Microscopy. The peptide/DNA complex was diluted to a final peptide concentration of 0.05 mg/mL with 10 mM HEPES buffer at pH 8.5. Then 0.1 mg/mL fluorescein 5-isothiocyanate (FITC) was added into the mixture for 1 h of reaction. The sample was dialyzed against 10 mM HEPES buffer at pH 7.0 overnight, and further centrifuged at 14000 rpm to pellet the particle and to remove any remaining FITC in the preparation. The modified particles were resuspended in 10 mM HEPES buffer at pH 7.0 and observed with a Nikon Eclipse TS100 fluorescence microscope (Kanagawa, Japan) with the excitation wavelength at 460−500 nm and emission wavelength at 510−560 nm.

Table 1: Secondary Structure Parameters of Peptides in the Presence of Detergent or TFE

	reconstitution conditions				
sample[a]	components	detergent or solvent	$[\theta]_{208nm} \times 10^{-3}$	$[\theta]_{222nm} \times 10^{-3}$	α-helix (%)[b]
1	32mer	pure TFE	−17.0	−15.0	41.8
2	32mer	20 mM SDS	−16.3	−14.5	40.1
3	32mer	0.2 M OG	−15.6	−15.3	42.8
4	PEG-32mer	pure TFE	−22.1	−17.9	51.4
5	PEG-32mer	0.2 M OG	−19.9	−18.8	54.3
6	pVIII	pure TFE	−19.0	−16.5	46.7
7	pVIII	0.2 M OG	−16.6	−15.2	42.4

[a] Samples 1, 4, and 6 were determined by dissolving 0.1 mg/mL peptide in pure TFE. Sample 2 at 0.1 mg/mL was assayed in the buffer of 20 mM SDS, 20 mM NaCl, and 20 mM Tris at pH 7.4. Samples 3, 5, and 7 were determined at 0.1 mg/mL in the buffer of 0.2 M OG and 10 mM HEPES at pH 7.0. [b] Calculation is based on $[\theta]_{222nm}$ by eq 2 (see Experimental Procedures).

RESULTS

Synthesis of Peptides. The pVIII (Figure 1A) and the 32mer peptide mimic of pVIII (Figure 1B) were synthesized using FMOC chemistry in good yield. Hydrophobicity and polarity calculations on pVIII (Figure 1C) indicate that the sequence of pVIII is hydrophobic and apolar, and the transmembrane domain is much more hydrophobic than the ends, which creates difficulties for synthesis and purification (*13*, *22*). The 32mer (Figure 1B) includes both the transmembrane domain and the DNA binding domain but lacks the amphipathic N-terminal domain (Figure 1). Stopar and co-workers recently proposed a new classification of the pVIII membrane-anchoring elements where the amino acid residues 1−6, defined as the acidic hydrophilic anchor, were classified as the fourth element in addition to the three domains described above (*23*). A glycine residue was added at the N-terminal of the 32mer peptide to prevent the acylation of N-terminal primary amine with the carboxyl group on the side chain of glutamate acid during subsequent chemical modification. This significantly enhanced the yield of the 32mer PEGylation (data not shown). Previous studies reported that both the C-terminal and N-terminal segments could be removed from the transmembrane domain of pVIII with no change to the secondary structure of the peptide or to the peptide−peptide interaction that is observed using the parental pVIII (*13*). This allowed us to investigate if the N-terminal amphipathic domain is necessary for peptide−DNA assembly.

A PEG chain was conjugated onto the N-terminal of the 32mer mimic before the protection groups on the peptide side chains were removed. PEG is a noncharged, hydrophilic and flexible molecule, and we hypothesized that the PEG would stabilize the peptide structure when the C-terminal DNA binding domain interacts with the DNA template. The 32mer and PEG-32mer are not water-soluble, like pVIII, which is usually dispersed into detergent micelles or membrane bilayers (*24*, *25*).

Secondary Structure of Free Peptide and Its Stability. The secondary structure of 32mer, PEG-32mer, and pVIII were examined using CD in pure TFE or detergent systems (Table 1). In pure TFE, OG, or SDS detergent solutions, the 32mer and PEG-32mer demonstrated typical α-helical structure (data not shown). The PEG-32mer had lower values at the

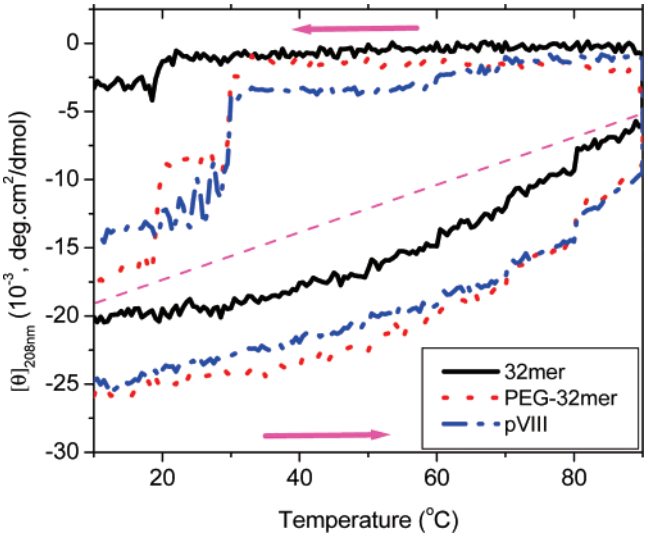

FIGURE 2: Comparison of the α-helix stability of pVIII and analogue peptides in TFE: 32mer (solid line), PEG-32mer (dot line), and pVIII (dot dash line). The ellipticity at 208 nm was monitored continuously from 10 °C to 90 °C (area below the dash line) and reversed from 90 °C to 10 °C (above the linear dash line).

minima shoulders at 208 and 222 nm than did the 32mer and pVIII in TFE or OG (Table 1). Calculation of the α-helix content using the ellipticity value at 222 nm, the 32mer, PEG-32mer, and pVIII have the α-helix ratio between 40% and 55% (Table 1). Removing the N-terminal amphipathic domain had little effect on the α-helical content of the 32mer and PEG-32mer (Table 1).

The thermal stability of pVIII and its peptide mimics was measured using CD to assess the stability of the α-helix (*13*, *26*, *27*). The ellipticity of PEG-32mer at 208 nm is more similar to that of pVIII than to the ellipticity of the 32mer in TFE (Figure 2). This suggests that the PEG-chain has a similar ability as the N-terminal amphipathic domain to stabilize the α-helix. The increase of ellipticity at 208 nm of pVIII and its mimic as the temperature was increased is due to a transition from α-helix to *β*-sheet (*13*, *26*, *27*). When the peptides were equilibrated at 90 °C for 30 min, their spectrum converted from one characteristic of a *β*-sheet to one characteristic of a random coil (data not shown).

When the temperature was decreased from 90 °C, the ellipticity at 208 nm fluctuated around a stable value from 90 °C to 30 °C (Figure 2). At 30 °C a striking difference was noted among the 32mer and the other two peptides. The secondary structure of the PEG-32mer and pVIII reappeared as the temperature was decreased from 30 °C to 10 °C whereas the secondary structure did not recover in the 32mer. The final ellipticities of PEG-32mer and pVIII at 208 nm after reaching 10 °C are 66% and 58% of the initial values at 10 °C. Contrasted to that, the ratio for 32mer is only 16% (Figure 2).

Peptide−DNA Particle Formation and Characterization. For the pVIII and 32mer, their poor solubility in aqueous buffer hinders their reconstitution with DNA. Thus, we employed an OG detergent removal method to assemble the peptide−DNA particle. OG is a nonionic detergent with the critical micelle concentration (CMC) ∼20 mM and an aggregation number ∼40 in aqueous buffers (*28*). The hydrophobic 32mer or PEG-32mer peptide was dissolved in the detergent buffer together with dsDNA. The detergent was gradually removed by dialysis against buffer, and the particles that formed depended upon the specific buffer conditions (Table 2).

The 32mer and DNA formed a visible precipitate (diameter greater than 1000 nm) at peptide/nucleotide ratio (P/N ratio) higher than 0.25 (Table 2). The P/N ratio 0.25 was the highest value for which the 32mer formed particle with diameter between 100 and 200 nm and with a high ratio of DNA encapsidation. The smaller diameter DNA-containing particles (diameter <200 nm) hold the potential to be used as a gene delivery vector with longer circulation time *in vivo*. For the PEG-32mer, particles with similar size to 32mer were found at the P/N ratio 0.25 (about 3000 copies of the peptide on one 6.1 kb dsDNA).

For pVIII, the best condition to obtain small diameter particles is at the P/N ratio 0.33 (about 4000 copies of the peptide on one 6.1kb dsDNA) where the DNA encapsidation is 97.8%. Further increases in the P/N ratio produced particles larger than 200 nm. Small particles were also found when the peptides were reconstituted with ssDNA.

Regardless of the peptide and DNA types used in the study, the overall ζ-potentials on the surface of particles are negative except the 32mer−DNA complex at P/N ratio 0.5. In these experimental conditions, the ζ-potential of the free DNA in the absence of peptide is not detectable, so the ζ-potential values in Table 2 reflect the charge on the particle surface.

Several factors are critical for the particle formation including the detergent type, the P/N value, the ionic strength, and the pH of the reconstitution buffer. Octanoic acid, an anionic detergent with a similar alkyl chain length as OG, was a good solvent for the peptide. But when used with the dialysis procedure, small particles were not formed and DNA was not encapsidated (Table 2). When the cationic agents 1,5-diaminopentane or 1,3-diamino-2-hydroxypropane were added into the 32mer−DNA reconstitution system, the resulting particles had similar encapsidation (∼90%) to the particles obtained with octanoic acid as additive (data not shown). However, these two cationic agents do not act as detergents and cannot dissolve the peptide−DNA mixture without OG prior to dialysis. At high salt concentration (such as when 0.15 M NaCl was used in the reconstitution and dialysis buffer), the 32mer peptide had a strong tendency to precipitate instead of forming small particles with DNA (data not shown) compared to when a low salt buffer (10 mM HEPES at pH 7.0) was used for dialysis. Reconstitution buffers with a low pH (pH 3.0−4.0) will also induce peptide precipitation (data not shown).

The major population of the peptide−DNA complexes had diameters that ranged from 50 to 100 nm, although particles above 200 nm were also found in some cases. A minor peak around 500 nm was sometimes observed (less than 10%; data not shown). This small percentage of large particles increased the ζ-average size to between 100 and 200 nm (Table 2). The typical ζ-average diameters for three evaluated particles are 143.4 ± 2.6 nm for 32mer/DNA at P/N ratio 0.25, 175.9 ± 1.2 nm for PEG-32mer/DNA at P/N ratio 0.25, and 133.4 ± 1.7 nm for pVIII/DNA at P/N ratio 0.33 using 0.2 M OG dialysis. The polydispersity index for these particle distributions are 0.43 ± 0.03, 0.38 ± 0.01, and 0.47 ± 0.03, respectively. The diameter distribution and polydispersity of the complexes formed from the three peptides are similar to

Table 2: Peptide−DNA Particle Formation by Detergent Dialysis

sample	reconstitution conditions: components	P/N ratio	detergent and additives	ζ-average particle size (nm)	ζ-potential (mV)	DNA encapsidation (%)
1	32mer/DNA	0.5	0.2 M OG	>1000	14.5 ± 1.3	99.8
2	32mer/DNA	0.33	0.2 M OG	>1000	−13.1 ± 9.7	95.3
3	32mer/DNA	0.25	0.2 M OG	143.4 ± 2.6	−23.6 ± 4.0	98.4
4	32mer/DNA	0.25	0.2 M octanoic acid	>1000	−16.3 ± 12.8	3.5
5	32mer/DNA	0.25	0.2 M OG/20 mM octanoic acid	168.6 ± 4.2	−24.6 ± 10.6	91.6
6	32mer/ssDNA	0.25	0.2 M OG	162.6 ± 1.6	−6.2 ± 3.5	-
7	PEG-32mer/DNA	0.25	0.2 M OG	175.9 ± 1.2	−11.3 ± 8.6	95.8
8	PEG-32mer/ssDNA	0.25	0.2 M OG	190.1 ± 1.4	−10.2 ± 5.3	-
9	pVIII/DNA	0.5	0.2 M OG	252.2 ± 1.1	−21.2 ± 1.1	97.9
10	pVIII/DNA	0.42	0.2 M OG	309.6 ± 3.8	−14.5 ± 1.2	99.3
11	pVIII/DNA	0.33	0.2 M OG	133.4 ± 1.7	−4.2 ± 4.2	97.8
12	pVIII/DNA	0.25	0.2 M OG	182.6 ± 17.9	−29.9 ± 2.6	80.4
13	pVIII/DNA	0.33	0.2 M OG /20 mM octanoic aicd	231.9 ± 5.0	−11.8 ± 5.2	54.9
14	pVIII/ssDNA	0.33	0.2 M OG	208.8 ± 1.5	−9.1 ± 6.0	-

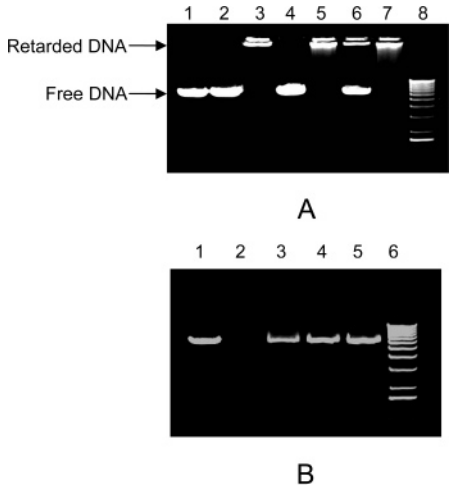

FIGURE 3: Gel retardation electrophoresis of peptide−DNA particles. (A) Lane 1, naked DNA; lane 2, 32mer aggregates mixed directly with DNA at P/N ratio 0.25; lane 3, the 32mer/DNA particle made by OG dialysis at P/N ratio 0.25; lane 4, PEG-32mer aggregates mixed directly with DNA at P/N ratio 0.25; lane 5, PEG-32mer/DNA particle at P/N ratio 0.25; lane 6, pVIII aggregates mixed directly with DNA at P/N ratio 0.33; lane 7, pVIII/DNA particle at P/N ratio 0.33; lane 8, 1 kb DNA ladder. (B) Analysis after DNase treatment of the sample. The sample was solubilized by the detergent of 10% SDS, which released the DNA from the particle, and EDTA addition to inactivate the DNase. Lane 1, naked DNA; lane 2, naked DNA after DNase digestion; lane 3, DNA released from 32mer/DNA particle after DNase digestion; lane 4, DNA released from PEG-32mer/DNA particle after DNase digestion; lane 5, DNA released from pVIII/DNA particle after DNase digestion; lane 6, 1 kb DNA ladder.

each other (data not shown), which indicates that the peptide−DNA interactions were not influenced when the N-terminal amphipathic segment was removed from 32mer.

Gel Electrophoresis and DNase Digestion of Peptide−DNA Particles. To confirm that DNA is in a complex with the peptide and the complex protects the DNA from degradation by nucleases, agarose gel electrophoresis was performed. When DNA was reconstituted with 32mer, PEG-32mer, or pVIII by the detergent dialysis method, the DNA encapsidated by peptides was fully retarded in the loading well (Figure 3A). The control samples, prepared by simply mixing the peptide aggregates with the same amount of DNA, have a large amount of free DNA which is not retarded by the peptides. These results suggest that the DNA was encapsidated inside the particles after detergent dialysis and the peptide−DNA reconstitution is different from the non-ordered attachment of DNA onto the surface of peptide aggregates.

The peptides when assembled with DNA using detergent dialysis effectively protected the DNA from DNase digestion (Figure 3B). This is a strong evidence for the peptide−DNA encapsidation and implies that the DNA in the particle is surrounded by the peptide.

Time Course of the Peptide−DNA Interaction in the Presence of Detergent. We examined the peptide CD spectrum as a function of time after adding DNA to learn if the peptide structure is an α-helix conformation. A transition from a high α-helix to a *β*-sheet structure occurred when DNA was added into the peptide−detergent system for all three peptides (Figure 4). The time course of the change in secondary structure after adding DNA indicated that DNA induced a structure change on the peptide (Figure 4). The ellipticity at 208 and 222 nm approached a stable value about 1 h after the DNA was added to the peptide detergent solution. In the near UV range between 270 and 280 nm, the ellipticity decreased slightly with time and became stable after 1 h (data not shown). This spectrum change is mainly due to the change of DNA structure when condensed by peptides.

In Table 3, the stable secondary structures of peptide reconstituted with DNA in OG solution under various conditions are compared. To learn if a charge interaction between the peptide and DNA results in the transition of peptide structure, octanoic acid was added into the OG peptide mixture prior to DNA addition. The ellipticities of the peptide at 208 and 222 nm with DNA were almost the same as the values for the peptides in 0.2 M OG without DNA (see samples 3, 5, and 7 in Table 1, samples 2, 7, and 10 in Table 3, and Figure 5).

When the cationic agents 1,5-diaminopentane or 1,3-diamino-2-hydroxypropane were added into the 32mer−DNA reconstitution system, the α-helix was also maintained (samples 3 and 4 in Table 3 and Figure 5). These results suggest that the change in peptide secondary structure is caused by the assembly of peptide on the DNA template. The peptide−DNA interaction was driven by the electrostatic interaction between the negative charges on the DNA phosphate backbone and the positive charges on the C-terminal DNA binding domain of 32mer, PEG-32mer, or pVIII and mediates helix−helix contact.

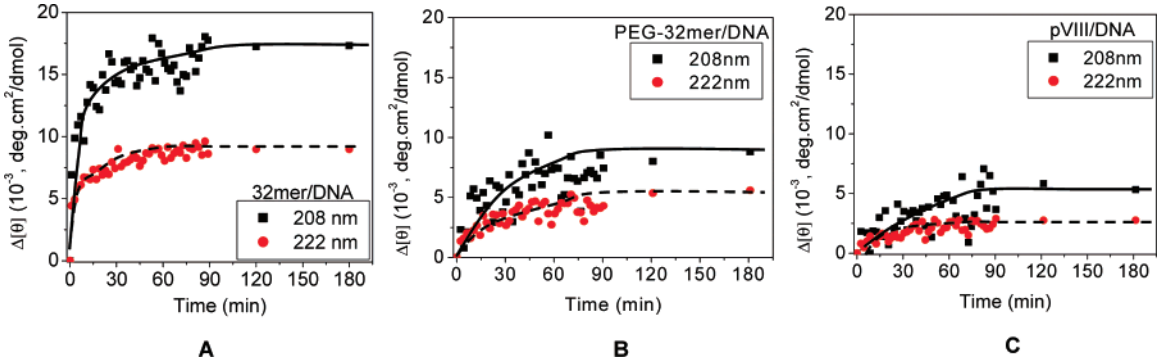

FIGURE 4: Time course of the peptide−DNA interaction in presence of detergent. The ellipticities at 222 and 208 nm of 32mer (A), PEG-32mer (B), and pVIII (C) are shown after adding DNA into the peptide solution in 0.2 M OG and 10 mM HEPES at pH 7.0. Samples were reconstituted with 50 μg/mL of dsDNA at P/N ratio 0.25 for 32mer and PEG-32mer, and P/N ratio 0.33 for pVIII without detergent removal step. For other details see Experimental Procedures.

Table 3: Secondary Structure Parameters of Peptides with DNA in the Presence of Detergent (OG)

	reconstitution conditions					
sample[a]	components	detergent or solvent	additives[b]	$[\theta]_{208nm}$ $\times10^{-3}$	$[\theta]_{222nm}$ $\times10^{-3}$	α-helix (%)[c]
1	32mer/DNA	0.2 M OG		−0.84	−5.36	10.0
2	32mer/DNA	0.2 M OG	20 mM octanoic acid	−17.1	−15.8	44.4
3	32mer/DNA	0.2 M OG	20 mM 1,5-diaminopentane	−13.6	−12.8	34.5
4	32mer/DNA	0.2 M OG	20 mM 1,3-diamino-2-hydroxypropane	−13.2	−12.1	32.2
5	32mer/ssDNA	0.2 M OG		−2.89	−8.88	21.6
6	PEG-32mer/DNA	0.2 M OG		−12.1	−14.7	40.8
7	PEG-32mer/DNA	0.2 M OG	20 mM octanoic aicd	−23.8	−20.6	60.3
8	PEG-32mer/ssDNA	0.2 M OG		−12.0	−15.0	41.8
9	pVIII/DNA	0.2 M OG		−11.3	−12.4	33.2
10	pVIII/DNA	0.2 M OG	20 mM octanoic aicd	−17.9	−16.2	45.7
11	pVIII/ssDNA	0.2 M OG		−12.9	−12.3	32.9

[a] Peptides were reconstituted with 50 μg/mL of dsDNA or ssDNA at P/N ratio 0.25 for 32mer and PEG-32mer, and 0.33 for pVIII in 10 mM HEPES at pH 7.0 plus detergent and additive according to the Experimental Procedures prior to the detergent dialysis. CD data were recorded after at least 1 h equilibration for the peptide−DNA interaction. [b] The anionic octanoic acid was mixed with peptide first, and then mixed with DNA. The cationic reagents, 1,5-diaminopentane and 1,3-diamino-2-hydroxypropane, were mixed with DNA first, and then mixed with peptide. [c] Calculation is based on $[\theta]_{222nm}$ by eq 2 (see Experimental Procedures).

The negatively charged octanoic acid has an alkyl chain which was suitable to be incorporated into OG micelles. The charge−charge interaction between the octanoic acid and 32mer interfered with the interaction of the 32mer with DNA in solution. Thus the secondary structure of the peptide in the micelle was not changed (Figure 5, curves d and e).

For the case of 1,5-diaminopentane or 1,3-diamino-2-hydroxypropane, they are cationic reagents which can bind to free DNA by charge−charge interaction. It should be noted that, in our conditions, both these two cationic reagents cannot precipitate DNA, and all the samples in Table 2 are clear solutions. So there was no phase separation to interfere with peptide access to DNA. When the charges on the DNA template were neutralized by cationic reagents, the charge−charge interaction between the 32mer and DNA was also inhibited and no change in secondary structure was obtained (Figure 5, curves c and d).

When the 32mer peptide was reconstituted with ssDNA, it retained higher α-helix contents than the peptide with dsDNA (Table 3). It was also found that the PEG-32mer and pVIII maintained a higher percentage of α-helix when interacting with DNA in detergent compared to the 32mer (Figure 4 and Table 3).

Secondary Structure of the Peptide Complexed with DNA after Detergent Removal. We investigated the secondary structure of the peptide−DNA complex formed after detergent dialysis (Table 4). The α-helical content of the 32mer in the complex went from 10.0% to 4.2% (also see Figure 5, curve a). Data on PEG-32mer and pVIII showed a similar loss of α-helical content, and the reconstitution did not inhibit the α-helix to β-sheet transition upon removal of the detergent. This is different from what is observed in the pVIII structure on the mature M13, phage which has a high α-helical content (*29*, *30*).

Table 4: Comparison of the α-Helical Contents (%)[a] of Peptides in Various Environments

	environment/DNA type				
peptide names	OG/ no DNA[b]	OG/ dsDNA[c]	OG/ ssDNA[c]	dsDNA[d]	ssDNA[d]
32mer	42.8	10.0	21.6	4.2	23.5
PEG-32mer	54.3	40.8	41.8	19.9	29.4
pVIII	42.4	33.2	32.9	20.7	19.7

[a] Calculation is based on $[\theta]_{222nm}$ by eq 2 (see Experimental Procedures). [b] Data were from Table 1 for peptides in 0.2 M OG. [c] Data were from Table 3 for peptides with dsDNA or ssDNA in the presence of 0.2 M OG. [d] Peptides were reconstituted with 50 μg/mL of dsDNA or ssDNA as particles at P/N ratio 0.25 for 32mer and PEG-32mer, and 0.33 for pVIII in 0.2 M OG and 10 mM HEPES at pH 7.0 after the detergent dialysis.

The PEG-32mer and pVIII complexed with DNA had a higher α-helical content than the 32mer, which might be due to the α-helical stabilization effect of the PEG chain or the N-terminal domain, respectively. The particles formed from

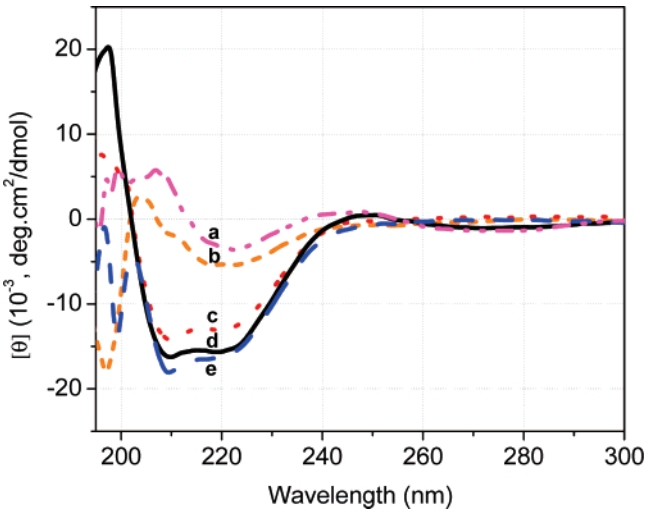

FIGURE 5: DNA template induced α-helix to *β*-sheet transition of peptides. The 32mer/DNA complex after 2 days of dialysis showed a typical *β*-sheet structure (a). The 32mer/DNA complex in the presence of 0.2 M OG after mixing the peptide and DNA together for 1 h but before dialysis (b). The peptide without DNA but in the presence of 0.2 M OG (d). The peptide α-helix to *β*-sheet transition was inhibited by negatively charged octanoic acid (e) and positively charged 1,5-diaminopentane (c), respectively in the presence of detergent. Sample a corresponded to the 32mer/dsDNA complex after dialysis in Table 4; samples b, c, and e corresponded to samples 1, 3, and 2 in Table 3; and sample d corresponded to sample 3 in Table 1.

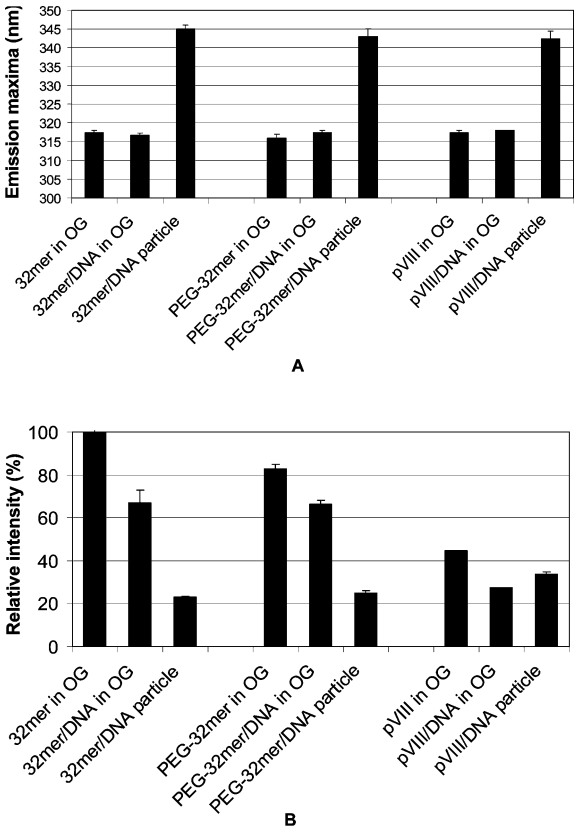

FIGURE 6: The tryptophan fluorescence emission wavelength and peak intensity at various stages of the reconstitution. The trytophan emission maxima (A) and the relative intensities (B) of peptides were measured in the presence of 0.2 M OG or after detergent removal. Samples are assigned as: "peptide in OG", pure peptides in 0.2 M OG and 10 mM HEPES at pH 7.0; "peptide/DNA in OG", DNA was added into the peptide in 0.2 M OG and 10 mM HEPES at pH 7.0; and "peptide/DNA particle", the peptide/DNA complex after detergent removal. For more details see Experimental Procedures. The P/N ratio for 32mer, PEG-32mer, and pVIII was 0.25, 0.25, and 0.33, respectively. The fluorescence intensity was divided by the molar concentration of the peptide to enable comparison of the intensities at a relative scale.

reconstitution of the 32mer and PEG-32mer with ssDNA had a higher percentage of α-helix than the dsDNA (Table 4).

The change in peptide structure before and after detergent removal was also monitored by the tryptophan emission spectrum of the single tryptophan residing in the middle of pVIII sequence (Figure 1A). The tryptophan excitation wavelength was set at 295 nm to avoid interference from tyrosine fluorescence (*31*). The 32mer, PEG-32mer, and pVIII have similar values of the emission maxima but different intensities (Figure 6). The peptides in 0.2 M OG have an intense emission with maxima at ∼317 nm, indicating that the tryptophan was localized in the hydrophobic micelle core and was not accessible to the hydrophilic environment. When DNA was added into the solution, the emission maxima were not changed (Figure 6A). However, the intensity of the emission decreased (Figure 6B). This is probably caused by interactions between tryptophans that were brought together on the DNA template (*13*). In addition, the fluorescence yield will also change by going into a water environment from micelles upon detergent dialysis. Removal of the detergent induced a large red shift of the emission maximum to ∼342 nm in the three peptide/DNA particles and a further decreased fluorescence intensity in the particles formed from the 32mer and PEG-32mer (Figures 6A and 6B). These results suggest that the tryptophans are more tightly packed when the detergent is removed but that the environment of the tryptophan is less hydrophobic in the particle than in the OG micelle.

Images of Naked DNA, Natural M13 Phage, and pVIII−DNA Particle after Detergent Removal. The interaction between M13 pVIII protein and DNA was examined by AFM (Figure 7). The linear DNA strand (Figure 7A) has an average height 0.52 nm and length ∼2 *μ*m when measured by AFM. This height, which should be the diameter of DNA, is much less than the theoretical diameter of the DNA double-helix (2.2 nm). This is due to the strong interaction of the DNA strand with the mica surface (*32*). The natural M13 phage (Figure 7B) is clearly visible as a ∼1 *μ*m long rod with average height of 2.0 nm under AFM. For the pVIII−DNA complex, the DNA was wrapped into irregularly shaped structures instead of a rodlike structure. The coated DNA domains have heights that ranged from 1.7 to 5.7 nm (Figure 7C). The image suggests that different DNA domains are cross-linked into a condensed aggregated structure. The AFM images of DNA particles formed with the 32mer or PEG-32mer are similar to the pVIII/DNA particles (Figure 7C) as condensed structures but also differ from the rodlike structure of M13 phage (data not shown).

The free amino groups on the N-terminal and lysine residues of pVIII in the particle can be modified with FITC to allow visualization of the features of the DNA-containing particles in a fluorescence microscope (Figure 7D). The particle size is below 1 *μ*m when viewed by fluorescence

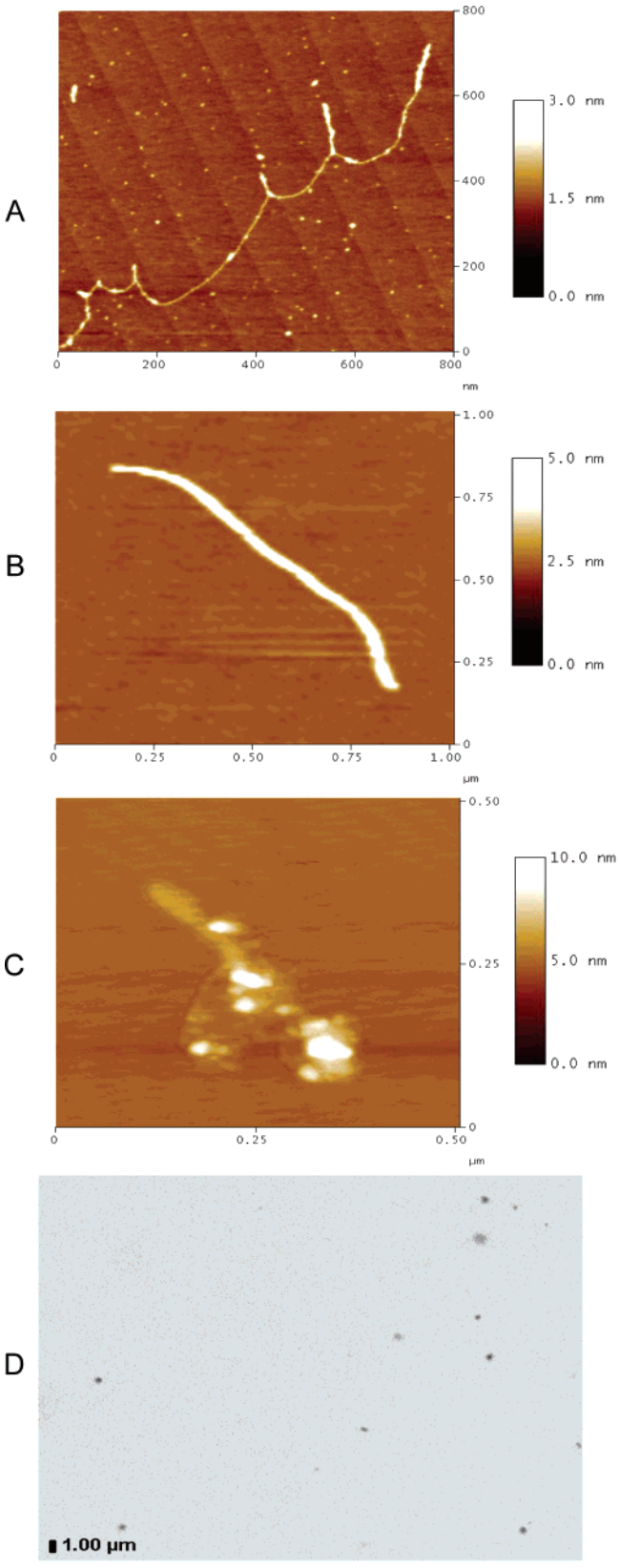

FIGURE 7: Representative images of DNA, M13 phage, and pVIII/DNA complex. Images of the linear dsDNA (A), M13 phage (B), and pVIII/DNA complex (C) and the fluorescence microscopic image of pVIII/DNA complex (D). The fluorescence image was converted to negative gray scale image for better visualization of the punctate particles. The pVIII/DNA complex was prepared using detergent dialysis at the P/N ratio of 0.33.

microscopy, which is consistent with the light scattering data (Table 2).

DISCUSSION

We have reconstituted dsDNA and ssDNA with pVIII and its analogues into a peptide coated nanoparticle that encapsidates the DNA using a neutral detergent dialysis protocol. Addition of DNA into the peptide detergent mixture induced an α-helical to β-sheet transition prior to detergent removal. Addition of reagents such as octanoic acid or cationic diamines can block the secondary structural transition in the pVIII in the presence of DNA by interfering with the peptide−DNA interaction. This transition from the α-helix to β-sheet may be the reason why a rod-shaped structure is not formed by detergent dialysis.

We became interested in viral reconstitution because it may provide a paradigm based upon structural principles for the assembly of bio-based components into better non-viral vectors (*5*, *33*, *34*). Particles with diameter <200 nm are thought to improve DNA delivery when vectors are injected into animals. Non-viral vectors employ peptides, lipids, proteins, or polymers as components of an "artificial virus" to encapsidate exogenous DNA and deliver the DNA into cells. Previous attempts to create non-viral vectors from peptides (*14*, *15*, *35*−*38*) were guided more by the membrane disrupting activity of the peptides than by their ability to form a particular type of structure with DNA.

In the case of filamentous bacteriophage, such as M13, there has been substantial progress on understanding the *in vivo* assembly of the bacteriophage (*4*, *9*, *39*−*41*) and extensive work on the conformation and structure of the coat protein, pVIII, in bilayer and detergent environments (*1*, *2*, *13*, *27*, *42*, *43*). However, there has been little progress on the *in vitro* reconstitution of the bacteriophage coat protein with DNA.

Knippers and Hoffmann-Berling used a urea dialysis procedure to assemble the coat protein from the fd filamentous bacteriophage with ssDNA to form an irregularly shaped particle when viewed by negative stain electron microscopy (*7*). Cavalieri et al. studied the morphology of pVIII aggregates formed upon dialysis from a detergent (20 mM sodium deoxycholate) solution without or with DNA and concluded that pVIII was not associated with DNA (*8*). This result is similar to what we observed when octanoic acid was used as the detergent, the DNA was not encapsidated (Table 2). This is because the negative charge on the detergent (either octanoic acid or deoxycholate) interacted with the positively charged residues in the peptide. Therefore the peptide could not assemble on the DNA and aggregated with other peptides via a hydrophobic interaction as the detergent was removed.

Given the numerous interactions among the phage components and the bacteria during phage assembly *in vivo*, it may not be a surprise that the urea reconstitution process did not result in a filamentous structure. In the mature phage, 2700 copies of pVIII in an α-helical conformation coat the phage ssDNA. The pVIII is organized in the filament so that the average tilt angle between the pVIII α-helix and the viron axis falls within the range of 13−20° (*44*). In M13 phage assembly, pVIII is thought to exist as a flexible hydrophobic monomer that associates with the cytoplasmic membrane (*4*). During assembly, the DNA first interacts with the gene V protein (pV) which directs the DNA into a channel (Figure 8). The DNA moves from the inner side of the host cell

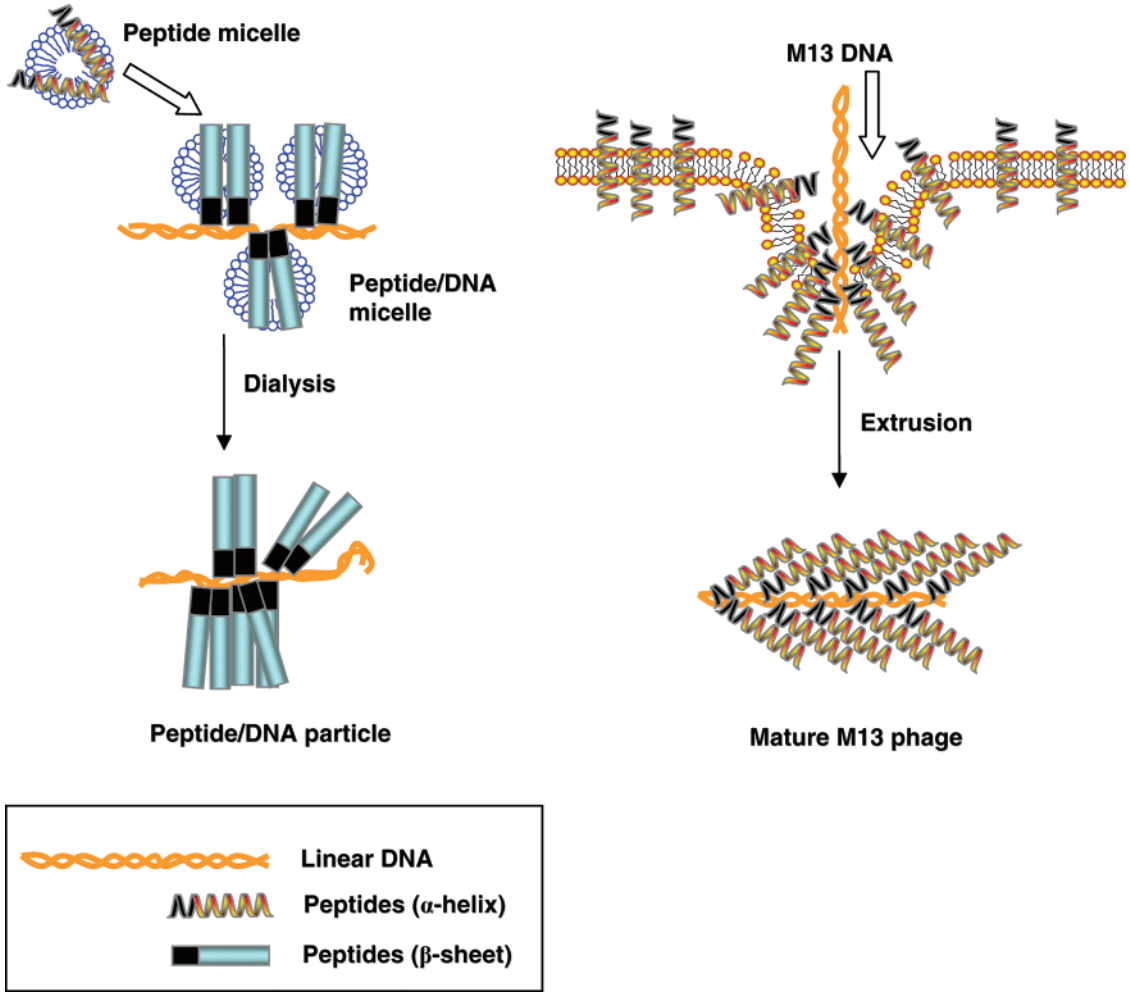

FIGURE 8: Possible pathways for pVIII peptide reconstitution on a DNA template with detergent removal compared to the M13 phage DNA extrusion process. The C-terminal DNA binding domains of the pVIII peptides are colored as black. A compact interaction model with tight peptide−peptide interaction along the long axis of DNA chain was proposed to describe the interaction of the M13 pVIII peptides with DNA in the *in vitro* reconstitution process using the detergent dialysis method (left side). The peptides in the micelles simultaneously approach the DNA template when DNA is added into the peptide detergent suspension, which induces the direct condensation between the peptide−peptide hydrophobic domains and results in the peptide α-helix to *β*-sheet transition. A simplified version of M13 phage maturation (right side) illustrates the peptides lying on the phage DNA template with an angle (13−20°) between the pVIII and the long axis of DNA (*44*). The extrusion process avoids the peptide α-helix to *β*-sheet transition as the hydrophobic domains move through the cell membrane. The peptide secondary structures in the different steps are indicated as α-helix or *β*-sheet.

membrane to the outside. During the translocation, the coat is formed when pV is replaced on the DNA by pVIII from the membrane (*45*, *46*). The interaction between DNA and pVIII occurs in a membrane-bound assembly site, which provides a protein environment to support the phage assembly and avoids exposing pVIII to an aqueous environment, and the α-helical conformation remains the final conformation of the pVIII in the coat. Because of this sequential "extrusion" process, the pVIII α-helix structure is preserved in the mature phage to form a rodlike coat on DNA.

In detergent or phospholipid bilayers, the M13 pVIII bound to lipid bilayers is well described as an α-helix. The membrane anchor domain is buried in the bilayer with the DNA binding domain exposed in the membrane−water interface (*23*). Furthermore, it was reported that pVIII (*45*) and its transmembrane mimic (*47*) could associate into noncovalent dimer or higher order oligomer in detergent or lipid bilayers. In the absence of DNA, helix−helix interactions can induce an α-helix to *β*-sheet transition of pVIII (*13*) as can elevated temperatures (*48*). Modifications of the pVIII transmembrane domain by the addition of several lysines on both the N-terminal and C-terminal improved the solubility of the peptide (*13*). To promote dimerization of these more soluble peptides, a single cysteine was placed at the N-terminal (*13*). This strategy revealed that in a detergent solution interactions between the transmembrane α-helices promote the transformation into *β*-sheet structures at elevated temperature (*13*, *27*).

These observations raised the question as to how pVIII can maintain an α-helix conformation in the phage filament; are other phage proteins needed to form a template to seed pVIII into an α-helical conformation in the viron, or are interactions between DNA and pVIII the factor that stabilizes the helix in the viron?

To learn if DNA is a template for the assembly of a rod-shaped structure with pVIII in an α-helix, we measured the secondary structure of pVIII in various environments with and without DNA. Three different peptides were synthesized (Figure 1): full length VIII, the C terminal 32mer analogue that contains the cationic DNA interacting segment plus the transmembrane domain, and the 32mer with a PEG segment

attached to the N terminal region. The latter was prepared in an attempt to stabilize the α-helical conformation of the 32mer as well as to improve the aqueous solubility of the 32mer. It also created an asymmetrical peptide that had a water soluble, but uncharged, N-terminal, which eliminates the possibility of charge interactions between the acidic N-terminal of pVIII and its basic C-terminal and, hence, reduces the possibility of antiparallel dimerization driving β-sheet formation. All three peptides had a significant α-helical content in TFE and in detergent solutions (Table 1).

pVIII and Its Peptide Can Form Nanoparticles with DNA Using the Detergent Dialysis Method. The detergent removal method enabled reconstitution of nanoparticles from non-ionic detergent suspensions of pVIII-based peptides with DNA that encapsidated the DNA and protected it from DNase digestion (Figure 3).

A variety of conditions, peptide/DNA nucleotide (P/N) ratios, and detergents were examined to learn their effect on peptide conformation as well as on particle formation (Table 2, Table 3). OG demonstrated good performance for the peptide−DNA reconstitution because of its non-ionic nature and high CMC which makes it easily removed from the suspensions.

Particles were formed between DNA and all three of the peptides studied when the detergent was removed. When the P/N ratios were 0.25 to 0.33, the particle diameters were about 175 nm when measured by dynamic light scattering (Table 2). The particles prepared from fluorescein labeled pVIII peptides could be visualized as discrete punctate objects by confocal microscopy (Figure 7D) and as globular aggregates using atomic force microscopy (Figure 7C) that differed from both the DNA used to form them (Figure 7A) and from a M13 bacteriophage (Figure 7B). The process that appears to occur during the reconstitution is outlined in Figure 8.

Particles could be prepared from either single or double-stranded DNA, which is different than the findings of Knippers and Hoffmann-Berling (*7*) with the fd coat protein, where a particle could only be formed with ssDNA from the urea removal process. This difference is most likely due to kinetic factors: urea is more rapidly removed from the protein−DNA mixture than is detergent, and short cationic segments bind more avidly to single-stranded DNA than to double-stranded DNA (*49*); hence peptide−peptide aggregation as the urea is removed may proceed more rapidly than does peptide−DNA assembly.

To reduce pVIII−DNA interaction, we used octanoic acid as the solubilizing detergent. The anionic micelle formed by octanoic acid can interact with the DNA binding domain of the 32mer, and when the octanoic acid was removed by dialysis, a large aggregate was formed that did not encapsidate DNA (Table 2).

The Transmembrane Segment of pVIII Has Peptide−Peptide and Peptide−DNA Interactions That Are Similar to Those of pVIII. By removing the amphipathic helix domain from pVIII, we obtain the 32mer that contains one helix domain, the transmembrane domain. Thus the change of the α-helical content in the presence of DNA can be solely attributed to the structural change of the transmembrane domain. The 32mer maintains a slightly lower α-helical content when compared to pVIII, in detergents or TFE. PEGylation on the N-terminal of 32mer increased the α-helical content to close to that observed in pVIII (Table 1 and Figure 2). The PEG-32mer also was similar to pVIII in terms of its thermostability and in the recovery of α-helical content after heating and cooling. These results are similar to the previous findings of peptides that contain the transmembrane domain and that were used to study the helix interactions in pVIII (*13*). The 32mer and PEG-32mer also formed a peptide/DNA particle (Table 2 and Figure 3). Since the PEG moiety removes the possibility of an electrostatic interaction between the N-terminal and DNA, the PEG-32mer must have a similar orientation on the DNA template as found in the intact M13 phage: the C-terminal interacts with the DNA. The PEG chain may reduce the peptide−peptide interactions at high temperature and allow refolding into an α-helix as the sample is cooled (Figure 2).

The Structural Change in the Peptide during Reconstitution Is Promoted by Nonspecific Electrostatic Interaction of the DNA-Binding Domain with DNA. The results indicate that all three peptides can bind to both dsDNA and ssDNA (Tables 2−4). Since pVIII in the native M13 phage is highly α-helical (*1*), the DNA induced α-helix to β-sheet transition of pVIII was not expected. The α-helix to β-sheet transition of pVIII observed in bilayers or detergent micelles in the absence of DNA was previously interpreted as evidence for strong pVIII−pVIII contacts (*24*, *43*). Furthermore, transmembrane mimics of pVIII convert to β-sheet from α-helical secondary structures at elevated temperature (*13*, *27*). Thus we think that interaction of DNA with the DNA binding domain in the micelle promotes peptide−peptide interactions which converts the α-helix to a β-sheet structure. This interaction occurs rapidly upon DNA addition; the transition is complete in about half an hour (Figure 4). The peptide remains in a relatively hydrophobic environment after the DNA is added into the detergent solution as indicated by the emission maximum of the tryptophan fluorescence (Figure 6). This suggests that the peptides are in the micelles when they interact with the DNA.

Removal of the detergent by dialysis further promotes the α-helix to β-sheet transition and places the tryptophan into a more hydrophilic environment as indicated by the red shift of the tryptophan fluorescence maximum (Figure 6). The effect of DNA addition and subsequent detergent removal on the conformation was similar for all three peptides.

When the anionic octanoic acid or the cationic 1,5-diaminopentane or 1,3-diamino-2-hydroxypropane was added into the 32mer/DNA reconstitution system, the transition into a β-sheet conformation did not occur when DNA was added (Table 3 and Figure 5). This result further supports the idea that the pVIII−pVIII interaction was provided by nonspecific electrostatic interaction between the DNA binding domain on the C-terminal of pVIII and DNA template. This result is consistent with the previous reports on M13 phage as nonspecific charge neutralization is also taking place in the phage particle (*50*, *51*).

The current dialysis procedure does not reconstitute a rod-shaped particle from the mixture of pVIII and DNA. This may be due to a kinetic effect caused by multiple peptides interacting with multiple positions on the DNA strand, a situation that cannot arise during the *in vivo* sequential assembly where only a few DNA binding sites are exposed to bilayer bound pVIII. The DNA-induced α-helix to β-sheet

transition *in vitro* might be due to "premature" peptide−peptide intermolecular hydrogen bonding which promotes a helix to sheet transition and impedes peptide alignment along the DNA long axis (Figure 8). The addition of the anionic or cationic agents can inhibit the binding of the peptides to DNA and subsequently inhibit peptide−peptide interactions on the DNA which drive the conformational change. With respect to M13 phage assembly, the assembly process is well controlled to allow only the peptides on the membrane-bound extrusion complex to coat on the circular ssDNA.

These results suggest that the assembly process to form a rod-shaped phage is a delicate balance to maintain pVIII in an α-helical conformation that requires either an oriented bilayer to solubilize pVIII prior to interaction with the DNA or other phage proteins to nucleate pVIII in the α-helical conformation on the DNA. One possible way to improve the α-helix content of pVIII in the DNA−detergent solution is to mutate amino acid residues in the sequence of pVIII to improve the stability of the α-helix, such as the mutation of valine to alanine in pVIII (*27*). By improving the α-helix stability of pVIII, the peptides might be packed on the DNA template into a configuration that more closely resembles the rod-shaped structure of the native M13 bacteriophage. Successful encapsidation of DNA in a peptide coat of defined structure would clarify issues related to bacteriophage assembly and could enable the design of improved DNA vectors for gene therapy. Previous peptide-based non-viral gene delivery systems using hydrophilic cationic peptides have shown promising gene transfer capabilities in cultured cells because of their bilayer destabilizing properties. However, their use in animals has been limited due to their highly positively charged nature which causes them to aggregate into larger structures when placed in blood which contains a high concentration of negatively charged proteins (*36*−*38*). The placement of positively charged residues in the peptide that can mediate the assembly of a vector containing DNA could be improved through investigating the viral peptide−DNA and peptide−peptide interaction. This study of pVIII−DNA interaction and assembly indicates that hydrophobic peptides can be used to form a peptide−DNA complex and that secondary structural measurements may provide useful information to guide the design of improved DNA vectors.

ACKNOWLEDGMENT

We gratefully acknowledge the helpful assistance of Vicky W. Pan and Dr. Jeff J. Sperinde in the preliminary experiments. Thanks are extended to the UCSF Macromolecular Structure Group (D. Agard and P. Hwang) for the use of their mass spectrometer and circular dichroism spectrometer and the UCSF Mass Spectrometry Facility (A. L. Burlingame, Director) supported by NIH NCRR RR01614. We thank Professor Jean M. J. Fréchet for the use of the atomic force microscope in his group at UC Berkeley.

REFERENCES

1. Marvin, D. A., and Wachtel, E. J. (1975) Structure and assembly of filamentous bacterial viruses, *Nature 253*, 19−23.
2. Marvin, D. A. (1998) Filamentous phage structure, infection and assembly, *Curr. Opin. Struct. Biol. 8*, 150−158.
3. Stassen, A. P., Folmer, R. H., Hilbers, C. W., and Konings, R. N. (1994) Single-stranded DNA binding protein encoded by the filamentous bacteriophage M13: structural and functional characteristics, *Mol. Biol. Rep. 20*, 109−127.
4. Russel, M. (1991) Filamentous phage assembly, *Mol. Microbiol. 5*, 1607−1613.
5. Fraenkel-Conrat, H., and Williams, R. C. (1955) Reconstitution of Active Tobacco Mosaic Virus from Its Inactive Protein and Nucleic Acid Components, *Proc. Natl. Acad. Sci. U.S.A. 41*, 690−698.
6. Fraenkel-Conrat, H. (1970) Reconstitution of viruses, *Annu. Rev. Microbiol. 24*, 463−478.
7. Knippers, R., and Hoffmann-Berling, H. (1966) A coat protein from bacteriophage fd. II. Interaction of the protein with DNA in vitro, *J. Mol. Biol. 21*, 293−304.
8. Cavalieri, S. J., Goldthwait, D. A., and Neet, K. E. (1976) The isolation of a dimer of gene 8 protein of bacteriophage fd, *J. Mol. Biol. 102*, 713−722.
9. Feng, J. N., Russel, M., and Model, P. (1997) A permeabilized cell system that assembles filamentous bacteriophage, *Proc. Natl. Acad. Sci. U. S. A. 94*, 4068−4073.
10. Thiaudiere, E., Soekarjo, M., Kuchinka, E., Kuhn, A., and Vogel, H. (1993) Structural characterization of membrane insertion of M13 procoat, M13 coat, and Pf3 coat proteins, *Biochemistry 32*, 12186−12196.
11. Papavoine, C. H., Christiaans, B. E., Folmer, R. H., Konings, R. N., and Hilbers, C. W. (1998) Solution structure of the M13 major coat protein in detergent micelles: a basis for a model of phage assembly involving specific residues, *J. Mol. Biol. 282*, 401−419.
12. Spruijt, R. B., Wolfs, C. J., Verver, J. W., and Hemminga, M. A. (1996) Accessibility and environment probing using cysteine residues introduced along the putative transmembrane domain of the major coat protein of bacteriophage M13, *Biochemistry 35*, 10383−10391.
13. Wang, C., and Deber, C. M. (2000) Peptide mimics of the M13 coat protein transmembrane segment. Retention of helix-helix interaction motifs, *J. Biol. Chem. 275*, 16155−16159.
14. Wyman, T. B., Nicol, F., Zelphati, O., Scaria, P. V., Plank, C., and Szoka, F. C., Jr. (1997) Design, synthesis, and characterization of a cationic peptide that binds to nucleic acids and permeabilizes bilayers, *Biochemistry 36*, 3008−3017.
15. Keller, M., Tagawa, T., Preuss, M., and Miller, A. D. (2002) Biophysical characterization of the DNA binding and condensing properties of adenoviral core peptide mu, *Biochemistry 41*, 652−659.
16. Morris, M. C., Vidal, P., Chaloin, L., Heitz, F., and Divita, G. (1997) A new peptide vector for efficient delivery of oligonucleotides into mammalian cells, *Nucleic Acids Res. 25*, 2730−2736.
17. Kyte, J., and Doolittle, R. F. (1982) A simple method for displaying the hydropathic character of a protein, *J. Mol. Biol. 157*, 105−132.
18. Zimmerman, J. M., Eliezer, N., and Simha, R. (1968) The characterization of amino acid sequences in proteins by statistical methods, *J. Theor. Biol. 21*, 170−201.
19. Chen, Y. H., Yang, J. T., and Martinez, H. M. (1972) Determination of the secondary structures of proteins by circular dichroism and optical rotatory dispersion, *Biochemistry 11*, 4120−4131.
20. Drin, G., Demene, H., Temsamani, J., and Brasseur, R. (2001) Translocation of the pAntp peptide and its amphipathic analogue AP-2AL, *Biochemistry 40*, 1824−1834.
21. Sperinde, J. J., Choi, S. J., and Szoka, F. C., Jr. (2001) Phage display selection of a peptide DNase II inhibitor that enhances gene delivery, *J. Gene Med. 3*, 101−108.
22. Liu, L. P., and Deber, C. M. (1998) Guidelines for membrane protein engineering derived from de novo designed model peptides, *Biopolymers 47*, 41−62.
23. Stopar, D., Spruijt, R. B., and Hemminga, M. A. (2006) Anchoring mechanisms of membrane-associated M13 major coat protein, *Chem. Phys. Lipids 141*, 83−93.
24. Spruijt, R. B., and Hemminga, M. A. (1991) The in situ aggregational and conformational state of the major coat protein of bacteriophage M13 in phospholipid bilayers mimicking the inner membrane of host *Escherichia coli*, *Biochemistry 30*, 11147−11154.
25. Yuen, C. T., Davidson, A. R., and Deber, C. M. (2000) Role of aromatic residues at the lipid-water interface in micelle-bound bacteriophage M13 major coat protein, *Biochemistry 39*, 16155−16162.

26. Li, Z., Glibowicka, M., Joensson, C., and Deber, C. M. (1993) Conformational states of mutant M13 coat proteins are regulated by transmembrane residues, *J. Biol. Chem. 268*, 4584−4587.
27. Deber, C. M., Khan, A. R., Li, Z., Joensson, C., Glibowicka, M., and Wang, J. (1993) Val−>Ala mutations selectively alter helix-helix packing in the transmembrane segment of phage M13 coat protein, *Proc. Natl. Acad. Sci. U.S.A. 90*, 11648−11652.
28. Neugebauer, J. (1987) *A Guide to the Properties and Uses of Detergents in Biology and Biochemistry*, Hoechst Celanese Corporation.
29. Nozaki, Y., Chamberlain, B. K., Webster, R. E., and Tanford, C. (1976) Evidence for a major conformational change of coat protein in assembly of fl bacteriophage, *Nature 259*, 335−337.
30. Stopar, D., Spruijt, R. B., Wolfs, C. J., and Hemminga, M. A. (2002) Structural characterization of bacteriophage M13 solubilization by amphiphiles, *Biochim. Biophys. Acta 1594*, 54−63.
31. Eftink, M. R., and Ghiron, C. A. (1975) Dynamics of a protein matrix revealed by fluorescence quenching, *Proc. Natl. Acad. Sci. U.S.A. 72*, 3290−3294.
32. Moreno-Herrero, F., Colchero, J., and Baro, A. M. (2003) DNA height in scanning force microscopy, *Ultramicroscopy 96*, 167−174.
33. Douglas, T., and Young, M. (2006) Viruses: making friends with old foes, *Science 312*, 873−875.
34. Manchester, M., and Singh, P. (2006) Virus-based nanoparticles (VNPs): platform technologies for diagnostic imaging, *Adv. Drug Delivery Rev. 58*, 1505−1522.
35. Murphy, E. A., Waring, A. J., Murphy, J. C., Willson, R. C., and Longmuir, K. J. (2001) Development of an effective gene delivery system: a study of complexes composed of a peptide-based amphiphilic DNA compaction agent and phospholipids, *Nucleic Acids Res. 29*, 3694−3704.
36. Legendre, J. Y., and Szoka, F. C., Jr. (1993) Cyclic amphipathic peptide-DNA complexes mediate high-efficiency transfection of adherent mammalian cells, *Proc. Natl. Acad. Sci. U.S.A. 90*, 893−897.
37. Li, W., Nicol, F., and Szoka, F. C., Jr. (2004) GALA: a designed synthetic pH-responsive amphipathic peptide with applications in drug and gene delivery, *Adv. Drug. Delivery Rev. 56*, 967−985.
38. Morris, M. C., Chaloin, L., Heitz, F., and Divita, G. (2000) Translocating peptides and proteins and their use for gene delivery, *Curr. Opin. Biotechnol. 11*, 461−466.
39. Russel, M. (1993) Protein-protein interactions during filamentous phage assembly, *J. Mol. Biol. 231*, 689−697.
40. Smilowitz, H. (1974) Bacteriophage f1 infection: fate of the parental major coat protein, *J. Virol. 13*, 94−99.
41. Russel, M. (1994) Phage assembly: a paradigm for bacterial virulence factor export?, *Science 265*, 612−614.
42. McDonnell, P. A., Shon, K., Kim, Y., and Opella, S. J. (1993) fd coat protein structure in membrane environments, *J. Mol. Biol. 233*, 447−463.
43. Sanders, J. C., Haris, P. I., Chapman, D., Otto, C., and Hemminga, M. A. (1993) Secondary structure of M13 coat protein in phospholipids studied by circular dichroism, Raman, and Fourier transform infrared spectroscopy, *Biochemistry 32*, 12446−12454.
44. Overman, S. A., Tsuboi, M., and Thomas, G. J., Jr. (1996) Subunit orientation in the filamentous virus Ff(fd, f1, M13), *J. Mol. Biol. 259*, 331−336.
45. Stopar, D., Spruijt, R. B., Wolfs, C. J., and Hemminga, M. A. (1997) In situ aggregational state of M13 bacteriophage major coat protein in sodium cholate and lipid bilayers, *Biochemistry 36*, 12268−12275.
46. Russel, M. (1995) Moving through the membrane with filamentous phages, *Trends Microbiol. 3*, 223−228.
47. Melnyk, R. A., Partridge, A. W., and Deber, C. M. (2002) Transmembrane domain mediated self-assembly of major coat protein subunits from Ff bacteriophage, *J. Mol. Biol. 315*, 63−72.
48. Spruijt, R. B., Wolfs, C. J., and Hemminga, M. A. (1989) Aggregation-related conformational change of the membrane-associated coat protein of bacteriophage M13, *Biochemistry 28*, 9158−9165.
49. Zhang, W., Bond, J. P., Anderson, C. F., Lohman, T. M., and Record, M. T., Jr. (1996) Large electrostatic differences in the binding thermodynamics of a cationic peptide to oligomeric and polymeric DNA, *Proc. Natl. Acad. Sci. U.S.A. 93*, 2511−2516.
50. Symmons, M. F., Welsh, L. C., Nave, C., Marvin, D. A., and Perham, R. N. (1995) Matching electrostatic charge between DNA and coat protein in filamentous bacteriophage. Fibre diffraction of charge-deletion mutants, *J. Mol. Biol. 245*, 86−91.
51. Hunter, G. J., Rowitch, D. H., and Perham, R. N. (1987) Interactions between DNA and coat protein in the structure and assembly of filamentous bacteriophage fd, *Nature 327*, 252−254.

BI700165M