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# Raman spectroscopic study on the conformation of a peptide fragment representing the DNA-binding domain of filamentous virus Pf3 coat protein

Takashi Miura*, Hideo Takeuchi and Issei Harada

*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan*

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Raman spectra have been measured of a nonapeptide which has an amino acid sequence identical to that of the C-terminal region of the major coat protein subunit of filamentous bacteriophage Pf3. The peptide shows a strong tendency to form a β-sheet structure in aqueous solution. The β-sheet formation is significantly promoted by complexation with single-stranded DNA but not with double-stranded DNA. It is suggested that the C-terminal region of the Pf3 coat protein binds to the single-stranded DNA genome in the virion with a β-sheet conformation, in sharp contrast with the α-helical binding in other filamentous bacteriophages.

Filamentous virus; Coat protein; Secondary structure; DNA–protein interaction; Raman spectroscopy

## 1. INTRODUCTION

Filamentous bacteriophages contain single-stranded DNA (ssDNA) as the viral genome, which is encapsulated in a shell made up of coat protein molecules. The major component of the coat protein is a large number of protein subunits of an identical amino acid sequence. Each subunit consists of acidic N-terminal, basic C-terminal, and middle hydrophobic regions [1–5]. In the best-studied filamentous bacteriophages, M13 and Pf1, the secondary structure of the coat subunit is predominantly α-helical and the helix is oriented roughly parallel to the axis of the filament with the positively charged C-terminal region abutting on the encapsulated DNA [6–10]. α-Helical interaction of coat protein with nucleic acid has also been suggested for the cylindrical tobacco mosaic virus [11] and for an icosahedral plant virus, cowpea chlorotic mottle virus [12]. The α-helical structure seems to be the main motif of the virus coat protein architectures, in particular at the interface with the nucleic acid.

The major coat protein of filamentous bacteriophage Pf3 is unique among those of filamentous phages [5]. Its subunit contains only two basic amino acid residues in the C-terminal region, in spite of the presence of 2.4 DNA phosphates per subunit in the virion. This is unusual because the major coat proteins of other viruses contain more basic residues than those required to neutralize the negative charges of nucleotides. Another unique feature is two successive Phe residues located at the C-terminus. Other filamentous phages do not have aromatic residues at these positions. The architecture of the Pf3 coat protein, particularly in the C-terminal DNA-binding region of the subunit, may differ from the usual α-helical motif.

In this work, we have studied the Raman spectra of a nonapeptide which has the same amino acid sequence as the C-terminal region of the Pf3 coat subunit. The Raman spectra recorded in aqueous solution and in complexes with DNA show that the nonapeptide has a strong tendency to assume a β-strand in aqueous solution and forms an ordered β-sheet structure when bound to ssDNA.

## 2. MATERIALS AND METHODS

DNA sodium salt (from calf thymus) was purchased from Sigma Chemicals, and purified by precipitation from 80% aqueous ethanol solution. ssDNA was prepared by denaturing double-stranded DNA (dsDNA) in boiling water for 5 min and then immediately cooling the solution in an ice bath. The nonapeptide, Ile-Arg-Trp-Ile-Lys-Ala-Gln-Phe-Phe (CP9), was synthesized on a solid phase peptide synthesizer (Pharmacia, Biolynx 4175) using the 9-fluorenylmethoxycarbonyl method. The peptide was cleaved from the resin with Reagent K [13] and purified on a Jasco 880 HPLC with a reversed-phase column (Nacalai 5C18-AR). To exclude trifluoroacetic acid, which came from Reagent K and the HPLC solvent, CP9 was precipitated from an aqueous solution at pH 10, redissolved at pH 7 by adding aqueous HCl, and then lyophilized. The complex of CP9 and DNA was obtained as a precipitate by mixing solutions of DNA sodium salt (6 mM in nucleotide) and CP9 hydrochloride (6 mM) at pH 7. Other concentration ratios of DNA/peptide also gave precipitates, but their Raman spectra were almost identical to each other. The number of

*Present address:* Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri at Kansas City, Kansas City, MO 64110-2499, USA.

*Correspondence address:* I. Harada, Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan. Fax: (81) (22) 263-9205.

nucleotides per peptide was estimated from the DNA/peptide Raman intensity ratio to be 2.0 and 1.8 for the complexes with ssDNA and dsDNA, respectively. Relative Raman scattering cross-sections of the peptide and DNA were measured in aqueous solution using the 981 $cm^{-1}$ band of $SO_4^{2-}$ added as an internal intensity standard.

Raman spectra were recorded on a Jasco CT-80D double monochromator equipped with a multi-channel detector (Princeton Instruments D/SIDA-700I/G) and an $Ar^+$ laser (488.0 nm, 200 mW). The spectral slit width was 5 $cm^{-1}$ and the peak wavenumbers of Raman bands were reproducible to within ±1 $cm^{-1}$. The scattering from solvent water was subtracted.

## 3. RESULTS AND DISCUSSION

Fig. 1 shows Raman spectra of aqueous solutions of CP9. In the spectrum of 5 mM solution (Fig. 1a), four peaks at 1258, 1250, 1235, and 1230 $cm^{-1}$ are seen in the amide III region. The 1258 and 1235 $cm^{-1}$ peaks are assignable to Trp side chain modes [14]. The remaining two bands disappear in $D_2O$ solution and they are assigned to the amide III modes of β-strand (1230 $cm^{-1}$) and irregular (1250 $cm^{-1}$) structures. As the peptide concentration increases (10 and 20 mM in Fig. 1b and c), the intensity at 1230 $cm^{-1}$ increases and that at 1250 $cm^{-1}$ decreases, suggesting that some molecules undergo conformational transition from the irregular to β-stranded form. Concomitant with the changes in the amide III region, the amide I band at 1666 $cm^{-1}$ becomes stronger and sharper, though the peak frequency remains unchanged. The sharpening of the amide I band is consistent with the irregular to β-strand conformational transition because irregular and β-stranded conformers usually give a broad band in the 1668–1662 $cm^{-1}$ region and a sharp band in the 1674–1665 $cm^{-1}$ region, respectively [14]. It is evident that CP9 has a strong tendency to form a β-strand and intermolecular association promotes the β-sheet formation.

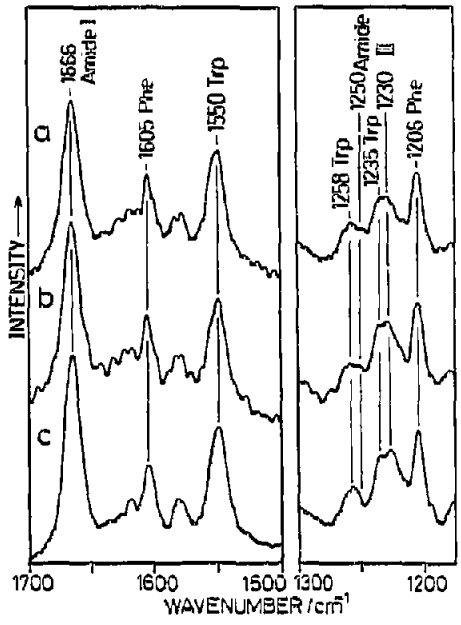


Fig. 1. Raman spectra of CP9 aqueous solution at pH 7: (a) 5 mM, (b) 10 mM, and (c) 20 mM.

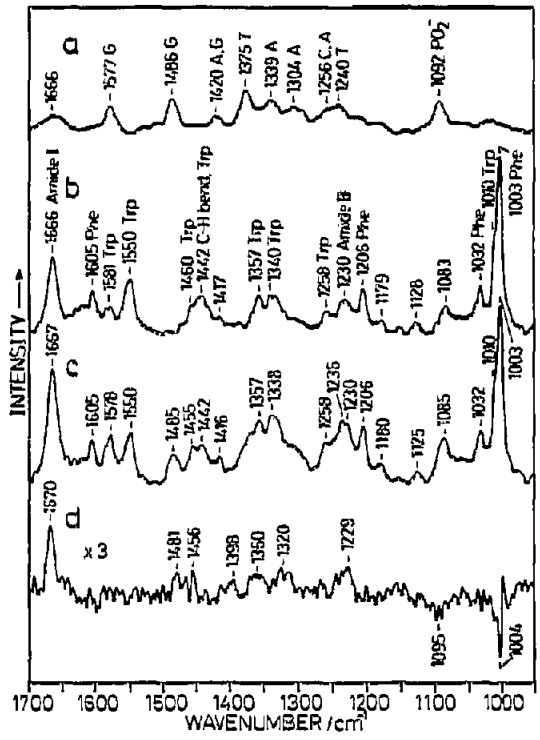


Fig. 2. Raman spectra of (a) thermally denatured calf thymus DNA (ssDNA) in aqueous solution, (b) CP9 in aqueous solution, (c) the CP9–ssDNA complex in the solid state, and (d) a difference spectrum: c−(a+b). The intensity of the difference spectrum is amplified by a factor of three.

Fig. 2a shows the Raman spectrum of thermally denatured DNA in aqueous solution. A prominent band at 1240 $cm^{-1}$ is due to a thymine base vibration and a marker of ssDNA [15]. The Raman spectrum of CP9 aqueous solution in the 1700–950 $cm^{-1}$ region is shown in Fig. 2b. The intensities of the two spectra are scaled such that the relative intensity corresponds to the 2:1 nucleotide/peptide mole ratio. Fig. 2c shows the spectrum of the CP9–ssDNA complex precipitated by mixing the peptide and DNA solutions. On the assumption that the total integrated intensity in the 1700–950 $cm^{-1}$ region is not affected by the complexation, we calculated a difference spectrum between the complex and the sum of components (Fig. 2c minus the sum of Fig. 2a and b). The difference spectrum in Fig. 2d shows significant intensity increases of the amide I (1670 $cm^{-1}$) and amide III (1229 $cm^{-1}$) bands upon complexation. These changes are analogous to those observed for CP9 alone when the concentration was raised (Fig. 1). CP9 must form a β-sheet structure in the complex with ssDNA.

Additional information on the peptide–ssDNA interaction can be drawn from the difference spectrum. A strong negative peak at 1005 $cm^{-1}$ is ascribed to an

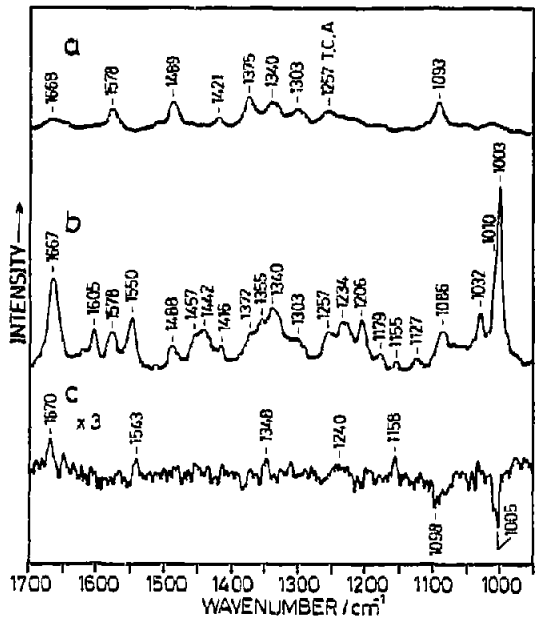


Fig. 3. Raman spectra of (a) intact calf thymus DNA (dsDNA) in aqueous solution, (b) the CP9-dsDNA complex in the solid state, and (c) a difference spectrum: the complex − the sum of components. The intensity of the difference spectrum is amplified by a factor of three.

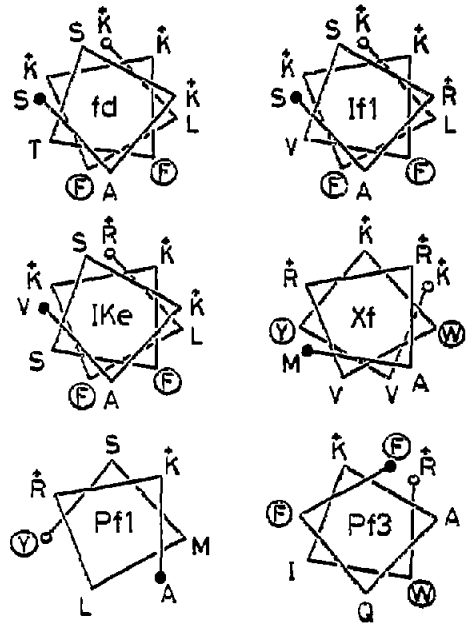


Fig. 4. Projection of residue positions along the helix axis for the C-terminal basic regions of the major coat subunits of filamentous viruses, fd, If1, IKe, Xf, Pf1, and Pf3. The basic region starts with a basic residue following the last common residue, Ile, of the hydrophobic region. The amino acid sequences are taken from [5] and represented by one-letter symbols. Open and filled circles indicate the beginning of the basic region and the C-terminal end, respectively. Basic residues are marked with + and aromatic residues are encircled.

intensity decrease of the Phe ring mode at 1003 $cm^{-1}$, probably due to ring stacking interaction with DNA [16]. The 1360 $cm^{-1}$ positive peak may be due to an intensity increase of the Trp W7 mode, which is known to intensify when the Trp indole ring is located in hydrophobic environments [17]. Positive and negative peaks around 1450 $cm^{-1}$ may be due to band shifts of Trp ring modes and/or C–H bending modes of aliphatic side chains. The 1320 $cm^{-1}$ peak is ascribed to a conformation-sensitive guanine ring mode, which appears strong at 1318 $cm^{-1}$ in the C3′*endo/anti* conformation [18,19]. This conformation is found for guanine residues in the genome of the Pf3 virion [19]. A positive peak at 1481 $cm^{-1}$ is also ascribed to changes in frequency and intensity of a guanine Raman band at 1486 $cm^{-1}$. Since strong hydrogen bonding at guanine N7 is known to decrease the frequency of this band [18], the N7 sites of some guanine bases may be hydrogen-bonded with strong proton donors, possibly with the Lys $NH_3^+$ or Arg $C(NH_2)_2^+$ groups of CP9. The appearance of a positive peak at 1398 $cm^{-1}$ is analogous to an intensity increase at 1402 $cm^{-1}$ accompanied by a conformational transition from C2′*endo/anti* to C3′*endo/anti* of thymine [20,21], the latter conformation being found in the Pf3 virion [19].

Fig. 3 shows the Raman spectra of dsDNA (a) and the precipitate obtained from a mixture solution of CP9 and dsDNA (b). The spectrum of the CP9–dsDNA complex is very close to the sum of CP9 (Fig. 2b) and dsDNA spectra. The difference spectrum calculated in the same way as for the CP9–ssDNA complex shows much smaller effects of complexation in the amide I and III regions as well as in the conformation marker regions of DNA, suggesting weaker interactions of CP9 with dsDNA. Accordingly, the $\beta$-sheet structure of CP9 in the complex with ssDNA is not attributable to a concentration effect associated with precipitation. Specific interaction between CP9 and ssDNA promotes the $\beta$-sheet formation. The present result strongly suggests that the Pf3 coat protein encapsidates the ssDNA genome with the C-terminal basic region of the subunit in a $\beta$-sheet structure. Previous Raman spectroscopic studies by Thomas et al. have shown that intact Pf3 virion contains 18% $\beta$-sheet and 82% $\alpha$-helical structures in coat protein subunits [16,22]. The percentage of $\beta$-sheet structure corresponds well to the percentage of amino acid residues composing the DNA-binding C-terminal basic region (8 residues of the total 44 residues in a subunit).

It is pointed out that amphiphilicity is one of the important factors that determine the conformations of peptides and proteins [23,24]. For instance, a peptide in which hydrophilic residues repeat every three or four residues will form an amphiphilic $\alpha$-helix. This is the case for the C-terminal basic regions of coat subunits of filamentous bacteriophages fd, If1, IKe, Xf, and Pf1 (see Fig. 4). Although the positive charges of basic residues are located in close spatial proximity of each other

and the α-helix may be energetically unfavorable by itself, the charge polarization can be neutralized by binding of the negatively charged phosphate groups of DNA, which results in stabilization of α-helical structure in the protein–DNA complex. In contrast to these filamentous viruses, the C-terminal domain of Pf3 will have poor amphiphilicity if it formed an α-helix (Fig. 4). Three aromatic amino acid side chains would be equally distributed all around the helical wheel and a hydrophobic Phe ring would lie between two basic residues, Lys and Arg. Thus, the α-helix formation cannot fully exploit the stabilization by charge neutralization in the Pf3 virion. Similarly, the β-strand conformation, in which two basic amino acid side chains of the Pf3 coat subunit stand out in opposite directions, also seems to be unfavorable to the interaction with DNA phosphates if charge neutralization is the dominant force to bind the protein and DNA. We speculate that forces other than electrostatic attraction operate in the protein–DNA interactions of Pf3.

Three aromatic amino acids, one Trp and two successive Phe's at the C-terminus, may play important roles in the interaction with DNA and in the formation of β-sheet structure. A Trp Raman band, whose frequency is a marker of the orientation of the Trp indole ring with respect to the peptide mainchain [25], is observed at 1550 $cm^{-1}$ for CP9 (Fig. 2) and at 1544 $cm^{-1}$ for intact Pf3 [16]. These frequencies are lower than the usual frequency (1552 $cm^{-1}$) of Trp side chains, suggesting strong interactions of the Trp ring with DNA and/or other amino acid residues. One of the roles of Phe residues may be to promote the β-strand formation. A CP9 analog peptide with a Leu residue replacing the C-terminal Phe did not form a β-strand in aqueous solution and gave a strong amide III Raman band at 1253 $cm^{-1}$ characteristic of irregular backbone structure (data not shown).

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