of the phage. The results shown in the table for coat protein mixed with sodium dodecyl sulfate (SDS) suggest that the structural change upon DNA binding may not be great, and that β-sheet is not involved.

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INVESTIGATION OF SECONDARY STRUCTURES AND MACROMOLECULAR INTERACTIONS IN BACTERIOPHAGE P22 BY LASER RAMAN SPECTROSCOPY*

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ABSTRACT Laser Raman spectra of the DNA bacteriophage P22 and of its precursor particles and related structures have been obtained using 514.5-nm excitation. The spectra show that P22 DNA exists in the B form both inside of the phage head and after extraction from the phage. The major coat protein (gp5) contains a secondary structure composed of 18% α-helix, 20% β-sheet and 62% irregular conformations. The scaffolding protein (gp8) in the phage prohead is substantially richer than gp5 in α-helical content. Among the amino acid residues which give prominent Raman lines, the spectra show that tryptophans are exposed to solvent and most tyrosines are hydrogen bonded to positive donor groups. The above features of phage DNA and protein structures are nearly invariant to changes in temperature up to 80°C, indicating a remarkable thermal stability of the phage head and its encapsulated DNA.

INTRODUCTION

The laser-Raman spectrum of bacteriophage P22 consists of numerous lines due to radiation scattered by the normal modes of vibration of protein and DNA molecules of the virion. Each line is identified by its frequency shift (in cm^{-1} units) from the laser excitation frequency, and its intensity relative to an arbitrary standard. Among the many factors which determine the frequency and intensity of a given Raman line are the intramolecular and intermolecular

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interactions of the atoms or groups of atoms whose vibrations generate the Raman scattering. The Raman spectrum is therefore a useful means of investigating such interactions.

In this paper we present Raman spectra of P22 phage, precursor (9⁻ and prohead) particles and protein-free DNA. The mature phage consists of a shell of coat protein (gp5) enclosing a tightly coiled double-stranded DNA molecule, and an appended tail plate consisting of the tail protein (gp9) (1). The 9⁻ particle differs from the mature phage by the absence of the tail structure. Proheads are the precursor shells in the DNA packaging process; they lack DNA but contain an inner shell of scaffolding protein (gp8) which is absent from the mature phage (2). Empty proheads have had the gp8 removed by treatment with 0.5 M guanidine·HCl, in vitro, so that they are essentially shells of coat protein (M. Fuller and J. King, this volume).

Most of the Raman lines of P22 phage are assigned to nucleotide residues of P22 DNA and amino acid residues of gp5. The spectral data permit conclusions about the kind and extent of secondary structure in P22 DNA and in gp5 of the virion. Comparison of these results with Raman spectra of the prohead also suggests fundamental differences in secondary structures of gp5 and gp8.

METHODS

Phage P22 and precursor particles were prepared as described previously (2). P22 DNA was purified from phage particles by phenol extraction, followed by ethanol precipitation.

For Raman spectroscopy, samples were loaded into glass capillary cells which were thermostatted in the spectrometer at the desired temperature (to ±0.5°C). Concentrations varied from 130 $\mu g/\mu l$ for phage to 40 $\mu g/\mu l$ for DNA. All spectra were excited with the 514.5-nm line of Ar^+ (Coherent Model CR2 laser, Coherent Inc., Palo Alto, California) and were recorded on a Spex Ramalog spectrometer (Spex Industries, Inc., Metuchen, N. J.). Further details of sample handling for Raman spectroscopy of viruses are as described previously (3).

RESULTS AND DISCUSSION

Fig. 1 shows Raman spectra of aqueous solutions of P22 and related structures. Standard abbreviations are used to indicate assignment of Raman lines to subgroup vibrations (3).

The spectrum of P22 was examined at 10°C intervals over the temperature range $0 < t < 80$°C. No major changes in the frequencies or intensities of Raman scattering were detected, indicating remarkable thermal stability of this viral structure when compared with other RNA and DNA phages examined previously (4, 5). Above 80°C the virus or its components apparently aggregate, as evidenced by increased turbidity of the solutions.

The Raman spectrum of free P22 DNA is also nearly invariant to temperature changes over the range $0 < t < 80$°C. This finding differs from the reported "pre-melting" behavior of calf-thymus DNA (6) and may be due to the specific base composition of P22 DNA or to the fact that a substantial concentration of Mg^{2+} ion (10 mM) has been employed here. At present conditions, P22 DNA does not exhibit a melting temperature below 95°C, as measured by A_{260}.

We have obtained Raman spectra of empty proheads over the range $0 < t < 55$°C, and these also are identical to the spectrum at 32°C shown in Fig. 1. Further study of the temperature dependence of Raman spectra of the normal and empty proheads is in progress.

The significant results so far obtained in these Raman studies and the structural information derived from them can be summarized as follows: (*a*) The Raman spectra of P22 and 9⁻ particles contain a prominent line near 835 cm^{-1}, similar to that found in free P22 DNA. Other Raman lines of the DNA bases exhibit intensities and frequencies which are similar for free and encapsulated states (7). These results indicate that DNA maintains a secondary

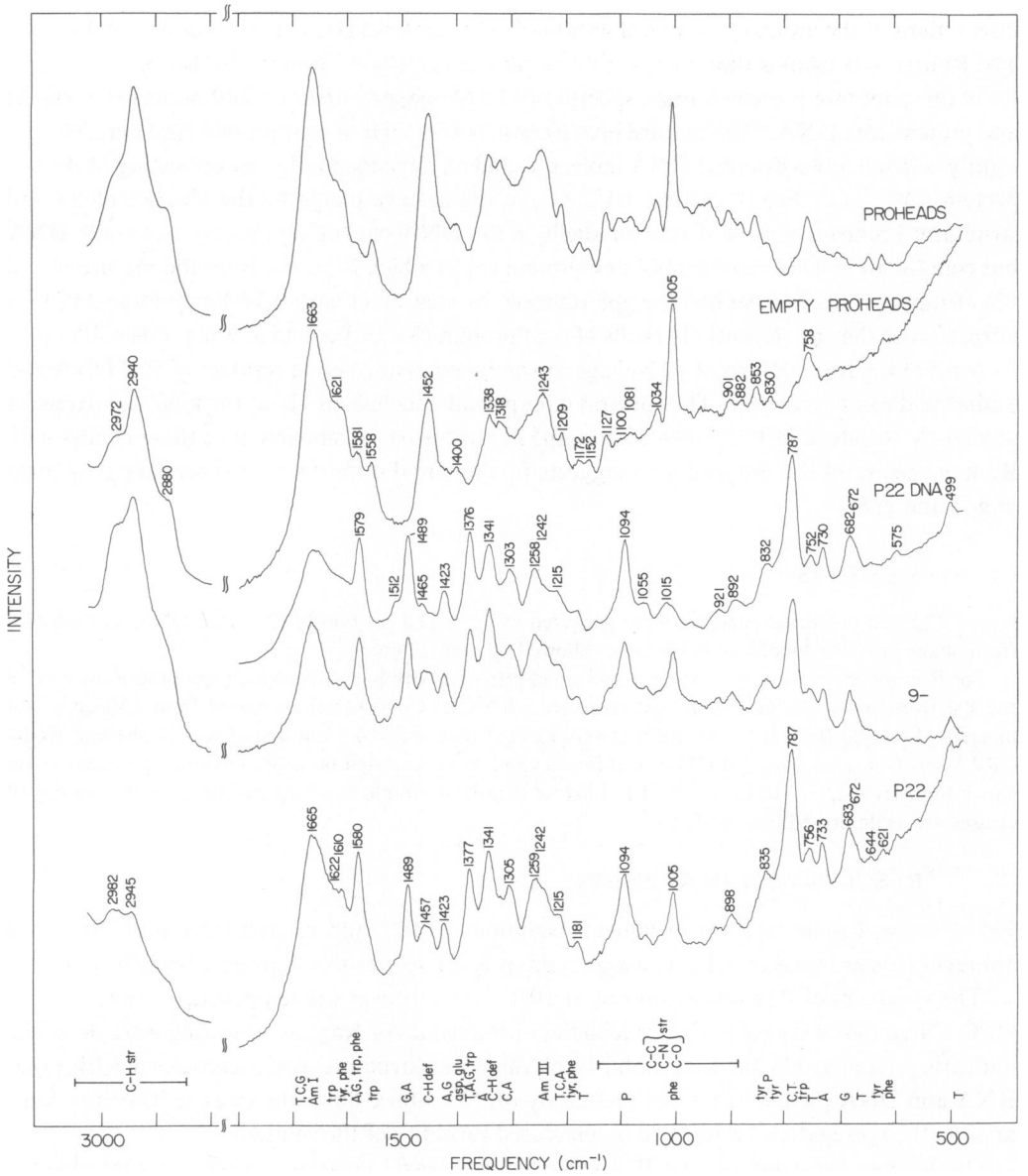

Figure 1 Raman spectra of P22 phage (4% wt/vol suspension), 9⁻ particles (13%), P22 DNA (4%), empty proheads (10%), and proheads (13%) in aqueous media contaning 10 mM Tris buffer (pH 7.5) and 10 mM $MgCl_2$. Sample temperature is 32°C in each case but spectra are essentially independent of temperature. (See text). Spectrometer conditions: λ = 514.5 nm, power = 300 mW, slit width = 10 cm^{-1}, scan speed = 25 cm^{-1}/min, rise time = 10 s.

structure of the B form both inside and outside the phage head. (*b*) Amide group frequencies of gp5 in the empty prohead occur at 1,665 cm^{-1} (amide I) and 1,243 cm^{-1} (amide III). The latter is broad and contains a poorly resolved shoulder on the high frequency side. The same amide group frequencies and intensities occur in P22 phage. Analysis of these intensities and those obtained after deuterium exchange (spectra not shown) by the method of Lippert et al. (8) indicates that the gp5 secondary structure contains ~18% α-helix, 20% β-sheet and 62% irregular conformation. (*c*) Amide group frequencies in the Raman spectrum of the prohead

occur at 1,665 and 1,245 cm^{-1}, the latter exhibiting a clearly resolved satellite peak at 1,274 cm^{-1} which indicates substantially more α-helix (~30%) at the expense of irregular conformations. An increase in α-helix is also indicated by the intensity of the line at 938 cm^{-1} in the spectrum of the prohead (8). Thus the protein gp8 is apparently richer than gp5 in α-helix. It is also possible that gp5 secondary structure is altered by the presence of gp8 in the prohead. Further investigations are in progress. (*d*) The Raman line at 758 cm^{-1} in the spectrum of empty proheads reveals the presence of tryptophan residues in gp5. Trp was not determined in previous amino acid analyses (M. Fuller and J. King, unpublished results). The ratio of trp:phe, as estimated from the Raman intensity ratio $I_{758}/I_{1,006}$, is about the same as in TMV coat protein, viz. 3:8 (S. R. Fish, K. A. Hartman, and G. J. Thomas, Jr., unpublished results). The absence of a sharp peak near 1,362 cm^{-1} indicates further that these trp residues are probably exposed to solvent. (*e*) Tyrosine residues of gp5 give Raman lines at 850 and 830 cm^{-1} with an intensity ratio of 1.9:1. This indicates that at least 54% of the para hydroxyl groups are involved as the acceptors of hydrogen bonding from relatively positive donor groups (9).

We are currently investigating whether the strong Raman scattering near 1,400 cm^{-1} in spectra of empty and normal proheads is due to exposed carboxyl groups of asp and glu residues (3).

The Raman spectrum of P22 is not sufficiently detailed to permit unambiguous detection of minor coat proteins, but the net effect of removal of the tail is evident from the diminished intensity of Raman scattering by specific amino acid residues. Future work will involve the isolation of individual proteins, including gp8 and gp9, to characterize further their secondary structures and interactions.

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