STRUCTURE OF FILAMENTOUS BACTERIOPHAGE Pf1

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The structure of filamentous bacteriophage Pf1 has been determined to 7 Å resolution by analysis of x-ray diffraction data from partially oriented fibers of virus particles (1). Continuous intensity distributions along layer lines were measured by numerically separating contributions from layer lines overlapping due to disorientation in the specimen. The data were phased by an iterative technique that utilized the known spatial extent and high α-helical content of the virus coat protein to refine structural models. Refinement of these models converges to a unique structural solution that is both consistent with the x-ray data and with information derived from physical and chemical studies. Examination of the resulting electron density map shows that the coat protein is made up of two α-helical segments. One helical segment, almost parallel to the particle axis, is centered at a radius of ~15Å. The other, at ~25Å radius, is tilted by 25° to the particle axis. This arrangement is consistent with every generalization about α-helical packing. The inner and outer segments interlock along most of their length with a crossing angle of 20.5°. The inner α-helical segments also interact with symmetry related copies of themselves as do the outer segments. The double layer of tightly packed, intricately interlocked α-helices forms a stable, 20-Å thick protein coat about the viral DNA. The arrangement of the α-helical segments of the coat protein is shown in Figs. 1 and 2.

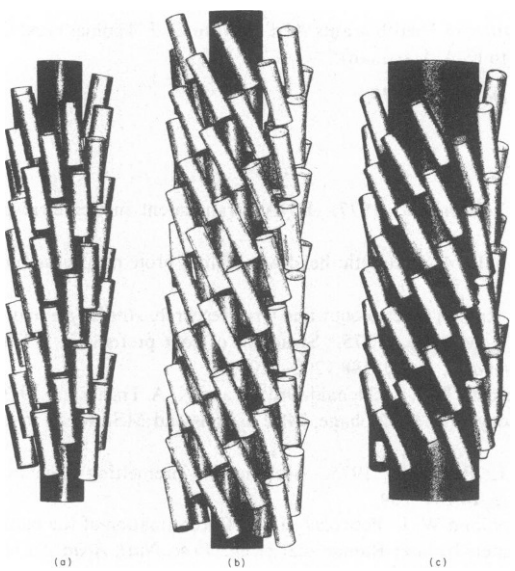

Figure 1 Diagrams showing the arrangement of α-helical segments in the Pf1 virus particle. Two axial repeats of the inner layer of rod segments are drawn in (*a*) and 2 repeats of the outer layer are shown in (*c*). About 2.5 axial repeats of both layers are shown n (*b*). The complete, 20,000 Å long Pf1 virus particle is more than 100 times as long as the section represented by this drawing. In these diagrams, the α-helical segments in the virus protein subunit are represented as cylinders about 30 Å long and 9 Å diam. The central core, occupied by DNA in the virus particle, is represented by a cylinder about 20 Å in diam. in (*a*) and (*b*). The outer layer of rads is arranged around a 40 Å diam. cylinder in (*c*) to create a simpler image of the relationships between the rods in that layer (from Makowski et al., 1980).

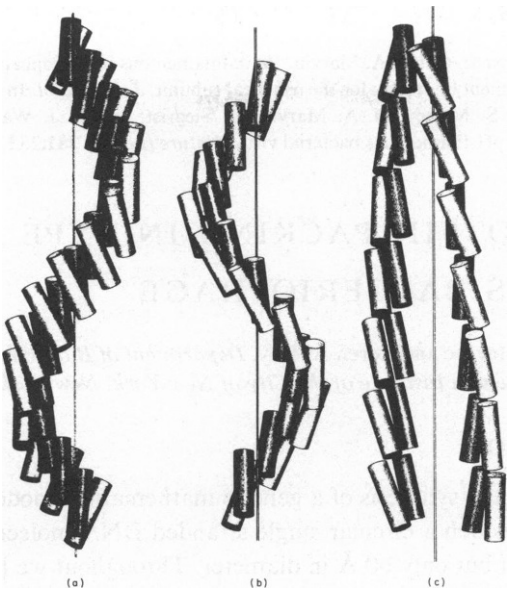

Figure 2 Diagrams illustrating the contact relations between structure units along the 5-start (*a*). 6-start (*b*), and 11-start (*c*) families of helices dissected from the particle surface lattice. For these drawings a structure unit was chosen that consists of one rod segment from the inner layer plus the segment from the outer layer that interlocks with it along most of its length with a coiled-coil crossing angle of 20.5°. The interaction between the 2 α-helical segments represented by this pair of rods accounts for over 40% of the helix helix interactions in the virus particle. This structure unit will represent a single peptide chain if the protein is in a hairpin configuration. If the protein is in an extended configuration, these 2 rod segments will correspond to α-helices in 2 different coat proteins. In either case, the entire structure can be generated from a single structure unit with the symmetry elements of the virus surface lattice (from Makowski et al., 1980).

The next stage of the analysis of the Pf1 virus structure is to begin model building to fit the 7-Å resolution map. With ordinary protein structures at this resolution, model building would be a futile undertaking. However, in Pf1 the positions of many residues are strongly constrained by their necessary interactions. The polar residues near the N-terminus must lie on the outside surface. Most of the nonpolar side chains will project into the region between the two layers of α-helices at ~20 Å radius. The basic residues and the tyrosin near the C-terminal end are likely to interact with the DNA. Each α-helix interacts with symmetrically related copies of itself, providing a very strong constraint for possible positions of the amino acid side chains. Therefore it may be possible to build a stereochemically plausible model giving the positions of all the side chains based on the 7 Å resolution map. Diffraction maxima can be observed to spacings smaller than 3 Å. The higher resolution data will provide a stringent constraint for refinement of the atomic resolution model. It should be possible to carry out this refinement by an extension of the iterative procedure used in the present analysis of the 7 Å data. This model will provide information about the interaction of protein with DNA, the forces important to virus stability, the structural rearrangements associated with the symmetry transition that occurs in the particle at ~8°C (2), and the kinds of forces acting during the membrane-associated virus assembly.

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DNA AND PROTEIN PACKING IN TYPE I FILAMENTOUS BACTERIOPHAGE

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INTRODUCTION

Our aim in this work is the synthesis of a generic mathematical model for type I filamentous phage (fd, IKe, Ifl) in which a circular single stranded DNA molecule is packed in a virion that is $\sim 10^4$ Å in length but only 60 Å in diameter. Throughout we have been guided by the principle that the structure must possess an intrinsic mechanical integrity which governs the relations of its parts. We present here a number of geometrical considerations and simple hypotheses that lead to a system of algebraic equations which relate the structure features.

A synthetic approach seems appropriate for fd since many fundamental data are available which impose constraints of different sorts on its structure, and its major coat protein consists of only 50 amino acids, ~80–90% of which are in α-helical conformation. Accordingly, each subunit is modeled as a flexible tube of ~10 Å diam. and 75 Å length. Mass-per-length data and x-ray diffraction studies suggest that fd has five-fold rotational symmetry, with five subunits every 16 Å along the structure axis (1–3). To maintain generality, we introduce N-fold rotational symmetry and locate protein subunits on an N-start helix with the N-mers of each level spaced by a distance T. Physicochemical data show that fd has 2.2– 2.4 nucleotides per major coat protein, so we require of the model a means whereby s (not necessarily an integer) nucleotides (~11or 12 in fd) can interact with N subunits (N = 5 in fd) over a distance T (T = 16 Å in fd).

CONSTRUCTING THE GENERAL MATHEMATICAL MODEL

We begin by advancing some trial assumptions which are reasonable but not inexorable. The radial electron density of Pf1 (a type II filamentous virus) suggests an inner layer and an outer layer of α-helices, each subunit contributing an α-helical segment to each layer (1), and we have assumed a structure of this general type for fd. N subunits originate in each level. In the inner layer the subunits rise through M_i levels, where M_i is a small integer, so the inner layer contains $N_i = M_i \times N$ tubes. Likewise, the outer layer contains $N_o = M_o \times N$ tubes, preserving N-fold rotational symmetry. The axis of each tube follows a helix of pitch P_i (P_o) in the inner (outer) layer and extends through an axial rise of M_iT (M_oT). Finally, we assume that the axes of the N_i helices in the inner layer continue smoothly between successive N-mers as do the axes of the N_o helices in the outer layer.

A concept we call the "pitch connection" between DNA and protein is given in the lattice diagram of Fig. 1, which shows s/2 nucleotides (from one of two antiparallel DNA chains) and N subunits in an axial distance T. Each x represents the same point on each subunit in a five-start helix of protein subunits. Each solid line represents the axis of an inner α-helix tube