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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

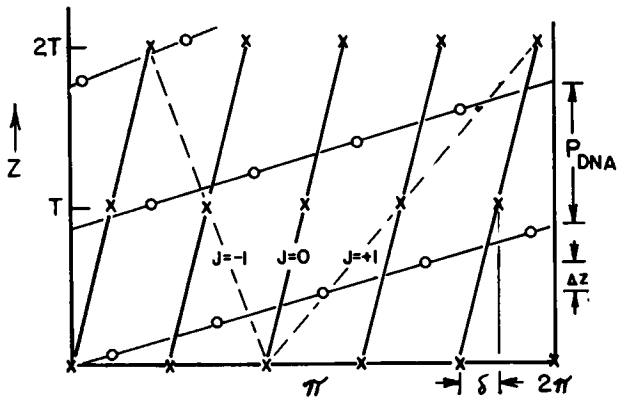

Figure 1 Lattice diagram of a N-start helix of subunits having a rotation per N-mer of δ. The principal solid lines represent axes of α-helix tubes with $J = 0$ and $P_i = 2\pi T/\delta$. Dashed lines show $J = \pm 1$ alternatives. Light solid lines represent one of two antiparallel chains of DNA of pitch P_{DNA} and axial nucleotide translation Δz. In this diagram $N = 5$, $s/2 \sim 6$, and $M_i = 1$.

of pitch P_i. The dots represent a point of each nucleotide located at the interface of the protein tubes and the DNA helix of pitch P_{DNA}. The dots are placed to have axial translations of Δz and to maintain invariant the relation between each dot and the surfaces of the two nearest tubes. The geometry of Fig. 1 leads to the pitch connection relation $P_{DNA} = \Delta z/(\pm 1/N_i + \Delta z/P_i)$, where a positive (negative) pitch is right (left) handed. Note that the inner and outer layers of α-helix tubes, connected via peptide cross chains, also form a pitch connected system. Applying the equation to fd with $\Delta z = 2.8$ Å, $N = 5$, $M_i = 1$, and anticipating that reasonable solutions will have $P_i > 100$ Å, we obtain 12 Å $< P_{DNA} <$ 17 Å. We have shown elsewhere that two-chain DNA structures with low pitches on the order of 15 Å and axial translations on the order of 3 Å or greater, as in Xf and Pf1 viruses, must have the phosphate groups located near the axis and the bases directed outward (4), so under these assumptions the DNA in fd must also have the phosphates in and the bases out. The bases, which fall on a helix of pitch P_{DNA} and extend into the inner layer of protein tubes of pitch P_i, form the mechanical connection between P_i and P_{DNA}.

The complete system of algebraic equations, to be presented in detail elsewhere, which encompasses the relations described above contains the quantities M_i, M_o, N, n, Δz, P_{DNA}, P_i, P_o, T, θ_i, and θ_o (polar angles for the inner and outer α-helix tube), R (the radius of the axis of the inner tube), n_i and n_o (the number of amino acids in the inner and outer tubes), δ (the rotation per N-mer), and finally, J and K, two indices (see Fig. 1) which label the possible relations between tubes within the inner and outer layers, respectively. A choice of J, K, M, N, n, and Δz is sufficient to determine the other variables.

SOLUTIONS

To model fd, we have used $n = 50$ and $M_i = 1$, and have tried $M = 2,3,4$ and $N = 4,5,6$. The $M = 3$, $N = 5$ solutions yield a diameter of ~60 Å and $T = 16$ Å. One choice of J and K yields a model with $\delta \sim 36°$, a value suggested by diffraction studies (1), which corresponds to $P_i =$ 160 Å. Preliminary fiber diffraction patterns synthesized from that solution by means of the CYLTRAN computer code, with each amino acid treated as a point, afford encouraging comparisons with the experimental fd patterns. A class of solutions exists for $N = 5$ and $M_i =$ 2, wherein the ten alpha helices of the inner layer are virtually parallel to the structure axis

and P_i approaches infinity. The pitch connection then yields $P_{DNA} = N_i\Delta z = 28$ Å for 2.3 nucleotides/subunit. This type of DNA structure, predicted by the pitch connection and five-fold rotational symmetry for fd, might account for the diffraction intensity recently observed on layer spacings of ~26 and 13 Å for magnetically oriented fibers of fd (5).

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PHOTOREACTIVATING ENZYME FROM *ESCHERICHIA COLI*

Interactions with DNA and Mechanism of Action

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In photoreactivation, photochemical damage produced in DNA by far ultraviolet radiation ($\lambda < 320$ nm) is repaired in an enzyme-mediated reaction using longer wavelength light ($310 < \lambda < 450$ nm for *E. coli*). Photoreactivating enzyme (PRE) acts on a single class of photoproducts, cyclobutyl pyrimidine dimers, in an otherwise normal DNA strand at least nine bases long. PRE is one of the few DNA repair enzymes which has been purified to homogeneity in quantities sufficient for physico-chemical studies.

The *E. coli* PRE is a single polypeptide of 32,500 d;[1] it is low in aromatics and appears to lack tryptophan.[1] The protein is associated with an RNA (roughly 10 nucleotides per protein monomer) which is required for activity;[1] its absorption spectrum thus has a peak near 257 nm due to its RNA. The measured spectrum has a trailing optical density at wavelengths > 320 nm. After correcting the measured spectrum for the effects of light scattering (1) we find no true absorption for wavelengths above 320 nm, the spectral region where light must be

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[1]Snapka, R., and B. M. Sutherland. Manuscript submitted for publicaton.