Fluorescence polarization study of tertiary structure of DNA within bacteriophage λ

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The fluorescence polarization of acridine orange-stained, oriented λ phages was measured. The parameters of DNA packing within the phage head $\cos^2\theta$ and $\cos^4\theta$ were calculated (θ, angle between the direction of a small segment of DNA and the phage axis). It is shown that simple models of λ phage DNA tertiary structure are not consistent with calculated values. A new model is proposed.

DNA packing *Phage λ* *Acridine orange* *Fluorescence polarization* *Bacteriophage λ*

1. INTRODUCTION

There is as yet no universally adopted concept of tertiary structure of DNA in heads of isometric bacteriophages. Some data argue in favour of the partial orientation of DNA along the symmetry axis of particles [1–3], others indicate a predominant perpendicular orientation [4, 5]. In some phages (S_d, DD7) no predominant DNA orientation was established at all [6]. It should be noted that in the above papers only one parameter of DNA orientation was measured, such as the mean value of $\cos^2\theta$ ($\overline{\cos^2\theta}$), where θ is the angle between the direction of a small segment of DNA and the phage symmetry axis. More definite conclusions may be drawn from the method of fluorescence polarization, successfully applied to determine the DNA orientation in bands of polytene chromosomes [7]. Two independent parameters of DNA orientation, for example $\overline{\cos^2\theta}$ and $\overline{\cos^4\theta}$ may be measured by this method.

Here, $\overline{\cos^2\theta}$ and $\overline{\cos^4\theta}$ were determined for DNA in the λ phage and a mode of DNA packing within the phage head is proposed.

2. MATERIALS AND METHODS

The preparation and purification of phage λCI857 was as in [9]. Samples obtained were dialyzed against 10 mM Tris–HCl (pH 7.4), 10 mM NaCl, 10 mM $MgCl_2$ and kept in this buffer at 4°C no longer than 2 weeks. Just before the experiment the phage suspension was dialyzed against 5 mM Tris–HCl (pH 7.4), 5 mM NaCl and 1 mM $MgCl_2$.

Phage preparations were stained with acridine orange by mixing equal volumes of the dye and the phage suspension with a final dye: nucleotide ratio of 2.5×10^{-4}. It was shown previously that at this ratio almost all dye molecules are bound to intraphage DNA and excitation energy migration between the bound dye molecules is negligibly small [8, 9].

Rectangular electrical pulses of 6 ms duration were applied to the phage suspension between two silver platinized electrodes to induce orientation of the phage particles. Fluorescence was excited by Hg-lamp radiation (λ = 365 nm) filtered through an FS-1 glass filter (USSR) and polarized by an Arens prism. Components of polarized fluorescence in-

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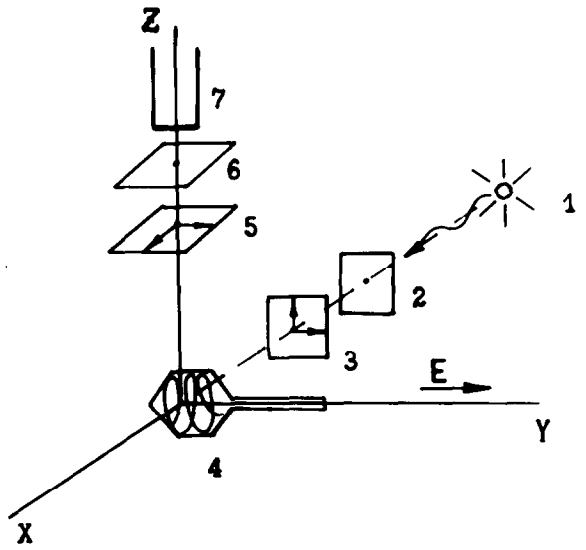


Fig. 1. Schematic representation of the experimental set-up. Light emerging from an Hg-lamp (1) passes through an FS-1 filter (2), a polarizer (3) and excites fluorescence of a suspension of acridine orange-stained oriented phage particles (4). Fluorescence intensities were measured by an FEU-29 photomultiplier (7), an analyzer (5) and an interference filter ($\lambda = 530$ nm) (6) being placed before it.

tensity I_{YY}, I_{ZY}, I_{YX} and I_{ZX} (fig.1) were measured by an FEU-29 photomultiplier (USSR) placed after the analyser and interference filter ($\lambda = 530$ nm). Electron microscopy and phage ability to bind EtBr [10] were used to check phage integrity.

3. RESULTS

While orienting phage particles under an applied electric field, marked changes of fluorescence intensity were noted for component I_{YY} only. Other components remained constant within the accuracy of measurement. As seen from fig.2, the intensity increment, $\Delta I_{YY}/I^0_{YY}$, was always positive and for strong fields it was inversely proportional to the electric field strength E. This dependence is typical [5] for phages bearing a permanent electrical dipole moment. The orientation of phage is unaffected by increase of the ionic strength (fig.2), confirming that this orientation results in a permanent moment.

A value of 6×10^4 debye for the dipole moment may be easily calculated from the slope of the increment $\Delta I_{YY}/I_{YY}$ corresponding to full orientation of phages at $E^{-1} \to 0$ (fig.2). For acridine orange-stained free DNA the extrapolated value of $\Delta I_{YX}/I_{YY}$ is 2-times larger in magnitude and negative in sign [11]. Hence DNA within the phage head cannot be aligned mainly along the phage symmetry axis. The polarization of fluorescence, $P = (I_{YY} - I_{YY})/(I_{YY} + I_{YX})$, is equal to 0.36 for the unoriented phage and 0.46 for the oriented phage. More definite conclusions may be drawn from values $\overline{\cos^2\theta} = 0.24 \pm 0.01$ and $\overline{\cos^4\theta} = 0.16 \pm 0.02$, calculated from experimental data using the formulae from [7].

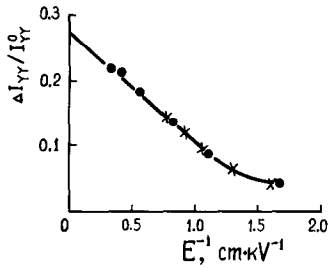


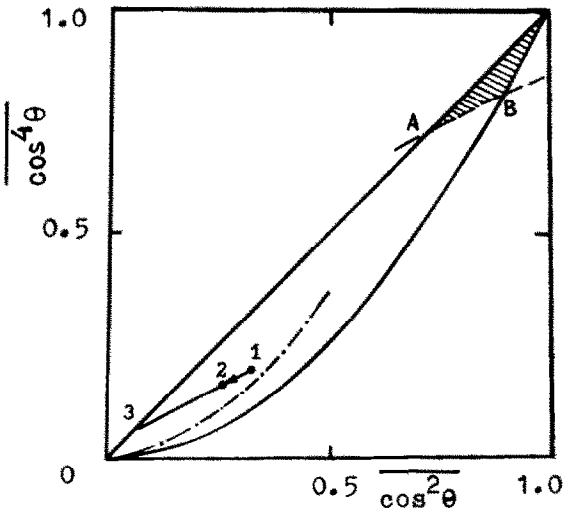
Fig. 2. Changes of fluorescence intensity I_{YY} induced by phage orientation during an electrical pulse. I^0_{YY}, intensity of fluorescence of the unoriented phage. (●) 5 mM Tris-HCl, 5 mM NaCl, 1 mM $MgCl_2$. (×) 5 mM Tris-HCl, 10 mM NaCl, 1 mM $MgCl_2$.

4. DISCUSSION

Fluorescence polarization data permit one to establish only the general features of DNA tertiary structure within the λ phage. Some other information should be taken into consideration for a description in more detail. To clarify this statement let us consider a phase plane every point of which corresponds to an axially symmetrical ensemble of DNA with known values $\overline{\cos^2\theta}$ and $\overline{\cos^4\theta}$. Two points of the plane correspond to the extreme modes of DNA orientation: point (1;1) to DNA aligned along the symmetry axis and point (0;0) to DNA aligned perpendicularly to the axis. The region of physically possible ensembles is limited. From above it is limited by a straight line $\overline{\cos^4\theta} = \overline{\cos^2\theta}$, corresponding to the superposition of ensembles (0;0) and (1;1). From below the region is limited by the parabola $\overline{\cos^4\theta} = \overline{\cos^2\theta}^2$, which corresponds to ensembles with constant θ, i.e. superhelices or zig-zags of DNA. Internal

points of the region have an unequivocal interpretation in terms of the DNA packing mode because the coordinates of each point may be presented as a sum of coordinates of any number of points chosen at will. This applies also to the point (0.16;0.24) which corresponds to the oriented phage particles (fig.3).

Modern X-ray diffraction and electron microscopy data [12-14] show that adjacent segments of the intraphage DNA molecule run locally parallel, like a thread on a solenoid spool.



Point	S	α	$\overline{\cos^2\theta_i}$	$\overline{\cos^4\theta_i}$
A	0.8	30°	0.71	0.71
B	0.8	23°	0.87	0.76
(1;1)	0.86	28°	1.0	1.0

Fig. 3. Phase plane of axially symmetrical ensembles of DNA molecules. θ, angle between the direction of a small segment of DNA and the phage symmetry axis. $\overline{\cos^2\theta}$ and $\overline{\cos^4\theta}$, mean values of $\cos^2\theta$ and $\cos^4\theta$. Different points correspond to: 1, unoriented phage (0.33;0.20); 2, oriented phage (0.24;0.16); 3, the end of the extrapolated trajectory of phage when samples contain phage particles without electrical dipole moments (0.1;0.1). The parabola (-----) corresponds to circular coils evenly inclined to the axis of orientation. Arc AB corresponds to inner part of phage DNA, assuming $S=0.8$. $\theta_i=\theta$ for inner part of intraphage DNA.

The solenoid contains more than 80% of the intraphage DNA hexagonally arranged with distances $d=27.4$ Å between parallel segments. For each layer of the solenoid one may calculate:

$$\overline{\cos^2\theta} = \cos^2\alpha\,\cos^2\delta + (1/2)\sin^2\alpha\sin^2\delta$$

$$\overline{\cos^4\theta} = \cos^4\alpha\cos^4\delta = (3/16)\sin^2 2\alpha\sin^2 2\delta + (3/8)\sin^4\alpha\sin^4\delta \quad (1)$$

where $\delta = \arccos(d/R)$, R is the radius of a layer, and α is the angle between axes of symmetry of the phage and of the solenoid (fig.4). Taking into account that the DNA molecule cannot be bent (even in the presence of polyamines) with a radius of curvature smaller than 140 Å [15], one obtains $d/R \ll 1$. Therefore, eq. 1 may be simplified to:

$$\overline{\cos^2\theta} = (1/2)\sin^2\alpha$$

$$\overline{\cos^4\theta} = (3/8)\sin^4\alpha \quad (2)$$

It follows that all DNA ensembles consisting of rings tilted to the phage symmetry axis at the same angle α form a parabola $\overline{\cos^4\theta} = (3/2)\overline{\cos^2\theta}^2$ in the phase plane (fig.3).

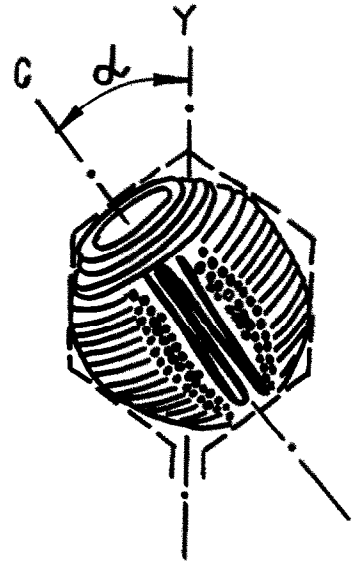


Fig. 4. A scheme of the tertiary structure of DNA in λ phage. Y, axis of orientation (phage symmetry axis). C, axis of DNA solenoid.

The point (0.16; 0.24) for the oriented phage does not belong to the parabola. This is very important, since only part of the intraphage DNA can be coiled into a solenoid. The other, smaller part of DNA is placed probably in the central cavity of the solenoid. How can it be packed?

To obtain an answer let us decompose $\overline{\cos^2\theta}$ and $\overline{\cos^4\theta}$ for the phage into the sum of 'solenoid' and 'inner' parts:

$$0.24 = (S/2)\sin^2\alpha + (1-S)\overline{\cos^2\theta_i}$$

$$0.16 = (3S/8)\sin^4\alpha + (1-S)\overline{\cos^4\theta_i} \quad (3)$$

where S is a part of DNA in the solenoid. Authors in [12] estimated S to be $\leqslant 0.8$. Substituting this value of S into eq. 3 one obtains the relation:

$$\overline{\cos^4\theta_i} = 0.26 + 0.9\overline{\cos^2\theta_i} - 0.37\overline{(\cos^2\theta_i)^2} \quad (4)$$

which is represented in fig.3 by a dotted curve. The calculation of S, α and parameters of inner DNA for the tops of a triangle A, B and (1;1) is presented in fig.3. From the data of the table of fig.3 one can conclude that the axis of the solenoid is inclined at an angle of about 30° to the phage symmetry axis, whereas the inner part can be represented as a linear or slightly wavy DNA segment oriented more or less along the symmetry axis.

It should be noted that the value of $\overline{\cos^2\theta} = 0.24$, measured here for λ phage, was found by authors in [5] for T7, P22, and ϕ_D-1 phages. This indicates the structural similarity of these phages. However, they neglected the inner DNA and hence obtained an overestimated angle value ($\alpha = 43.5 \pm 2.5°$). Meanwhile the whole DNA cannot be packed within the phage head because in this case the radius of curvature of inner coils would be too small (estimated value about 3 nm).

4.1. *Analysis of possible errors*

(i) While calculating the above packing parameters the dye molecules along DNA in a phage were assumed to be evenly distributed. However, the left end of DNA is known to be more available to an intercalating probe [16]. Moreover, some data [9] confirm the fact that the most stainable DNA is located on the periphery of a DNA-rich region within a phage head. Thus, the inner part of DNA should be less stainable. This means that we could only overestimate S and, consequently, the point (1;1) of a phase plane is more probable than the points A and B. (ii) If some factors prevented the perfect orientation of phage [for example, if: (1) the axis of phage orientation deviates from the axis of phage symmetry, (2) some particles lack dipole moments, etc.] the point of perfect orientation should be [7] shifted from point 2 towards point 3. This shift diminishes the angle θ_i, because point 3 corresponds to the phage inner part of which DNA (comprising 10%) is oriented along the axis of phage symmetry.

The above discussion can be summarized for the tertiary structure of DNA within the λ phage. The main part (80–90%) of the DNA molecule is coiled into a solenoid whose axis is deflected from the phage symmetry axis by an angle smaller than 30°. External coils of the solenoid are adjacent to the protein shell of the phage head, while inner coils form a cylindrical cavity with radius about 10–12 nm. The other, smaller part of the phage DNA is roughly oriented along the axis of the cavity (fig.4).

4.2. *Model*

The pathway for DNA packaging into the phage head can be represented as follows. Our data on the higher stainability of the left DNA end within the phage indicate that the first layer of DNA coils is likely packed on the inner surface of a head [16]. The second layer cannot be a simple continuation of coiling (fig.5a), because it does not produce a

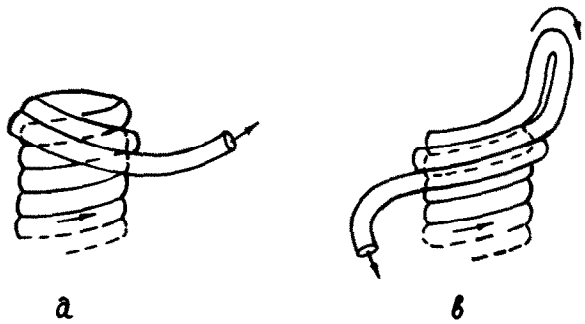


Fig. 5. Two possible orders of coiling of DNA molecule in subsequent layers of a solenoid. (a) The external layer is the continuation of the inner layer (both coiled clockwise). (b) The external layer (anticlockwise coiling) is connected to the inner layer (clockwise coiling) with a backward loop.

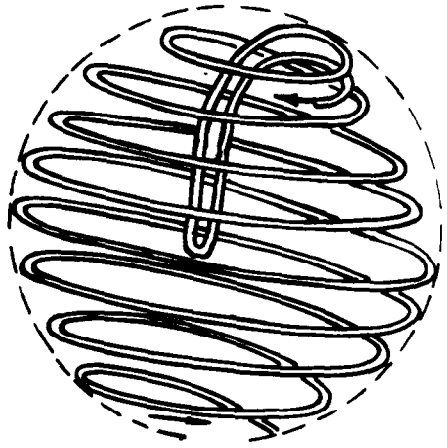


Fig. 6. Model of DNA packing into λ phage head.

hexagonal order of packing (DNA segments are non-parallel) as is proved by X-ray diffraction [12]. To obtain a hexagonal order the second layer should be coiled in an opposite direction and both layers should be connected by the loop, as shown in fig.5b. Such loops are located at every point of transition from one layer of a solenoid to another.

It has been shown earlier [18] that about 1% adenines of intraphage λDNA belong to the single-stranded or denatured regions. The proposed loops seem to be the most probable sites for denaturation.

Such loops must be also located at the ends of segments of inner DNA if they are packed in a chain-folded structure (fig.6). We assume, however, that every loop between the layers of the solenoid has a long neck, the set of which constitutes the inner part of phage DNA. If this is the case, it is easy to calculate the number of denatured regions and their distribution along λDNA (fig.7). Indeed, assuming the length of λDNA to be about 49 000 base pairs [17] and the radius of the DNA–protein boundary in the phage head to be 294 Å [12], one finds that the solenoid

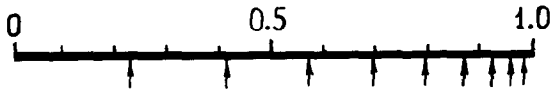


Fig. 7. Predicted distribution of the denatured regions (↑) along intraphage λDNA.

should have 10 layers and the inner part should contain 11 segments of DNA comprising about 4% of the whole λDNA. The last value is in qualitative agreement with experimental data (see above).

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