Mapping of 8-metoxypsoralen binding sites in DNA within phage λ particles

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DNA, packing in phage λ *Intraphage DNA, availability to 8-MOP*
Intraphage DNA, surface localisation

1. INTRODUCTION

Using the small-angle X-ray diffraction method, it has been established that the major part of DNA within phage λ has the B-form and forms layers concentrically converging from the protein shell to the phage particle center [1].

However, how DNA strand is packed in layers is not yet known.

This investigation is an extension of the series of studies aimed at elucidating the geometry of DNA packing in phage λ with intercalating probes. Our goal was to determine:

(i) The distribution of the intercalating probe molecules on the intraphage DNA;

(ii) The spatial arrangement of probe molecules in the phage head.

In [2,3] the majority of intraphage DNA was found available to the intercalating probe acridine orange; these molecules are rather irregularly located in the phage head. It was also shown that the arrangement of acridine orange molecules on the external layers of the packed DNA is the most probable one.

Here, we have determined that the left half of the intraphage DNA is more available to the 8-methoxypsoralen (8-MOP) intercalating probe. The region of DNA adjacent to the left end is likely to localize on the surface of the DNA core within phage λ.

2. MATERIALS AND METHODS

The preparation and purification of phage λ1857 was performed as in [4]. The phage preparations obtained were dialyzed against the solution 0.01 M NaCl–0.001 M $MgCl_2$–0.01 M Tris–HCl (pH 7.4). DNA was isolated from phages by the phenol extraction method [5]. Phage or DNA solutions were mixed with the solution of 8-MOP and the mixtures (50 μg DNA/ml, 20 μg 8-MOP/ml) were incubated for 15 min.

8-MOP is known to crosslink the complementary DNA strands at the binding sites after UV irradiation (365 nm) [6].

To achieve crosslinking, phage or DNA solutions mixed with 8-MOP were irradiated with a mercury lamp through filters UVS-6 and SZS-7 (light transmission range 320–380 nm). Unreacted 8-MOP was removed by dialysis against the solution of 0.01 M Tris–HCl (pH 8) – 0.001 M EDTA.

To detect crosslinks, DNA was denatured in the

Fig.1. DNA λ photoreacted with 8-MOP within phage for 10 min and spread for electron microscopy under totally denaturing conditions. The length of the bar corresponds to 1000 bases. The arrows indicate crosslinks.

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presence of glyoxal [7]. Preparations were spread for electron microscopy by the formamide–protein method [8], followed by dusting with platinum. After irradiation, the photoreacted DNA appears as a series of single-stranded bubbles (fig.1). The bubbles are interpreted to be uncrosslinked stretches of DNA bound by covalent crosslinks. To count the number of crosslinks, only full-size molecules of λDNA varying in length by $\leq 5\%$ were considered. Circular denaturated ψ x 174 DNA was used as a length standard.

3. RESULTS AND DISCUSSION

To estimate the distribution of crosslinks, each DNA molecule seen in the electron micrograph was divided into 10 equal segments, then the number of crosslinks on each segment counted (fig.3a). About 100 crosslinks/segment are needed for reliable evaluation of the distribution; i.e., 1 crosslink/50 basepairs. However, in our experiments we could distinguish crosslinks divided by ≥ 400–500 basepairs. Therefore, to obtain reliable data, it is necessary to add crosslinks found in many DNA molecules.

Adding up the crosslinks in even 2 DNA molecules is difficult because it is unknown which is the left or the right end of DNA molecules in question.

However, if it is known a priori that the arrangement of psoralen crosslinks is asymmetrical relative to different halves of DNA, then orientation becomes possible.

Let us denote probability of the presence of a crosslink in a given (i.e., more available for modification by 8-MOP) half of DNA as P. Then, the probability of erroneous conclusion on the orientation of a given DNA molecule, according to Bayes [9], will be:

$$q_i = \left[\frac{1-P}{P}\right]^{K_{i1}-K_{i2}} \Big/ \left[1+\left(\frac{1-P}{P}\right)^{K_{i1}-K_{i2}}\right] \leq \left[\frac{1-P}{P}\right]^{K_{i1}-K_{i2}}$$

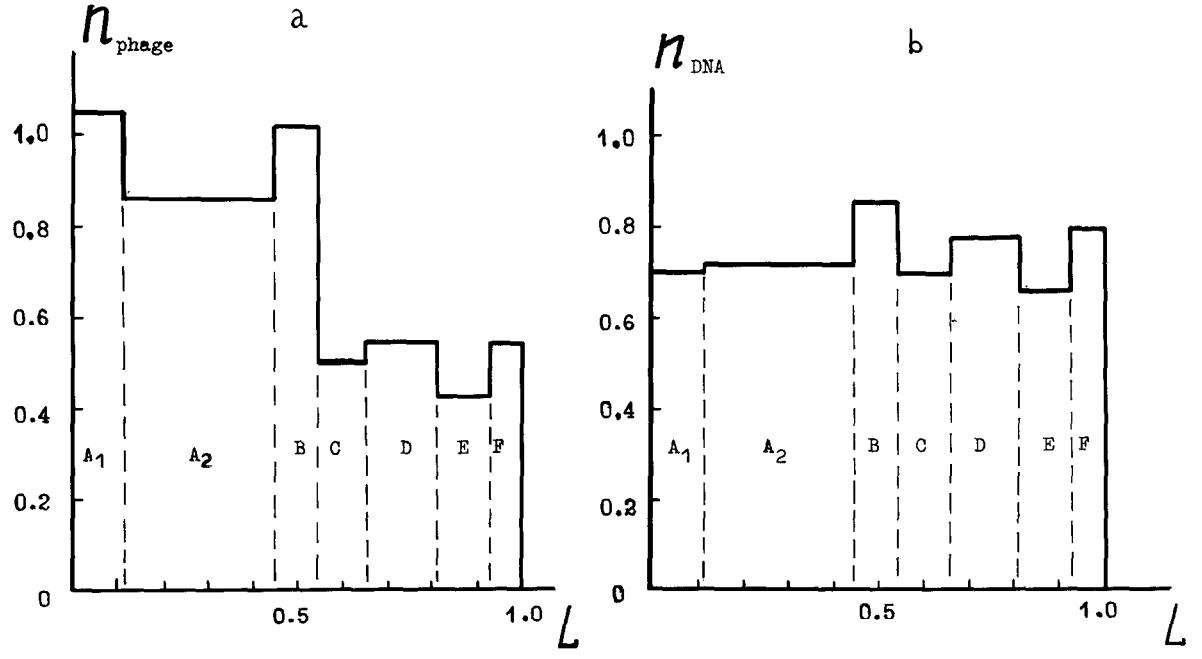

Fig.2. The distribution of crosslinks in the restriction fragments: (a) for the intraphage DNA, UV for 30 min; (b) for free DNA, UV for 4 min. N_{DNA} and N_{phage} = average number of crosslinks per 1% DNA length in free DNA and in intraphage DNA, respectively; L = the distance from the left DNA–λ end. Fragments B–F were obtained by hydrolysis of DNA with *Eco*RI nuclease; A_1 and A_2 after successive hydrolysis with *Eco*RI and *Bam*HI. 50–100 molecules were used for each restriction fragments to calculate the average number, mean-square deviation $\leq 5\%$.

where K_{i1} and K_{i2} are numbers of crosslinks in the 2 halves of DNA ($K_{i1} > K_{i2}$). The probability of making this error l times in the orientation of m molecules will be:

$$\Theta^l \cdot \exp(-\Theta)/l!$$

where

$$\Theta = q_1 + q_2 + \ldots + q_m$$

To estimate independently the unknown value P, which is the measure of asymmetry in the distrubution of 8-MOP in intraphage DNA, modified DNA was isolated from the phage and digested by nucleases *Eco*RI and *Bam*HI; the restriction fragments were separated by gel-electrophoresis, and the numbers of crosslinks in them were counted using electron microscopy.

The conclusion of [3], that the intercalating probe binds with the external layers of the intraphage DNA, may be reasonably interpreted. The interaction of such probes with DNA in the middle of the tight homogenous packing region must be restricted [12]. Conversely, the external layers of DNA must be more accessible. Thus the accessibility of DNA bases to the probes reflects its arrangement in the phage head.

8-MOP is 7-times less reactive towards intraphage DNA compared to free DNA because of the tight packing of DNA and the screening effect of the protein shell.

As seen in (fig.2a), the left half of the intraphage DNA, coding for the structural genes of the phage, is modified more extensively than the right half ($P > 0.64$). Control experiments with free DNA did not exhibit any considerable asymmetry of crosslinks (fig.2b).

Fig.3a shows the distribution of the psoralen crosslinks in intraphage DNA obtained by averaging over 23 oriented molecules.

8-MOP is known to crosslink only thymines in the $\begin{matrix}-AT-\\-TA-\end{matrix}$ sites [10], so that the density of crosslinks is proportional both to the accessibility of the intraphage DNA bases to the probe, and to the content of $\begin{matrix}-AT-\\-TA-\end{matrix}$ sites.

The distribution in fig.3b is proportional of the ratio $N_{0.1}/n_{DNA}$. This distribution is approximately proportional to the degree of accessibility of the different intraphage DNA sites to the probe (fig.3b).

Based on these data we conclude: The region of DNA adjacent to the left end is more accessible to 8-MOP, therefore this site is presumably localized on the surface of the DNA core within phage λ.

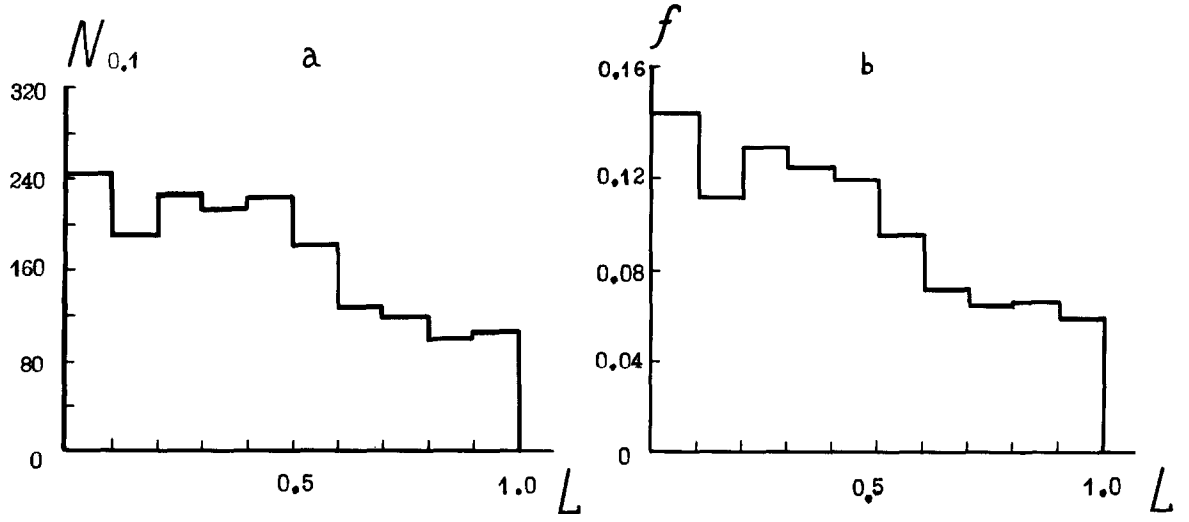

Fig.3. The distributions of: (a) 8-MOP crosslinks in intraphage DNA. (This distribution is plotted for 23 DNA molecules. The probability of correct orientation of all the molecules is >0.97.); (b) 8-MOP binding sites in intraphage DNA. $N_{0.1}$ is the total number of crosslinks/10% DNA length; f is proportional of the ratio $N_{0.1}/n_{DNA}$ ($\int_0^1 f dL = 1$).

Approximate monotonous decrease of the density of 8-MOP binding sites from the left to the right DNA end suggests that the greater the separation of the DNA site from the left end the larger the distance of this site from the phage shell.

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