Studies on the Secondary Structure of Intraphage T-7 DNA

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Secondary structure of intraphage DNA of coliphage T-7 was studied by spectrophotometry and electron microscopy. T-7 DNA in vitro showed 7% hyperchromicity, on the average, compared to DNA released by heating the phase 30 min at 55—60 °C. The intraphage DNA when melted by heating the phase for 15 min at 95 °C and then rapidly chilled, was found to be deficient in hyperchromic rise by about 10% compared to the in vivo denatured DNA. Electron micrographs of T-7 DNA released by osmotic shock revealed that the incompletely released molecules show a pattern of denatured regions in the form of single-stranded loops. Phenol extracted T-7 DNA molecules as well as T-7 genomes completely released by osmotic shock showed fastening to any pattern and possessed no denatured zones. These results helped to infer that the intraphage T-7 DNA is partially denatured.

It was shown earlier by spectral studies that the conformation of intraphage DNA of several bacteriophages differed from that of Watson-Crick duplex. No electron microscopic evidence supporting the above feature of intraphage DNA has so far been obtained. The present study by electron microscopy shows that the intraphage T-7 DNA has denatured zones. Spectrophotometric data also indicates a partially denatured state of the intraphage DNA.

Material and Methods

Coliphage T-7 was prepared, concentrated and purified as described elsewhere. The phage solvent used in all the experiments was standard saline citrate (pH 7.4). DNA from T-7 was extracted by phenol. Viscosimetric and phage-titer measurements of the phage samples, heated 30 min at different temperatures, showed complete disruption of the phages at 55—66 °C. Intra-phage DNA was, therefore, released by heating the phase-suspension as above and then fast-chilled or cooled slowly to room temperature. Formaldehyde, used in the experiments, was freshly neutralized, prior to use, following the method of Davison and Freifelder. Optical absorbance of the samples was measured at room temperature by a PMQ II Zeiss spectrophotometer. The samples for electron microscopy were prepared by the osmotic shock method. One part of the phage solution (titer ~10^10/ml) was mixed with four parts of 15 M-NO_2 (pH 7). The mixture was kept at 4 °C for 15 min and then mixed with equal volume of 0.04 M-cytochrome-c (solvent 1 M-NO_3) and spread onto a clean surface of 3-distilled water containing 2% neutralized HCHO. The film was then picked up on carbon coated grids, dried in ethanol for 15—20 sec and shadowed (rotatory) with 5 A 10% Ir-Pt at an angle of 11°. Electron micrographs were recorded at an electronic magnification of 8,000 × by a Siemens Elmiskop I operating at 60 kV.

Measurement of true optical densities of intraphage DNA was done by extrapolation method. The general procedure of extrapolation was followed here taking the appropriate and adequate caution as adopted by Tichonenko and co-workers. These authors showed that the true values of O.D. of a virus suspension, calculated by the use of extrapolation method, did not depend upon the conditions of measurement and were not affected by the change in the contribution of light scattering. The similar evidence was also obtained by us using cells having optical path-lengths of 1 cm and 0.5 cm, and various concentrations of T-7 phages.

Results

The experimental results are shown in Figs. 1—6 and Table I. The slopes of the O.D. versus wavelength curves for the disrupted phages (curves 1—3, 4) were not affected by the change in the contribution of light scattering. The similar evidence was also obtained by us using cells having optical path-lengths of 1 cm and 0.5 cm, and various concentrations of T-7 phages.

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SECONDARY STRUCTURE OF INTRAPHAGE T-7 DNA

Table I. UV absorbance measurements on different preparations of coliphage T-7.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Condition of sample preparation</th>
<th>Percentage hyperchromicity (Δλ) and hypochromicity (−) at 260 nm relative to true O.D. of intraphage DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T-7 heated between 55—60°C for 30' and slowly cooled to room temperature</td>
<td>−6</td>
</tr>
<tr>
<td>2</td>
<td>T-7 + 0.02% SDS heated 30' between 55—60°C and slowly cooled to room temperature</td>
<td>−8</td>
</tr>
<tr>
<td>3</td>
<td>T-7 heated 30' between 55—60°C and rapidly chilled</td>
<td>−7</td>
</tr>
<tr>
<td>4</td>
<td>T-7 + 2% neutralized HCHO heated for 30' between 55—60°C and slowly cooled to room temperature</td>
<td>−6</td>
</tr>
<tr>
<td>5</td>
<td>T-7 heated for 13' at 95°C and fast chilled</td>
<td>+22</td>
</tr>
<tr>
<td>6</td>
<td>Phenol extracted T-7 DNA heated at 55—60°C for 30' and either slowly cooled or rapidly chilled</td>
<td>+ nil</td>
</tr>
<tr>
<td>7</td>
<td>Phenol extracted T-7 DNA heated for 13' at 95°C and rapidly chilled</td>
<td>+32</td>
</tr>
<tr>
<td>8</td>
<td>T-7 DNA (excreted by heating the phages for 30' at 55—60°C) — heated 13' at 95°C and chilled quickly</td>
<td>+31</td>
</tr>
</tbody>
</table>

Fig. 1) were slightly higher than those (curves 4—6) for the intact particles. The scattering coefficients in all the cases were round about 4, indicating that the scattering followed the well known Rayleigh's law.

Curves 1—5 of Fig. 2 have been described in the legends. The absorption maximum at 260 m\(\mu\) for the intraphage DNA (curve 3) was found to shift to 259 m\(\mu\) when the DNA was released from the phage coat by heat (curves 4 and 5). Tikhonenko et al.\(^2\) reported a similar shift of absorption maximum from 262 m\(\mu\) (intact phage) to 259 m\(\mu\) (disrupted phage) for SD phage. Such a shift of the absorption maximum possibly reflects a change of the DNA conformation when DNA is released from the in vivo state. The spectral difference of curve 3 from curves 4 and 5 within the wavelength range of 250—300 m\(\mu\) indicated that the intra-phage DNA (curve 3) was hyperchromic compared to the excreted DNA (curves 4 and 5). There was a small spectral difference between curves 4 and 5. This arose possibly from the difference in degree of DNA release in the two cases: the DNA release in the presence of 0.02% SDS was better than that without
SDS. The spectrum of the melted T-7 phage (curve 2) was found to be typical for denatured DNA. The above characteristics indicated that the T-7 DNA in vivo was, to some extent, denatured as compared to T-7 DNA in vitro.

Quantitative data of hyperchromicity are given in Table I. In comparison with the excreted DNA (samples 1—4) the intraphage DNA was found to be hyperchromic by 6—8 percent. Heat treatment of the phenol extracted T-7 DNA (sample 6) did not show any absorption difference, indicating a difference of its secondary structure from that of the intraphage T-7 DNA. Further, it was found that upon melting, the T-7 DNA in vivo (sample 5) showed hyperchromicity less, by 9—10%, than the phenol extracted (sample 7) and the heat released (sample 8) T-7 DNA, subjected to identical melting conditions. These observations lead to the conclusion that the T-7 DNA in situ is partially denatured.

An attempt was made to investigate the state of the intraparticle T-7 DNA by electron microscopy. For the following reasons, the osmotic shock method was thought to be effective, at least to some extent, to demonstrate the denatured zones, if any, of the intraphage DNA: both the water hypophase and the low temperature (~8 °C) selected for sample-preparation for electron microscopy are unfavourable for renaturation of a denatured DNA; the presence of formaldehyde in the hypophase creates further obstacle to renaturation. It may be mentioned that pre-incubation of SD phages with formaldehyde was found to arrest the efficient release of DNA. Also treatment of SD phages with HCHO was found ineffective to react with the free amino groups of the intraphage SD DNA. We, therefore, hoped that the conditions of the osmotic shock method would probably be favourable for the reactibility of HCHO with the free amino groups, as indicated by the spectrophotometric data, of the intraphage T-7 DNA.

Figs. 3 to 6 show electron micrographs of osmotically disrupted T-7 phages. Three types of DNA-release were visible: the DNA molecules completely free from the phage coat (Fig. 3*), the completely released DNA with rosette-like appearance (Fig. 4), and the incompletely released DNA molecules showing a pattern (Figs. 5 and 6) as well as distinct denatured zones (arrow-marks). It was interesting to note that almost all the incompletely released DNA molecules possessing patterns (type 3) also possessed denatured zones while those without pattern did not. Such denatured zones could not be formed due to the water hypophase itself, because they were absent from phenol extracted DNA molecules prepared under identical hypophase condition. The three types of DNA-release indicate that phages of the same preparation were not equally susceptible to the same osmotic shock; shock-susceptibility was perhaps the least for the incompletely released T-7 DNA molecules. The least shockability also means mildest effect on the conformation of the intraphage DNA. It is reasonable to assume, therefore, that these patterned molecules were more closely of intraphage nature than those without pattern. The observed denatured zones on the patterned molecules, therefore, represent the conformational characteristics of the intraphage DNA.

It would have been significant if location of the loops could be determined with respect to the ends of the T-7 DNA molecules. This was not possible, because, the patterned DNA molecules were incompletely released. However, we found a few completely released DNA molecules which possessed a number of denatured zones. For four such molecules, the location of the denatured zones has been shown in Fig. 7. This figure shows that grossly one half of a full T-7 DNA molecule is apparently richer in denatured zones than the other half. But it is difficult to reach at a definite conclusion about the distribution of such denatured zones from end to end of the molecules out of the few measurements.

Thus the present results show that intraphage T-7 DNA is 6—8 per cent hyperchromic and this hyperchromicity is believed to have been reflected

\[ T. I. Tichonenko \text{ and E. N. Dobrokov J. molecular Biol. 42, 119 [1969].} \]

* Figs. 3—6 see Table p. 1290 a.
Figs. 3–6. Electron micrographs of T-7 DNA released by the osmotic shock on protein monolayer: (3) completely released T-7 DNA with the detached protein coat of the phage possessing no denatured zones; (4) rosette-like appearance of DNA molecules without denatured zones; (5–6) two partially released T-7 DNA molecules showing a pattern of release as well as denatured zones (arrows). Magnification: 80,000 X.
in the observed denatured zones on the molecules. If we assume that the hyperchromicity of intraphage DNA is due to strand separation alone, then 6 – 8 per cent hyperchromicity indicates that 20 – 25 per cent base pairs are free (assuming that 31 – 32 per cent hyperchromicity represents complete strand separation as shown by samples 7 and 8 in Table I). Consonant with this the electron micrographs also showed a good amount of separated strands.

**Discussion**

Earlier studies by several authors (see review by Thomas\textsuperscript{12}) led to the supposition that DNA inside a virus particle is partially denatured. Recent spectrophotometric and deamination studies by Tikhonenko et al.\textsuperscript{2} and more recently circular dichroism studies by Minchenko et al.\textsuperscript{4} on T-2 and SD phage indicated that the conformation of their DNA in vivo differed from that of their free DNA and resembles the state of a partially denatured DNA. Spectrophotometric and luminiscence spectral investigation of Klebsiella phages\textsuperscript{3} revealed the similar nature of the intraphage DNA. Luminiscence spectral investigation\textsuperscript{5} of dye-bound intraphage DNA from all T-coliphages including T-6, Klebsiella phages L\textsubscript{1} and 380, Mycobacteriophages R\textsubscript{1} and Leo, and from \textlambda{} and SD, showed that the DNA inside these phages possessed single-stranded regions. Maestre and Tinoco\textsuperscript{13} also studied a large number of bacteriophage including T-2, T-6 and T-7 by ORD spectra, and found that the intraphage packing of all the bacteriophages DNA had similar characteristics and that the conformation of the intraphage DNA's was not normal. However, Bonhoeffer and Schachman\textsuperscript{1}, from their spectrophotometric investigation, showed that the intraparticle T-6 DNA was not hyperchromic. We do not see any reason that the conformation of intraparticle T-6 DNA would be different from that of T-2, T-4 or other coliphages.

From all these studies it emerges that the intraparticle DNA's of all the T-coliphages and many other phages have got an abnormal secondary structure. For T-7 DNA in vivo, it appears from our studies, that this abnormality arises from the presence of denatured zones in the form of single-stranded loops.

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\textsuperscript{13} M. F. Maestre and I. Tinoco, JR., J. molecular Biol. 23, 323 [1967].