Biophys Struct Mech (1982) 9:1–9

Biophysics of Structure and Mechanism


# Temperature Dependent Structural Changes of Intraphage T7 DNA

A. Fekete[1], Gy. Rontó[1], L. A. Feigin[2], V. V. Tikhonychev[3], and K. Módos[1]

[1] Institute of Biophysics, Semmelweis Medical University, P.O. Box 263, H-1444 Budapest, Hungary
[2] Institute of Crystallography, Academy of Sciences of the USSR, SU-117333 Moscow, USSR
[3] Department of Physics, Moscow State University, SU-117234 Moscow, USSR

**Abstract.** The phenomena connected with the first phase transition step of the native T7 phage at 40°–65° C have been studied using various methods. In this temperature range a) the optical melting curve shows an absorption decrease, b) the maximum of the small-angle X-ray scattering characteristic for DNA packing disappears, c) there is a drop of biological activity and d) there are changes in the structure of the difference absorption spectra of native phages versus isolated DNA. All data are interpreted assuming a structural change of the DNA due to the release of its protein coat towards the end of the first phase transition step (at 60°–65° C in the case of M9 buffer). Above this temperature the intraphage DNA packing appears to be destroyed and the DNA structure seems to be similar to that in DNA solution.

**Key words:** Bacteriophages – Phase transition – Optical melting curve – X-ray scattering

## Introduction

The nucleoproteins may be thought of as liquid crystals (Sipski et al. 1977) thus their phase transitions can give information on the supramolecular structure and on some details of the nucleic acid-protein interaction. A suitable object for such studies is the T7 phage investigated earlier in our laboratory under various other aspects including UV damage as well (e.g., Rontó et al. 1967; Monaselidze et al. 1978; Fekete et al. 1980). In addition to the well known hyperchromic melting step due to helix-coil transition of DNA various phage systems ($S_d$, T2, T7) showed also a hypochromic step in the 40°–65° C range (Tikchonenko et al. 1966; Monaselidze et al. 1978).

In this paper additional data obtained by various physical and biological methods are presented concerning this first melting step of T7 phages. An

0340-1057/82/0009/0001/$ 01.80

attempt is made to interpret the structural changes taking into account all our presented experimental results.

## Materials and Methods

The cultivation of T7 phages relayed upon our earlier experiences on *E. coli* B/r bacteria obtained in a chemostat (Gáspár et al. 1979). For the purification and concentration of phages a modified method of Strauss et al. (1963) was applied. The T7 DNA was prepared according to Mandell (1969). The purity of DNA was characterized by $E_{260}/E_{280} = 1.85-1.90$. In the optical and biological measurements 0.1 mol/l phosphate buffer (M9), in the X-ray studies Tris buffer (0.1 mol/l NaCl) at pH = 7 was used. The concentration of phages consumed for the various measurements varied from 1 μg/ml to 90 mg/ml according to the requirements of the applied method.

The optical melting curves and the difference spectra were determined on a Perkin-Elmer 200 spectrophometer equipped by a Perkin-Elmer 0570-0710 type temperature program controller. The heating rate was 0.4° C/min (slower heating did not change the results; Monaselidze et al. 1978), the accuracy of the temperature measurement was ± 0.1° C. The melting curves were determined at 260 nm by continuous heating.

In each case the correction for light scattering was determined independently for the measured samples from their absolute spectra using the relation $\mathbf{k} \cdot \lambda^{-\mathbf{n}}$ with empirical fitting for **k** and **n** starting from OD values at 350 and 400 nm. This method has been adopted by a number of authors for similar cases (Tikchonenko et al. 1966; Inners et al. 1969; Gratton, 1971; Sinha et al. 1971; Dore et al. 1972). Though the necessity of a better correction procedure is evident (e.g., Inagaki et al. 1974; Demangeat et al. 1978) the accuracy of the potential methods are not expected to excide ours (Tóth et al. 1980; Földvári et al. 1981).

In the difference spectroscopic measurements the reference and the sample cuvette contained isolated T7 DNA and T7 phage (or "heated" phage) respectively. The concentration of the measured samples was adjusted so as to obtain exactly the same optical density (0.500) at 260 nm, as for the reference. The choice of the 260 nm point was arbitrary, however, being the maximum of DNA absorption it seemed to be a good reference. It must be mentioned that the shape of difference curves apparently depends on the choice of the fitting point. Without using any fitting the hypochromicity and the structural changes in the spectra appear simultaneously. In the present work, however, we intended to deal only with structural changes. Recently Brakke (1979) published similar difference spectra for RNA phages, the elaboration of these data, however, seems to be less reliable.

The small-angle X-ray scattering data were measured on an "AMUR" diffractometer and some details of the method were described by Rontó et al. (to be published). In the present experiments the phages were heated in a glass capillary (external diameter 1.0 mm, wall thickness 0.01 mm) to 40°, 50°, 55°, 60° C temperatures respectively. After an incubation of $^1/_2$ h the quenched

samples were measured at room temperature in the 2°–5° scattering angle region by steps of 0.05°. These angles correspond to the scattering vector (**S**) range 0.142–0.356 $Å^{-1}$. **S** is determined by

$$\mathbf{S} = \frac{4\pi \sin\theta}{\lambda},$$

where $2\theta$ corresponds to the scattering angle and $\lambda$ denotes the wavelength of X-rays, 1.54 Å in our case. A correction was carried out for the background scattering and for the absorption difference between the solution and the solvent. Data processing was accomplished with the aid of a computer program.

The changes in biological activity of T7 phages were investigated using the same heat treatment as in the case of optical melting curves. The number of active phages in samples unheated ($N_0$) or heated to 42°, 50°, 55°, 60°, 61°, 62° C (N) were determined by a modified Gratia-titration method.

## Results and Discussion

Figure 1 shows the *optical melting curve* of the T7 phage after the correction for light scattering. The first melting step between 50°–60° C is a hypochromic one and the same was found earlier in Tris buffer (Monaselidze et al. 1978), nevertheless in the hyperchromic range (over 80° C) the shapes of the two curves are somewhat different.

To study changes at the end of the first melting step the phage suspension was heated for 2 h at 65° C where this step is already accomplished ("heated phage"). After cooling the samples with different velocities to room temperature

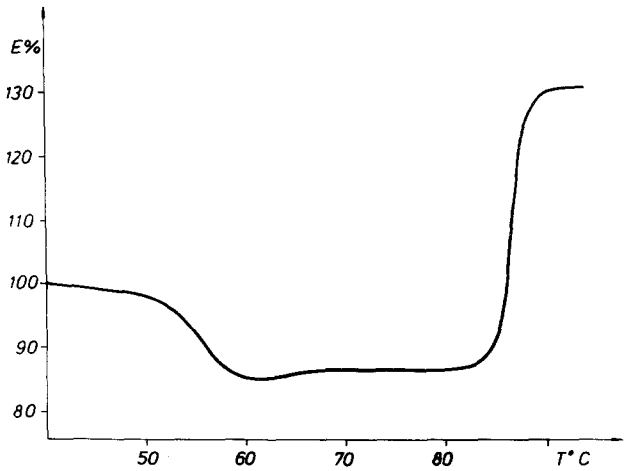


**Fig. 1.** The optical melting curve (measured at 260 nm) of T7 phage in 0.1 mol/l phosphate buffer. Heating rate: 0.4° C/min; DNA concentration 20 μg/ml. E is the absorbance relative to the original one (at 25° C)

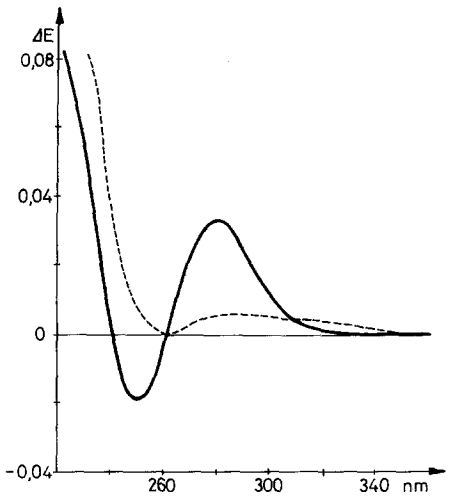


**Fig. 2.** Difference spectra (———) T7 phage–T7 DNA, (-----) heated phage–T7 DNA. ΔE: differences in absorbance after light scattering correction (M9, DNA conc.: 20 μg/ml)

the absorption did not change, indicating that the process is irreversible. Therefore the further measurements on this sample could be performed at room temperature.

Figure 2 shows the *difference spectra* of T7 phage and "heated phage" after correction for scattered light using the same T7 DNA solution in the reference path in both cases. The difference absorption spectrum of T7 phage with respect to DNA solution (full line) shows positive peaks at ~ 280 nm and below 220 nm, and a negative peak at ~ 250 nm. The 280 and 250 nm peaks practically disappeared in the case of heated phage (dashed line). The hypochromic effect which was shown in Fig. 1 cannot be seen in the figure because both curves were fitted at 260 nm to the DNA solution. The strong positive absorption below 220 nm in both difference spectra is probably due to the phage protein.

Earlier reports have shown that during the first melting step the intensity of scattered light decreases due to the change of diameter and refractive index of phage particles originating from the collapse of protein envelope (Tikchonenko et al. 1966; Permogorov et al. 1977; Tóth et al. 1980). The empty capsid itself has negligible absorption at 260 nm and does not show any transition in the UV region (Tikchonenko et al. 1966). Permogorov et al. (1977) tried to explain the whole decrease in optical density as a light scattering difference, but their experimental data do not correlate with the other results. This is probably due to the fact that for estimating the light scattering losses Permogorov et al. used the optical density value at 320 nm where the absorption was not negligible. According to our results the light scattering of native phage was about 15% of the original optical density at 260 nm and its value decreased to 4%–5% at 65° C thus the hypochromicity in the first melting step was about 10%. The difference spectra obtained reflect strutural changes which may be derived from the differences in the higher order structure of DNA between intraphage and "heated phage" states. On the other hand, the spectra showed that the DNA structure in the heated phage is very similar to that of isolated DNA. The same

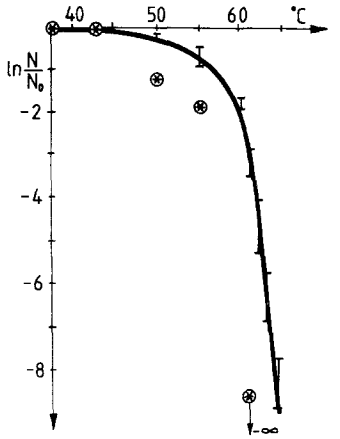


**Fig. 3.** Heat inactivation of T7 phages. Activity determinations were carried out at the indicated temperatures. $N/N_0$ is the survival ratio; ⊛: ratio of non-destructed phages estimated from the X-ray scattering data

conclusions can be drawn from the CD spectra obtained in similar circumstances (Tóth 1981). The residual weak absorption in the heated phage-DNA difference spectrum near 280 nm may be due either to the absorption of protein and/or to the remaining weak interaction of DNA with protein.

The disappearance of the original form of DNA-protein interaction over 60° C drastically changes the *plaque forming ability* of T7 phages. Figure 3 shows the loss of biological activity of T7 phages in the temperature range of the first melting step. The biological function of the phages suddenly ceases because the necessary condition of this function is the maintenance of the intact structure.

The first step of the phase transition was studied by *small-angle X-ray scattering* as well. Figure 4 shows the scattered intensity (I) as a function of the scattering angles in the angular range ($2\theta$) 2°–5°, [scattering vector (S) range 0.142–0.356 $Å^{-1}$]. In the case of the native phage (20° C) the curve shows a distinct maximum at $2\theta = 3.5°$ ($S = 0.25\ Å^{-1}$). Increasing the temperature this maximum becomes smaller and shifts to lower angle, at 55° C it takes the form of a shoulder and at 60° C practically vanishes. This maximum corresponding to the Bragg-spacing of 2.4 nm was detected by several authors in the scattering patterns of phages T2, T7, $\lambda$ and $S_d$ (North et al. 1961; Dembo et al. 1965; Earnshaw et al. 1976) and also for DNA gels (Maniatis et al. 1975) whereas it was never found in the case either of DNA solution or empty phage head or prohead (Earnshaw et al. 1976). To follow the real change on each scattering curve the maximum was separated from the general background. This is demonstrated on Fig. 5. The characteristics concerning the changes of these maxima are summarized in the Table 1. The first column contains the incubation temperatures, the second and third the scattering angles ($2\theta$) and vectors ($S_{max}$) belonging to the maxima respectively, the fourth and fifth the half-widths ($\beta_{2\theta}$) of the separated peaks, and the last one contains the areas ($B_{max}$) under the peaks. As a result of heating the heights of the maxima as well as the areas of the peaks diminish. At 40° C the maximum shifts to the left but its position is slightly uncertain because of the increasing half-with.

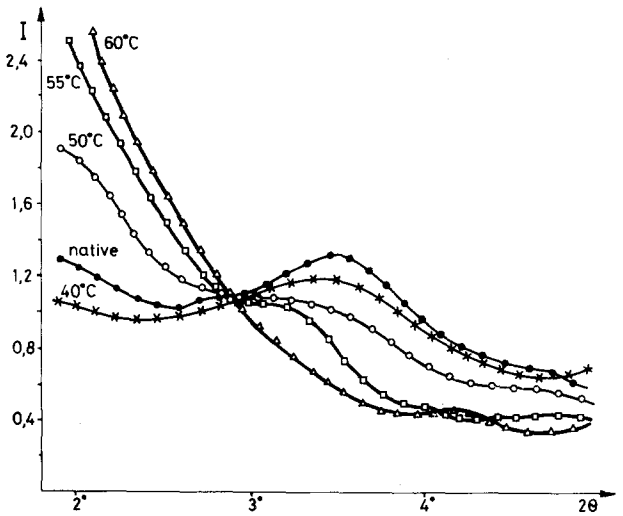


**Fig. 4.** The X-ray scattering pattern of T7 phage in the 2°–5° range of scattering angles ($2\theta$); I: scattered intensity (in arbitrary units). The superscripts indicate the incubation temperatures before the X-ray measurement

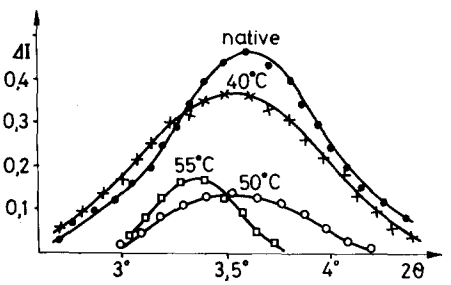


**Fig. 5.** The maxima separated from the general background of Fig. 4. $2\theta$: scattering angle, $\Delta I$: scattered intensity in the maximum. Superscripts indicate the incubation temperatures

**Table 1.** Measured and calculated characteristics for T7 DNA packing. For the notations used see the text

| Incubation temperature [°C] | $2\theta_{max}$ [degree] | $S_{max}$ [Å$^{-1}$] | $\beta_{2\theta}$ [degree] | $\beta_{2\theta}$ [rad.] | d [nm] | a [nm] | L [nm] | $r_m$ [nm] | $B_{max}$ [a. u.] |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 3.60 | 0.256 | 0.83 | 0.0145 | 2.45 | 2.83 | 11 | 17 | 1.00 |
| 40 | 3.55 | 0.252 | 0.88 | 0.0154 | 2.50 | 2.88 | 10 | 16 | 0.95 |
| 50 | 3.55 | 0.252 | – | – | 2.50 | 2.88 | – | – | 0.25 |
| 55 | 3.36 | 0.239 | – | – | 2.62 | 3.02 | – | – | 0.17 |

Assuming that the area of the peak ($B_{max}$ in the Table 1) is unity in the case of native phage and at other tmperatures its value corresponds to the proportion of phages remaining structurally and consequently functionally intact in solution, these proportions should be similar to the ratios $N/N_0$ determined by biological activity measurements. In fact these values approximately agree with

those obtained from biological measurements as shown in Fig. 3 where the stars indicate the logarithms of $B_{max}$ values. We think this matching to be acceptable.

The sixth column of the Table 1 summarizes the values of the Bragg-spacing (**d**) which is connected according to several authors (North et al. 1961; Dembo et al. 1965; Earnshaw et al. 1976; Maniatis et al. 1974) to the packing periodicity of intraphage DNA. The next column contains the lattice constants (**a**) characterizing the DNA crystallite size assuming a hexagonal packing of DNA filaments, and the eighth column shows the size of so called DNA crystallites (**L**) calculated on the basis of half-widths according to the Debye-Scherrer relation:

$$\mathbf{L} = \frac{\lambda}{\cos\,\theta \cdot \beta_{2\theta}} ,$$

where $\beta_{2\theta}$ is the half-width of the maximum, $2\theta$ is the scattering angle and $\lambda$ denotes the wavelength of X-rays (North et al. 1961). In the ninth column the interaction radius ($\mathbf{r}_m$) is listed (Vainshtein 1963; Dembo et al. 1965). The value for $\mathbf{r}_m$ is given by

$$\mathbf{r}_m = \frac{2}{2.5^2} \cdot \frac{\lambda}{\beta_{2\theta}} .$$

For distances $\mathbf{r} > \mathbf{r}_m$ the probability of localization does not depend on the distance **r** from the given molecule and $\mathbf{r}_m$ has a constant value determined by the packing density only. In the case of a densely packed chain molecule the interaction radius characterizes the range of order.

It can be seen from the Table 1, that raising the temperature the values characterizing the regular packing of the intraphage DNA i.e., the Bragg spacing (**d**) and the lattice constant (**a**) increase, whereas the size of DNA crystallites (**L**) and the interaction radius ($\mathbf{r}_m$) decrease. These data suggest that the DNA packing density decreases which can be explained in two ways. The structure of the highly hydrated intraphage DNA may either loosen without any destruction of the protein capsid, or alternatively, the DNA may be released stepwise from the capsid with the formation hereby of an intermediate stage, where only a part of the DNA is located in the phage head which becomes less densely packed. It is not easy to choose between these two interpretations. There are no pecularities in the melting curve of T7 phages which could be ascribed to intermediate disruption stages. However, an internal protein body revealed by electron microscopy (Serwer 1976) and by small angle neutron scattering (Rontó et al. to be published) may cause intermediate stages during phage disurption.

According to the theoretical considerations of Sussmann et al. (1978) concerning chromatin we assume that this transition in our much simpler case corresponds to the cessation of the "distorted" conformation of intraphage DNA. From our earlier results (Monaselidze et al. 1978) we could estimate the enthalpy of this phase transition step: $\sim 3$ kJ/mole of base pairs, which approximately agrees with the values of Sussmann et al. (1978).

We may summarize that the first step in the phase transition process of T7 phages can be explained as a structural change of DNA due to its release from the protein coat. This process is irreversible, the packing of DNA inside the phage particle appears to be destroyed and the conformation seems to be similar to that of isolated T7 DNA.

*Acknowledgements.* The authors express their gratitude to Prof. B. K. Vainstein and Prof. I. Tarján for their support in the work and for their helpful discussion in preparing the manuscript.

## References

Brakke M (1979) Ultraviolet absorption spectra and difference spectra of barley stripe mosaic and tobacco mosaic viruses in buffer and sodium dodecyl sulfate. Virology 98: 76–87

Demangeat JL, Chambron J (1978) Correction des effects de diffusion dans les spectres d'absorption de suspensions. J Fr Biophys Med Nucl 1: 9–19

Dembo AT, Dobrov EN, Lednev VV, Tikchonenko TI, Feigin LA (1965) Packing of intraphage DNA in $D_d$, $T_2$ and $S_d$ Bacteriophages. Biofiz 10: 404–407 (in Russ)

Dore E, Frontali C, Gratton E (1972) Physico-chemical description of a condensed form of DNA. Biopolymers 11: 443–459

Earnshaw WC, Harrison SC (1977) DNA arrangement in isometric phage heads. Nature 268: 598–602

Fekete A, Rontó Gy (1980) The role of DNA – protein interaction in the UV-damage of T7 bacteriophage at high fluence. Studia Biophys 80: 165–171

Földvári I, Fekete A, Corradi G (1981) Prospects and difficulties in the far – and vacuum – ultraviolet spectroscopy of DNA. J Biochem Biophys Methods 5: 319–327

Gáspár S, Rontó Gy, Müller G (1979) Determination of the biological parameters of bacterium-phage complexes. Z Allg Microbiol 19: 163–169

Gratton E (1971) Method for the automatic correction of scattering in absorption spectra by using the integrating sphere. Biopolymers 10: 2629–2634

Inagaki T, Hamm RN, Arakawa ET, Painter RL (1974) Optical and dielectric properties of DNA in the extreme ultraviolet. J Chem Phys 61: 4246–4250

Inners D, Bendet IJ (1969) Thermal stability of T2 DNA in situ. Virology 38: 269–277

Mandell ID, Hershey AD (1969) A fractionating column for analysis of nucleic acids. Anal Biochem 1: 66–77

Maniatis T, Venable IH, Lerman LS (1974) The structure of $\psi$ DNA. J Mol Biol 84: 37–64

Monaselidze DR, Chanchalashvili DzI, Mgeladze GN, Madjagaladze GV, Fekete A, Rontó Gy (1978) Thermodynamical properties of phage T7. Studia Biophys 70: 213–220

North ACT, Rich A (1961) X-ray diffraction studies of bacterial viruses. Nature 191: 1242–1245

Permogorov VJ, Tyaglov BV, Bogush VG, Minaev VE (1977) Scattering contribution to the optical density during melting of T7 and $S_d$ phages. Molekul Biol 11: 134–138 (in Russ)

Rontó Gy, Agamalian MM, Drabkin GM, Feigin LA, Lvov YuM (1981) Structure of bacteriophage T7. Small-angle X-ray and neutron scattering study. Biophys J (to be published)

Rontó Gy, Sarkadi K, Tarján I (1976) Zur Analyse der UV-Dosiswirkungskurven der T7 Phagen. Strahlentherapie 134: 151–157

Serwer P (1976) Internal proteins of bacteriophage T7. J Mol Biol 107: 271–291

Sinha RK, Misra DN (1971) Studies on the secondary structure of intraphage T7-DNA. Z Naturforsch 26: 1288–1291

Sipski ML, Wagner LT (1977) Probing DNA quaternary ordering with circular dichroism spectroscopy: Studies of equine sperm chromosomal fibers. Biopolymers 16: 573–582

Strauss JrJH, Sinsheimer RL (1963) Purification and properties of bacteriophages MS2 and of its ribonucleic acid. J Mol Biol 7: 43–54

Sussmann JL, Trifonov EN (1978) Possibility of nonkinked packing of DNA in chromatin. Proc Natl Acad Sci USA 75: 103–107

Tikchonenko TI, Dobrov EN (1966) Pecularities of the secondary structure of phage DNA in situ. J Mol Biol 18: 58–67

Tóth K, Fekete A (1980) Effect of microenvironment on the structure of bacteriophage solution (light scattering studies). Acta Biochim Acad Sci Hung 15: 130

Tóth K (1981) CD and UV-absorption Study of T7, MS2 and $\phi$X 174 bacteriophages. Proving of the effect of low-dose UV irradiation "In Vivo" T7 bacteriophage. Thesis of Univ. P. et M. Curie, Paris (in French)

Vainstein BK (1963) X-ray diffraction of chain-molecules. Publ. Acad Nauk SSR, Moscow (in Russ)

Received February 9, 1982/Accepted May 11, 1982