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UV-Induced Small Structural Changes in the T7 Bacteriophage Studied by Melting Methods

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Abstract. UV optical absorption and circular dichroism (CD) properties (spectra and melting curves) of T7 bacteriophage were investigated to detect „in situ" structural damages which can be related to the biological inactivation due to UV irradiation. UV doses (0.2–1.2 kJ/m^2 at 254 nm) near to the biologically effective minimal dose were applied where the initial genetic damage (~ 10 events/phage) was observed. The decrease of the melting temperature of the helix-coil transition and the broadening of the transition range indicate the destabilization of the intraphage structure due to the presence of about 0.1–0.6% damaged base concentration.

Key words: Absorption and CD melting – Thermal stability of DNA and nucleoproteins – UV irradiation effect – Bacteriophage

Introduction

Numerous studies have been made in an attempt to understand the alterations which take place in T7 bacteriophage and in its isolated DNA under UV irradiation. At lower doses (below 0.1 kJ/m^2 which corresponds to 10^{16} incident photons/cm^2), with $\lambda = 254$ nm, only the functional damage is detectable (Rontó and Tarján 1967; Bräutigam and Sauerbier 1973, 1974) since the biological functions, e. g., plaque-forming ability, template activity for transcription, are very sensitive to damage to one or two nucleic acid bases. At higher UV doses (above 1 kJ/m^2) various physical and chemical methods are used for detection and identification of photoproducts in phage-nucleoprotein as well as in its isolated DNA. For phage T7 Patrick and Gray (1976) and Patrick (1977) found that at 4 kJ/m^2 the predominant photoproducts were two types of pyrimidine dimers. Sutherland et al. (1979) explained that thymine dimer formation leads to a break of the T–A pair H-bonds in the irradiated DNA molecules. The induction of dimers and other photoproducts was followed by Fekete and Rontó (1980) with the help of UV-differential absorption spectrometry.

The detection of UV damage in the transition dose range of 0.1–1 kJ/m^2 is an interesting problem because the "concentration of damage" is very low and only certain physical methods are able demonstrate the subtle structural changes caused by irradiation (Jensen et al. 1976). These changes are mainly conformational variations and they can be detected only by extremely sensitive methods, such as microcalorimetry, CD, vibrational spectrometry etc. Monaselidze et al. (1978) used a differential scanning microcalorimeter to establish that the formation of photoproducts reinforced the destabilizing effect of phage proteins on the "in situ" T7-DNA with UV doses in the above-mentioned transition dose range. The high sensitivity of CD allowed us to use a similar "damage concentration" per phage but samples five to 10 times more diluted than those of the preceding study (Monaselidze et al. 1978). The CD-melting methods are widely used for investigation of the structure of isolated DNA as well as for detection of the photoproducts (Toulmé and Helene 1977).

In this study a parallel analysis of two melting profiles obtained by optical density and CD measurements of UV-irradiated T7 phage is made to follow the changes of the conformational stability of the low-dose irradiated phage.

Materials and Methods

T7 bacteriophages were grown on *Escherichia coli* B host cells. The cultivation was carried out according to the method based on experiments on chemostat-cultures (Gáspár et al. 1979). The purification and concentration were carried out by a modified method of Strauss and Sinsheimer (1963). The samples were separated finally in CsCl gradient. Before the measurements the solutions were dialysed three times through an Amicon XM300 filter into phosphate buffer "M9" which is composed of

$MgSO_4$: 10^{-3} mol/l
NH_4Cl: $2 \cdot 10^{-2}$ mol/l
Na_2HPO_4: $4 \cdot 10^{-2}$ mol/l and
KH_2PO_4: $4 \cdot 10^{-2}$ mol/l at pH = 7.

The bacteriophage concentration was 50–100 μg/ml, determined by optical density measurements (OD = 0.5–1) using light-scattering corrections.

The UV irradiation was performed by a 2.5-kW Xenon lamp, using a Jobin-Yvon monochromator. Samples were irradiated in quartz cells with continuous stirring. The incident doses at 254 nm changed from 3×10^{16} photons/cm^2 (0.2 kJ/m^2) to 18×10^{16} photons/cm^2 (1.2 kJ/m^2). For the melting measurements samples were heated slowly (0.5° C/min) between 30 and 95° C using an automatic temperature programmer (Perkin-Elmer 0570–0710 type). Changes of the optical density at 260 nm were measured by a Perkin-Elmer 200 UV-*vis* spectrophotometer. Digital data were recorded every 0.02° C for computer analysis. In the case of melting measurements the light-scattering correction was not made. Changes in the CD at 280 nm were recorded by a Jobin-Yvon Mark III dichrograph. The analogue CD-melting curves were digitized every 0.05° C by a Hewlett-Packard 983 OA desk-calculator. The first

derivatives of the melting curves were calculated by polynomial-fitting on 0.5° C wide regions around every point.

Results

1. Non-Treated T7 Bacteriophage

Figure 1 presents the UV absorption and CD spectra of non-treated T7 bacteriophage. Data are presented as ε or $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ per mole of bases, where ε, ε_L, and ε_R are the molar extinction coefficients for non-polarized and for left and right circularly polarized light, respectively. To obtain the real absorption spectrum shown in Fig. 1 the light-scattering contribution was subtracted from the measured optical density values using a semi empirical method (Tóth and Fekete 1980). An attempt was also made to diminish the light-scattering effect in the CD spectrum by choosing an optimal position for the measuring cell, close to the detector.

For the molar absorptivity of the bases inside the intact phage the absorption spectrum gives the value

$$\varepsilon_{260} = 7{,}300 \pm 200\ \mathrm{l} \cdot \mathrm{mol}^{-1} \cdot \mathrm{cm}^{-1}\ .$$

The comparison to the corresponding value of isolated DNA

$$\varepsilon_{260} = 6{,}600 \pm 200\ \mathrm{l} \cdot \mathrm{mol}^{-1} \cdot \mathrm{cm}^{-1}\ .$$

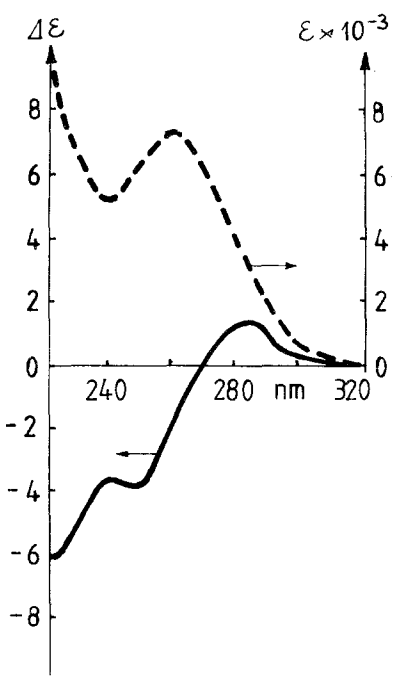

Fig. 1. UV absorption (ε – – –) and CD ($\Delta\varepsilon$ ———) spectra of non-treated T7 bacteriophage in phosphate buffer M9 (see Materials). ε and $\Delta\varepsilon$ are expressed as averaged molar extinction of the nucleotide bases ($\mathrm{l} \cdot \mathrm{mol}^{-1} \cdot \mathrm{cm}^{-1}$)

(Davison and Freifelder 1962) indicated the presence of a hyperchromic effect. It cannot be due to proteins because the protein absorption is much smaller than the value observed here. In the CD spectrum a double Cotton effect is observed which was shown to be due to the optical activity of the DNA (Tinoco 1968). The positive maximum is situated at 285 nm and the value of

$$\Delta\varepsilon_{285} = 1.47 \pm 0.05\ \mathrm{l \cdot mol^{-1} \cdot cm^{-1}}$$

for intraphage DNA is about the half of the value measured for isolated T7 DNA in the same buffer (Tóth 1981). The negative band of the Cotton effect at 247 nm is partially hidden by a second negative band at 222 nm, the latter comes from the optical activity of proteins. Therefore only the positive band in the CD spectrum [Fig. 1 (———)] can be considered to be characteristic of the intraphage DNA conformation. Since the maximum of this band shifts from 285 to 275 nm during heating (Tóth 1981), an intermediate value (280 nm) is taken for the CD-melting measurements.

Figure 2 shows the optical density (OD) melting curve and the CD-melting curve of non-treated T7 phage (Fig. 2a) together with their first derivatives (Fig. 2b). The hyper- or hypo-chromicity values related to the native-state (30° C) values are plotted against temperature. H_{OD} is related to the change of the optical density; it is given by

$$H_{OD} = \frac{OD(T) - OD(30^\circ\,C)}{OD(30^\circ\,C)} \times 100\ (\%)$$

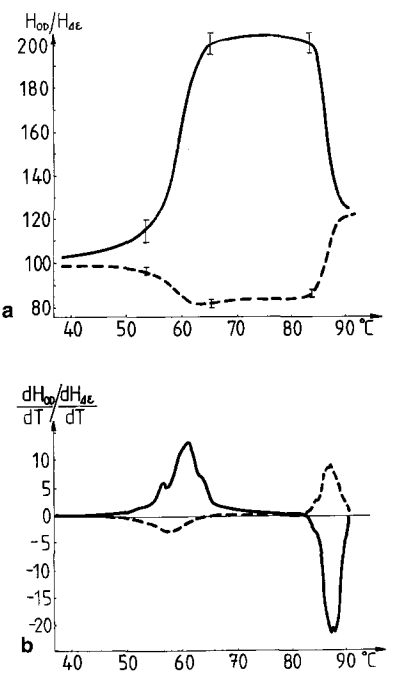

Fig. 2. Optical density (H_{OD} - - - - at λ = 260 nm and) CD (H ——— at λ = 280 nm) melting curves (**a**) and their first derivatives (**b**) of non-treated T7 bacteriophage. H_{OD} and $H_{\Delta\varepsilon}$ are expressed in percentage of the room temperature values, the first derivatives are given in percentage/° C units. Phosphate buffer M9 used as solvent (see Materials)

and $H_{\Delta\varepsilon}$ is similar, for the CD values:

$$H_{\Delta\varepsilon} = \frac{\Delta\varepsilon(T) - \Delta\varepsilon(30^\circ\,\mathrm{C})}{\Delta\varepsilon(30^\circ\,\mathrm{C})} \times 100\ (\%)\ .$$

The first derivatives $\left(\frac{dH_{\mathrm{OD}}}{dT}, \frac{dH}{dT}\right)$ of both melting curves are expressed in percent of change per degree Celsius.

The melting profiles show two main transitions. The first takes place between 50 and 65° C and the second at 83–90° C.

The first transition is indicated by a week decrease (~ 20%) of the optical density and a very definite increase of the CD values (~ 100%).

The change of the optical density can be separated into two components (Table 1). The scattering change was discussed in an earlier work (Tóth and Fekete 1980). The scattering diminishes by 60% of its room temperature value: about two-thirds of the OD change comes from this factor, and only the remaining one-third can be ascribed to the change of the real absorptivity of the bases.

The great increase (~ 100%) in the CD value at the DNA conformation sensitive wavelength (280 nm) presents the development of a more regular double helical order of the bases after this transition. The resulting CD spectrum at 65° C in the band characteristic of the DNA (250–300 nm) is very close to the free T7 DNA spectrum (Tóth 1981). No indication for significant change in the differential scattering has been found in the long wavelength region[1]. The first transition is totally irreversible.

The second transition is sharper in both curves and the direction of the changes of the optical parameters are opposite to the first transition. The temperature range of the second transition coincides with the helix-coil transition of the DNA. The optical density values show an increase of ~ 40% and do not show any real saturation. The simultaneous increase in light scattering has been measured at a wavelength outside the absorption band (at 320 nm, not shown here). The CD intensity returns to almost its original value, with saturation. Significant differential scattering has not been observed. This transition is partly reversible.

Table 1. Separation of the measured optical density into scattering and real absorption at different temperatures. S/OD: the scattering ratio; A/OD: the absorptivity ratio

	Temperature	
	30° C	60° C
S/OD	20 ± 2%	10 ± 2%
A/OD	80 ± 2%	90 ± 2%

1 This is in accordance with the recently published results of Bustamente et al. (1983) Proc Natl Acad Sci USA 80: 3568–3572

Both transitions are characterized by parameters obtained from the melting curves and from their first derivatives as follows
T_m: the transition temperature (melting temperature) where the derivative has an extreme value;
E: the extreme value of the first derivative;
W: the width of the transition (between 15.9% and 84.1% of the total change);
A: the integrated area below the derivative curve, determined numerically from the digitized data or by planimetry.

(A corresponds to the total change of the H_{OD} or $H_{\Delta\varepsilon}$ values).
To obtain those parameters, the derivative bands were considered without any band-resolution. The data are listed in Table 2.

Table 2. Optical density and CD melting parameters of non-treated T7 phage. (Definitions in the text)

	First transition		Second transition	
	OD	CD	OD	CD
T_m (°C)	58.0 ± 0.1	61.0 ± 0.25	87.5 ± 0.1	87.5 ± 0.5
E $\frac{1}{°C}$	−.0 ± 0.2	13.0 ± 0.2	10.0 ± 0.2	− 21.0 ± 0.2
W (°C)	7.0 ± 0.5	7.0 ± 0.5	4.5 ± 0.5	4.0 ± 0.5
A	24.0 ± 0.5	90.5 ± 0.5	30.5 ± 0.5	62.5 ± 0.5

2. *UV-Irradiated T7 Bacteriophage*

Since the expected photoproducts, the pyrimidine dimers, can influence the helix-coil transition of the DNA, we examined the second transition range of the melting curves in function of the UV irradiation dose.

Figure 3 shows the first derivative curves of the optical density (Fig. 3a) and CD-melting (Fig. 3b) of UV-irradiated phages in the second transition range, for various irradiation doses.

The curves obtained by each method show a decrease in melting temperatures, broadening of the melting regions, and decrease in the extreme values of the derivative bands. The decrease of the melting temperature could be correlated to the loss of the conformational stability in the presence of the photoproducts, whereas the broadening of the melting region and the decrease of the extreme values could be related to the decrease of the co-operativity during the transition. The obtained melting-parameters are listed in Table 3 as a function of the UV dose. The values are compared to the parameters of the non-treated phage. Some of the parameters listed in Table 3 as a function of the UV dose are shown in Fig. 4. The decrease of the extreme values (E) and that of the reciprocal values of the half width (W^{-1}) are about 50% at a dose of 1.2 kJ/m^2. Both parameters show similar profiles in the optical density and CD

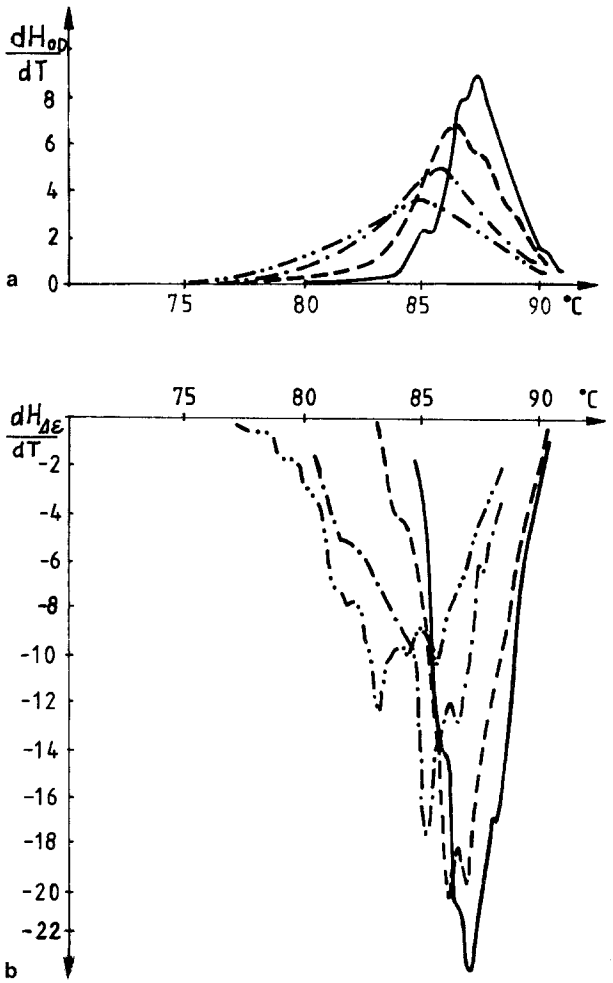

Fig. 3. First derivatives of the optical density at $\lambda = 260$ nm (**a**)and CD at $\lambda = 280$ nm (**b**) melting curves in the second transition range, for UV (254 nm) irradiated T7 bacteriophages. Doses are: O (———); 0.2 kJ/m^2 (- - - -); 0.6 kJ/m^2 (— · — · —) and 1.2 kJ/m^2 (— ·· — ·· —). Phosphate buffer M9 used as solvent (see Materials)

Table 3. Changes in the melting parameters (for the second transition range) at different doses of UV irradiation. A: Area of the derivative band related to the non-treated case; E: Height of the extreme value of the derivative in relation to the non-treated case; W^{-1}: Reciprocal value of the width of the transition relative to the non-treated case; ΔT: The shift of the melting temperature

Dose (kJ/m^2)	A (%)		E (%)		W^{-1} (%)		T (°C)	
	OD	CD	OD	CD	OD	CD	OD	CD
0.2	95	86	83	86	90	88	− 1.0 ± 0.1	− 1.0 ± 0.5
0.6	87	90	57	68	82	72	− 2.0 ± 0.1	− 2.0 ± 0.5
1.2	68	86	44	50	70	53	− 3.0 ± 0.1	− 3.0 ± 0.5

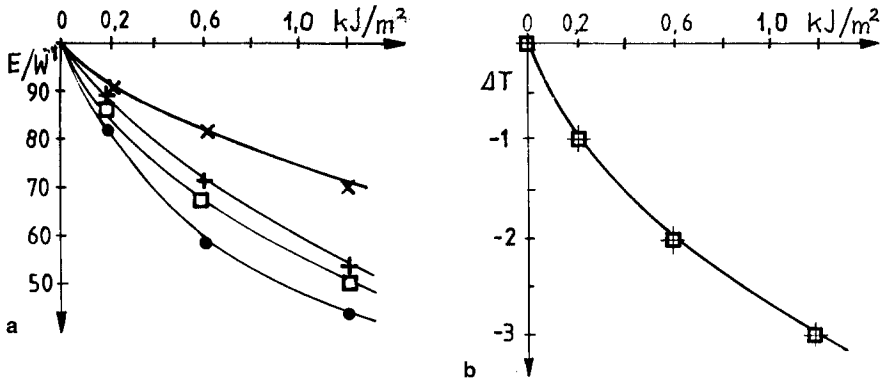

Fig. 4. Changes of some melting parameters in the second transition range of UV irradiated bacteriophage in the function of the incident dose. (The values are taken from Table 3.) The measurements have been made in phosphate buffer M9 (see Materials) at $\lambda = 260$ nm for the optical density and at $\lambda = 280$ nm for the CD melting. **a** *E*: ratio of the height of the extreme value of the derivative, to the non-treated phage; in optical density (− ● − ● −) and in CD (− □ − □ −); W^{-1}: ratio of the reciprocal of the half-width of the transition to the non-treated phage; in optical density [× − × − ×] and in CD [+ − + − +]. **b** ΔT: the shift of the melting temperature determined from the position of the extreme value of the first derivatives in optical density (− □ − □ −) and in CD (+ − + − +)

curves. The melting temperature shifts to lower temperatures, thus at 1.2 kJ/m^2 the decrease is about 3° C.

Discussion

Spectra of the Phages

The optical absorption and the CD spectra of T7 bacteriophage show a region (250–300 nm) containing information about the *intraphage DNA conformation.* Considering the molar ε and $\Delta\varepsilon$ values of the bases, the intraphage DNA conformation seems to be different from the B- and C-type conformations which are usually observed for isolated DNA in such buffer conditions (e. g., Sprecher and Johnson 1978). The conformational difference is more pronounced in the chirality parameter, i.e., in the positive CD band. Neither the absorption nor the CD spectra showed significant changes after a low-dose UV irradiation of the phages. In the literature different changes have been reported in the CD spectrum of UV irradiated DNA. A new negative band appeared at about 320 nm (Lang and Luck 1973) and the change of the optical activity at 275 nm (Toulmé and Helene 1977) was observed but in each case the isolated DNA was irradiated by doses about 10 times higher than in this work.

Melting of Non-Treated Phages

To discuss the optical density melting curve and the CD melting curve we have to take into consideration some earlier results.

First the heat-absorption experiments showed the different transition-stages of the T7 phage (Monaselidze et al. 1978). However, from this one cannot distinguish whether it is the protein or the DNA which absorbs the heat at a certain temperature. The optical density melting curve is more characteristic of the DNA modification because of the well knwon hyperchromic change at the helix-coil transition. In our case, this method is not unambiguous because in nucleoproteins the light-scattering changes are superimposed on the hyper- or hypochromic effects (Permogorov et al. 1977; Tóth and Fekete 1980). However, the change of the CD value at 280 nm is much more specific for the DNA conformational modifications. Therefore, in addition to the usual optical density melting method we used the CD melting method to get information about the intraphage DNA transitions.

The observed *first transition* between 50 and 65° C is strongly evidenced by the CD melting curve (Fig. 2). At 65° C both the absorption and the CD properties of the phage in DNA-sensitive spectral regions are very close to those of isolated DNA in solution, which is assumed to be in the B conformation. From earlier (electronmicroscopic) studies (Serwer 1978) it is known that the DNA is released from the capsid at the same temperature (~ 65° C). The loss of the higher order organization of the intraphage DNA was also demonstrated in that temperature range by small angle X-ray scattering (Fekete et al. 1982). To these, we can add from our results that the released DNA has helicity and base stacking characters similar to that of the isolated DNA in solution.

For the original intraphage DNA conformation we therefore conclude that it contains special base-base, base-sugar or base-protein interactions which might decrease the optical activity, by changing the overall helicity of the DNA. The characteristic base-base distances in the intact phage head correspond to the B-type conformation as indicated by X-ray diffraction (North and Rich 1961), thus it is not responsible for the helicity change. We admit that this change can be caused either by some angular torsions between the overlapping bases, more by some special interactions between the superturns of the wrapped DNA as has been suggested by Sussman and Trifonov (1978) and by Kosturko et al. (1979).

During the second transition (83–90° C) the increase in optical density and the decrease in the CD-value indicate that the helix-coil transition of the released DNA is predominant. Using the first derivative curve of the optical density we can distinguish a little resolved fine structure on this second transition. Fluorescence-labelling of the DNA and of the coat proteins showed changes of the protein conformation in this temperature range (Fidy et al. 1983). These changes could influence the optical density melting profile because of the capsid induced light scattering.

The CD melting values at 280 nm show a very sharp change in this temperature range. The decrease of the optical activity is very great and finally it reduces to almost the original value. This change is about twice that of the recently reported results of Sprecher and Johnson (1982) in the case of *E. coli* DNA which has the same G-C content. Thus we can conclude that the CD metlting curve of the T7 phage can be used to indicate selectively and sensitively the helix-coil transition of the released DNA.

Dose Effect on the Melting Process

Absorption and CD spectra are not sensitive enough to the induced changes in the T7 phage under low-dose UV irradiation, but the optical density and the CD melting curves can be used as a sensitive tools for detection of such structural modification. Thus the observed changes of the melting parameters during the second transition (listed in Table 3) could be related to the decrease in the degree of co-operativity in the helix-coil transition in the presence of the photoproducts which influence the thermal stability of the double-helix. It has been shown (Pavlov et al. 1977) that if the DNA is more regular in its primary, secondary or higher conformation the co-operativity of the transition is stronger. Even at low concentration, the photoproducts can disturb the ordering of the DNA structure, reducing the co-operativity during the helix-coil transition. The presence of a few damaged bases provokes in their neigbourhood a strand separation at lower temperature due to their lower stability. Thus the co-operativity will amplify the effect of the damaged bases, which can be manifested by a lowering of the melting temperature of the whole DNA. In such cases the photoproducts could play a more important role during the melting process than in the other concentration-dependent processes. In the present study the photoproduct concentration was estimated to be about 0.1–0.6% in comparison of all of the normal bases (Rontó 1979). This indicates that a low damage-concentration can be detected by the melting technique. At higher doses the direct detection methods can be used as we mentioned in the introduction. With the buffer M9 used in these experiments the changes of the parameters of the optical density and CD melting curves increase with the irradiation dose. Nevertheless it is necessary to use the two methods simultaneously in cases when the protein and the DNA changes need to be separated.

Our recent results concerning the effect on the denaturnation of the ion composition and ionic strength of the solution revealed that with appropriate ionic conditions one can separate into different temperature ranges the conformational changes of the nucleic acid and of the proteins. This fact helps us to study the changes in stability due to chemical or physical agents acting separately on the nucleic acids and proteins or on the whole complex.

We believe that the capability of the CD melting method to detect the low damage concentrations in DNA could also be applied to the study of other environmental agents which produce small structural changes of biological interest.

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