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Domenico Grasso[a]; Salvatore Fasone[a]; Carmelo La Rosa[a]; Victor Salyanov[b]
[a] Dipartimento di Scienze Chimiche, Università di Catania, Catania, ITALY [b] Institute of Molecular Biology, Academy of Sciences, Moscow, U.S.S.R.

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PRELIMINARY COMMUNICATION

A calorimetric study of the different thermal behaviour of DNA in the isotropic and liquid-crystalline states

by DOMENICO GRASSO*, SALVATORE FASONE
and CARMELO LA ROSA
Dipartimento di Scienze Chimiche, Università di Catania,
Viale A. Doria, 8-95125 Catania, Italy

and VICTOR SALYANOV
Institute of Molecular Biology, Academy of Sciences, Moscow, U.S.S.R.

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A difference between the thermal behaviour of the isotropic and liquid-crystalline state of sonicated DNA in aqueous salt solution containing poly(ethyleneglycol) (PEG) has been demonstrated. On cooling, a different degree of renaturation of thermally denaturated DNA is observed between samples which form the isotropic state and more concentrated samples which on cooling form the cholesteric state.

Naturally occurring DNA is double stranded, the two strands being linked by hydrogen bonds. The two strands can be separated by physical or chemical methods. Heating results in denaturation, that is the breaking of the hydrogen bonds and the separation of the two strands. This process is often described as 'melting'. It can be partial, where the two strands are separated only over isolated regions of the DNA molecule, or complete, where all of the interchain bonds are broken and the two strands are free to move apart. On cooling, the interchain hydrogen bonds tend to reform, renaturating the DNA. The extent to which renaturation occurs depends very much on the external experimental conditions as temperature or annealing time and on characteristics of the system such as concentration and length of DNA fragments, ionic strength and pH.

It has been found that natural DNA in concentrated aqueous solution forms hexagonal and cholesteric lyotropic mesophases [1, 2]. It is also known that DNA in dilute aqueous solution can be 'condensed' by the addition of salts and a neutral polymer such as polyethyleneglycol (PEG) to form a suspension of liquid-crystalline microphase regions [3–5]. Because of its helicoidal structure this mesophase shows a very pronounced circular dichroism and a number of previous CD studies of liquid-crystalline DNA have been reported [6]. In particular previous spectroscopic investigations of the thermal stability of liquid-crystalline DNA showed that heating causes the disappearance of the intense negative band at 260 nm in the CD spectrum of the microphase as a result of the transition from the ordered cholesteric liquid-

* Author for correspondence.

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crystalline state to the disordered isotropic state [7]. An increase of the PEG concentration in the original solution leads to a widening of the temperature range over which this phenomenon occurs. For a PEG concentration of 170 mg/ml this temperature range is from 80 to 85°C [8]. The 'condensing' effect of the PEG, can be explained in terms of its very high hydrophilicity. In aqueous solution PEG becomes heavily hydrated and so reduces the amount of water accessible to the DNA. The addition of PEG is, therefore, a way of effectively increasing the concentration of DNA, thus encouraging its condensation out of solution. The concentration of the condensed phase (i.e. the DNA/water ratio) is determined by the amount of PEG added to the original solution.

The properties of the liquid-crystalline phases of the DNA have been extensively investigated [8–11], however, to the best of our knowledge, no calorimetric studies have been reported. In the present work the first attempt to study these phases by means of a microcalorimetric approach using a differential scanning calorimeter is described.

Our preliminary calorimetric experiments were carried out directly using the DNA microphase suspension prepared for the CD measurements, but no heat effect was observed, because the quantity of DNA present was too small (50 μg/ml). In order to increase the amount of the DNA studied in the microcalorimeter, we have used the bulk mesophase obtained by low speed centrifugation of the microphase suspension [12]. The preparation of samples is described in particular in the last part of this preliminary communication.

Figure 1 shows a typical example of the DSC curve (C_p versus T) of a DNA sample in the range of PEG concentration from 0 to 90 mg/ml (i.e. in the region where the liquid-crystalline state of DNA is not formed). In particular curve (*a*) refers to the first heating of the sample and (*b*) refers to the second heating, carried out after cooling the sample to room temperature. The endotherm peak corresponds to the DNA melting, i.e. the denaturation process where the hydrogen bonds which link the two DNA

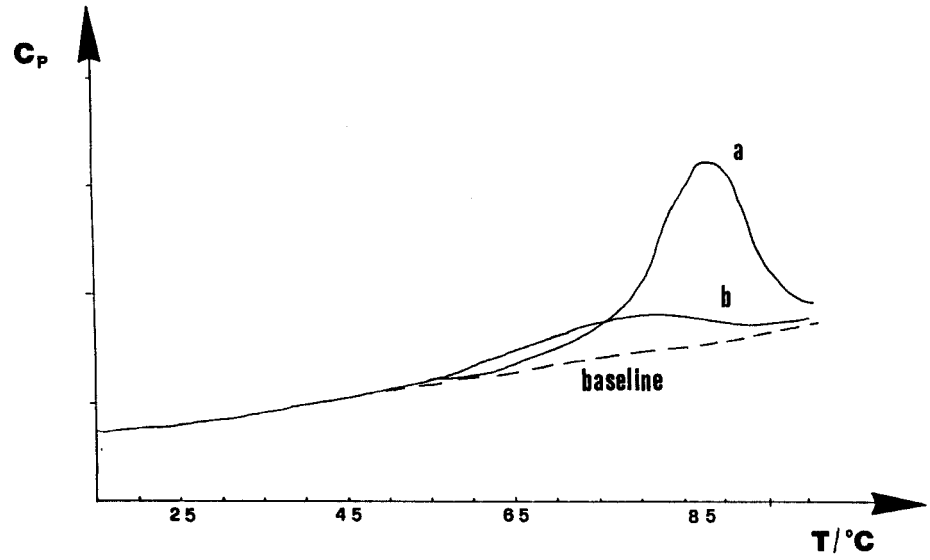


Figure 1. The heat capacity C_p of DNA as a function of temperature in 50 mg/ml PEG aqueous solution. Curves (*a*) and (*b*) refer to the first and second heating, respectively.

strands are broken and the two chains are able to separate. The temperature of the maximum is an indication of the stability of the system and the area of the band is a measure of the enthalpy involved in the melting process. We can see that both the maximum temperatures and the area of the peaks of the two curves are completely different. Figure 2 shows the typical DSC curves for the first and second heating of a sample in the range of PEG concentration from 130 to 170 mg/ml where the liquid-crystalline phase occurs. We note that the two curves are very similar for this sample.

Figure 3 shows the dependence of the maximum temperature (T_m) of the peak of the DSC thermograms on the PEG concentration. Between 0 and 90 mg/ml of PEG, T_m is practically constant at about 88°C, while from 110 to 170 mg/ml of PEG T_m increases slowly from 93·8 to 95°C. From 90 to 110 mg/ml, where the liquid-crystalline phase begins to form, a sharp jump in T_m is observed. These results might be related to the different thermal stability of two different initial phases of DNA molecules in the PEG containing solutions. The same figure shows the isotropic/cholesteric phase boundary as determined by polarized optical microscopy. In the same phase diagram are drawn the thermal paths relating to the heating and cooling cycles for samples above and below the critical PEG concentration. In the latter case (path A) only the DNA melting and partial renaturation occurs. When the more concentrated samples are heated (path B) two distinct phenomena happen:

(i) the DNA melts as the inter-base hydrogen bonds break and the two chains separate;
(ii) a phase change from the cholesteric to the isotropic phase occurs.

Figure 4 shows the renaturation percentages obtained from the calorimetric experiments for several samples at different PEG concentrations. It is evident that the behaviour of those samples in which the liquid-crystalline phase exists is different from those which are in the isotropic state. In particular, samples originally in the condensed liquid-crystalline state renature to about 80 per cent on cooling, whilst samples in the

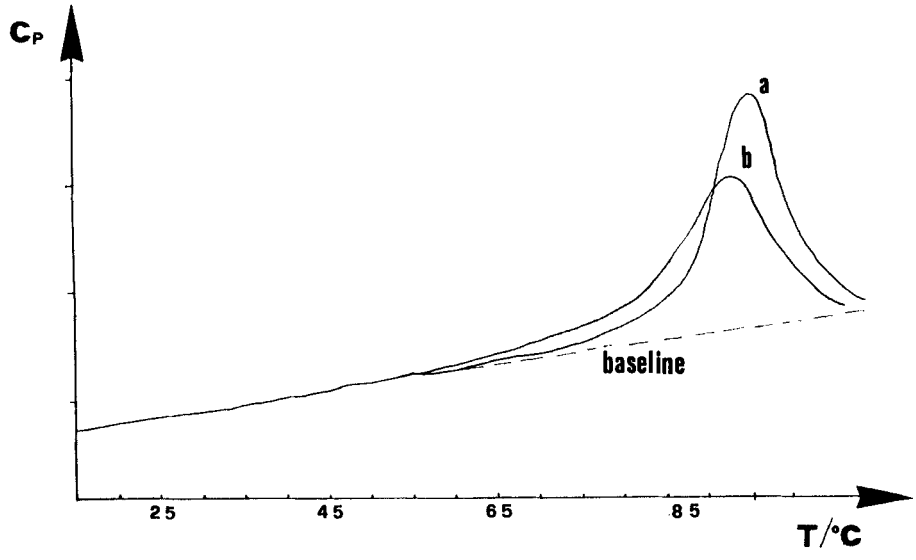


Figure 2. The heat capacity C_p of DNA as a function of temperature in 170 mg/ml PEG aqueous solution. Curves (*a*) and (*b*) refer to the first and second heating, respectively.

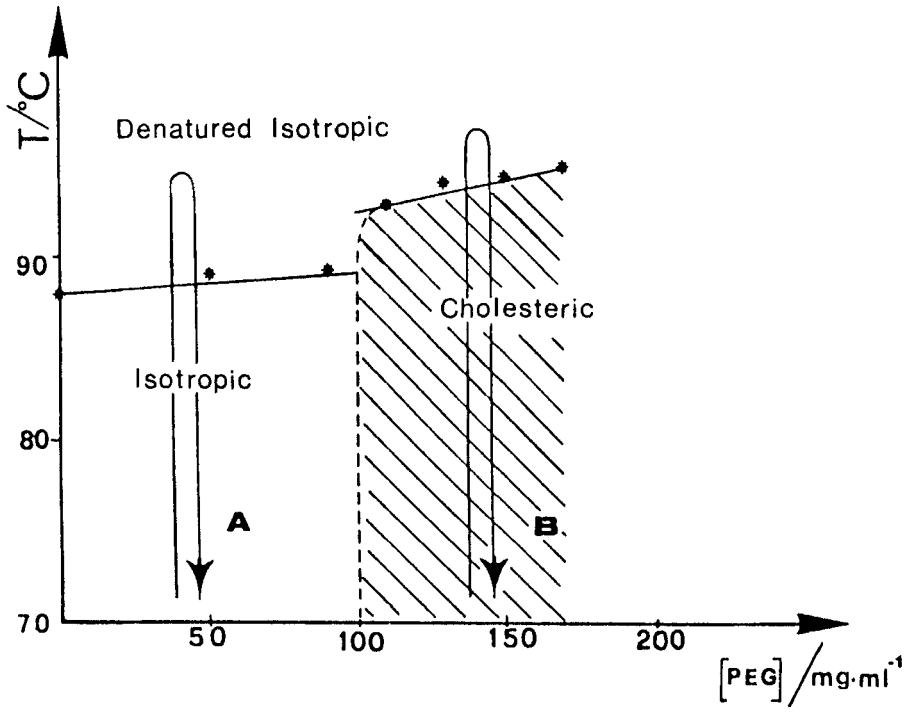


Figure 3. The temperature/composition phase diagram of sonicated DNA solution showing the isotropic/cholesteric phase boundary as determined by optical microscopy, and the temperatures T_m corresponding to chain melting inferred from calorimetric studies (*). (A) Heating/cooling path from the isotropic phase. (B) Heating/cooling path from the cholesteric phase.

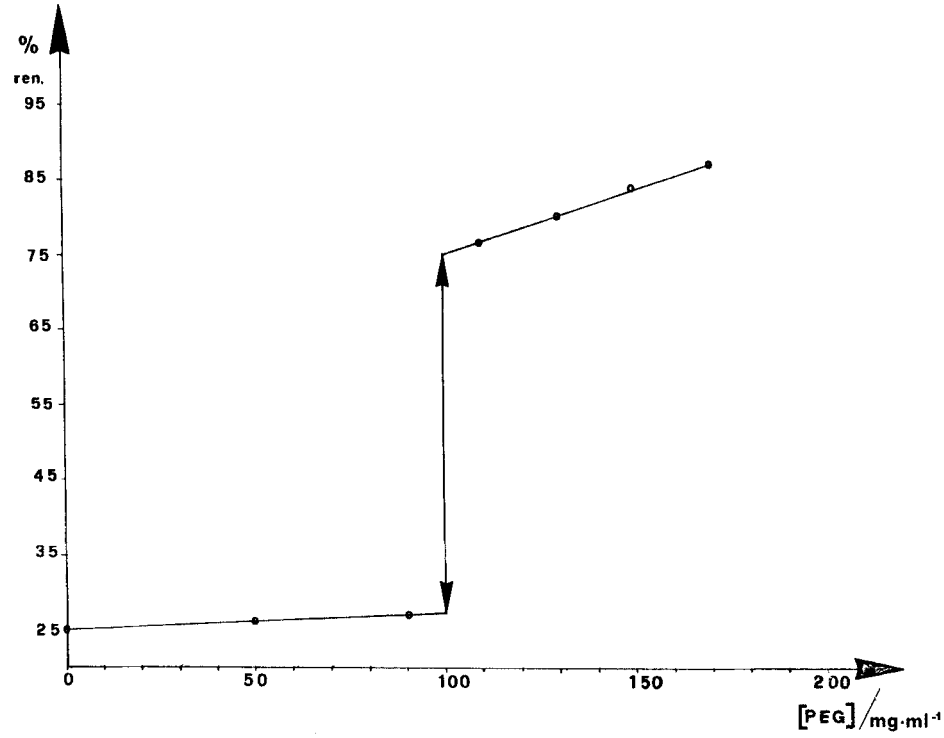
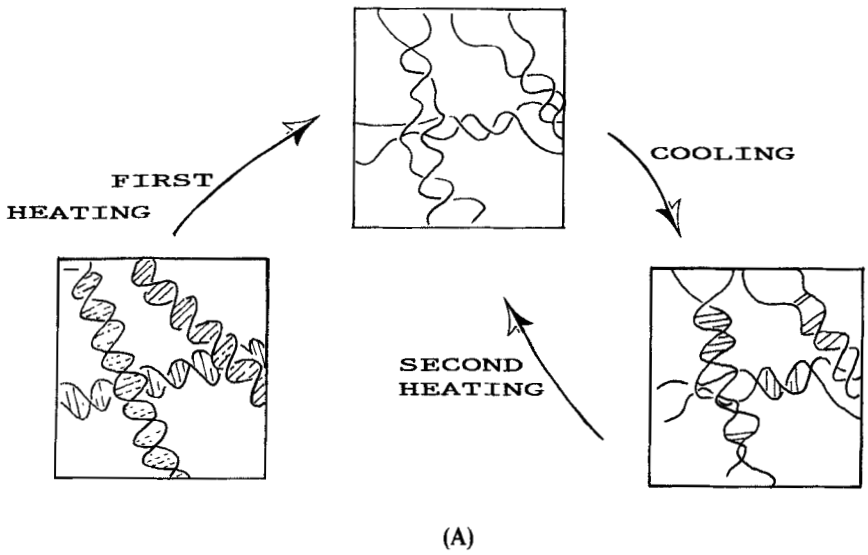
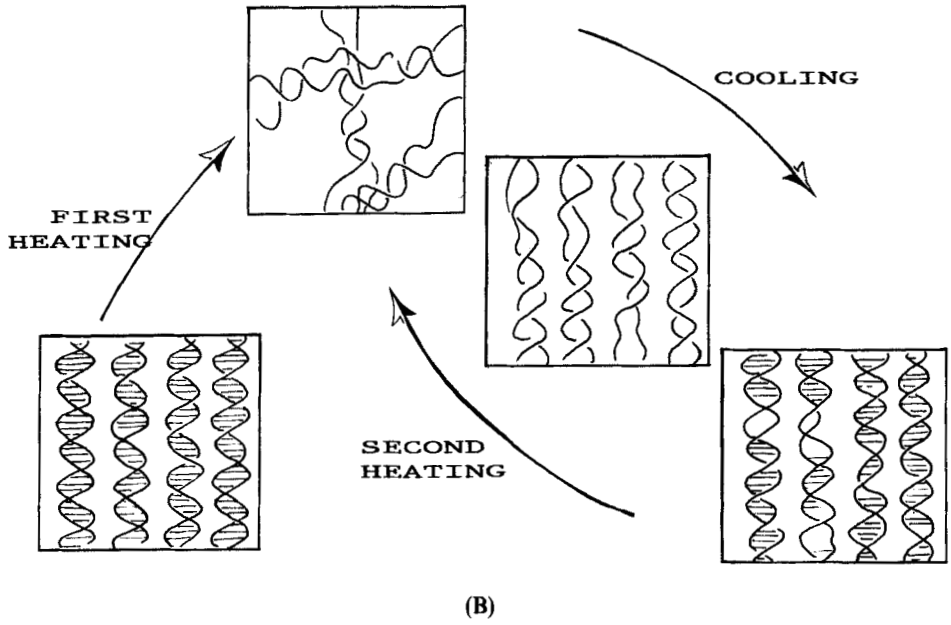


Figure 4. Renaturation percentage of DNA against PEG concentration in solution.



(A)



(B)

Figure 5. Diagrammatic representation of the two denaturation and renaturation processes corresponding to (A) and (B) thermal paths in figure 3. (A) For samples originally in the isotropic state, where the cooled sample is only 25 per cent renatured. (B) For samples originally in the cholesteric liquid-crystalline state where the reformation of the cholesteric state on cooling gives samples which are 80 per cent renatured.

initial isotropic state renature only to about 25 per cent. Figure 5 shows a diagrammatic representation of the two denaturation and renaturation processes related to thermal paths (A) and (B) of figure 3. Heating the initial isotropic solution (figure 5(A)) a denaturated (melted) isotropic solution of DNA is formed which, on cooling, only partially renatures (25 per cent). Reheating this renatured phase (second heating), the denatured isotropic solution is formed again. Heating the initial cholesteric phase (figure 5(B)) the same denaturated (melted) isotropic solution is formed. On cooling this last phase, a cholesteric state is formed, which guides the complementary DNA strands together, enabling a higher level of renaturation to occur (80 per cent).

In order to confirm the temperature at which the liquid crystal phase transforms to the isotropic phase, a set of polarized microscopy measurements of thin layers (about 20 μm) of the DNA liquid-crystalline phases was carried out at different temperatures. The results showed that the specific fingerprint texture (characteristic of the cholesteric phase) disappears in the range 92–98°C. This indicates that the transition from the cholesteric to the isotropic state takes place over the same temperature range as suggested by our calorimetric investigations considering the characteristic of the device used for heating. Moreover, it should be noted that after cooling, the sample again shows the cholesteric texture. These first results, obtained by a microcalorimetrical approach, demonstrate that the reversibility of the liquid crystal phase of DNA may have an appreciable biological significance and go some way towards explaining the resistance of DNA molecules to external destructive factors.

The naturally occurring DNA which we have investigated was extracted from salmon sperm and has a molecular mass of about $8{\cdot}0 \times 10^6$ D. This corresponds to a sequence of about $12{\cdot}3 \times 10^3$ paired bases and a polymer chain length of about $43{\cdot}1 \times 10^2$ nm. Material of this kind shows a tendency towards mesophase formation, but if it is fragmented into units of about $6{\cdot}0 \times 10^5$ D by sonication, it gives a phase which (with the addition of salt and the neutral hydrophilic polymer PEG) can readily form a well-defined cholesteric phase if sufficiently concentrated. Because of its helicoidal structure this phase exhibits a very pronounced circular dichroism as shown by a large number of CD spectra [7]. Equal volumes of sonicated nucleic acid aqueous salt solution (NaCl 0·3 M, DNA 50 μg/ml) and PEG solution (NaCl 0·3 M, PEG 0–170 mg/ml, molar mass 4000) were mixed in order to obtain a dispersed phase consisting of microscopic droplets of nucleic acid [8]. Sets of different preparations were made to determine the effects of changing PEG concentration. The stability of the preparations was also checked by recording their CD spectra at different times. The molecular weight of PEG and the NaCl concentration were chosen to allow direct comparison with our previous results [13]. Samples used in polarizing microscopy and microcalorimetry were prepared by centrifuging the microphase dispersion at 4°C for 1 hour at 4500 rpm. Calorimetric measurements were carried out with a micro differential scanning calorimeter SETARAM (France). About 0·7 ml of aqueous salt solution containing PEG and 1–2 mg of DNA phase was used in the sample vessel (with the same quantity of solution in the reference cell). DSC runs were carried out with a heating rate of 0·5°C/min in the 30–99°C range. The apparatus was previously calibrated for temperature and energy, using high purity naphthalene as the standard.

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