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Microcalorimetric measurements of thermal denaturation and renaturation processes of salmon sperm DNA in gel and liquid crystalline phases [1]

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Abstract

Thermally induced denaturation and subsequent renaturation of salmon sperm DNA in gel (G) and liquid crystalline (LC) phases have been studied by a microcalorimetric approach, using differential scanning calorimetry. An unusual very high renaturation percentage of the denatured LC-DNA and a greater thermodynamic stability of the LC phase were observed.

INTRODUCTION

It is commonly supposed that naturally occurring DNA are double-stranded. The forces that link the two strands are mainly due to the hydrogen bonds between the pair of bases, but the stacking energies and the external environment also play an important role. The two strands can be separated by physical or chemical methods. Thermal denaturation, in particular, breaks the hydrogen bonds and thus separates the strands [1–5].

This "melting" process may be partial or complete and the temperature at which it occurs depends on the base sequences. In complete melting, the two strands are free to move apart. On cooling, the hydrogen bonds tend to re-form, and renature the DNA to an extent that depends on the experimental conditions, such as temperature, annealing time, ionic strength, pH, etc. [6].

The enthalpy of denaturation can be considered as the sum of three contributions from the stacking, hydrogen bonds and medium interactions, though this separation is not without problems. The total enthalpy of denaturation–renaturation, however, can be obtained directly by micro-

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calorimetric measurements using a differential scanning calorimetry technique (DSC).

DNA in dilute aqueous solution can be "condensed" by addition of a neutral polymer, such as polyethyleneglycol (PEG), to form a suspension of liquid crystalline microphase regions [7–9]. This particular state of DNA has been chiefly studied by polarized light microscopy [10,11] and chiro-optical techniques, such as circular dichroism (CD) and linear dichroism (LD) [12–15], as well as by X-ray diffractometry [16,17]. The influence of different operating parameters has been assessed to find the best conditions to provide the DNA liquid crystalline phase [14]. Our microcalorimetric study of salmon sperm DNA has shown that its thermal behaviour is different in the isotropic gel (G) and liquid crystalline (LC) phases [18]. In particular, renaturation is more extended when DNA is denatured in its LC state rather than in its G state. This paper reports our first quantitative assessment of the denaturation and renaturation process in salmon sperm DNA in these two states. A very simple model explaining the high renaturation percentage of the denatured DNA in the LC phase is also proposed.

EXPERIMENTAL

Salmon sperm DNA, supplied by the Institute of Molecular Biology, Academy of Sciences of Moscow, was dispersed in water–salt solution (NaCl, 0.3 M) and fragmented by sonication. Its final average molecular weight, checked by electrophoresis, was about 6.0×10^5 g mol^{-1}. A PEG water–salt solution (170 mg ml^{-1}) was added to this phase to give an LC microphase. Because of its helical structure, the formation of this phase was checked by circular dichroism (CD). The LC-DNA phase used in our microcalorimetric measurements was obtained from this microphase by centrifugation. The calorimetric measurements were carried out with a MICRO-DSC differential scanning calorimeter (Setaram, France). About 0.7 ml of water–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 at a heating rate of 0.5°C min^{-1} from 30 to 99°C. The reproducibility showed that the experimental error was 10–15% as a consequence of the characteristics of the apparatus and the procedures for preparing the samples. The apparatus was previously calibrated for temperature and energy, using high-purity naphthalene as standard.

RESULTS AND DISCUSSION

Preliminary measurements of PEG–DNA solutions at different PEG percentages have shown that at a DNA concentration of 50 μg ml^{-1}, a threshold PEG concentration of 100 mg ml^{-1} is needed to establish the LC

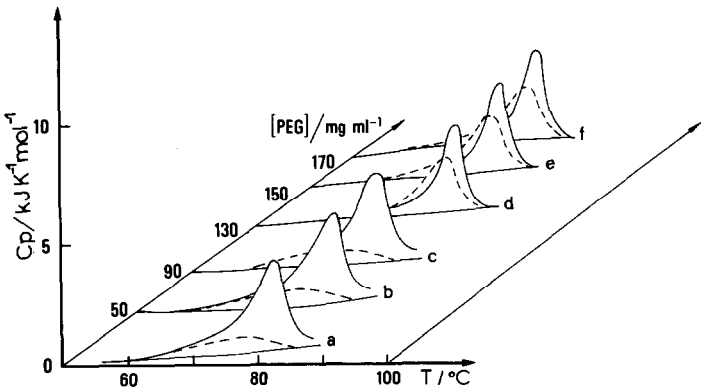

Fig. 1. DSC curves for the six samples at different PEG concentrations. Full line refers to the first heating and broken line to the second heating.

phase. Our starting solutions were samples at different PEG concentrations above and below the threshold value. Figure 1 shows the DSC curves for six samples with a PEG concentration from 0 and 170 mg ml^{-1}: curves (a), (b) and (c) refer to the G state; curves (d), (e) and (f) refer to the LC state. The full line refers to the first heating of the sample, the dotted line to the second heating after maintaining the sample at room temperature for 1 h. In each case, the curves represent the apparent specific heat (C_p) value plotted against the temperature in the range 30–99°C. Several thermodynamic parameters, such as melting temperature (T_m), and denaturation enthalpy ($\Delta_m H$), were obtained from this figure and by using eqns. (1) and (2) reported below.

Denaturation was regarded as a simple two-step process [19–21]

$$\Delta_m H = \int_{T_1}^{T_2} C_p \, dT \tag{1}$$

$$\Delta_m H_T = \Delta_m H_{T_m} + \int_{T_m}^{T} \Delta_m C_p \, dT \tag{2}$$

where C_p is the apparent specific heat during the transition, T_1 and T_2 are the initial and final temperatures of the transition and $\Delta_m C_p = C_{p(\text{denatured})} - C_{p(\text{non-denatured})}$. In addition, the free energy variation ($\Delta_m G$) and entropy variation ($\Delta_m S$) relative to the thermal denaturation were calculated using the standard thermodynamic equations. During the thermal process, the system was assumed to be not far from the thermodynamic equilibrium as a consequence of the small heating rate chosen.

The thermodynamic data for the two heatings are collected in parts (a) and (b) of Table 1 respectively. The results for the first denaturation show a different behaviour in the G and LC phases. The LC phase denatures at a higher temperature (5°C on average) than the G phase. The enthalpy of

TABLE 1

Thermodynamic parameters related to the thermal denaturation of salmon sperm DNA in salt solution [a]

(a) First heating

[PEG]	$\Delta_m H_{T_m}$	$\Delta_m S_{T_m}$	T_m	$\Delta_m G_{310}$	r
0	32.8	78.1	87.9	4.6	20
50	35.1	83.2	88.6	5.0	21
90	37.2	87.7	89.4	5.4	22
130	41.1	95.0	93.0	6.3	81
150	43.7	100.0	94.6	6.9	85
170	42.3	96.7	95.0	6.7	89

(b) Second heating

[PEG]	$\Delta_m H_{T_m}$	$\Delta_m S_{T_m}$	T_m	$\Delta_m G_{310}$
0	6.7	16.8	79.0	0.8
50	7.4	18.4	79.5	0.9
90	8.2	20.4	80.1	1.0
130	33.29	77.5	91.9	5.0
150	37.12	85.9	92.8	5.7
170	37.62	87.0	92.9	5.8

[a] Units: [PEG] in mg ml^{-1}; $\Delta_m H_{T_m}$ in kJ per nucleotide pair equivalent; $\Delta_m S_{T_m}$ in J per nucleotide pair equivalent K^{-1}; T_m in °C; $\Delta_m G_{310}$ in kJ per nucleotide pair equivalent; r in %.

this denaturation is higher than that for the G state by about 8 kJ per nucleotide pair equivalent. The $\Delta_m S$ values are less indicative, although the remarkable increase in denaturation of the LC phase is in any event a measure of the higher order of the LC-DNA. Lastly, the $\Delta_m G$ values show that at room temperature the LC phase is more stable — about 1.5 kJ per nucleotide pair equivalent — than the G phase. Quantitative evidence is thus offered of the higher stability of the LC phase. A remarkable difference between the two states was also noted for the second thermal denaturation. Renaturation evidently depends to a great extent on the nature of the phase. Only about 20% of denatured G-DNA renatures, whereas denatured LC-DNA renatures almost entirely (81–89%, depending on the PEG concentration).

The renaturation percentages were calculated from the expression

$$r\% = \Delta H_I 100/\Delta H_{II} \tag{3}$$

Because the macrochirality of the LC-DNA depends strictly on the experimental conditions and the inner structure of the DNA, we carried out two sets of experiments to see whether true renaturation recovery of the primitive structure, and reassociation of the two complementary single strands, really occurred, as opposed to recovery of the enthalpic effects.

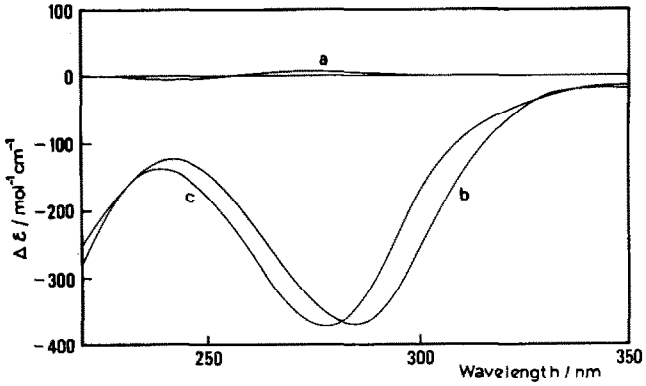

Fig. 2. Circular dichroism profiles of buffer solutions of LC-DNA microphases (curve a, "native" state; curve b, renatured state) and G-DNA (curve c).

A set of polarized microscopy measurements of thin layers (about 20 μm thick) of LC-DNA phases was carried out at different temperatures [18]. The results showed that the specific fingerprint texture characteristic of the cholesteric phase [22] disappears when the temperature reaches 95°C and appears again on cooling.

The CD spectra of the native and renatured forms of the LC-DNA at room temperature are reported in Fig. 2 with the CD curve of G-DNA. The similarity between the curves is evidence of macrochirality, and, hence, of true renaturation.

The trend of the renaturation percentage, reported in Fig. 3, is independent of the PEG concentration when its value changes below the critical threshold value. At higher PEG concentrations, when the DNA is an LC arrangement, the percentage increases linearly against PEG. These results

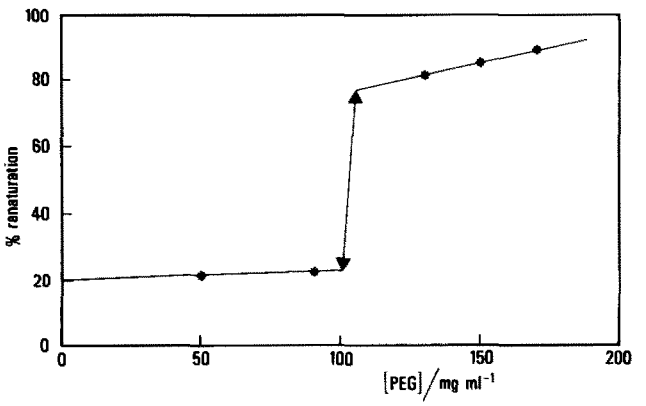

Fig. 3. Renaturation percentages as a function of the PEG concentration.

are consistent with the progressive decrease of the solution dielectric constant value [23] allowing contiguous DNA molecules to interact more strongly as the PEG concentration increases. This high interaction causes close packing of the biopolymeric molecules as shown by the X-ray pictures of different LC-DNA [24]. The high renaturation percentage of LC-DNA seems to be a consequence of phase maintenance between the two strands, even after denaturation. This behaviour can be tentatively explained by supposing restriction of the diffusional freedom degrees and/or possible "outliving" of "locks" between the two packed strands from which quasi-quantitative renaturation begins.

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