THE THERMAL TRANSITION OF 'PSI' DNA MONITORED BY CIRCULAR DICHROISM

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1. Introduction

The Watson-Crick model for nucleic acid secondary structure fails to provide a basis for understanding the extreme degree of compactness exhibited by DNA molecules in vivo. Purified high-molecular-weight native DNA in vitro adopts a 'stiff coil' conformation whose dimensions – even at high ionic strength – far exceed those of the biological structure (virus particle, bacterial nucleoid, eukaryotic chromosome) from which the DNA was originally isolated [1–3]. The recent discovery by Lerman [4] that above a critical ionic strength high concentrations of an inert macromolecule such as poly(ethylene oxide) (PEO) cause DNA to collapse into a rapidly sedimenting form strongly suggests that 'exclusion interaction' between DNA and other macromolecules plays a role in generating compact DNA conformations in vivo. Profound changes in the circular dichroism (CD) spectrum accompany this 'psi' or ψ (polymer- and salt-induced) transition [5,6] and we have accordingly employed CD to investigate the thermal stability of the ψ state of calf thymus DNA. Our results show that ψ-DNA possesses a fairly sharp thermal transition whose mid-point (T_m') – which lies far below the helix-coil transition temperature (T_m) – depends strongly upon PEO concentration, but only weakly on ionic strength. Above the transition region the CD spectrum resembles that of the ordinary B-form DNA – though with somewhat reduced intensity. This fact, combined with the reversibility of the transition, demonstrates – contrary to an earlier report [7] – that ψ-DNA melts to give B-DNA, not denatured DNA. The melting behavior which we report here is compatible with the identification of ψ-DNA as a cholesteric liquid crystal [8].

2. Materials and methods

PEO ('Carbowax 6000') obtained from Union Carbide Chemical Co. was used without further purification. All experiments were carried out in 0.015 M potassium phosphate buffer, pH 7.0. Standard 'PEO-salt solutions' were prepared by dissolving weighed amounts of PEO and KCl in buffer. Calf thymus DNA from Sigma Chemical Co. was dissolved in buffer (0.13 mg/ml) and sonicated for 30 sec with a Branson Sonifier to reduce viscosity [9]. For the preparation of ψ-DNA the stock solution was diluted with a standard 'PEO-salt solution' and buffer to yield a final DNA concentration of about 4.5×10^{-5} M (phosphate residues). We observed no turbidity in these solutions. Spectra were recorded no sooner than 30 min after mixing 'PEO-salt solution' with the stock DNA. ψ-DNA solutions were prepared fresh each day and 'PEO-salt solutions' were never used for more than two weeks. Stock DNA solution was stored frozen at $-20°$C. CD spectra were taken on a Cary 60 spectropolarimeter equipped with a 6002 CD attachment using a jacketed cell with a 1-cm light path. To obtain a melting curve the circulating thermostat bath was allowed to equilibrate for about 15 min at each desired temperature after which the CD spectrum was run. Repeated spectra confirmed that equilibrium had been attained. In the case of the cooling curve each successive point represents a CD spectrum taken

15 minutes after the previous point. We recorded 'fluorscat' spectra using standard procedures [10].

3. Results and discussion

Fig. 1 shows the effect of temperature on the CD spectrum of ψ-DNA. The initial large negative ellipticity band centered at 263–264 nm (curve a), the most distinguishing characteristic of the spectrum of the ψ state [5–7], weakens in intensity as temperature increases (curves b and c) and eventually becomes a positive band (curve d) which closely resembles the CD spectrum of B-DNA (curve e), though with reduced intensity. Plotting the ellipticity at a single wavelength (265 nm) versus temperature yields an S-shaped melting curve. Fig. 2a shows a series of such

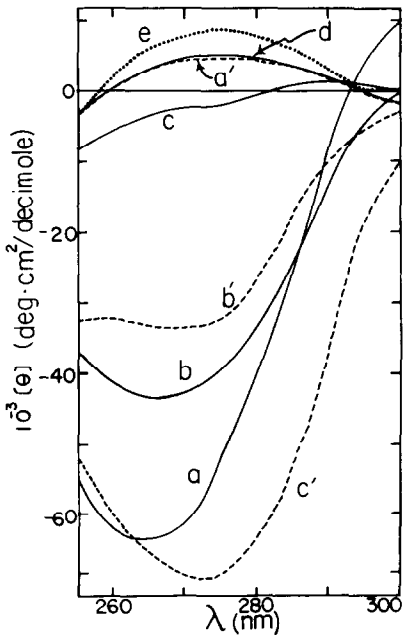

Fig. 1. Temperature-dependent circular dichroism spectra of calf thymus DNA in PEO-salt solution. Solid lines correspond to spectra taken successively at a) 31.5°C, b) 47.9°C, c) 50.2°C and d) 52.7°C. Dashed lines give spectra recorded by cooling the same solution to a') 50.2°C, b') 46.7°C and c') 35.5°C. Concentrations were: DNA, 4.35 × 10^{-5} M (nucleotide residues); PEO, 105.6 mg/ml; and KCl, 0.39 M. The dotted line (curve e) gives the spectrum of DNA (4.7 × 10^{-5} M) in buffer without added PEO or KCl at 59.3°C.

melting curves obtained at different PEO concentrations. The transition from a ψ-type CD spectrum to that closely resembling the CD spectrum of B-DNA always occurs over a fairly narrow temperature range (5–10°C) and appears to be quite symmetrical about the midpoint (which we have designated T_m' by analogy to T_m, the midpoint of the thermal transition of DNA secondary structure). T_m' depends strikingly on PEO concentration (fig. 2b), increasing by almost 20°C when [PEO] increases by 20%. In contrast, a 50% increase in ionic strength effects only a 5–6°C increase in T_m' (fig. 2c).

If a DNA-PEO-salt solution at elevated temperature (where the ψ structure has entirely melted out) is slowly cooled the CD spectrum returns to that characteristic of the ψ state (fig. 1, curves a', b', and c'). After a complete cycle of heating and cooling the principal band of the final spectrum actually has a somewhat greater negative amplitude than that of the original ψ-DNA and the wavelength of maximum ellipticity has shifted about 10 nm to the red (compare curves a and c' in fig. 1). The cooling curve (fig. 2d) does not exactly follow the melting curve, but displays hysteresis over most of the transition region followed by a crossover at lower temperatures corresponding to the more intense negative ellipticity mentioned above.

The rather sharp transitions which we have observed in the melting of ψ-DNA strongly suggest an ordered structure in agreement with the conclusion of Jordan et al. [5] based on kinetic data. One would expect a mere random condensate of double-stranded DNA, either unimolecular or aggregated, to unfold over a broad temperature range (all the more so considering the molecular heterogeneity of calf thymus DNA). Other results from this laboratory indicate that under suitable conditions double- and triple-stranded polyribonucleotides also adopt ψ-like conformations whereas single-stranded ones do not [11]. Denatured DNA likewise remains unaffected by high concentrations of PEO and salt [7]. Taken together these findings support the view that helical nucleic acids have available a reasonably well-defined compact ordered arrangement which they adopt in the presence of a suitable perturbant such as PEO.

Maniatis et al. [12] have employed X-ray scattering to characterize the structure of ψ-DNA. By comparison of their data with computed scattering

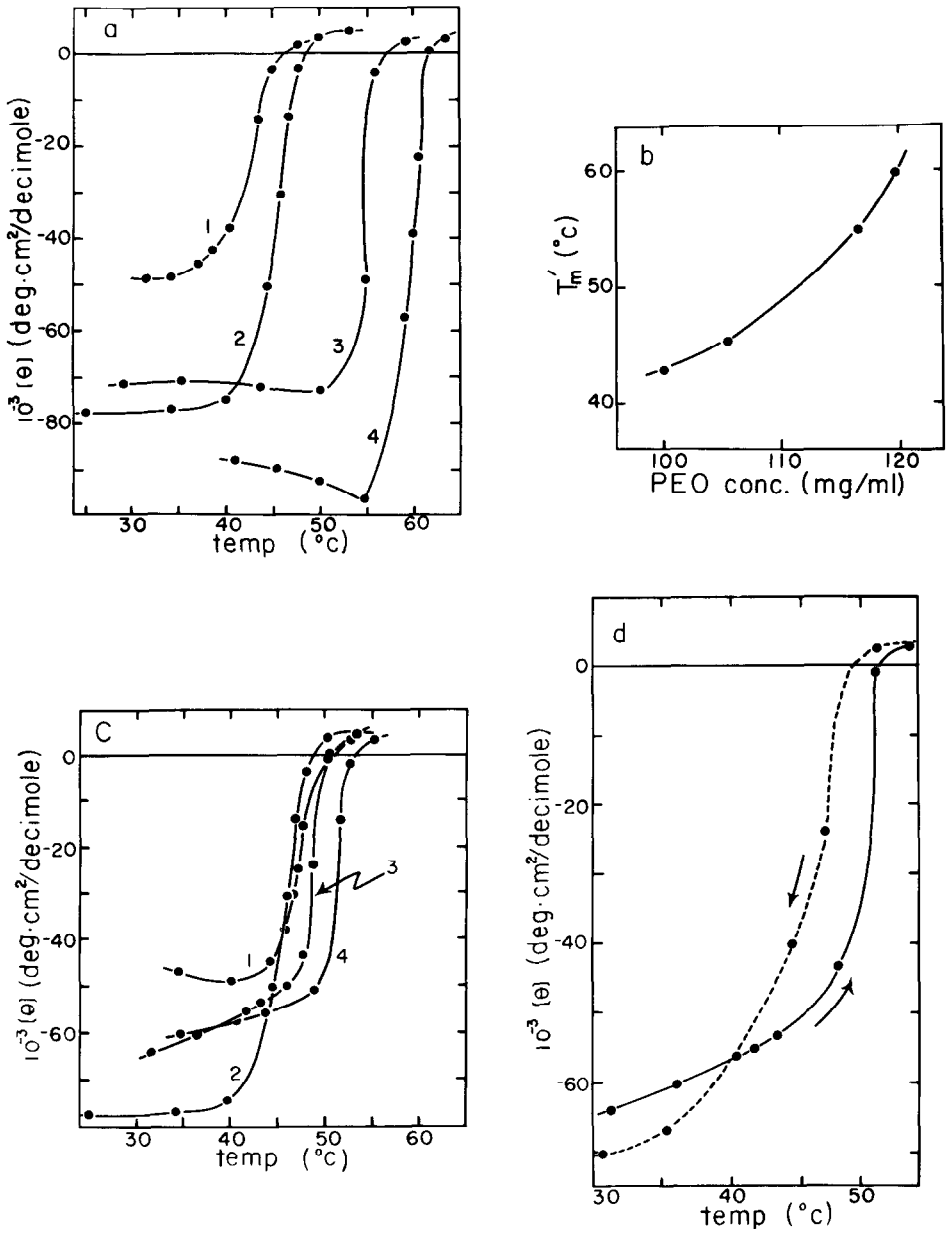

Fig. 2. Thermal transition curves for ψ-DNA. a) Ellipticity at 265 nm as a function of temperature for DNA in PEO-salt solutions at constant salt concentration ([KCl] = 0.35 M) with PEO concentrations of 1) 100 mg/ml, 2) 105.6 mg/ml, 3) 116.7 mg/ml and 4) 120 mg/ml. b) Dependence of the mid-point (T_m') of the thermal transition on PEO concentration (– –) data from the curves given in a). c) Ellipticity at 265 nm as a function of temperature for DNA in PEO-salt solutions at constant PEO concentration (106 mg/ml) with KCl concentrations of 1) 0.30 M, 2) 0.34 M, 3) 0.39 M and 4) 0.44 M. d) Reversibility of the thermal transition. Ellipticity at 265 nm as a function of temperature for heating (——) and cooling (– – –). The composition of the solution was the same as in fig. 1.

curves for the A-, B- and C-forms of DNA they conclude that ψ-DNA most nearly resembles the B-form, although some slight differences exist between the computed and experimental curves. An additional scattering maximum corresponding to intermolecular spacings of 25–40 Å (depending upon PEO concentration) occurs, but no peaks are present which could correspond to a supercoiled tertiary structure.

Whatever the exact geometry of the ψ structure our data shows that it does not involve complete disruption of the double helix since the ψ structure obviously reforms upon cooling. Had the DNA in 'PEO-salt solution' undergone denaturation of secondary structure upon heating as suggested by Evdokimov et al. [7] strand separation would have occurred, precluding reformation of the ψ state. Destabilization of the ψ state by dilution to subcritical PEO concentrations likewise regenerates B-form, not denatured DNA [4,7]. Neither these results, however, nor those of Maniatis et al. [12] preclude some localized helix denaturation in the ψ state.

The hysteresis which we observe in cooling curves probably reflects the necessity for nucleation of the ψ state [5]. The more intense negative ellipticity which results after a cycle of heating and cooling (fig. 1, curve c′) presumably comes about because of annealing which produces a more highly ordered final state. Sponar and Fric [13] have observed a similar effect with certain f1 histone-DNA complexes.

Histone f1 [13,14], poly-L-lysine [15], and protonated poly-L-histidine [16], form complexes with DNA which exhibit CD spectra strikingly similar to that of ψ-DNA. Haynes et al. [15] have suggested that such complexes constitute a liquid crystalline state of DNA comparable to the cholesteric liquid crystal phase of α-helical poly-γ-benzyl-L-glutamate (PBLG) [17,18]. Such liquid crystals display relatively enormous circular dichroism (and optical rotation) particularly at wavelengths comparable to the pitch of the cholesteric helix. Because the enhanced CD of histone–DNA complexes involves only minor shifts in the positions of the band maxima relative to free DNA, and therefore almost certainly does not arise from the pitch band of a cholesteric helix, Adler and Fasman [14] and Sponar and Fric [13] have disputed the liquid crystal explanation for this effect. Recent work in the area of liquid-crystal-induced circular dichroism ('LCICD'), however, indicates that the rotational strengths of the electronic transitions of PBLG *itself* undergo 10^2-10^3-fold enhancement upon formation of the lyotropic mesophase [19]. A number of other systems exhibit similar behavior and Holzwarth and Holzwarth [20] have developed a comprehensive theory to explain this effect. We believe that the greatly enhanced CD spectrum of ψ-DNA, as well as that of polycation–DNA complexes, primarily reflects the LCICD phenomenon and that the thermal transition which we have monitored corresponds to the melting-out of a lyotropic liquid crystalline phase. Brunner and Maestre [21] have recently reported intense, non-conservative ψ-like CD spectra from DNA films at relative humidities < 92% which they likewise attribute to liquid crystal effects.

Since the present study does not attempt to analyze in detail the CD spectrum of ψ-DNA, but rather employs CD to monitor the thermal transition, we have confined our presentation (fig. 1) to a relatively narrow spectral range. Various features of the spectra such as the presence of long-wavelength 'tails' in non-absorptive regions, which is indicative in part of artifactual scattering, demonstrate that a detailed analysis will not be simple.

To the suggestion that the CD spectrum of ψ-DNA represents a mere artifact caused either by scattering or some other effect of aggregation, however, we can reply that although some scatter-distortion is present, by comparison with other systems [22] it comprises no more than a small fraction of the total CD changes observed. We find that CD spectra of ψ-DNA samples taken at 27°C both in a standard cuvette and in a fluorscat cell, which largely corrects for scattering artifacts [10], show only minor differences, primarily a diminution of the long-wavelength 'tail' above 300 nm and a slight enhancement and 1–2 nm blue shift of the 263–264 nm band. As for aggregation, it undoubtedly occurs at the DNA concentrations which we have used [4], but aggregation by itself does not generate CD enhancement of an order of magnitude or more. Rather, the observation of *greatly* enhanced CD in a system known to contain aggregates implies that they are *ordered* in some way which is the point of significance in the first place [23,24]. Appreciable enhancement of the spectra by Mie scattering from

aggregates [25] can, of course, be excluded on the basis of the fluorscat result. Absorption flattening artifacts, which are *not* correctable by the fluorscat technique, would be expected to enhance the CD only slightly and at wavelengths away from the absorption maxima [25].

We have no explanation for the strong dependence of T_m' on PEO concentration, although it would seem to favor the exclusion interaction theory of the ψ transition [4] as opposed to the theory that the ψ state results from dehydration of the double helix [6]. The recent viscosimetric study of Akimenko et al. [26] also adds weight to the exclusion interaction argument. In this regard it is of interest to compare our results with those of Laurent et al. [27] who have demonstrated an exclusion interaction effect of PEO (and dextran) on the T_m for DNA *secondary* structure at low ionic strength.

We cannot at present ascribe any concrete biological significance to ψ-DNA as neither whole bacteriophage [22], nor chromatin [28], nor intact rat thymus nuclei [29] exhibit CD spectra in the DNA region corresponding exactly to that of ψ-DNA. Nevertheless, the ψ-form offers a reversibly attainable state of great compactness available to bihelical (and trihelical [11]) nucleic acids and it would seem unwise to dismiss it prematurely as irrelevant to their in vivo condition. At the very least one might ask whether its CD spectrum should not be included in the basis set (along with A-, B-, and C-form spectra) when attempting to interpret observed CD spectra of biological materials as linear combinations of standard spectra [30]. (The CD difference spectrum between intact and disrupted T2 bacteriophage [22] which strikingly resembles the ψ-DNA spectrum provides a case in point.) Finally we note that since the ψ transition can be effected quite easily in vitro the absence of a significant amount of ψ-DNA in vivo may imply that some factor(s) actively inhibits this transition. Fasman et al. [14,31] have observed apparently antagonistic effects of different histones on DNA conformation which are very suggestive in this regard.

Work in progress in this laboratory will characterize the thermal transition of ψ-DNA with regard to size and composition of the DNA sample.

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