Conformational transitions of poly(dA-dC)-poly(dG-dT) induced by high salt or in ethanolic solution

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Received 16 November 1981; Accepted 4 January 1982

ABSTRACT: Poly(dA-dC)-poly(dG-dT) was studied by circular dichroism in the presence of high CsCl concentrations and in ethanolic solutions. This alternating purine-pyrimidine duplex may undergo two conformational transitions from a B-type to a novel structure and subsequently into an A-form. Cs⁺ ions or increasing ethanol concentrations induced a change of the B-type CD spectrum and an inversion of the long wavelength CD band. Lowering the temperature below 0°C or addition of small amounts of Ca²⁺ ions were particularly potent in producing a large negative CD band. A modified B-type structure or a conversion into a left-handed Z-form is considered for this conformational transition.

The variability and dynamics of the DNA conformation is dependent on the nucleotide sequence (1-5). Discovery of left-handed Z-DNA in crystals of alternating d(G-C)ₙ sequences (1-3) has recently focused attention on sequence controlled conformational transitions of DNA between the right-handed B- and A-forms and Z-type or B-type left-handed structures (4-6). Poly(dG-dC)·poly(dG-dC) may assume a left-handed Z-helix in fibres (6,8) and in solution (9) under conditions of reduced water activity. Drastic CD changes of this alternating duplex had first indicated a left-right conversion in solution (9) at high salt concentrations. ³¹P-NMR studies (10) presented support that the high salt form of poly(dG-dC)·poly(dG-dC) may be a Z-DNA. Formation of Z-DNA is strongly favoured by modification of deoxyguanosine residues (11-13) or deoxycytidine residues (14). Poly(dG-₅dC)·poly(dG-₅dC) undergoes a B to Z transition close to physiological ionic strength conditions (14). X-ray fibre diffraction data (6,8) showed that other alternating purine-pyrimidine sequences, like poly(dA-dC)·poly(dG-dT) may adopt the left handed Z-helix. For poly(dA-dT)·poly(dA-dT) which is believed to exist mainly in the B-form (6), changes in its geometry have been suggested (15) and were observed in solution at high CsF concentrations (16).

In the present paper, first results by circular dichroism (CD) on conformational transitions of poly(dA-dC)·poly(dG-dT) induced by high salt or...
ethanol and/or low temperature are reported.

MATERIAL AND METHODS

Poly(dC-dC)-poly(dG-dC), poly(dA-dT)-poly(dA-dT) and poly(dA-dC)
poly(dG-dT) were purchased from Boehringer Mannheim; extinction coefficients
used were $\varepsilon_{254} = 8400$, $\varepsilon_{260} = 6800$ and $\varepsilon_{258} = 6500 \text{ M}^{-1}\text{cm}^{-1}$, respectively. The oli-
gomers p(dC-dA)$_4$ and p(dT-dG)$_4$ were synthesized by Drs. Birsch-Hirschfeld and
Weiss (17). Solutions were prepared by diluting appropriate amounts of stock
solutions of the polymers into the required salt or ethanolic solutions. Poly-
nucleotide concentrations were 0.02 mM to 0.05 mM, if not otherwise stated.

CD spectra were recorded on a Jobin-Yvon Mark III dichrograph using
1 cm or 0.5 cm cells. Low temperature measurements were made in a thermo-
static cuvette (1 cm) branched to a cryostat; temperature measurements were
made in the solution by a thermocouple branched to a digital temperature read-
out device. UV absorbance was measured on a Zeiss DMR 10 spectrophotometer.

RESULTS

1) B to Z conformational change? CD spectra of poly(dA-dC)-poly(dG-dT) were
measured under a variety of environmental conditions of decreased water activ-
ity: in high salt media of NaCl, NaClO$_4$, CsCl, CsF, CaCl$_2$, MnCl$_2$ and in
ethanolic solutions at various temperatures.

Most effective CD changes occurred in the presence of Cs$^+$ ions. In
fig. 1 the CD spectra of poly(dA-dC)-poly(dG-dT) in different Cs$^+$ salts are
compared with those of poly(dG-dC)-poly(dG-dC) and poly(dA-dT)-poly(dA-dT).
Addition of 6M CsCl or 3.7 M CsF caused a decrease of the positive CD band
around 280 nm and resulted in an inversion of the B-type spectrum (in 0.1 M
NaCl) into one with a negative long wavelength band. This negative CD band
at 278 nm was more intense in ethanolic solution in presence of 1.5 mM CaCl
and 0.02 mM Ca$^{++}$ (fig. 1a). Lowering of the first positive CD band of
poly(dA-dC) poly(dG-dT) and inversion into a negative one was also observed
in 5 M NaCl or in methanolic solution, but the decrease in intensity was less
pronounced (results not shown). In addition, we compare in fig. 1 the CD
spectra of poly(dG-dC)-poly(dG-dC) and poly(dA-dT)-poly(dA-dT) in 60% ethanol
in the presence of Ca$^+$. These spectra correspond to a strongly altered CD
with negative bands at 290 nm as already known for poly(dG-dC) poly(dG-dC)
(7,9,16) and at 278 nm for poly(dA-dT)-poly(dA-dT) (18,19) in high salt media
or in ethanolic solutions.

The intensity of the negative band at 278 nm of poly(dA-dC)-poly(dG-dT)
Figure 1: CD spectra of poly(dA-dC)-poly(dG-dT), poly(dG-dC)-poly(dG-dC) and poly(dA-dT)-poly(dA-dT) at different solution conditions.

in 60% or 70% ethanol is strongly influenced by the presence of trace amounts of Ca\(^{2+}\) (fig. 2). Titration of the duplex in 60% ethanol in the presence of CaCl\(_2\) with CaCl\(_2\) increased the CD signal at 278 nm from $\Delta \varepsilon \sim -5$ to $\Delta \varepsilon \sim -12$ (fig. 2) at a final concentration of 0.011 mM Ca\(^{2+}\). Presence of 0.05 mM Ca\(^{2+}\) caused the appearance of a negative CD band of poly(dA-dC)-poly(dG-dT) even in 80% ethanolic solution, where a transition to the A-type form occurred when Ca\(^{2+}\) ions were removed by EDTA (see results below).

Addition of Ca\(^{2+}\) ions to a solution of poly(dA-dC)-poly(dG-dT) in the absence of Ca\(^{+}\) ions, did not give rise to an inversion of the Cotton effects however (fig. 3a). Instead, a general decrease of the CD spectrum over the entire wavelength range was observed, indicative of a partial denaturation of the duplex (20). On the other hand, Mn\(^{2+}\) ions at high concentrations induced an inversion of the Cotton effect at 278 nm (fig. 3b). No significant effects were observed with MgCl\(_2\) up to molar concentrations.

The dependence of the CD changes in poly(dA-dC)-poly(dG-dT) on the ethanol concentration is demonstrated in fig. 4. There is a gradual change of the positive CD band and inversion into a negative one between 0% and 40% ethanol indicating a conversion of the B-type conformation to another form. Between 40% and 60% ethanol this negative band becomes more intense and below the isodichroic point at 251 nm, CD changes towards positive values (fig. 4a).
Figure 2: CD spectra of poly(dA-dC)·poly(dG-dT) in 60% ethanol as a function of increasing CaCl₂ concentration. (a) curve 1: 1.5 mM CsCl, 0.002 mM CaCl₂, 0.005 mM EDTA, curve 2: 1 plus 0.009 mM CaCl₂; curve 3: 2 plus 0.1 mM EDTA. (b): titration of curve 1 with CaCl₂.

This last transition between 40% and 60% ethanol exhibits cooperativity (fig. 4b).

Fig. 5 displays the CD spectra of poly(dA-dC)·poly(dG-dT) in 6 M CsCl as a function of temperature. The negative band at 278 nm strongly increased on lowering the temperature to ~8.5 C. Again, below the isodichroic point at 254 nm, the CD changed towards positive values resulting in an enhancement of the positive CD band at 220 nm (fig. 5a). This effect paralleled the spectral changes found on increasing the ethanol concentration (fig. 4a) or on addition of Ca²⁺ ions (fig. 2a). The negative CD band showed a gradual increase by decreasing the temperature to minus degrees (fig. 5b). Presence of Ca²⁺ ions had a stabilizing effect on the conformation characterized by a large negative CD signal. The CD of poly(dA-dC)·poly(dG-dT) in 6 M CsCl at ~8.5 C is similar to that in 60% ethanol at 0 C in the presence of 1.5 mM CsCl plus 0.002 mM Ca²⁺ (fig. 5, a and b). The transition indicated by the appearance of the negative long-wavelength CD band gives rise to two alternative suggestions - a modified B form or a Z-type conformation (see discussion).
Figure 3: CD spectra of poly(dA-dC)-poly(dG-dT) as a function of Ca$^{2+}$ concentration (a) and Mn$^{2+}$ concentration (b). All solutions were in Tris buffer 40 mM, KCl 0.1 mM.

Figure 4: (a) CD spectra of poly(dA-dC)-poly(dG-dT) in 1.5 mM CsCl, 0.002 0.002 mM CaCl$_2$, 0.03 mM EDTA as a function of ethanol concentration. (b) Titration at 278 nm.
Figure 5: Variation of CD spectra of poly(dA-dC)-poly(dG-dT) as a function of temperature; (a) 6 M CsCl, 10^{-3} M EDTA; the CD in 60% ethanol 1.5 mM CsCl, 0.002 mM CaCl₂ at 0°C is shown for comparison; (b) plots of CD at 278 nm versus increasing temperature: curve 1, 6 M CsCl; curve 2, 0% ethanol, 1.5 mM CsCl, 0.01 mM EDTA.

2) Transition to A-type conformation: Poly(dA-dC)-poly(dG-dT) undergoes a B to A conformational change in solution of 60% to 80% ethanol at mM NaCl (20). It was of interest to study CD changes of the modified B conformation of the duplex polymer at higher ethanol content in the presence of Cs⁺ ions. Fig. 6 shows the variation of the CD spectrum of poly(dA-dC)-poly(dG-dT) observed on decreasing the ethanol concentration from 80% to 60% (V:V) in 1.5 mM CsCl plus 0.05 mM EDTA. The transition between the putative Z conformation and the A-type conformation is strongly cooperative as judged by the CD at 278 nm (fig. 6b). The A-form is indicated by the non-conservative spectrum and the large positive CD band around 260 nm which agrees with previous solution data of this duplex polymer (20) and of natural DNA's (21-23). No intermediate B-type spectrum was observed in the course of this transition. Different from the behaviour of the modified B structure in 60% ethanolic solution, the A-type spectrum of poly(dA-dC)-poly(dG-dT)(fig. 6, 80% ethanol) was unaffected by the exposure to low temperature (-12°C).

3) CD of the duplex octamer: As a comparative study, the CD spectra of an equimolar structure of the octamers (dA-dC)₄ and (dG-dT)₄ were recorded in 6 M CsCl at various temperatures (fig. 7). In high CsCl medium the octamers formed a duplex. Its CD spectrum at 25°C showed large alterations compared
Figure 6: Transition to A conformation of poly(dA-dC)·poly(dG-dT) induced by 80% ethanol at 1.5 mM CsCl, 0.005 mM CaCl$_2$ plus 0.01 mM EDTA.

Figure 7: (a) CD spectra of the duplex octamer (dA-dC)$_4$·(dG-dT)$_4$ in 4 M NaClO$_4$ at 20°C, in 6 M CsCl as a function of temperature. (b) CD spectra of poly(dA-dC)·poly(dG-dT) in 0.1 M NaCl and in ethanol solution 3 mM NaCl.
with that in 4 M NaClO₄ (for comparison the CD of the octamer duplex in 0.1 M NaCl showed similar signals at the same wavelength maxima - not shown). In 6 M CsCl lowering of the temperature caused a significant decrease and large shift of the positive CD band towards longer wavelengths as well as a change of the negative CD band at 245 nm. The low-temperature spectrum of the oligomer (-11 C) closely resembled the intermediate CD spectrum of the duplex polymer when it underwent a transition from the B-type to the putative Z-type structure in ethanolic solution (fig. 7, e.g. at 50% ethanol). This similarity supports the occurrence of a conformational change as suggested for the duplex polymer.

DISCUSSION

The data presented here show that poly(dA-dC)·poly(dG-dT) undergoes two conformational transitions: one from B to another modified B-form or to a Z-type conformation and a second one from this latter structure to an A-type conformation induced by decreasing water activity in solution. A third direct transition from B- to A-type structure had been reported previously (20). At high CsCl salt concentration or in ethanolic solutions, the positive CD band of the B-type spectrum is lowered and subsequently inverted into a negative long-wavelength CD band (figs. 1 to 5). Cs⁺ ions are highly effective in favouring these conformational changes. Ca²⁺ ions are most potent in causing CD changes from B-type spectrum to a novel spectrum characterized by an intense negative CD band (fig. 2). The conformational change of the B-type structure induced by increasing ethanol content shows a gradual transition in going from 0% to 60% while 20% further increase of the ethanol concentration exhibits cooperativity (fig. 4).

Previously, intense negative CD bands of DNA's in poly(ethylene glycol) were ascribed to Ψ-type structures (24-27) formed by condensation of DNA into a compact form as evidenced by light-scattering (26), electron microscopy (27) or by large enhancement of the sedimentation coefficient (24). The Ψ-structure of T4 DNA showed S values > 200 (24) and suggested that DNA is collapsed into compact particles.

A Ψ-type structure cannot account for an interpretation of our present data: Contrary to Ψ-DNA, poly(dA-dC)·poly(dG-dT) shows no significant light scattering in 60% ethanol solution (A₂₅₈ = 0.63, A₃₄₀ = 0; in 6 M CsCl A₂₅₈ = 0.63, A₃₄₀ = 0.03). The CD spectra of poly(dA-dC)·poly(dG-dT) are strikingly different from that of Ψ-DNA in the short-wavelength range: the compact Ψ-DNA structure exhibits an extremely large negative CD over the en-
tire wavelength region (25-27) and shows no isodichroic point. On the contrary, poly(dA-dC)-poly(dG-dT) shows an isodichroic point around 252 nm and a CD change towards positive values below this wavelength (fig. 2 to 4). Furthermore, the sedimentation coefficient of the duplex polymer in 60% ethanol had a value of $S_{20,w} = 27.8 \pm 0.3$ (corrected) and of $S_{20,w} = 11.9 \pm 0.3$ (corrected) in the presence of 1 mM EDTA, compared with $S_{20,w} = 8.3$ in 0.1 M NaCl without ethanol. These values do not account for any compactixation of the polymer like V-DNA.

Our data (fig. 1 to 5) reflect thus primarily conformational changes in poly(dA-dC)-poly(dG-dT). By analogy with poly(dG-dC)-poly(dG-dC) (9-12), the inversion of the positive CD band into an intense negative long-wavelength CD band of poly(dA-dC)-poly(dG-dT) (fig. 1) could indicate a B to Z transition. A left-handed polynucleotide helix found in fibers of this duplex (8) would support this interpretation. Such a B to Z conversion is, however, not entirely conclusive from the present CD data. An alternative explanation could be a change of the geometry within the B-type family as regard to the sugar-phosphate conformation in alternating purine-pyrimidine nucleotides in the polymer. In this case poly(dA-dC)-poly(dG-dT) would behave similar to poly(dA-dT)-poly(dA-dT) (18,19).

The nature of the cation (or combination of cations) and water coordination apparently play an important role in the induction of the B → Z-like transitions (7,9,12,14,16). While Ca$^{2+}$ alone only decreases the intensity of the CD spectrum of poly(dA-dC)-poly(dG-dT) (fig. 3,a), it is highly efficient in inducing a B → "Z" transition in combination with 1.5 mM CsCl (fig. 2). Also, a given cation may have very different effects on different systems. It was shown (28) that Tb$^{3+}$ ions are very efficient at $10^{-5}$ M to induce the Z form in poly(dG-dC)-poly(dG-dC), in poly(dA-dC)-poly(dG-dT) they cause a decrease of the CD spectrum similar to that in fig. 3,a. Similarly, differential effects of various salts have been observed for the B → Z transition of poly-(dG-$m^5$dC).poly(dG-$m^5$dC) (14).

The fact that these transitions to Z-like structures can be reproduced in ethanol (12) or trifluoroethanol (7) for poly(dG-dC)-poly(dG-dC) and poly(dA-dC)-poly(dG-dT) (fig. 4) clearly shows that these effects are not accountable by polyelectrolyte theory (29), but are related to the water activity (30). The recent localization of water molecules in a dodecamer DNA helix (5) between base pairs demonstrated a higher degree of ordered hydration between A-T base pairs than between G-C pairs. This difference in hydration was suggested to be responsible for the ease of conformational transitions.
of G-C rich regions in DNA (5). Further decrease of water activity transforms the putative Z conformation of poly(dA-dC)•poly(dG-dC) into the A form (fig. 4).

The ability of poly(dA-dC)•poly(dG-dT) to undergo transitions between three different classes of conformations in solution corroborates recent findings on poly(dG-dC)•poly(dG-dC) in the presence of trifluoroethanol (7). If a transition from the right-handed B helix to the left-handed Z-type form is present as it was found in fibres (8) and for poly(dG-dC)•poly(dG-dC) in solution (7,9-12), the purine-pyrimidine dinucleotide may have a 2'-endo pucker at the pyrimidine and a 3'-endo or 1'-exo pucker at the purine site. Insertion of A-T pairs between C-G pairs introduces alterations in the duplex geometry compared with poly(dG-dC)•poly(dG-dC) which can explain deviations in the inverted CD spectra of the two duplex polymers (fig. 1). The alternating purine-pyrimidine structure has been regarded as essential for a possible B → Z transition (1,4,8). Whether the structure of poly(dA-dC)•poly(dG-dT) is a left-handed Z-compatible sequence requires, however, further investigation. Our findings expand the conformational properties of an alternating purine-pyrimidine DNA sequence which may have a role in structural transitions of the helix in recognition and genetic regulation.

Note: During the preparation of this paper similar work has been initiated by Vorličková et al. (31). These data include the demonstration of the effect of Ca²⁺ ions and a large temperature effect on the negative band of the putative Z form. No data on the divalent cations or on oligomers are presented, however.

ACKNOWLEDGMENTS

We thank Dr. M. Vorličková for sending us their manuscript prior to publication. Thanks are also due to Dr. R. Weiss and E. Birsch-Hirschfeld for deoxy-oligomers and to Dr. H. Triebel for establishing the sedimentation data.

Sylvie Tymen died suddenly in the laboratory on July 17th, 1981 at the age of 26. The coauthors and colleagues of this laboratory will remember her kindness, intelligence, enthusiasm and sense of humour. This paper is an affectionate tribute to her memory.

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