Volume 10 Number 3 1982 Nucleic Acids Research

A Z-like form of poly(dA-dC).poly(dG-dT) in solution?

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Received 24 November 1981; Accepted 4 January 1982

ABSTRACT

Circular dichroism was used to study changes in conformation of poly(dA-dC).poly(dG-dT) caused by a high concentration of various monovalent salts. It was found that CsF induced the gradual appearance of a negative band in the long wavelength part of the CD spectrum of poly(dA-dC).poly(dG-dT), which might reflect a transition of this DNA toward a Z-like structure.

INTRODUCTION

The discovery of the left-handed Z-DNA (1,2) showed that the double helix of DNA, in accord with theoretical predictions (3,4) and experimental indications (5,6), possesses much greater conformational flexibility than had originally been supposed.

So far, conformational flexibility is best understood for synthetic DNA's with the alternating G-C sequence of bases. Poly(dG-dC).poly(dG-dC) can assume the forms B-, A-, and Z-DNA both in solution (7,8) and in oriented fibres (9), depending on the environment. Transitions between the particular forms are highly cooperative and reversible (7,8).

A natural question connected with the discovery of the left-handed Z-DNA is the possibility of its biological relevance. It has been shown (10) that a high concentration of NaCl induced a B-Z transition in oligo(dG-dC).oligo(dG-dC) inserted in random sequence DNA restriction fragments. The length of oligo(dG-dC).oligo(dG-dC) should, however, be greater than 20 base pairs. A negative influence of neighbouring regions on the capability of a fragment with the alternating G-C sequence of bases to turn into the Z-DNA form

0305-1048/82/1003-1071$2.00/0

has been demonstrated by a study on the dodecamer d(CpGpCpGpApApTpTpCpGpCpG) (11). This dodecamer forms a double helix very similar to the right-handed B-DNA form in crystals obtained from a high-salt solution, while the isolated tetramer d(CpGpCpG) makes up a left-handed double helix Z-DNA (12) in the crystal under identical conditions. Scepticism on the question of whether the Z-DNA form is used in vivo has been mitigated by the finding that methylation of cytosine stabilizes the Z-form of poly(dG-dC).poly(dG-dC) (13) so that the B-Z transition takes place even in low-salt solution.

We reported in previous papers that not only poly(dG-dC).poly(dG-dC) but, under favourable conditions, also poly(dA-dT).poly(dA-dT) had an extreme conformational flexibility (14,15). The course of the conformational transitions and the forms of poly(dG-dC).poly(dG-dC) and poly(dA-dT).poly(dA-dT) which arise were shown to be different, though both exhibited an expressive polydinucleotide architecture of the double helix (15,16). Poly(dA-dC).poly(dG-dT) shares a characteristic property of poly(dG-dC).poly(dG-dC) and poly(dA-dT).poly(dA-dT), an alternating purine-pyrimidine sequence of bases. It contains A•T and G•C base pairs, being thus a useful model for studies of the influence of their interactions on conformational changes in poly(dA-dC).poly(dG-dT) under conditions which induce formation of the distinct polydinucleotide arrangements of poly(dG-dC).poly(dG-dC) and poly(dA-dT).poly(dA-dT).

Oriented fibres of poly(dA-dC).poly(dG-dT) gave diffraction patterns corresponding to A-, B-, and Z-DNA form (9). In low-salt solution, this DNA has a CD spectrum like natural DNA's (17). It changes in a cooperative manner after addition of 50-60% ethanol, to become very close to the CD spectrum of poly(rA-rC).poly(rG-rT) (17). However, conditions giving rise to the Z-form of poly(dA-dC).poly(dG-dT) in solution have still not been reported. Therefore we attempted to look for them using CD, which has been shown to trace the B-Z transition of poly(dG-dC).poly(dG-dC) very distinctly (2).

MATERIAL AND METHODS

Poly(dA-dC).poly(dG-dT) was produced by Böhringer (Mann-

heim, GFR). Some batches of this DNA were kindly provided by Professor W. Guschlbauer. CD spectra were recorded with a Roussel-Jouan Dichrograph, Model CD 185, and with a Cary 61 apparatus, using 1 cm cells. The instruments were calibrated using a 0.1% (w/v) aqueous solution of (+) 10-camphorsulphonic acid in 1 cm cell checking the ellipticity of the band centered at 290 nm. For measurements of temperature dependence a thermostated cell holder and a Haake ultrathermostat filled with ethanol were used. The temperature was controlled within the limits of 0.2°C after equilibration with a thermometer inserted in a separate cell, which was removed before recording the CD curves. Solutions with high CsF concentration could not be measured at temperatures above 40°C because of etching the cell windows.

Absorption spectra were recorded using a Perkin-Elmer 340 spectrophotometer in the wavelength range from 350 to 210 nm. Concentration of poly(dA-dC).poly(dG-dT) was determined from the absorbance at 260 nm using a molar extinction coefficient 6.5×10^3 cm^{-1} (18). The absorbance values at 260 nm were read from the digital output of Perkin-Elmer Datahandler or measured using a Zeiss VSU-P spectrophotometer.

Stock solution of the polynucleotide (optical density about 1) was prepared in 10^{-2} M sodium phosphate, pH 7, salt concentration was increased by addition of solid NaCl, $NaClO_4$, NH_4F, CsCl and 13.6 M solution of CsF.

CsCl density gradients of poly(dA-dC).poly(dG-dT) were run in an analytical ultracentrifuge Spinco, Model E, at 44 330 rev/min for about 48 hours using a high molecular DNA Streptomyces chrysomallus as a marker.

RESULTS AND DISCUSSION

Changes in chiroptical properties of poly(dA-dC).poly (dG-dT) caused by high concentration of various monovalent salts were studied. Figure 1 shows CD spectra of poly(dA-dC). poly(dG-dT) measured at various concentrations of NaCl, $NaClO_4$, CsCl, and CsF. The presented salt-induced changes are quite reversible. It can be seen that both the salt concentration and the nature of the cation and anion profoundly affect

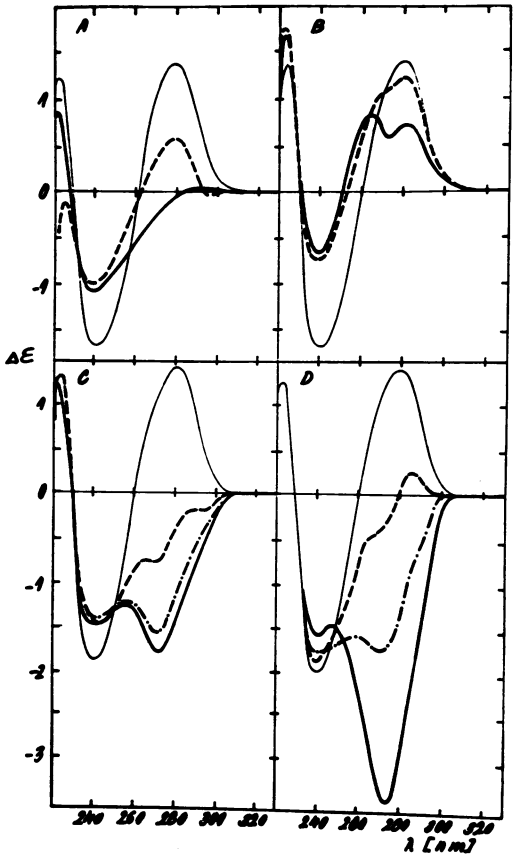

Figure 1:

CD spectra of poly (dA-dC).poly(dG-dT) in dependence on concentration of various monovalent salts at 27°C; ——— 10^{-2} M sodium phosphate, pH 7 with A: NaCl --- 2.7 M, ——— 5.0 M; B: $NaClO_4$ ---2.0 M, ——— 4.0 M; C: CsCl --- 2.0 M, —·— 4.0 M, ——— 5.6 M; D: CsF --- 1.2 M, —·— 2.3 M, ——— 4.3 M.

chiroptical properties of this DNA. In the presence of a high concentration of caesium ions in solution a negative long wavelength band appears in the CD spectrum. The negative band is very distinct, especially if the anion in the caesium salt is fluorine. It is of interest that, in contrast to poly(dG-dC). poly(dG-dC), the Cs^+-induced conformational transition of poly (dA-dC).poly(dG-dT) displays a low degree of cooperativity. In the case of CsF, $\Delta\mathcal{E}_{275}$ of poly(dA-dC).poly(dG-dT) is a linear function of ionic strength up to 4 M concentration of the salt (27°C). It declines one $\Delta\mathcal{E}$ unit per 1 M increase of the concentration of CsF.

The use of CsF among common monovalent salts for the study of conformational properties of poly(dA-dC).poly(dG-dT) was

motivated by our previous results on poly(dA-dT).poly(dA-dT). We found that unlike other salts CsF induced distinct conformational changes of poly(dA-dT).poly(dA-dT) which were manifested by a deep negative band in the long wavelength part of the CD spectrum (14) and by two well-separated resonances in the ^{31}P NMR spectrum (15). These conformational changes were non-cooperative, as in the case of poly(dA-dC).poly(dG-dT).

The fact that cations and anions cooperate in affecting the conformation of poly(dA-dC).poly(dG-dT) is obvious from Figure 2. This Figure shows that the depth of the negative long wavelength CD band at about the same concentration of CsCl and NH_4F is clearly smaller than with a sample which was prepared by mixing these two solutions. The long wavelength part of the CD spectrum of the mixed sample and of a solution where CsF was added to poly(dA-dC).poly(dG-dT) so that concentrations of caesium and fluoride ions were the same in both cases was identical within the experimental error (the short wavelength part was rather different). Thus it is apparent that both the nature of the cation, which was shown (19) to determine the winding angle between adjacent base pairs in the

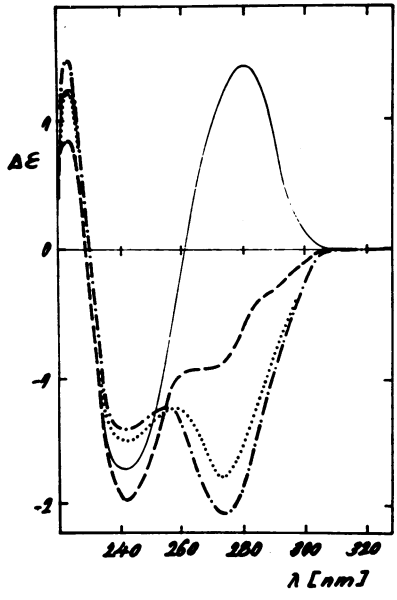

Figure 2:

CD spectra of poly (dA-dC).poly(dG-dT) in ——— 10^{-2} M sodium phosphate, pH 7, measured at 27°C with ——— 5.4 M NH_4F, ···· 5.6 M CsCl and —·— 2.7 M NH_4F and 2.8 M CsCl.

double helix of random sequence DNA, and the nature of the anion play an important role in establishing the observed distinct changes in the CD spectrum of poly(dA-dC).poly(dG-dT). There are few references in the literature regarding the influence of anions on the DNA conformation. It has been reported (12) that chlorine anions are bound to amino groups of bases in the Z' form of d(CpGpCpG) in crystal, being an essential factor for its formation. It is possible that binding of anions to the amino groups of bases is also a factor stabilizing the high-salt form of poly(dA-dC).poly(dG-dT). Small fluorine anions, which have a high surface charge density and occupy the same space in the water lattice as a water molecule (20), can be supposed to bind to the amino groups more tightly than chlorine anions. This might be a reason for the difference between CsF and CsCl in the influence on the structure of poly(dA-dC).poly(dG-dT).

The negative long wavelength CD band of poly(dA-dC).poly (dG-dT) at high concentrations of CsF and CsCl becomes very deep especially at low temperatures (Fig. 3). Circular dichroic spectra of this DNA measured at various temperatures pass through an isoelliptic point at 255 nm which is common to the sample in both CsF and CsCl. A shift of 30°C of the temperature scale in 4.2 M CsCl relative to that in 4.2 M CsF allows one to connect smoothly temperature dependences of the depths of the negative long wavelength CD bands of poly(dA-dC). poly(dG-dT) obtained for the two cases (Figure 3, inset). The presented temperature-induced changes in chiroptical properties of poly(dA-dC).poly(dG-dT) are quite reversible.

In some cases the appearance of an intensive negative band in the 260-280 nm range of DNA CD spectra was shown to be due to intermolecular associations of DNA molecules (ψ form of DNA), which could obscure the interpretation of the spectra in terms of the secondary structure (21). However, two types of experiments have shown that there is no association of poly (dA-dC).poly(dG-dT) in high concentrations of Cs^+ salts. First, the polynucleotide was run in an analytical CsCl density gradient together with a pure high molecular bacterial DNA. A sharp band of the bacterial DNA was observed after 20 hours'

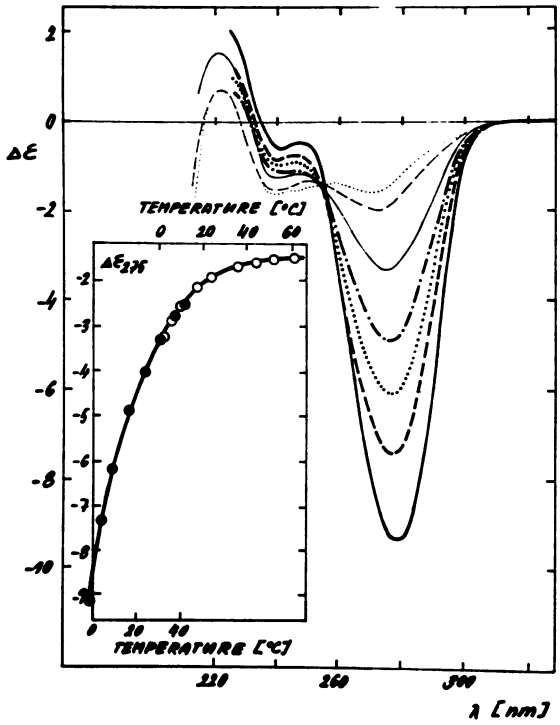

Figure 3:

CD spectra of poly (dA-dC).poly(dG-dT) in 4.2 M CsF at ——— -1°C, – – – 4°C, ···· 8°C, —·— 16°C, and in 4.2 M CsCl at ——— 1°C, – – – 35°C, ···· 61°C.

Inset: Temperature dependence of ellipticity at 275 nm of poly (dA-dC).poly(dG-dT) in ●-● 4.2 M CsF (bottom scale) and o-o 4.2 M CsCl (top scale).

running, but 48 hours' banding was needed to observe a well-developed band of poly(dA-dC).poly(dG-dT), which was still broader than that of DNA. This clearly excludes any polynucleotide association at CsCl concentrations as high as 6-7 M. Secondly, the UV spectra of poly(dA-dC).poly(dG-dT) at high concentrations of both CsCl and CsF against pure water used as a blank were recorded. Typical absorbance values in 4.2 M salts were: CsCl, A_{350} = 0.033, A_{258} (max) = 0.968, A_{234}(min)= = 0.485; CsF, A_{350} = 0.046, A_{258} (max) = 0.851, A_{239} (min) = 0.615, indicating a slightly lower optical purity of CsF in the low wavelength region, but essentially no light scattering above 300 nm in both cases. These data and also the similarity of the poly(dA-dC).poly(dG-dT) transition with that of poly (dA-dT).poly(dA-dT) in CsF, where aggregation has been excluded on the basis of NMR relaxation data (22), strongly suggest that the CD changes of poly(dA-dC).(dG-dT) in CsF are also entirely due to changes in the secondary structure of DNA.

Circular dichroic spectra of poly(dA-dC).poly(dG-dT) at high concentrations of CsF are strikingly different from those observed for its B- and A-DNA form (17). It can therefore be supposed that either CsF induces the formation of a structure in poly(dA-dC).poly(dG-dT) which has not still been observed in oriented fibres, or the observed changes reflect a non-cooperative transition of poly(dA-dC).poly(dG-dT) toward a polydinucleotide Z-like arrangement of the double helix observed in fibres (9). The B-A transition in DNA (19) and the B-Z transition of poly(dG-dC).poly(dG-dC) (2), which involve changes in puckering of the sugar residues, are highly cooperative. Hence it is probable that no substantial changes in puckering occur during the non-cooperative CsF-induced transition of poly(dA-dC).poly(dG-dT). If one admits that the observed changes correspond to a transition toward the Z form, then the requirement of a constant geometry of sugars might be fulfilled, for example, during a transition of the type alternating B-DNA (23) ⟷ Z-DNA, where both initial and final conformation have a C3′- endo sugar puckering at the purine bases and a C2′- endo sugar puckering at the pyrimidine bases. Another possibility is a transition between the B-form found in the dodecamer d(CpGpCpGpApApTpTpCpGpCpG) (11) and the Z′- form of the tetramer d(CpGpCpG) (12) with a C1′- exo sugar puckering at the purine bases in both forms.

It is interesting to compare the influence of the particular ions on the appearance of the negative long wavelength band in the CD spectrum of poly(dA-dC).poly(dG-dT), poly(dG-dC).poly(dG-dC), and poly(dA-dT).poly(dA-dT). As far as the cations are concerned, Na^+ exerts a greater influence on poly(dG-dC).poly(dG-dC) than Cs^+ (2,14). On the contrary, with poly(dA-dC).poly(dG-dT), poly(dA-dT).poly(dA-dT) (14) and native DNA (24, 25), Cs^+ is much more effective in inducing the formation of negative long wavelength CD bands than Na^+. The anions influence the conformation of poly(dA-dC).poly(dG-dT) in the order $F^- > Cl^- > ClO_4^-$ just like the conformation of poly(dA-dT).poly(dA-dT) (14). With poly(dG-dC).poly(dG-dC), the situation is quite the reverse again (2,14). It can thus be concluded that the salt-induced transition of poly(dA-dC).poly(dG-dT) is by the

low degree of cooperativity, ionic specificity and kinetics much more similar to that of poly(dA-dT).poly(dA-dT) (14) than to poly(dG-dC).poly(dG-dC) (2).

The fact that the extreme changes in conformation of synthetic molecules of DNA with alternating purine-pyrimidine base sequences were observed in solutins with extremely high concentrations of salts and, moreover, in a medium with unphysiological ions like Cs^+, does not exclude the possibility of their existence in living organisms. It is plausible that agents other than high salt may effect such transitions in DNA in vivo. The non-cooperative nature of the changes in conformation of poly(dA-dC).poly(dG-dT) and poly(dA-dT).poly(dA-dT) (14) allows one to expect that deviations from the regular B-form can be encountered even in short regions of native DNA with a particular base sequence.

ACKNOWLEDGEMENTS

After this work had been completed we learnt that Guschlbauer and Zimmer had independently performed a similar analysis. Their and our results are consistent. We thank Dr. Ch. Zimmer for sending us a preliminary version of their manuscript.

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