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Salt-induced conformational changes of poly(dA-dT)

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ABSTRACT

Conformational changes of poly(dA-dT).poly(dA-dT) induced by increasing ionic strength were studied using CD spectroscopy. It was found that a pronounced noncooperative inversion of the long-wavelength part of the CD spectrum of poly(dA-dT).poly(dA-dT) occurred at high concentrations of CsF in solution. It was suggested that a great difference between the geometries of the purine and pyrimidine residues in the helix was characteristic of the structure of poly(dA-dT).poly(dA-dT) in concentrated CsF solutions.

INTRODUCTION

DNA is known to be capable of different structural rearrangements with no destruction of complementarity: the cooperative transitions from the B to A form and a continuous winding of the helix within the families of the A-like and B-like structures (1). Nevertheless, both experiment and theory indicate (2-8) that the conformational possibilities of DNA are not restricted to A and B families only.

Recently, Wang et al. (2) have found that the DNA fragment d(CpGpCpGpCpG) crystallises as a left-handed double helix, where the repeating unit is a dinucleotide. This structure was called Z-DNA. Its characteristic feature is a marked difference in the conformations of the purine and pyrimidine residues. Arnott et al. (3) detected the left-handed Z-DNA conformation in oriented fibres of poly(dG-dC).poly(dG-dC), poly(dA-dC).poly(dG-dT) and poly(dA-ds^{4}T).poly(dA-ds^{4}T).

It has been suggested that poly(dA-dT).poly(dA-dT) as-

sumes an alternating B-DNA conformation with the repeating unit of two nucleotides (7). This structure exhibits A-DNA type sugar pucker and glycosidic torsion angle at the purine residues but changes to B-DNA type sugar pucker and glycosidic torsion angle at the pyrimidine residues. The internucleotide phosphates linking purine(3'-5')pyrimidine adopt ω', ω backbone O-P rotation angles similar to B-DNA but ω' changes for those linking pyrimidine(3'-5')purine. An evidence for the existence of an alternating phosphodiester backbone conformation of poly(dA-dT) duplex in solution of low ionic strength was provided by the ^{31}P NMR results of Shindo et al. (4).

^{1}H and ^{31}P NMR results suggest the existence of an alternating structure also in oligo(dG-dC)$_8$ (5) and poly(dG-dC) (9) duplexes at 4.0 M NaCl while in low salt concentration the oligomer as well as polymer were reported to adopt the regular B-DNA conformation (5,10). These NMR spectra were recorded to evaluate the structural aspects of the salt-induced transition of oligo(dG-dC) duplex which was reported (6) to be accompanied by the inversion of the CD spectrum. By contrast, no significant changes in the ORD spectrum of poly(dA-dT).poly (dA-dT) due to an increased salt concentration up to 4.0 M $NaClO_4$ were observed (6).

We demonstrate a marked non-cooperative inversion of the long-wavelength part of the CD spectrum of poly(dA-dT). poly(dA-dT) at high concentrations of CsF in solution. A possible explanation of this fact is suggested, based on similarities in the shapes of the CD spectra of poly(dG-dC).poly (dG-dC) and poly(dA-dT).poly(dA-dT) at high CsF concentrations.

MATERIALS AND METHODS

Poly(dG-dC).poly(dG-dC) was purchased from Böhringer (Mannheim, GFR), samples of poly(dA-dT).poly(dA-dT), poly(dA). poly(dT) and poly(dG).poly(dC) were from P-L Biochemicals (St.Goar, GFR).

Absorption spectra were recorded with a spectrophotometer Unicam SP 700. CD spectra were obtained with a Roussel-Jouan Dichrograph, Model CD 185. All measurements were carried out

at 23.5 °C. Stock solutions of polynucleotides (optical density about 1) were prepared in 0.05 M sodium phosphate buffer, pH 7.0. Salt concentration in solutions of polynucleotides was increased by adding solid NaCl or CsCl and 16 M solution of CsF.

RESULTS

We investigated the influence of various salts on the CD spectra of poly(dA-dT).poly(dA-dT), poly(dG-dC).poly(dG-dC), poly(dA).poly(dT), and poly(dG).poly(dC). The room temperature CD spectrum of poly(dA-dT).poly(dA-dT) was shown (11) to be incompatible with those of natural DNA's. This fact may be connected with its alternating B-DNA structure. In accord with the results of Studdert et al. (12) and Zimmer and Luck (13) we observed a continuous decrease of the long-wavelength part of the CD spectrum of poly(dA-dT).poly(dA-dT) with increasing NaCl or CsCl concentration in solution. At high concentrations of these salts the ellipticity in the vicinity of 280 nm changed the sign and a negative band appeared (Fig.1).

CD spectral changes of poly(dA-dT).poly(dA-dT) considerably more pronounced than in NaCl or CsCl were observed in CsF solutions (CsF was chosen because of the special properties of

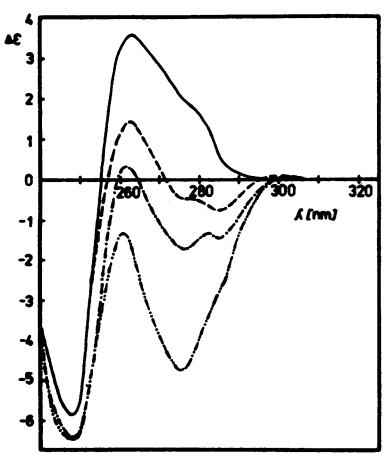

Fig.1. CD spectra of poly (dA-dT).poly(dA-dT) in low and high-salt solutions. 0.05 M sodium phosphate, pH 7.0, with ——— no salt added, – – – – 5.0 M NaCl, –·–·– 4.7 M CsCl and —·· 4.8 M CsF.

a CsF-water system (14)) (Figs.1,2). For high concentrations of CsF an inversion of the long-wavelength part of the CD spectrum of poly(dA-dT).poly(dA-dT) appeared analogically to that observed for poly(dG-dC).poly(dG-dC) in NaCl (6) and in CsF (Fig.3). The short-wavelength part of the CD spectrum of poly(dG-dC).poly(dG-dC) in 4.8 M CsF (Fig.3) is rather different from that in high concentration of NaCl (6). After heating the sample to 89 °C and slow cooling to 23.5 °C we have obtained a CD spectrum identical to that reported by Pohl and Jovin (6). By contrast, the temperature dependence of poly(dA-dT).poly(dA-dT) up to 89 °C in 4.8 M CsF was fully reversible. The CD spectral changes of poly(dA-dT).poly(dA-dT) within 0.1 - 6.6 M CsF are also fully reversible. Unlike poly(dG-dC).poly(dG-dC) the salt-induced transition was non-cooperative for poly(dA-dT).poly(dA-dT) (Fig.2).

UV absorption spectra showed no changes which would indicate denaturation or aggregation of poly(dA-dT).poly(dA-dT) at high concentrations of CsF in solution. Recently, no changes in the tertiary structure of DNA at high salt concentrations have been reported to occur (15).

The formation of the negative long-wavelength CD band was not detected for poly(dG).poly(dC) and poly(dA).poly(dT) at high concentrations of CsF in solution (Figs.4,5). Thus this effect seems to be specific for alternating purine-pyrimidine sequences.

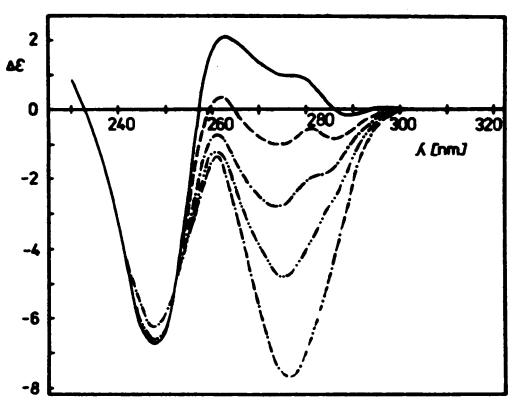

Fig.2. CD spectral changes of poly(dA-dT).poly(dA-dT) induced by increasing CsF concentration.
0.05 M sodium phosphate, pH 7.0, with ——— 0.1 M, – – – 1.2 M, —·— 2.8 M, —··— 4.8 M, ——· 6.6 M CsF.

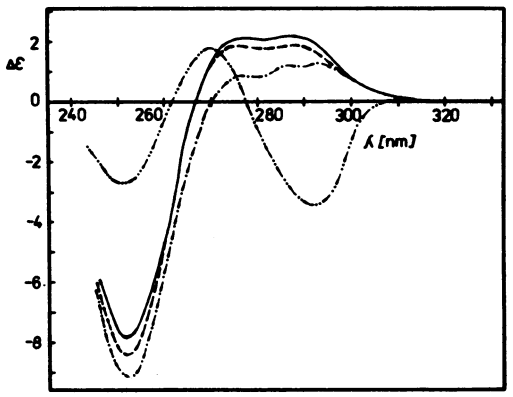

Fig.3. CD spectral changes of poly(dG-dC). poly(dG-dC) induced by increasing CsF concentration.
0.05 M sodium phosphate, pH 7.0, with ——— 0.1 M, — — — 1.2 M, —·— 2.8 M, and —— · 4.8 M CsF.

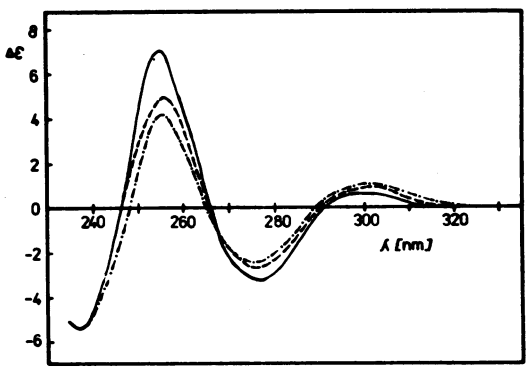

Fig.4. CD spectral changes of poly(dG). poly(dC) induced by increasing CsF concentration.
0.05 M sodium phosphate, pH 7.0, with ——— 0.8 M, — — — 4.2 M, and —·— 6.0 M CsF.

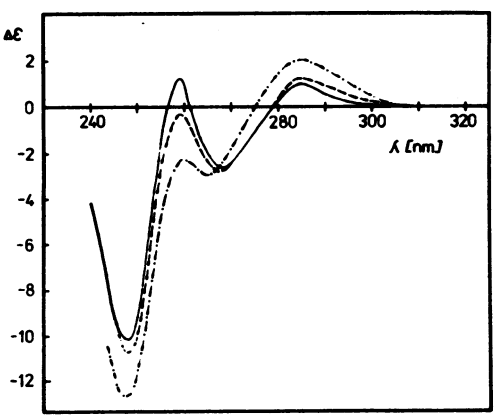

Fig.5. CD spectral changes of poly(dA). poly(dT) induced by increasing CsF concentration.
0.05 M sodium phosphate, pH 7.0, with ——— 0.8 M, — — — 4.9 M, and —·— · 6.0 M CsF.

DISCUSSION

Conformational changes in DNA induced by increasing the salt concentration in the solution have been inferred from CD spectra by various authors (12,13,16,17). It has been demonstrated (16,17) that a salt-induced decrease of the positive CD band in the vicinity of 280 nm reflects a non-cooperative winding of the DNA double helix within the family of B forms in the direction B→C.

More or less perfect mirror inversions of the CD spectra of native DNA (especially in the long-wavelength spectral region) were observed under many different conditions (e.g., in water-poly(ethylene glycol) (18), water-poly(ethylene oxide) (19), or water-methanol (16) mixtures, in very high LiCl concentrations (20), and in complexes of DNA with histones (21, 22), poly-L-lysine (23), or poly-L-histidine (24)). Changes in the tertiary structure have often been presented as a possible explanation of these inversions. In all cases given above one can expect a decreased water activity and/or a pronounced electrostatic shielding of the DNA backbone phosphates. The question is whether the inversions of the CD spectra of DNA under the conditions mentioned above and the inversions observed in the CD spectra of poly(dG-dC).poly (dG-dC) and poly(dA-dT).poly(dA-dT) at high salt concentrations might not reflect similar conformational changes originating in the secondary structure.

In this paper we demonstrate pronounced changes in the CD spectrum of poly(dA-dT).poly(dA-dT) with increasing CsF concentration in solution dominated by the conspicuous negative long-wavelength CD band analogous to the negative long-wavelength CD band of the high-salt form of poly(dG-dC).poly (dG-dC) (Figs.2,3). This analogy prompted us to suggest that conformations of both polynucleotides have common features at high CsF concentration in solution.

It follows from literary data (6,13) as well as from our results (Figs.4,5) that neither analogous polyribonucleotides nor complexes of homopolydeoxyribonucleotides yield an inversion of CD spectra at high salt concentrations. The formation

of the negative long-wavelength CD band induced by a high concentration of salt seems to be specific for alternating polydeoxyribonucleotides poly(dR-dY).poly(dR-dY), where R is a purine residue and Y a pyrimidine residue complementary to R. (poly(dI-dC).poly(dI-dC) has a negative CD band at 285 nm even at low ionic strength (25) and cooperative salt-induced transitions of the CD spectra have also been reported for poly (dA-ds^{4}T).poly(dA-ds^{4}T) (26) and poly(dI-br^{5}dC).poly(dI-br^{5}dC) (5)). Thus it can be expected that poly(dR-dY).poly (dR-dY) may assume a conformation characterised by a specific structure of the sugar residues, which is not, under the conditions discussed, accessible for analogous polyribonucleotides (an alternating C3'endo(3'-5') C2'endo sugar puckering pattern has been suggested for the high salt form of poly(dG-dC).poly(dG-dC) (9)). Moreover, a unique vertical arrangement of the bases which is made possible by the stacking interactions only in alternating purine-pyrimidine sequences should be expected.

For alternating polynucleotides poly(dR-dY).poly(dR-dY) one must expect that the conformation of the nucleotide dR will generally differ from that of the nucleotide dY. The symmetry of the primary structure of the alternating polynucleotide duplexes requires that conformations of all purine monomers and of all pyrimidine monomers are identical (hairpin structures and other deviations from the regular double-helical structure are not considered, as they have been reported (4) to involve a negligible portion of base pairs). As a consequence of these facts, a dinucleotide should be taken as a repeating unit in polynucleotides poly(dR-dY).poly(dR-dY).

The difference in conformations of the nucleotides dR and dY can be partially inferred from the separation of the ^{31}P NMR resonances of the phosphorus atoms in the sequences dR (3'-5')dY and dY(3'-5')dR because ^{31}P NMR chemical shift is known to be sensitive to the backbone O-P rotation angles (27). Patel showed (9) that poly(dG-dC).poly(dG-dC) exhibited only a single ^{31}P NMR signal in low-salt but two in high-salt solution. The separation of these two resonances is 1.25 ppm (9). The difference between the chemical shifts of

the ^{31}P NMR resonances of poly(dA-dT).poly(dA-dT) in 0.1 M NaCl is 0.23 ppm only (4). These results indicate that the difference between the conformations of purine(3'-5')pyrimidine and pyrimidine(3'-5')purine phosphodiester linkages is small for poly(dA-dT).poly(dA-dT) in low-salt solution compared to that in the high-salt form of poly(dG-dC).poly(dG-dC). We suppose that the gradual inversion of the long-wavelength part of the CD spectrum of poly(dA-dT).poly(dA-dT) induced by increasing the CsF concentration in solution accompanies the formation of a structure with different geometries of the purine and pyrimidine residues. Our very recent ^{31}P NMR results strongly support this conclusion (28). A great difference between the conformations of the purine and pyrimidine residues is also characteristic of the left-handed Z-DNA structure. Whether the high-salt form of poly(dA-dT).poly (dA-dT) is the left-handed Z-DNA remains still obscure.

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