Conformational stability of alternating d (CG) oligomers in high salt solution

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ABSTRACT

The conformation of d(CG)n oligomers with n = 2, 3 has been studied in aqueous solution in the presence of high salt concentration. A minimum n value of three is necessary to obtain a left-handed Z-helix. When d(CG)3 is flanked by three non Z-helicogenic alternating AT sequences the left-handed helix is unstable and a B-type conformation is obtained also at high salt concentration.

INTRODUCTION

The discovery of left-handed zig-zag structures (Z-helix) in the solid state for oligomers of alternating CG sequence (1-4) has again developed interest in the possibility of sequence-controlled DNA local conformations. The question whether the new conformation has a biological significance has been raised (1-5).

Very recently the X-ray structure of the self-complementary dodecamer d(CGCGAATTCCGG) in high salt has been carried out (6). Contrary to expectations for the Z-DNA structure a B-type right-handed double helix has been found, thus indicating the low stability of a Z-helix when associated with short B-helicogenic self-complementary sequence.

We have begun a study of several oligonucleotides in high salt aqueous solutions with the aim of finding a) how long a d(CG) alternating sequence should be to stabilize, in appropriate conditions, a Z-helix, b) how long a d(CG) alternating sequence should be to stabilize a Z-helix when flanked by sequences non Z-helicogenic.

In this note we report the results obtained in aqueous solution on d(CG)2, d(CG)3 and d(AT)3(CG)3.
All these compounds together with \( d(AT)_2 \) and \( d(AT)_3 \) have been synthesized by the phosphotriester method according to the procedure reported previously (7). Purification of the unblocked oligonucleotides was performed by high performance liquid chromatography on anionic exchange (Permaphase AAX, Dupont) and reverse-phase resins (Bondapak C18, Waters Ass.). The concentration of the oligonucleotides was calculated by taking the following mean extinction coefficient per base in the coiled form at high temperature: \( \varepsilon_{d(CG)_2} = 8550 \), \( \varepsilon_{d(A)7} = 12200 \), \( \varepsilon_{d(AT)_2} = 10100 \). These values have been calculated after enzymic hydrolysis with pancreatic DNAse (Sigma) following the procedure described by Bollum (8).

All solutions contained 1 mM phosphate buffer, pH 7.2. Absorption spectra were run with a Cary 219 spectrophotometer equipped with a thermostatable cell holder. When melting curves were run increase in temperature was programmed by using a Haake F3 thermostat with a PG 10 programmer. The rate of temperature change was generally 0.1°C/min. In these cases the X axis of the spectrophotometer recorder was linear with temperature.

CD spectra were obtained on a Jobin-Yvon CD 185 dichrograph with thermostatable cell holder. Stopped quartz cells of 1, 5 or 10 mm pathlength were used throughout. Evaporation during melting curves was minimized by overlayering with paraffin oil. All the transitions were fully reversible.

RESULTS

Fig.1 shows the absorption spectrum for \( d(CG)_2 \) dissolved in 5M NaCl, 4M LiCl at low and high temperatures. The spectra at low temperature are both typical of ordered structure and are in fairly good agreement with those shown by Pohl and Jovin (9) for \( d(G-C)_m \) (with \( m \) about 13) at low and high salt. Our extinction values are higher, probably because of a larger end effect.

Fig.2 shows the melting profile for \( d(CG)_2 \) in both salts. The data are plotted as increment in absorbance at 270 nm, where the same extinction is observed when either NaCl or LiCl is added. The derivative curves plotted in the insert show a single step melting process with \( T_m \) of about 40° and about 47° for \( d(CG)_2 \) in 5M NaCl, 4M LiCl.
Fig. 1 Absorption spectra of d(CG)$_3$ in high salt solution at 0°C (-----) (----) and at 70°C (-----).

5M NaCl and in 4M LiCl, respectively. A clear isosbestic point at 288 nm is observed when the spectra of d(CG)$_3$ in 5M NaCl are recorded on increasing temperature.

Fig. 3 shows the CD spectra of d(CG)$_3$ at 25°C for low and high NaCl concentration. The curve obtained at low NaCl is similar to those obtained at any LiCl concentration (not reported) and to that obtained at low NaCl concentration by Pohl and Jovin with poly d(CG)$_3$.poly d(CG) (9,10). This type of spectrum was assigned by these authors to a B conformation.

On increasing NaCl content the negative 253 nm band decreases in intensity while a new negative band (reminiscent of the Z-conformation) appears at 297 nm. In the insert the intensity of the negative 253 nm band is plotted against NaCl concentration. The midpoint of the transition is located at about 3M NaCl which is in good agreement with previous results on poly d(CG)$_3$.poly d (CG) (9). When d(CG)$_3$ in 5M NaCl is cooled to 0°C the CD spectrum
Melting profiles for $d(CG)_3$ in $5\text{M NaCl}$ and $4\text{M LiCl}$. The derivative curves are shown in the insert. $[d(CG)_3] = 2.5 \times 10^{-4} \text{ monomol/l.}$

The spectrum changes gradually to that of the coil form on increasing temperature with an isoelliptic point at 277 nm.

When $d(CG)_2$ is investigated in the appropriate conditions (low temperature and high NaCl concentration) no evidence for Z-conformation is obtained using the CD technique. The absorption spectrum at high ionic strength and low temperature is only slightly lower than that at high temperature thus showing that little, if any, ordered conformation is obtained. The same is true for $d(AT)_2$ and $d(AT)_3$.

However, $d(AT)_3$ (CG)$_3$ when dissolved in $5\text{M NaCl}$ at low temperature shows an ordered structure which melts when temperature is raised. The melting temperature is highly dependent on the concentration of the dodecamer. This behavior is illustrated.
Fig. 3 CD spectra of d(CG)₃ at 25°C at low and high NaCl concentration. The insert shows the variation of the ellipticity at 253 nm with the salt concentration. [d(CG)₃] = 2.5 x 10⁻⁴ monomol/l.

In Fig. 5 when the increase in absorption maximum (λ = 258 nm) is plotted at three different concentrations as a function of the temperature. The behavior shown is indicative of the formation in solution of concatamers
5'...ATATATCCCGCG ATATATCCCGCG ATATATCCCGCG ... 3'
3'...CCCGCTATATA CCCGCCTATATA CCCGCCTATATA ... 5'
as already reported for non self-complementary nucleotide oligomers (11).

The three series of absorption spectra from which data of Fig. 5 were derived show an isosbestic point at 288 nm (as d(CG)₃) only above a temperature value which depends on the dodecamer concentration. This finding suggests a two step
process which is also reflected by the shape of the melting curves of Fig. 5 which depart from the typical sigmoidal profile. This is better shown in Fig. 6 where relative absorption at 258 nm (maximum) and 288 nm (isosbestic point) is plotted. The two derivative curves are shown in the insert. The increase in absorption at 288 nm is typical of a single step melting process with a Tm of about 23°C. The increase in absorption at 258 nm and its derivative evidence a two-step melting process with Tm's of about 23°C and about 40°C. These data are fairly well explained by attributing the first melting (23°C) to less stable AT duplexes with consequent breakdown of the concatamers and the second one (40°C) to CG duplexes. Further support to this interpretation comes from the presence of the same isosbestic point and melting temperature found when d(CG)3 in 5M NaCl is heated. If the same experiment is carried out in 4.2M LiCl the melting curves at 258 and 288 nm are more symmetric and almost coincident thus suggesting that AT and CG duplexes melt in the same temperature range. No isosbestic points are observed as the AT portions

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Fig. 4 CD spectra of d(CG)3 in 5M NaCl as a function of temperature. \([d(CG)_3] = 2.5 \times 10^{-4} \text{ monomol/l}\).
Fig. 5 Melting profiles for d(AT)$_3$(CG)$_3$ in 5M NaCl at different oligonucleotide concentrations. (□): 1.75 x 10$^{-5}$ monomol/l; (O): 8x10$^{-5}$ monomol/l; (△): 8x10$^{-4}$ monomol/l.

of the concatamer melt with an absorption increase in the entire wavelength range. The slight difference in T$_m$ observed at the two wavelengths (see insert of Fig.7) can be due to a very small difference in stability in these conditions for the two alternating sequence duplexes.

The CD curves for d(AT)$_3$(CG)$_3$ in 5M NaCl at three different temperatures corresponding to the completely ordered structure (concatamer), the semiordered one (mainly dimer) and the coiled conformation (monomer) are shown in Fig.8. The low temperature CD curve is characteristic of a B-family conformation whereas that at 25°C (mainly dimer) shows a small negative band at 295 nm which could be attributed to the presence of appreciable amounts of Z-helices, formed by the CG duplexes after disruption of the concatamer. At 52°C the CD of the coil (monomer) is obtained. When the high ionic strength is obtained with LiCl the CD curve at low temperature is again of the B-family type but increas-
Fig. 6 Melting profiles recorded at two different wavelengths for d(AT)$_3$(CG)$_3$ in 5M NaCl. The insert shows the derivative curves. $\left[d(AT)_3(CG)_3\right] = 8 \times 10^{-5}$ monomol/1.

At higher temperature a continuous transformation to coil form is obtained (Fig. 9).

**DISCUSSION**

These results can be summarized and interpreted as follows:

As in the case of poly d(CG).poly d(CG), high concentration of sodium salt stabilizes the Z-conformation of d(CG)$_3$ (at low temperature), whereas high concentration of lithium salt stabilizes the B-family helix (10). Contrary to what was found for the polymer ethanol does not induce a Z or A conformation for d(CG)$_3$. 

Melting profiles recorded at two different wavelengths for d(AT)$_3$(CG)$_3$ in 4.2M LiCl. The insert shows the derivative curves. [d(AT)$_3$(CG)$_3$] = 9x10$^{-5}$ monomol/l.

In fact at ethanol concentration above 50% (w/w) only a CD spectrum corresponding to the coil conformation is obtained (data not shown).

A sequence of three CG units is the shortest one necessary to obtain a full Z-conformation in high salt (NaCl) solution and at low temperature. d(CG)$_2$ in the same conditions shows only a slight hypochromism and no negative band at 295 nm in the CD spectrum. This conclusion is obviously valid only for oligomers having cytosine as first base in the 5' position. It has in fact been shown that for alternating deoxynucleotide oligomers the nature of the first base causes some differences in physical properties.

When an alternating d(CG)$_3$ duplex is flanked by an alternating d(AT)$_3$ duplex, as in the case of the concatamer obtained at high
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Fig. 8 CD spectra of d(AT)$_3$(CG)$_3$ in 5M NaCl at different temperatures. $[d(AT)_3(CG)_3] = 8 \times 10^{-5}$ monomol/l.

Ionic strength (NaCl) and low temperature, the Z-conformation is destabilized and the CG sequence assumes a B-family conformation. This result is in agreement with the finding of Wing et al. (6) on the solid state conformation of the self complementary dodecamer CGCGAATTCCGG. Both these results reduce noticeably the biological significance of the left-handed Z-conformation only to longer CG sequences, which are indeed not so frequent in natural DNA. On the other hand, the results obtained in high salt (NaCl) solution on d(AT)$_3$(CG)$_3$ show that it is difficult for AT alternating sequences to assume the left-handed conformation contrary to what Arnott et al. (5) postulated. It must be noted, however, that a further difficulty to obtain a Z helix
at high salt can be due to the pyrimidine-pyrimidine sequence at the joining of AT and CG portions of the dodecamer.

More data have to be accumulated in this field to know what sequence conditions must be fulfilled to obtain a stable Z-helix within a DNA chain. Work is in progress along this line.

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