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Conformation of the synthetic DNA poly(amino^{2}dA-dT) duplex in high-salt and aqueous alcohol solutions

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ABSTRACT

It has previously been demonstrated by other workers that the duplex of a synthetic DNA poly(amino^{2}dA-dT) undergoes a salt-induced conformational isomerization. We show in the present work using circular dichroism that the same isomerization is induced in poly(amino^{2}dA-dT) by various alcohols. The isomerization was originally identified as the B-to-Z and then B-to-A conformational transition of DNA but we demonstrate that the high-salt or alcohol conformation of poly (amino^{2}dA-dT) is the non Z-DNA zig-zag double helix we have previously observed with poly(dA-dT) and called X-DNA. X-DNA is a cesium cation specific conformation of poly(dA-dT) while no similar cation specificity is observed with poly(amino^{2}dA--dT). Thus it appears that the extra amino group attached to A and cesium cations make the same thing; they probably dehydrate the double helix minor groove and relieve its conformational variability. Poly(amino^{2}dA-dT) is exceptionally stable in X-DNA and conditions inducing it are mild, which opens the door to assess its molecular structure.

INTRODUCTION

AT and GC base pairs contained in DNA confer different properties on it in several respects. One difference has been suggested by a single crystal X-ray diffraction study of a DNA dodecamer (1). The amino group of G interferes with hydration of the B-DNA minor groove so that the groove is heavily hydrated in its AT rich regions but not where GC base pairs predominate. This observation indicates a molecular basis of the different hydration of AT and GC base pairs in DNA known for years (2). The minor groove hydration stabilizes B-DNA (1) and this accounts for the poor inclination of AT rich DNA molecules to isomerize into A- (3) and Z-DNA (4) conformations.

The amino group attached to purines at their minor groove

edge is obviously very important for establishing DNA conformational properties. Hence there is an evident interest in the synthesis and conformational analysis of poly(amino^{2}dA-dT). It was first demonstrated on the oligonucleotide level that (dT-amino^{2}dA)$_3$ underwent a salt-induced conformational transition similar to the B-to-Z transition observed for (dC-dG)$_n$ oligonucleotides (5). Later on, this phenomenon has also been observed on the polynucleotide level and identified as the B-to-Z transition again, on the basis of CD, IR and ^{31}P NMR data (6). However, this interpretation proved to be incompatible with the results of subsequent two-dimensional NMR studies (7,8). As they excluded a possibility that the high-salt form of poly(amino^{2}dA-dT) is B- or Z-DNA the authors interpreted the salt-induced changes in terms of a B-to-A conversion of the polynucleotide. In the present work we report circular dichroism data demonstrating that rather than A-DNA the high-salt form of poly(amino^{2}dA-dT) is the X form we have previously observed and characterized with poly(dA-dT) (9-11).

MATERIALS AND METHODS

2-Amino-2'-deoxyadenosine (V, beta-isomer) was prepared by condensation of 9-N-(trimethylsilyl)-2,6-dichloropurine (I) with protected halogenose in the following way. To 1.55 g (8.18 mmol) of 2,6-dichloropurine 5 ml of hexamethyl-disilazane and 4 drops of trimethylchlorosilane were added. The mixture was refluxed at 150 °C for 5 hours and then evaporated in vacuum. 2.1 g of crystalline residue, I, was obtained. 2.1 g of I (7.67 mmol) and 3.28 g (8 mmol) of 2-deoxy-3,5-di-O-p-chlorobenzoyl-D-erythro-pentafuranosyl chloride in 10 ml of anhydrous chloroform were added to the suspension of 0.2 g of $ZnCl_2$ in 32 ml of anhydrous chloroform. The resulting clear solution was stirred for 30 min at room temperature, then evaporated in vacuum and the residue dissolved in 50 ml of ethylacetate. The solution was extracted with 3 x 10 ml of water, organic phase dried over $MgSO_4$ and evaporated in vacuum. The oily residue was dissolved in a 5 ml mixture of chloroform-diethylether (7:3), purified by column chromatography using 200 ml of silica gel and eluted with the former mixture. The product, 2,6-dichloro-9-(3;5'-di-O-p-chlorobenzoyl-2'-deoxy-alpha-beta-D-ribofuranosyl)-purine (II) was 1.8 g, m.p. was 136 - 140 °C.

1.8 g of II and 60 ml of dry methanol saturated with ammonia at 0 °C were sealed in a tube and left for 4 days at 60 °C. The clear solution was then evaporated, residue dissolved in 15 ml of methanol and applied on a column filled with 100 ml of silica gel. Elution was carried out with a mixture

of ethylacetate-methanol (8:2). Evaporated fractions were treated with diethylether and the crystalline material obtained was 850 mg (71 %) of 2-chloro-6-amino-9-(2'-deoxy-alpha-beta-D--ribofuranosyl)purine (III). Its m.p. was 210 °C, calculation gave 12.4 and 24.5 % while experiment 12.55 and 24.35 % for C and N, respectively.

650 mg (2.27 mmol) of III was added to 50 ml of dry hydrazine and the sealed flask was held at room temperature for 20 hours. The mixture was then evaporated in vacuum under 35 °C. 2 ml of 1 N NaOH and 10 ml of isopropyl alcohol were added to the residual syrupy material and evaporation was repeated. Since the product, 2-hydrazino-6-amino-9-(2'-deoxy-alpha-beta-D-ribofuranosyl)purine (IV) is unstable, it was used up without further purification.

1 g of Raney-nickel and then compound IV in 20 ml of the former mixture were added to 80 ml of 50% aqueous methanol. Hydrogenation was carried out at 45 psi for 18 hours at room temperature. Suspension was then filtrated and the filtrate evaporated. The residue was dissolved in methanol and applied on the top of a silica gel column (100 ml). The gel was then washed with 300 ml of ethylacetate. Products, 2,6-diamino-9--(2'-deoxy-D-ribofuranosyl)-purines (V) were eluted with ethylacetate-methanol (8:2). In this way 165 mg of beta-isomer, 92 mg of alpha-beta mixture and 250 mg of alpha-isomer of V were obtained. Total yield was 67 %. The beta-isomer of V was recrystallized from ethylalcohol-ethylacetate-diethylether mixture, m.p. was 146 - 148 °C (lit. value was 146 - 148 °C in ref. 12). The compound was further characterized by UV (12) and ^{1}H NMR ($CDCl_3$ + DMSO-d_6; 1:1) spectral parameters : 2.28 (1H, ddd, J_{gem}: 13.3 Hz, $J_{1';2'_A}$: 6.0 Hz, $J_{2'_A,3'}$: 2.5 Hz; 2'-H_A), 2.81 (1H, ddd, $J_{1';2'_B}$: 8.0 Hz, $J_{2'_B,3'}$: 5.7 Hz; 2'-H_B), 3.72 (1H, dd, J_{gem}: 12.0 Hz, $J_{4';5'_A}$: 3.4 Hz, 5'-H_A), 3.82 (1H, dd, $J_{4';5'_B}$: 3.0 Hz; 5'-H_B), 4.07 (1H, ddd, $J_{3';4'}$: 2.5 Hz; 4'-H), 4.57 (1H, ddd; 3'-H), 5.1 and 6.2 (2x2H; 2xNH_2), 6.26 (1H, dd; 1'-H), 7.71 (1H, s; 8-H) ppm.

2-amino-dATP was prepared from the beta-isomer of compound V in a similar way as described by Howard and Miles (13). dTTP was from USB Corp., Cleveland, Ohio and E. coli DNA polymerase I Klenow fragment enzyme (7000 units/mg) from Boehringer-Mannheim GmbH. Synthesis of the alternating copolymer poly (amino^{2}dA-dT) was carried out by a step-wise scale-up method. Reaction mixture contained 60 mM potassium phosphate (pH 7.4), 6 mM $MgCl_2$, 0.4 mM EDTA, 0.4 mM dTTP and 0.36 mM 2-amino-dATP. 300 units of DNA polymerase enzyme were used in the final volume 50 ml. The reaction was started in 0.3 ml that contained 0.63 units of highly activated poly(dA-dT) ($S^{250}_{25,w}$ was 0.5). Progress of the synthesis was followed by hypochromic changes, in a Specord UV VIS recording spectrophotometer (GDR). After no decrease in absorbancy (generally 23 - 28 % hypochromism) was observed, the mixture was scaled up 2 - 3 times, until 50 ml was reached but no more poly(dA-dT) was added. Purification of the product was carried out essentially as described

earlier (14). Protein-free material was applied onto a column of Bio-Gel A-5m (Bio-Rad Labs., Richmond, California) and ultraviolet-absorbing material of the void volume was only collected, dialyzed against two changes of 10 mM phosphate buffer (pH 7), 1 mM EDTA and then against three changes of redistilled water. Finally, it was freeze-dried. The product was 71 OD_{260} units (29 % yield), which corresponds to a 112-times net synthesis over the starting poly(dA-dT) template-primer. In this way dA-content of the product poly(amino^{2}dA-dT) has to be under 1 % of the amino^{2}dA content. No transition indicating the presence of poly(dA-dT) tracts was observed in the thermal denaturation curve of poly(amino^{2}dA-dT). UV spectrum of our product was identical to that shown by Borah et al. (8). Sedimentation velocity experiments reflected its high-molecular weight character, $S^{25^o}_{25,w}$ value was 14.59. Poly(dA-dT) was a product of PL Biochemicals.

Circular dichroism measurements were carried out in thermostated 1 cm pathlength cells using a Jobin-Yvon dichrograph Mark IV calibrated with isoandrosterone. For low temperature measurements ultracryostat VEB MLW MK 70 was used. Lyophilized polynucleotides were dissolved in a sodium phosphate/EDTA buffer to make the stock solutions. Aliquots of the stock solutions, salts and alcohols were mixed to make the solution conditions stated in particular Figure captions.

RESULTS AND DISCUSSION

Fig. 1 shows CD spectra of poly(amino^{2}dA-dT) during its NaCl-induced conformational transition, which are essentially identical to the spectra obtained previously by other authors (6). Low-salt spectrum of this polymer is unusual by a global redshift of the bands if compared to the spectra of other DNAs but we do not address this aspect here. It is more relevant to the main point of this article that the isomerization takes place at a relatively low-salt concentration, and that it is fast (repeatedly recorded spectra are identical at any point of the transition). The transition shows little, if any, particular salt specificity (not shown). Note the isodichroic point at 268.5 nm indicating that two distinct conformations of the polymer coexist in solution during the isomerization. Its cooperativity is much less than with the B-to-Z isomerization of poly(dG-dC) (4). What we mainly deal with in this article is a recent suggestion that this phenomenon is a salt--induced B-to-A conformational isomerization (7,8).

A common inducer of A-DNA is not high-salt but ethanol

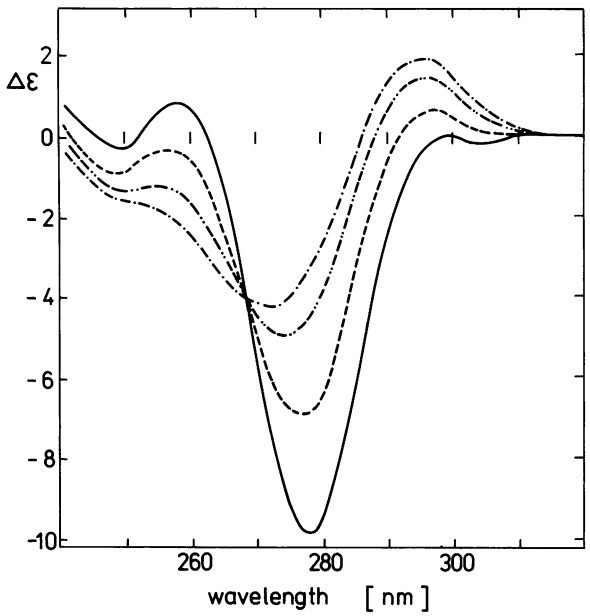

FIGURE 1: CD spectra of poly(amino^{2}dA-dT) in 5 mM sodium phosphate, pH 6.7, 0.5 mM EDTA and NaCl concentrations: -.- 0.14, --.. 0.59, ---- 1.14, and —— 2.24 M. Temperature 27 ^{o}C.

(15,16), though a B-to-A isomerization in poly(dG).poly(dC) gels used for Raman studies has recently been reported to be induced by a salt content elevation in the absence of alcohol (17,18). We wondered what the CD spectra of poly(amino^{2}dA-dT) looked like in aqueous ethanol solutions under conditions inducing A-DNA in poly(dA-dT) (19). The result is shown in Fig. 2 - the high-salt and ethanol conformations of poly(amino^{2}dA-dT) are identical. The spectra identity extends to short wavelengths, up to where we were able to get a reasonable CD signal. The short wavelength spectral region shows the presence of two additional isodichroic points, one at 207.5 nm and the other at 236.5 nm. The transition is reversible and fairly cooperative, its midpoint is unusually low, 47% v/v ethanol, compared to 69 % ethanol in the midpoint of the B-to-A transition of poly(dA-dT) (Inserts in Figs. 2,3), the kinetics is faster than can be measured by CD. Note the dramatic ellip-

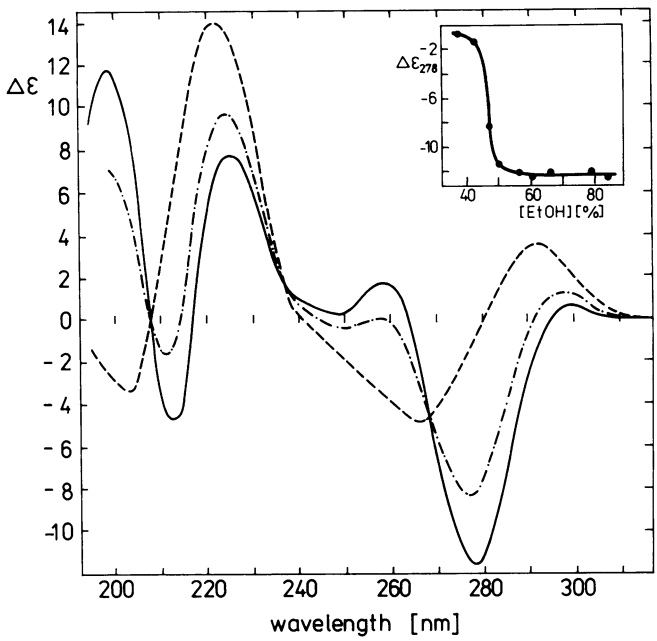

FIGURE 2: CD spectra of poly(amino^{2}dA-dT) in ethanol-water mixtures at 1 ^{o}C. 96% ethanol was added to the polynucleotide in 37% ethanol, 0.2 mM sodium phosphate, pH 6.8, and 0.02 mM EDTA to the resulting concentrations: --- 42.5, -·- 47.5, and —— 50.1 %. Insert: Dependence of ellipticity at 278 nm on ethanol concentration.

ticity change around 200 nm during the isomerization of poly (amino^{2}dA-dT) because this part of the spectrum is exceptionally sensitive to relative base pair disposition in the double helix (20,21).

Although the isomerization midpoint is unusually low and the CD spectrum of the putative A form is rather peculiar, there is no serious reason up to this point to doubt that poly(amino^{2}dA-dT) goes to A-DNA in high-salt or ethanol solutions. However, we know from our previous studies (9-11) that poly(dA-dT) adopts the same type of CD spectrum as poly(amino^2 dA-dT). Yet, in addition, poly(dA-dT) displays a completely different type of spectrum when it undergoes the transition to A conformation (19). To demonstrate this point, the B-to-A isomerization of poly(dA-dT) is shown in Fig. 3a and we point

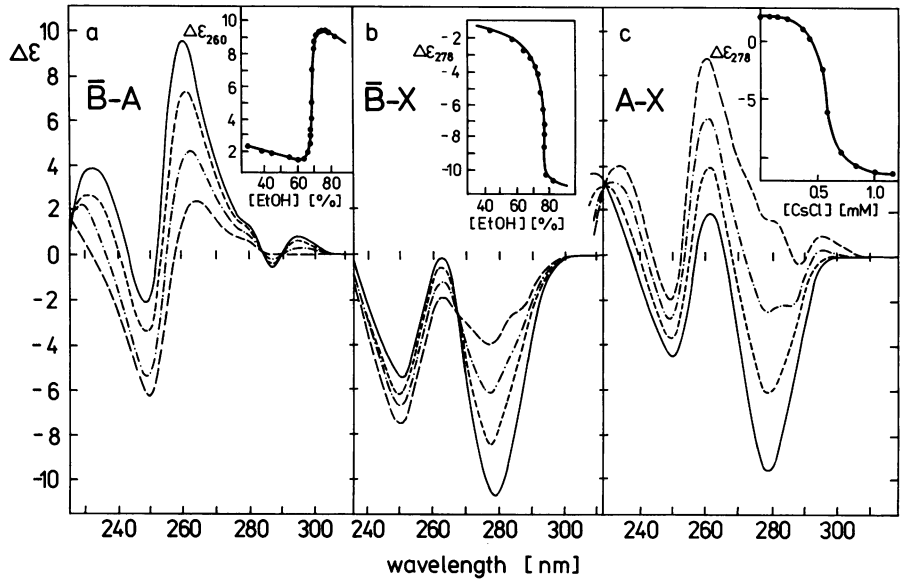

FIGURE 3: CD spectra of poly(dA-dT) in ethanol-water solutions:
a) During the A-to-B transition induced by decreasing ethanol concentration in 0.19 mM sodium phosphate, 0.048 mM EDTA to values: —— 68.6, ---- 66.8, —·— 66.2, and ———— 62.1 %. Insert: Dependence of ellipticity of the positive maximum on ethanol concentration. Temperature 10 °C.
b) During the X-to-B transition induced by decreasing ethanol concentration in 0.15 mM sodium phosphate, pH 6.8, 0.038 mM EDTA, and 1.3 mM CsCl to values: —— 79.4, ---- 77.1, —·— 75.4, and ———— 71.4 %. Insert: Dependence of the long wavelength band amplitude on ethanol concentration. Temperature 4 °C.
c) During the A-to-X transition induced by CsCl additions to the polynucleotide in 82% ethanol, 0.15 mM sodium phosphate, pH 6.8, and 0.04 mM EDTA to concentrations: — — 0.23, —·— 0.53, ---- 0.59, and —— 0.68 mM. Temperature 4 °C. Insert: Dependence of ellipticity at 278 nm on CsCl concentration.

out that not only poly(dA-dT) but many other synthetic and natural polydeoxynucleotide duplexes so far examined displayed the same resulting spectrum shape upon adopting A-DNA conformation (16,22-24). However, besides A-DNA poly(dA-dT) also goes to X-DNA in ethanol solutions if sodium cations stabilizing A-DNA are replaced by cesium cations (19). X-DNA CD spectrum

and the course of its appearance from $\overline{B}$-DNA (by $\overline{B}$-DNA a family of conformations is denoted which arise from low-salt aqueous B-DNA through gradual non-cooperative changes) are shown in Fig. 3b. The type of the resulting spectrum, kinetics and cooperativity of the CD changes and reversibility of the isomerization are the same as those observed with poly(amino^{2}dA--dT).

The present data do not exclude that X-DNA is a variant of A-DNA. This possibility is, however, eliminated by the experiment shown in Fig. 3c where an A-to-X transition of poly(dA--dT) is induced by the addition of cesium cations to its A form. The transition is clearly a cooperative two-state process during which A and X forms coexist in the polynucleotide molecules so that they are not mutually convertible in a gradual fashion. Hence the high-salt or alcohol form of poly(amino^{2}dA-dT) cannot be A-DNA.

Trifluoroethanol (TFE, 25) is a better inducer of A-DNA in poly(dA-dT) than ethanol. Using it we were, however, unable to induce its X form (unpublished data). But in poly(amino^{2}dA-dT) even trifluoroethanol induced X-DNA (Fig. 4). Midpoints of the transition induced in poly(amino^{2}dA-dT) by ethanol and trifluoroethanol are identical, which is interesting in the light of their distinct effectivity to induce X form of poly(dA-dT). Methanol does not induce A form in DNA (26), and this is especially the case with its AT rich molecules (27). However, the transition of poly(amino^{2}dA-dT) is also induced by methanol with a midpoint of 69%. Under these conditions no transition is observed with poly(dA-dT). But if one adds a little bit of cesium cations and omits EDTA from the solution to let trace amounts of divalent cations operate, then the same transition is observed (27, Fig. 4). The experiments in trifluoroethanol and methanol solutions indicate how crucial the amino group in the double helix minor groove is for DNA conformational variability and, simultaneously, how deeply the variability is controlled by hydration and ion binding. This work illuminates the mysterious dependence of poly(dA-dT) conformational variability on the presence of cesium cations.

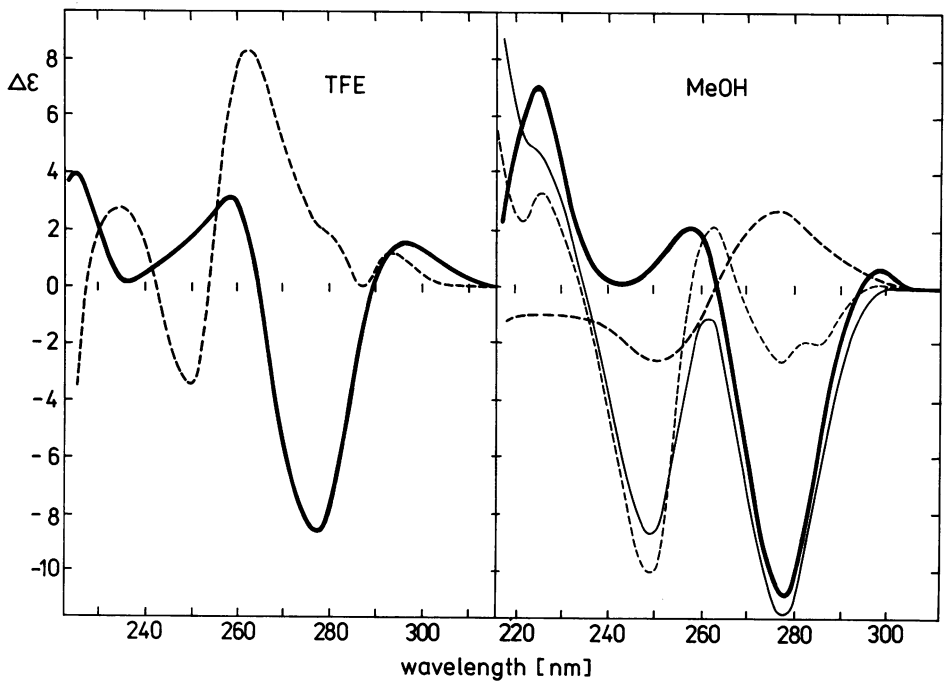

FIGURE 4: CD spectra of poly(amino^{2}dA-dT) and poly(dA-dT) in aqueous trifluoroethanol (left) and methanol (right) solutions. Left: ——— poly(amino^{2}dA-dT) in 81.6 % TFE, 0.067 mM sodium phosphate, 0.0067 mM EDTA, temperature 0 °C, ---- poly(dA-dT) in 80 % TFE, 0.66 mM sodium phosphate, 0.0066 mM EDTA, temperature 16 °C. Right: ——— poly(amino^{2}dA-dT) in 73.8 % methanol, 0.08 mM sodium phosphate, 0.008 mM EDTA, – – – poly (dA-dT) in 81.6 % methanol, 0.08 mM sodium phosphate and 0.02 mM EDTA, ---- poly(dA-dT) in 71.4 % methanol and 0.14 mM sodium phosphate without EDTA, ——— poly(dA-dT) in 71.4 % methanol, 0.14 mM sodium phosphate, and 0.28 mM CsCl.

No similar effect is observed with poly(amino^{2}dA-dT) so that the extra amino group attached to A and cesium cations are very likely to make the same thing - dehydrate the minor groove, this way destabilize B form of the polynucleotide and relieve its conformational variability. The presence of cesium cations in B-DNA minor groove has been demonstrated experimentally (28).

It follows from the data presented in this article that it is not a change in optical activity due to the extra amino group of dA but rather an unusual double helix conformation

which causes the peculiar shape of the CD spectrum of poly(amino^{2}dA-dT) in high-salt or alcohol solutions. Furthermore, other analogs of poly(dA-dT) having ethyl or butyl in place of thymine methyl, which substitution does not change the polymer optical properties (29), behave like poly(amino^{2}dA-dT) in ethanol solutions (our unpublished data), i.e. they isomerize into X-DNA under conditions inducing A-DNA in poly(dA-dT).

This work demonstrates that the duplex of poly(amino^{2}dA-dT) adopts X-DNA conformation in high-salt or alcohol solutions. This unusual conformation was first observed with poly (dA-dT) but under extremely stringent conditions, which did not allow its structure determination. Nevertheless, we know that it is similar to Z-DNA in the zig-zag character of the backbone but their dinucleotide repeats are inverted (11). Zig-zag backbone of the high-salt form of poly(amino^{2}dA-dT) is suggested by its recognition by Z-DNA specific antibodies (30) and by two well separated ^{31}P NMR resonances (6) whose assignment is opposite to Z-DNA and the same as with the X form of poly(dA-dT) (11). Stability of poly(amino^{2}dA-dT) in the X form and the ease with which it can be induced is what is needed for its more detailed characterization that is already in progress.

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