Articles

Thymine Methyl Groups Stabilize the Putative A-Form of the Synthetic DNA Poly(amino^{2}dA-dT)

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ABSTRACT: Poly(amino^{2}dA-dT) easily isomerizes into a non-B conformer which most authors think is an A-form. We synthesized new DNA analogs poly(amino^{2}dA-ethyl^{5}dU) and poly(amino^{2}dA-dU) to show that they do not prefer this conformer. Hence the putative A-form is, like Z-DNA of poly(dG-dC) but unlike A-DNA, strongly stabilized by the methyl group in position 5 of the pyrimidine base. In addition, the putative A-form is induced by divalent cations while it does not need any alcohol to be stable, both properties being typical for Z-DNA again but quite unusual with A-DNA. Despite these similarities, the putative A-form is also distinct from Z-DNA, as poly(amino^{2}dA-dT) is shown to isomerize into a Z-form in the NaCl + $NiCl_2$ solvent system like poly(dA-dT). The present data indicate that the putative A-form of poly(amino^{2}dA-dT) differs in a significant way from all canonical conformers of DNA. Furthermore, the studies of the poly(amino^{2}dA-dT) family of polydeoxynucleotides reveal a novel type of conformational switch in DNA. We also report the B–Z transitions of poly(amino^{2}dA-ethyl^{5}dU) and poly(amino^{2}dA-dU) and their transitions into the putative A-form in aqueous alcohol solutions.

Nucleic acids contain four canonical bases. Three of them, i.e. adenine, cytosine, and guanine, are shared by RNA and DNA, but the fourth is specific because uracil occurs in RNA but 5-methyluracil, i.e. thymine, occurs in DNA. Two complex enzymes, thymidylate synthase and uracil glycosylase, participate in the processes whose result is the presence of thymine instead of uracil in DNA. Inactivation of any of the enzymes is lethal to indicate that the thymine methyl group is essential for DNA to function properly. However, the role of the thymine methyl group is unknown. On the other hand, position 5 of cytosine is also methylated in the genomes of many organisms and the methylation has profound biological consequences in higher eukaryotes including humans.

The methyl group in position 5 of the pyrimidine bases exerts profound effects on DNA conformation. Poly(dA-dT) coexists in two conformers (Kypr et al., 1990) while poly(dA-dU) adopts a single conformation with some properties resembling those of RNA (Kypr & Vorlíčková, 1985). In addition, methylation of a synthetic RNA poly(rA-rU) gives poly(rA-rT), which possesses some properties of DNA (Vorlíčková et al., 1990). Furthermore, poly(dA-dT), unlike poly(dA-dU) (Wu & Behe, 1985), has an alternating backbone (Klug et al., 1979; Shindo et al., 1979) while cytosine methylation, which also makes the regular B-DNA conformer of poly(dG-dC) alternating (Chen et al., 1983), strongly promotes Z-DNA (Behe & Felsenfeld, 1981). Interestingly, while methylation promotes the B–Z transition of poly(dG-dC), it inhibits the A–Z transition of its RNA analog (Jovin et al., 1987). In order to understand the conformational effects of the methyl group, we have previously synthesized many DNAs containing ethyl and other substituents in position 5 of uracil (Sági et al., 1977, 1979, 1980, 1982, 1986, 1987, 1992; Ötvös et al., 1987; Sági & Ötvös, 1979, 1980) and cytosine (Vorlíčková & Sági, 1989; Sági et al., 1991) and studied their biophysical and biochemical properties. The studies demonstrate that the methyl group is optimum to stabilize B-type conformations of the alternating purine–pyrimidine DNAs against thermal melting because its replacement by both hydrogen, ethyl, and other aliphatic substituents leads to the duplex destabilization (Sági et al., 1979, 1991).

Here we extend our previous effort by a synthesis and analysis of new DNA analogs poly(amino^{2}dA-ethyl^{5}dU) and poly(amino^{2}dA-dU) to understand the conformational behavior of poly(amino^{2}dA-dT), an alternating copolymer of thymine and 2-aminoadenine, a rare base which replaces adenine in the genome of S-2L cyanophage (Kirnos et al., 1977; Khudyakov et al., 1978). This polydeoxynucleotide undergoes a facile conformational transition induced by either high salt concentrations (Gaffney et al., 1982, 1984; Jovin et al., 1983; Howard et al., 1984), various alcohols (Vorlíčková et al., 1988a), submillimolar concentrations of divalent magnesium cations in low-salt aqueous solution (Vorlíčková et al., 1988b), or polyamines (Garriga et al., 1993). The molecular structure of the resulting conformer has not yet been determined, but the results of most studies were interpreted in terms of an A-form (Borah et al., 1985, 1986; Alexeev et al., 1992; Garriga et al., 1992, 1993; Mojzeš et al., 1992; Konyukhov et al., 1992). Though other interpretations of the isomerization also exist (Vorlíčková et al., 1988a,b), there is one important conclusion on which all of the previous studies agree. The conclusion is that the polydeoxynucleotide

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Table 1: UV Spectroscopic and Thermal Melting Properties of the Polydeoxynucleotides Used in This Study

	UV[a]				thermal denaturation[b]		
polydeoxynucleotide	max (nm)	min (nm)	280/260	ϵ_{260} (M^{-1} cm^{-1})	T_m (°C)	H_{260} (%)	ΔT^c (°C)
poly(dA-dU)	261	233	0.53	6660	56.3	50.0	2.2
poly(dA-dT)	262	235	0.57	6640	59.8	52.1	2.0
poly(dA-ethyl^{5}dU)	262	235	0.55	6680	48.8	51.0	3.0
poly(amino^{2}dA-dU)	258	238	0.60	5920	73.6	44.6	4.7
poly(amino^{2}dA-dT)	260	239	0.74	5220	80.7	35.8	2.8
poly(amino^{2}dA-ethyl^{5}dU)	260	239	0.71	5630	67.7	36.9	4.9

[a] Spectra were measured at 25 °C in buffered 0.1 M Na^+, pH 7, after a heat denaturation–renaturation cycle in a Shimadzu UV-160 spectrophotometer. [b] Thermal transitions were determined in buffered 0.1 M Na^+, pH 7. [c] Half width of the denaturation.

isomerizes into a non-B conformer, which in connection with the possibility to induce the isomerization by divalent cations in low-salt aqueous solution (Vorlíčková et al., 1988b) or polyamines (Garriga et al., 1993) makes both the isomerization and the resulting conformer of considerable interest.

Here we analyze the isomerization and the resulting conformer using poly(amino^{2}dA-ethyl^{5}dU), poly(amino^2-dA-dU), and other related DNAs to show that stability of the putative A-form of poly(amino^{2}dA-dT) is strongly promoted by the thymine methyl group. This property and several others, which are summarized in the Discussion part of this article, are quite opposite to properties of A-forms exhibited by most other DNAs. That is why we will call the A-like conformer of poly(amino^{2}dA-dT) a putative A-form until its molecular structure is conclusively determined.

MATERIALS AND METHODS

Synthesis of Triphosphates. The triphosphates dUTP, dTTP, and dATP were from Sigma GmbH. Amino^{2}dAT and ethyl^{5}dUTP were synthesized as described earlier (Vorlíčková et al., 1988a; Sági et al., 1977).

Synthesis of Polydeoxynucleotides. The novel, alternating polydeoxynucleotides poly(amino^{2}dA-ethyl^{5}dU) and poly-(amino^{2}dA-dU) were synthesized by a step-wise scale-up method as described earlier for poly(amino^{2}dA-dT) (Vorlíčková et al., 1988a). Poly(dA-dU) was the starting template primer for both polymers. The template primer constituted less than 0.7% of the final, pure, high molecular weight polydeoxynucleotide products. The total volume of the reaction mixture was 10 mL, and Klenow DNA polymerase reactions yielded 20 and 33 OD_{260} units of pure polydeoxynucleotides, respectively. Their compositions were determined by HPLC (ISCO) analysis of the enzymatic hydrolysates (DNase I, PDE, and alkaline phosphatase, 0.5 mg each in a 0.6-mL mixture of 10 mM potassium phosphate, pH 7.4, 5 mM $MgCl_2$, and 0.05 mg/mL polydeoxynucleotide), as described (Vorlíčková et al., 1993). The hydrolysis yielded equimolar purine–pyrimidine deoxynucleoside contents within the experimental error. ϵ_{260}-values (pH 7) of 10 100 for dU, 8750 for dT (*CRC Handbook of Biochemistry and Molecular Biology*, 1975) and ethyl^{5}dU (Sági et al., 1977), 15 200 for dA (*CRC Handbook of Biochemistry and Molecular Biology*, 1975), and 8590 for amino^{2}dA (Sági, J., unpublished) were used. Molar extinction coefficients of the polydeoxynucleotides at 260 nm were determined from the total hyperchromic change upon enzymatic hydrolysis to deoxynucleosides and by using their above ϵ_{260}-values. Poly(amino^{2}dA-dT), poly-(dA-dU), poly(dA-dT), and poly(dA-ethyl^{5}dU) were prepared as described (Vorlíčková et al., 1988a; Sági et al., 1977).

UV Absorption and CD Spectroscopy Measurements. UV absorption spectra were recorded on a Philips PU 8750 (Brno laboratory) spectrophotometer or a Hewlett-Packard HP8452A spectrophotometer (Budapest laboratory). Thermal transition profiles were determined as described (Vorlíčková et al., 1993) except that a 1 cm path length cell and a 0.7-mL volume were used.

A Jobin–Yvon, Mark IV, dichrograph was used to measure the CD spectra. The measurements were carried out in 1 cm path length cells placed in a thermostated holder. The instrument was calibrated with isoandrosterone. The polydeoxynucleotide concentrations (about 0.1 mM DNA phosphates) were determined using the molar extinction coefficients summarized in Table 1.

RESULTS

Duplex Thermostabilities, UV Absorption, and CD Spectra. Table 1 contains UV absorption spectral characteristics of the polynucleotide duplexes studied. The duplex thermostability of poly(dA-dU) and poly(dA-ethyl^{5}dU) strongly increased with the replacement of dA by amino^{2}dA, as in the case of poly(dA-dT) (Sági et al., 1989, and references therein). The melting temperatures were higher by 17.3, 18.9, and 20.9 °C, respectively, while the highest increase in T_m (20.9 °C) caused by the substitution of 2-aminoadenine for adenine was observed with the polydeoxynucleotide containing the methyl group in position 5 of the pyrimidine base, i.e. with poly-(amino^{2}dA-dT).

UV absorption spectra of double-stranded poly(amino^2-dA-dU), poly(amino^{2}dA-dT), and poly(amino^{2}dA-ethyl^{5}dU) are shown in Figure 1, along with the UV absorption spectra of the corresponding counterparts containing adenine instead of 2-aminoadenine. Generally, the replacement of adenine by 2-aminoadenine in the alternating purine–pyrimidine DNAs causes a depression of the major UV absorption band at 260 nm while a shoulder simultaneously appears above 280 nm. This modification of the UV absorption properties is reflected in the marked red shift of their CD spectra (Figure 1). However, differences in the CD spectra of the adenine- and 2-aminoadenine-containing polydeoxynucleotide duplexes do not only originate from their different chromophores because they assume rather different B-forms. This follows from studies of mixed copolymers of poly(dA,amino^{2}dA-dT) containing various proportions of adenine and 2-aminoadenine (Kypr, J., et al., unpublished). On the other hand, the aliphatic substituents in position 5 of the pyrimidine base influence both UV and CD spectra much less.

We measured how the polydeoxynucleotide CD spectra change with temperature and found that, prior to denaturation, the changes were smaller with the present duplexes containing 2-aminoadenine than with the duplexes containing adenine. It seems that the substitution of adenine by 2-aminoadenine gives rise to a rigid B-type conformation which does not much depend on whether a hydrogen atom or methyl or ethyl group is placed in position 5 of the pyrimidine base. The CD spectra of the 2-aminoadenine-containing polydeoxynucleotides pass through isodichroic points (not shown) during the thermal

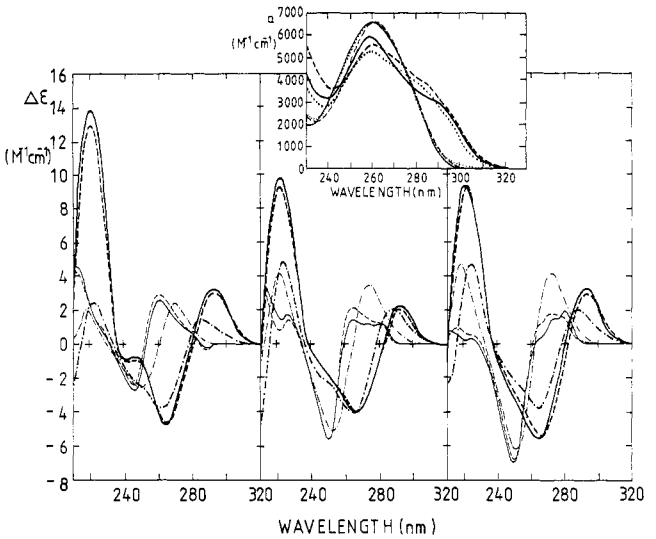

FIGURE 1: CD spectra of the polydeoxynucleotides measured in 5 × 10^{-4} M potassium phosphate and 5 × 10^{-5} M EDTA, pH 7.2, at low temperature (—) and at temperatures before (- - -) and after (·· ·· ··) melting. The spectra of the 2-aminoadenine-containing polydeoxynucleotides are drawn in bold. Left: poly(dA-dU) 0.2, 5.6, and 41.6 °C; poly(amino²dA-dU) 4.4, 33.7, and 63.3 °C. Middle: poly(dA-dT) 0.9, 18, and 38.6 °C; poly(amino²dA-dT) 3.7, 36.2, and 51.3 °C. Right: poly(dA-ethyl⁵dU) 0, 14.5, and 25.3 °C; poly(amino²dA-ethyl⁵dU) 0, 14.5, and 25.3 °C; poly(amino²dA-ethyl⁵dU) 4.7, 34.3, and 64.5 °C. Inset: UV absorption spectra of (thin lines) (—) poly(dA-dU), (···) poly(dA-dT), (- - -) poly(dA-ethyl⁵dU); (bold lines) (—) poly(amino²dA-dU), (···) poly(amino²dA-dT), and (- - -) poly(amino²dA-ethyl⁵dU) measured in 5 × 10^{-4} M potassium phosphate and 5 × 10^{-5} M EDTA, pH 7.2, at temperatures before melting.

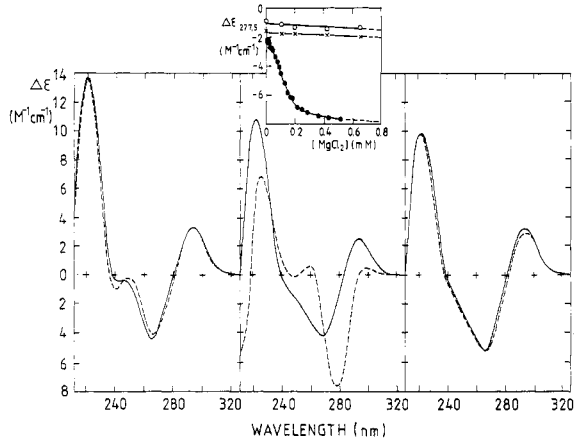

FIGURE 2: Effects of $MgCl_2$ on the CD spectra of the polydeoxynucleotides in 5 × 10^{-4} M potassium phosphate and 5 × 10^{-5} M EDTA, pH 7.2. Left: poly(amino²dA-dU) (—) 0 M $MgCl_2$, (- - -) 0.66 mM $MgCl_2$. Middle: poly(amino²dA-dT) (—) 0 M $MgCl_2$, (- - -) 0.44 mM $MgCl_2$. Right: poly(amino²dA-ethyl⁵dU) (—) 0 M $MgCl_2$, (- - -) 0.65 mM $MgCl_2$. Inset: Dependences of ellipticity at 277.5 nm on the concentration of $MgCl_2$: (○) poly(amino²dA-dU); (●) poly(amino²dA-dT); (×) poly(amino²dA-ethyl⁵dU).

denaturations which are S-shaped, reversible, but less cooperative than thermal transitions of the analogs not containing 2-aminoadenine (Table 1).

Conformational Transition Induced by Low Concentrations of $MgCl_2$. Poly(amino²dA-dT) easily isomerizes into the putative A-form. For example, the isomerization is induced by submillimolar concentrations of $MgCl_2$ in low-salt aqueous solution (Vorlíčková et al., 1988b; Figure 2). During the transition, a strong negative CD band appears close to 280 nm. The transition is quite cooperative. However, it is peculiar by being switched immediately after the first salt addition.

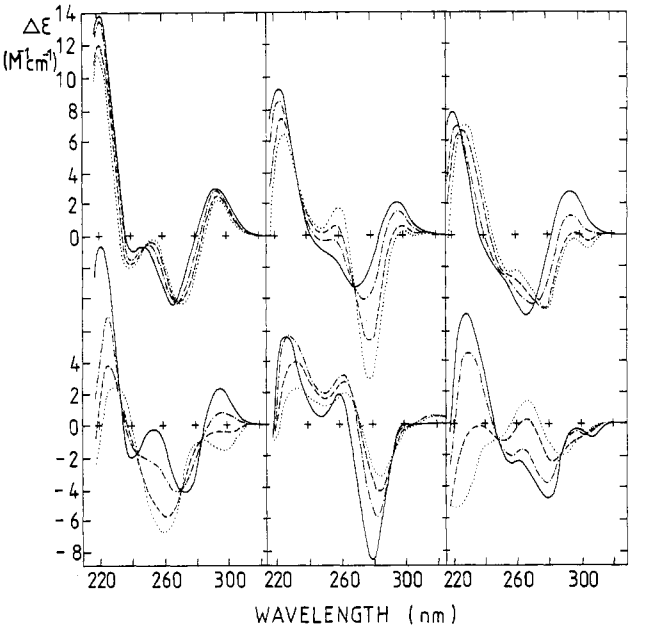

FIGURE 3: Effects of NaCl (top) and NaCl + $NiCl_2$ (bottom) on the CD spectra of poly(amino²dA-dU), poly(amino²dA-dT), and poly(amino²dA-ethyl⁵dU). Top left: poly(amino²dA-dU) in 10 mM sodium phosphate and 0.1 mM EDTA, pH 6.9, and (—) 0 M NaCl, (·· ·· ··) 2.8 M NaCl, (- - -) 4.2 M NaCl, and (···) 5 M NaCl; then 2 M $NiCl_2$ was added up to the concentrations (bottom left) (—) 0 mM, (·· ·· ··) 12.5 mM, and (- - -) 12.5 mM after 10 min and (···) 12.5 mM after 65 min. Top middle: poly(amino²dA-dT) in 10 mM Tris-HCl and 0.1 mM EDTA, pH 7.6, and (—) 0 M NaCl, (·· ·· ··) 0.75 M NaCl, (- - -) 1.5 M NaCl, and (···) 4 M NaCl; then 2 M $NiCl_2$ was added up to the concentration (bottom middle) 21.2 mM, measured after (—) 0, (·· ·· ··) 60, (- - -) 210, and (···) 560 min. Top right: poly(amino²dA-ethyl⁵dU) in 10 mM Tris-HCl and 0.1 mM EDTA, pH 7.5, and (—) 0 M NaCl, (·· ·· ··) 3.3 M NaCl, (- - -) 4.0 M NaCl, and (···) 5 M NaCl; then 2 M $NiCl_2$ was added up to the concentrations (bottom right) (—) 0 mM, (·· ·· ··) 14.9 mM, (- - -) 16.9 mM, and (···) 16.9 mM after 75 min.

There is no preparatory step typical of cooperative phenomena, so that it appears that the isomerizing conformations already coexist in the polydeoxynucleotide before the extra salt is added.

We did parallel experiments with poly(amino²dA-dU) and poly(amino²dA-ethyl⁵dU) and found that neither underwent this transition up to 1.5 M $MgCl_2$ (Figure 2). Similarly, nearly no changes were observed in the CD spectra of poly(dA-dU), poly(dA-dT), and poly(dA-ethyl⁵dU) up to 1.5 M $MgCl_2$ (not shown). Hence the amino group in the minor groove and the thymine methyl group in the major groove both play a central role in stabilization of the putative A-form of poly(amino²dA-dT).

Conformational Transitions in NaCl and NaCl + $NiCl_2$. Poly(amino²dA-dT) isomerizes into the putative A-form in concentrated aqueous solutions of NaCl as well (Figure 3). This NaCl-induced conformational transition was first reported a decade ago (Gaffney et al., 1982, 1984; Jovin et al., 1983; Howard et al., 1984). In contrast, poly(amino²dA-dU) displays only small and gradual NaCl-induced CD changes which mainly include a shift of the CD spectrum to the red. The spectral changes may reflect conformational alterations of the polydeoxynucleotide in the direction toward the putative A-form, which, however, is very far from being achieved even in 5 M NaCl. Poly(amino²dA-ethyl⁵dU) is perhaps more prone to adopt the putative A-form than poly(amino²dA-dU). High NaCl concentrations induce slightly cooperative CD spectral changes with this polydeoxynucleotide, but the putative A-form is hardly adopted as well (Figure 3). The

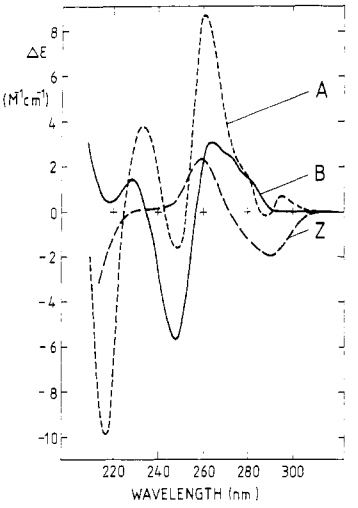

FIGURE 4: CD spectra of the B-, A-, and Z-forms of double-stranded poly(dA-dT). B-form was measured in 1 mM sodium phosphate and 0.3 mM EDTA, pH 7, A-form in 0.18 mM sodium phosphate, 0.05 mM EDTA, pH 7, and 79% ethanol, and Z-form in 10 mM Robinson–Britten buffer and 5 M NaCl, pH 5.7, and 54 mM $NiCl_2$. The A-form CD spectrum was obtained at 4 °C; the other CD spectra were measured at room temperature.

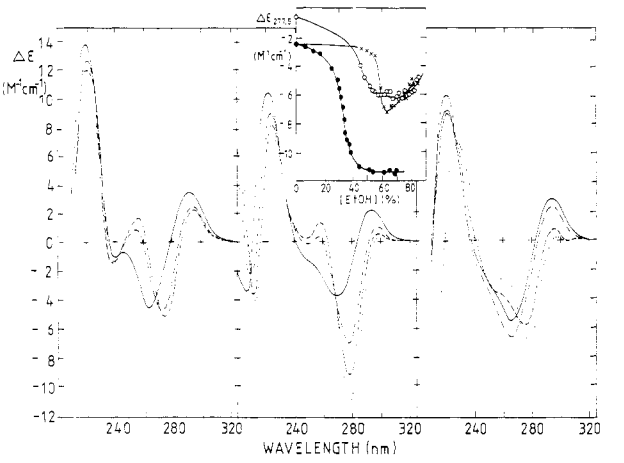

FIGURE 5: Ethanol effects on the CD spectra of (left) poly(amino²-dA-dU), (middle) poly(amino²dA-dT), and (right) poly(amino²dA-ethyl⁵dU). Ethanol (96%) was added (at 0 °C) to the polydeoxynucleotides dissolved in 1 mM potassium phosphate and 0.1 mM EDTA, pH 7.2, up to the concentrations (left) (—) 0%, (-· -· -·) 45.4%, (- - -) 47.3%, and (···) 57.6; (middle) (—) 0%, (-· -· -·) 32.6%, (- - -) 36.0%, and (···) 44.3%; (right) (—) 0%, (-· -· -·) 54.9%, (- - -) 58.4%, and (···) 61.4% (v/v). Inset: Dependences of ellipticity at 277.5 nm on the ethanol concentration: (○) poly(amino²dA-dU); (●) poly(amino²dA-dT); and (×) poly(amino²dA-ethyl⁵dU).

shift of the spectrum to the red is accompanied by a relatively extensive depression of the positive long-wavelength band, and a small negative band appears at about 300 nm. In contrast, NaCl only induces gradual changes in the CD spectra of the B-forms of poly(dA-dU), poly(dA-dT), and poly(dA-ethyl⁵dU). The CD spectra are all depressed in the whole spectral region, and a negative band appears in the long-wavelength region like the case with poly(amino²dA-ethyl⁵dU). However, the bands are not shifted, so that the CD spectra in the absence and presence of 5 M NaCl share the minimum at 245–250 nm, the maximum at 261–263 nm, and a small negative minimum at 285–280 nm (not shown).

We managed to transform all three 2-aminoadenine-containing polydeoxynucleotides into Z-form at high NaCl concentrations upon the addition of divalent nickel cations, i.e. under conditions when poly(dA-dT) isomerizes into Z-form (Adam et al., 1986; Bourtayre et al., 1987; Ridoux et al., 1988). The CD spectrum of the Z-form of poly(dA-dT) is shown in Figure 4. While 90 mM Ni^{2+} induced the transition of poly(dA-dT) at high NaCl concentrations and neutral pH, considerably lower Ni^{2+} concentrations were needed to induce the Z-form with the analogs containing 2-aminoadenine (Figure 3). Though the CD spectra of poly(dA-dT) and poly(amino²dA-ethyl⁵dU) are quite dissimilar in 5 M NaCl, they become very similar as soon as the polydeoxynucleotides assume the Z-form (compare Figures 3 and 4). This similarity is surprising because the two polydeoxynucleotides have different chromophores and their B-form CD spectra are very different. Both B–Z transitions have slow kinetics.

About the same Ni^{2+} concentrations as with poly(amino²-dA-ethyl⁵dU) induce a time-dependent two-state isomerization of poly(amino²dA-dU). The arising spectrum is an inversion of the original spectrum in the sense that the maxima become minima and vice versa, but it is considerably different from the CD spectra of the Z-forms of poly(dA-dT) and poly(amino²dA-ethyl⁵dU). Nevertheless, the two-state nature of this process leading to a CD spectrum inversion, its long kinetics, and the characteristic conditions of induction make it probable that it indeed is a B–Z transition. Probably poly(amino²dA-ethyl⁵dU) and poly(amino²dA-dU) isomerize into different variants of Z-form. Poly(dA-dU) and poly(dA-ethyl⁵dU) also isomerize into a different variant of Z-form than poly(dA-dT) (M. Vorlíčková, unpublished).

Poly(amino²dA-dT) isomerizes into Z-DNA less easily than poly(amino²dA-dU) and poly(amino²dA-ethyl⁵dU). Addition of Ni^{2+} to poly(amino²dA-dT) in 5 M NaCl leads to a polydeoxynucleotide aggregation hindering its B–Z transition. However, poly(amino²dA-dT) undergoes the transition as soon as the NaCl concentration is lowered while the concentration of Ni^{2+} is comparable to those inducing the transition with poly(amino²dA-ethyl⁵dU) and poly(amino²dA-dU). Yet the kinetics is substantially slower, and the polydeoxynucleotide still tends to aggregate, as indicated by a nonzero CD signal at wavelengths longer than 310 nm (Figure 3). The Ni^{2+}-induced CD changes of poly(amino²dA-dT) take place in two steps, and the aggregation is strong in the preparatory step while it no longer increases during the isomerization. Perhaps the stability of poly(amino²dA-dT) in the putative A-form and the corresponding unwillingness to leave this conformation stand behind this behavior.

Conformational Transitions in Aqueous Ethanol Solutions. Various alcohols including methanol, ethanol, and trifluoroethanol also transform poly(amino²dA-dT) into the putative A-form (Vorlíčková et al., 1988a; Figure 5). However, the A-form CD spectrum of poly(dA-dT) (Vorlíčková et al., 1988a) is quite distinct by containing the canonical positive band at 260 nm and a deep negative band at 220 nm (Figure 4). Poly(amino²dA-dU) shows a stronger tendency to undergo the transition into the putative A-form in the alcohol solutions (Figure 5) than in aqueous solutions containing various salts. Yet, the resulting CD spectrum is much weaker than with poly(amino²dA-dT). The same is true with poly(amino²dA-ethyl⁵dU). Midponts of the transitions are 52, 33, and 58% ethanol (v/v) with the the amino²adenine-containing polydeoxynucleotides containing U, T, and ethyl⁵U, respectively. The midpoint differences also indicate the importance of the thymine methyl group in stabilization of the putative A-form. In contrast, the canonical A-form appears at a lower ethanol concentration with poly(dA-dU) as compared to poly(dA-dT) while poly(dA-ethyl⁵dU) cannot adopt the A-form at all (Vorlíčková et al., 1991).

DISCUSSION

The present study extends the previous work of our and other laboratories on poly(amino^{2}dA-dT) by a synthesis and biophysical studies of poly(amino^{2}dA-dU) and poly(amino^2-dA-ethyl^{5}dU). The most interesting result of this work is the demonstration that the thymine methyl group strongly stabilizes the putative A-form of poly(amino^{2}dA-dT). Neither poly(amino^{2}dA-ethyl^{5}dU) nor poly(amino^{2}dA-dU) isomerizes into the putative A-form in the low-salt aqueous solution containing divalent magnesium cations; their isomerizations into this conformer in concentrated NaCl solutions are doubtful while the CD spectral changes of these two polydeoxynucleotides are rather small even in aqueous ethanol solutions. It follows from our previous studies that aliphatic substituents in position 5 of the pyrimidine bases destabilize A-DNA (Vorlíčková et al., 1991; Vorlíčková and Sági, 1991; Sági et al., 1991). We therefore expected that if the conformer into which poly(amino^{2}dA-dT) isomerizes so easily is actually A-DNA, then poly(amino^{2}dA-dU) will adopt it even constitutively. However, the present experiments unambiguously speak against this notion, so that either the experience following from studies of the corresponding poly(dA-dT) (Vorlíčková et al., 1991), poly(dI-dC) (Vorlíčková & Sági, 1991), and poly(dG-dC) (Sági et al., 1991) families cannot be extrapolated to the family of poly(amino^{2}dA-dT), which is unlikely, or their A-forms are significantly different.

The B–A transition has been studied extensively in the past, and the conclusions of these studies relevant to the present work are the following: (i) High ethanol or trifluoroethanol but not methanol concentrations induce the B–A transition (Malenkov et al., 1975). In contrast, the putative A-form of poly(amino^{2}dA-dT) is stable in aqueous methanol solutions (Vorlíčková et al., 1988a) or even needs no alcohol or high-salt concentration to be stable (Vorlíčková et al., 1988b; Garriga et al., 1993). (ii) Other than very low salt concentrations destabilize A-DNA (Ivanov et al., 1974). In contrast, the putative A-form of poly(amino^{2}dA-dT) is even stabilized by high-salt concentrations (Howard et al., 1984). In this respect, the behavior of poly(amino^{2}dA-dT) supports a theory predicting stability of A-DNA at high-salt concentrations (Soumpasis et al., 1987). (iii) Even traces of divalent cations including magnesium ones destabilize A-DNA (Ivanov et al., 1974) while the putative A-form of poly(amino^{2}dA-dT) is stabilized by traces of divalent cations (Vorlíčková et al., 1988b).

We have found previously that the isomerization of poly(amino^{2}dA-dT) takes place at solution conditions, both low salt and high salt, identical to those for the B–Z isomerization of poly(dG-methyl^{5}dC) (Vorlíčková et al., 1988b). In addition, the conformational isomerizations are strongly promoted by the amino group in position 2 of the purine base and by the methyl group in position 5 of the pyrimidine base because only poly(dG-methyl^{5}dC) but not poly(dI-methyl^{5}dC) and poly(dG-dC) adopt Z-form easily. The same is true regarding the putative A-form of poly(amino^{2}dA-dT) as compared to poly(dA-dT) and poly(amino^{2}dA-dU). The analogy further continues by the fact that both conformers provide widely separated ^{31}P NMR resonances, suggesting similar zigzag backbones (Chen et al., 1983; Howard et al., 1984). So it seems that the amino groups in the double-helix minor groove cooperate with the methyl groups in the double-helix major groove and with the divalent cations to stabilize the zigzag backbones of both Z-form and the putative A-form.

A recent paper (Guenther et al., 1992) reports on a loop geometry rearrangement in a DNA hairpin induced by a cooperation of divalent magnesium cations with the methyl group in position 5 of the pyrimidine base. This observation demonstrates, like the present communication, that the methyl group can cooperate with divalent cations to cause various structural rearrangements in DNA.

The base pairs adopt the inverted topology in Z-DNA (Wang et al., 1979) as compared to the canonical B- and A-forms. The ease of the transition of poly(amino^{2}dA-dT) from its B-form into the putative A-form suggests that their base-pair topologies are the same. However, the putative A-form has a zigzag backbone, which is a feature discriminating it significantly from the A-forms of poly(dA-dT) and natural DNAs, whose backbones are regular (Shindo et al., 1985; Kypr et al., 1986). This picture is complemented here by the attempts to induce the B–Z transition of the present polydeoxynucleotides. The Z-form is adopted by poly(amino^2-dA-dU), poly(amino^{2}dA-dT), and poly(amino^{2}dA-ethyl^{5}dU), so that the methyl group in position 5 of the pyrimidine base is not necessary for Z-form stability. In fact, the methyl group rather destabilizes Z-form in this family of polydeoxynucleotides because the Z-form is unwillingly adopted by poly(amino^{2}dA-dT). Presumably, the destabilization is caused by the high polydeoxynucleotide stability in the putative A-form. Poly(amino^{2}dA-dU) and poly(amino^{2}dA-ethyl^{5}dU) exhibit just the opposite preference, being unstable in the putative A-form but relatively stable in the Z-form. This observation indicates that the Z-form and putative A-form are mutually exclusive; i.e., if a polydeoxynucleotide easily adopts one of them, then it is unstable in the other and vice versa. The two structures share significant backbone features and are even stabilized by the same combination of base exocyclic groups and divalent cations, but nevertheless, they are substantially different. The substantial difference is also suggested by the long-lasting kinetics of the putative A–Z transition of poly(amino^{2}dA-dT).

Studies of poly(amino^{2}dA-dT) and its analogs extended our knowledge about the conformational possibilities of DNA by a demonstration that there is a mechanism to induce a non-B/non-Z conformer in linear DNA under conditions which do not denature proteins of DNA metabolism. This result opens the door for a deeper understanding of how DNA works.

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