Conformational transitions of poly(dA-bromo\textsubscript{5}dU) and poly(dA-iodo\textsubscript{5}dU) in solution

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ABSTRACT

Extensive circular dichroism studies have been conducted with the title polynucleotides under various solution conditions. The studies provided the following information: (i) The halogen atoms in place of thymine methyl hinder the isomerization into X-DNA. (ii) The brominated but not iodinated polynucleotide isomerizes into Z-DNA in concentrated NaCl + NiCl\textsubscript{2}. The transition takes place at lower NiCl\textsubscript{2} concentrations than with poly(dA-dT). (iii) The iodinated polynucleotide forms an unusual conformation in aqueous solution in which it is very stable. It isomerizes from this conformer into the usual B-type double helix in concentrated ethanol solutions. The isomerization is a two-state cooperative process. (iv) Both title polynucleotides undergo still another two-state cooperative transition in trifluorethanol solutions presumably into A-DNA showing a rather unusual circular dichroism spectrum.

INTRODUCTION

Bromo\textsuperscript{5}uracil is a biologically interesting analog of thymine whose mutagenicity is believed to be due to its ability to mispair with guanine during DNA replication (for review see ref. 1). The alternating copolymer poly(dA-br\textsuperscript{5}dU) has been analyzed regarding its duplex melting and pre-denaturation changes monitored by UV absorption spectroscopy and viscometry (2), replication (3), hybrid formation with poly(dA-dT) (4), conformation by fibre X-ray diffraction (5) and phosphorus NMR (6), and binding of proflavin (7), lac repressor (8, 9) and CAP (9). Natural DNAs or oligonucleotides containing br\textsuperscript{5}U have been used to study the alkaline denaturation of E. coli DNA and its implication for bacterial chromosome replication (10), DNA interactions with restriction enzymes (11), structure and dynamics of a DNA fragment containing A:br\textsuperscript{5}U pairs (12), base mispairing during replication (13), and interactions of lac repressor with br\textsuperscript{5}U substituted operator DNA (14). In contrast, we are not aware of any relevant published studies of poly(dA-iodo\textsuperscript{5}dU). In the present work we study conformational properties of these two halogenated polynucleotides by CD spectroscopy in order to improve our understanding of the conformational properties of a related synthetic DNA poly(dA-dT) which is in focus of research in our laboratory (for reviews, see 15, 16).

MATERIALS AND METHODS

The polynucleotides used in this work were produced by P-L Biochemicals. Their extinction coefficients (M\textsuperscript{-1}cm\textsuperscript{-1}) were taken 5950 at 266 nm—poly(dA-br\textsuperscript{5}dU) (2), 4650 at 266 nm—poly(dA-iodo\textsuperscript{5}dU), 6640 at 266 nm—poly(dA-dT) (17) and 6660 at 260 nm — poly(dA-dU) (17). CD measurements have been performed as described previously (18).

RESULTS

Figure 1 shows CD spectra of the duplex and single-stranded conformers (induced by high temperature) of the title polynucleotides. Poly(dA-dU) is included as a control. CD spectrum of poly(dA-dU) is characteristic by its very strong positive and small negative band (at 260 and 245 nm, respectively), which is rather unusual with DNA and reminds RNA (19) though the global conformation of poly(dA-dU) is IS-DNA in low-salt aqueous solution (20). The positive band at 260 nm is strongly depressed on going from poly(dA-dU) through poly(dA-br\textsuperscript{5}dU) to poly(dA-iodo\textsuperscript{5}dU) and a small shoulder at about 280 nm turns negative with the halogenated polynucleotides. As a result, CD of poly(dA-iodo\textsuperscript{5}dU) displays negative values above 240 nm. The depression is accompanied by a red-shift of the spectrum.

A part of the above differences results from different chromophores of the polynucleotides as indicated by the UV absorption spectra (Fig. 1, insert). CD spectra of the single-stranded polynucleotides are also different though the differences are smaller in comparison with their duplexes. Thus, not only the altered chromophores but also different conformations are responsible for the dramatic differences in the CD spectra of the polynucleotide double helices.

The halogenated duplexes are more thermostable than the parent polynucleotides. Melting temperatures are 46.7, 50.5, 58.0 and 55.5°C under the conditions of Fig. 1 for poly(dA-dU), poly(dA-dT), poly(dA-br\textsuperscript{5}dU) and poly(dA-iodo\textsuperscript{5}dU), respectively, while the thermal melting of poly(dA-iodo\textsuperscript{5}dU) is much less cooperative than with the remaining three polynucleotides. Both poly(dA-br\textsuperscript{5}dU) and poly(dA-iodo\textsuperscript{5}dU) melt obeying the two-state mechanism as indicated by the isoelliptic points in the spectra recorded in the course of melting. Prior to melting, temperature induces only very slight changes in the CD spectrum of poly(dA-iodo\textsuperscript{5}dU) while, similar to poly(dA-dT), the changes are extensive with poly(dA-br\textsuperscript{5}dU).
The halogenated polynucleotides provide negative long wavelength bands, which made us expect their easy isomerization into X-DNA (for reviews, see 15, 16) that is characteristic by a very deep negative CD band around 280 nm (21, Fig. 2). This type of CD spectrum is reached non-cooperatively with poly(dA-dT) at 3 M CsF (Fig. 2, insert) or saturated concentrations of CsCl (21). The corresponding conformation is very stable (22). It is the conformer from which poly(dA-dT) isomerizes into X-DNA upon further increase of CsF concentration. In case of poly(dA-br^dU), CsF concentrations higher than 3 M, however, aggregate the polynucleotide.

The ellipticity of poly(dA-io^dU) at 280 nm is about -3 even in the absence of CsF while this salt induces almost no changes in the polynucleotide CD spectrum unless scattering, suggesting a polynucleotide aggregation, appears around 2 M CsF.

NaCl gradually depresses the negative band of poly(dA-br^dU) at 280 nm like CsF. The addition of NiCl\(_2\) to the polynucleotide solution in concentrated NaCl induces time-dependent CD spectral changes resulting in a distinct red-shift of the negative band at 280 nm while the band at 260 nm increases and that at 250 nm disappears (Fig. 3). Very similar spectral changes are observed with poly(dA-dT) (Fig. 3). These changes have been interpreted to reflect a transition into the opposite conformation of Z-DNA (23). The same inducing agents, slow kinetics, high cooperativity and the very similar but even more expressed spectral changes suggest that poly(dA-br^dU) is undergoing the same conformational transition. The transition of poly(dA-br^dU) appears at lower NiCl\(_2\) concentrations as compared to poly(dA-dT) (Fig. 3, insert).

NaCl induces no changes in the CD spectrum of poly(dA-io^dU). Addition of NiCl\(_2\) in concentrated NaCl leads to a polynucleotide aggregation so that its secondary structure cannot be assessed.

Because of the relatively mild conditions of the B-Z transition of poly(dA-br^dU), we tried to induce it by high temperature in 5 M NaCl without NiCl\(_2\). No transition was, however, observed up to 64.4°C where we stopped these experiments. Nevertheless, lower NiCl\(_2\) concentrations were necessary to induce the putative Z-DNA in poly(dA-br^dU) at high temperatures than at low temperatures. However, the transition induced by NiCl\(_2\) at 40°C was not reversible, the polynucleotide remained in Z-DNA upon cooling the sample down to room temperature.

Further experiments were conducted in aqueous alcohol solutions. Ethanol induces a two-state cooperative transition of poly(dA-br^dU) (Fig. 4) during which the polynucleotide CD spectra intersect in isoelectroinc points at 230, 272.5 and 297 nm. The CD changes are most extensive at 220 nm and presumably reflect a transition into A-type conformation though its CD spectrum does not contain the characteristic strong positive band at 260 nm. On the other hand, the observed spectral changes in the short wavelength region, namely the appearance of the deep negative band at 220 nm, are similar to those accompanying the B-A transition of poly(dA-dT) (16, 24). Trifluoroethanol but not methanol induces the same transition in poly(dA-br^dU) as well as poly(dA-dT). No characteristic CD changes were observed in poly(dA-br^dU) in ethanol solutions containing 1 mM CsCl which conditions destabilize A-DNA and induce X-DNA in poly(dA-dT) (25, 26). The X-form therefore arises in poly(dA-br^dU) neither in concentrated CsF nor ethanol-millimolar Cs\(^+\) solutions.

Poly(dA-io^dU) exhibits different conformational transitions in ethanol and trifluoroethanol solutions. The ethanol-induced...
transition of poly(dA-io5dU) proceeds in an opposite direction to that of poly(dA-br5dU) (Fig. 4) while the resulting CD spectrum is similar to that of poly(dA-br5dU) in the absence of ethanol. The transition has a two-state nature to indicate that poly(dA-br5dU) and poly(dA-io5dU) adopt fairly different conformations in low-salt aqueous solution and that these two conformations are separated by an energy barrier. TFE induces the same conformational transition in poly(dA-br5dU) and poly(dA-io5dU) as indicated by the similarity of changes in the negative bands close to 220 nm (Fig. 4, insert B), their identical inducing TFE concentrations and cooperativities. The above experiments demonstrate that poly(dA-io5dU) forms an unusual B-type conformation in aqueous solution which is transformed into another B-conformation in ethanol and into an A-type conformer in TFE solutions. This interpretation is in accordance with the fact that TFE is a better inducer of A-DNA than ethanol.

**DISCUSSION**

This study brings new information not only about conformational properties of poly(dA-br5dU) and poly(dA-io5dU) but also about poly(dA-dT). First of all, it demonstrates that bromine and iodine in place of thymine methyl destabilize the unusual and yet not fully characterized X-DNA conformation. In contrast, long aliphatic substituents in position 5 of the pyrimidine base stabilize X-DNA (26, 27). This difference indicates that the substituent protrusion into the double helix major groove and its interactions with hydrating water molecules and ions are of secondary importance for the stability of X-DNA while its primary

**Figure 2.** CD spectra of poly(dA-br5dU) and poly(dA-io5dU) in CsF solutions. Solid CsF was added to the polynucleotides dissolved in 0.01 M MTris-HCl and 3 mM EDTA, pH 7. The measurements were performed at 23°C. Poly(dA-br5dU): — 0, — 1.7 M CsF. Poly(dA-io5dU): — 0, — 1.8 M CsF. Insert: Left: CsF-induced changes in the CD spectra of poly(dA-dT): — 0.05 M sodium phosphate, pH 7, without CsF, — plus 3.4, and — 6.3 M CsF. Right: CsF-induced changes in the CD spectra of O—O poly(dA-dT), — A—A poly(dA-br5dU), and — A—A poly(dA-io5dU). The changes were monitored at 275 nm with poly(dA-dT), and at 280 nm with the halogenated polynucleotides. Interrupted lines connect the points when the spectra indicate light scattering.

**Figure 3.** NaCl + NiCl2-induced B-Z transitions in poly(dA-dT) and poly(dA-br5dU) dissolved in 0.01 M sodium phosphate and 3 mM EDTA, pH 7.0. Drops of 2 M NiCl2 were added to the polynucleotides in the presence of 5 M NaCl. Temperature 25°C. Poly(dA-dT): — no NaCl + no NiCl2, — 5.0 M NaCl, — 5 M NaCl + 91 mM NiCl2. Poly(dA-br5dU): — no NaCl + no NiCl2, — 5.0 M NaCl, — 5 M NaCl + 46.5 mM NiCl2. Insert: The B-Z transitions of O—O poly(dA-dT) and A—A poly(dA-br5dU) monitored at 252 nm.

**Figure 4.** Conformational transitions of poly(dA-br5dU) and poly(dA-io5dU) in water-ethanol solutions. Ethanol (96%) was added to the polynucleotides in 1 mM sodium phosphate and 0.3 mM EDTA, pH 7.0, to get the following concentrations: Poly(dA-br5dU): — 55.5, 61.3, 74.0% (v/v); Poly(dA-io5dU): — 58.4, 66.0, 72.9% (v/v). Temperature 0°C. Insert A: The ethanol-induced transitions of O—O poly(dA-br5dU) and A—A poly(dA-io5dU) monitored at 220 nm. Insert B: CD spectra of poly(dA-io5dU) in — 58.9 and — 72.5% (v/v) trifluoroethanol. Temperature and the buffer are the same as in the figure.
stabilizing factor is the base stacking which is strongly influenced by the highly polarizable halogen atoms. Base pairs stack unevenly in the alternating purine—pyrimidine sequences of AT pairs (28, 29) and this property is likely to make the isomerization into X-DNA possible (15, 16). The highly alternating B-DNA (characterized by ellipticity —3 at 280 nm, Fig. 2) from which the isomerization into X-DNA starts is easily adopted by poly(dA-br^dU) while poly(dA-io^dU) even adopts this structure at low salt. High stability of the halogenated polynucleotides in this structure probably causes their incapacity to isomerize into X-DNA.

This work demonstrates that poly(dA-io^dU) is very stable in a conformation which is not the usual B-DNA. Existence of this conformer indicates that the family of B-DNA contains a number of discrete conformations which are separated by energy barriers. These barriers are relatively small and the various B-type conformers may differ in rotamers around the backbone bonds, including the BI-BII bimorphism of the phosphodiester bond (30), the orientations around the C4-C5 bond and puckering of sugars attached to the purine and pyrimidine residues. No doubt, the different stacking properties in the purine-pyrimidine and pyrimidine-purine steps is the primary cause of the extensive polymorphism within the B-family of conformations of the alternating purine-pyrimidine DNAs. There are many possibilities to solve the Calladine’s clashes (31, 32) in the alternating purine-pyrimidine sequences because poly(dl-dC) even coexists in two related B-type conformers in pyrimidine sequences because poly(dl-dC) also undergoes a two-photon effect.

The last result of this work shows that Z-DNA is more easily adopted by poly(dA-br^dU) than by poly(dA-dT). This finding is not surprising because the bromination also stabilizes Z-forms (28, 29) and this property is likely to make the isomerization into X-DNA possible (15, 16). The highly alternating B-DNA (characterized by ellipticity —3 at 280 nm, Fig. 2) from which the isomerization into X-DNA starts is easily adopted by poly(dA-br^dU) while poly(dA-io^dU) even adopts this structure at low salt. High stability of the halogenated polynucleotides in this structure probably causes their incapacity to isomerize into X-DNA.

REFERENCES