A polycationic amine that induces unique conformational changes in poly(dA-dT) in low salt

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ABSTRACT

Circular dichroism was used to examine alterations in the secondary structure of poly(dA-dT)-poly(dA-dT) upon binding polymer X, a polycationic CD probe for aspects of DNA structure. Stable complex formation is evidenced by increasing T_m and the appearance of large extrinsic bands in the > 300 nm, region which increase proportionally with r (ratio of polymer charge to DNA phosphate), in the range 0.0 to 0.32. At relatively low values of r ($\leq .32$), CD spectra of the poly(dA-dT)-poly(dA-dT)-polymer X complex show a gradual non-cooperative inversion in the long wavelength portion (275 nm) of the intrinsic band in low salt solutions suggesting structural and conformational flexibility in poly(dA-dT)-poly(dA-dT) and further implicating polymer X as a potential probe for variations in DNA secondary structure. The dinucleotide repeat configuration of poly(dA-dT)-poly(dA-dT) is presumed to play a role in the observed intrinsic CD changes. NMR data support an "alternating B" conformation for the complex.

INTRODUCTION

In recent years, the investigation of nucleotide sequence and its influence on DNA conformation and flexibility has played an important role in understanding DNA polymorphism. A major contribution to this understanding was the discovery of Z-DNA in crystals (1-4) and fibers (5) of alternating poly(dG-dC)-poly(dG-dC) sequences. Prior to this, observations of structural anomalies associated with poly(dG-dC)-poly(dG-dC) sequences were evidenced by cooperative inversions in the intrinsic CD bands under conditions of high salt (6) or 60% ethanol (7). Since then, similar inversions in the 275 nm CD band of poly(dA-dT)-poly(dA-dT) (8,9) and poly(dA-dC)-poly(dG-dT) (10, 11) have been observed in high salt solutions of CsF and CsCl; however, NMR (^{31}P chemical shifts) of poly(dA-dT)-poly(dA-dT) (12) were not the same as in the Z-form. An "alternating-B" structure had been proposed (14,15) with A-T as the repeating unit whereby a conformational rearrangement occurs in the phosphodiester linkage between purine and pyrimidine bases. A similar "alternating-B" structure was suggested to occur in high salt solutions of oligo(dG-dC) (16); however, results could not be reproduced in low salt conditions (16). Recent x-ray fiber diffraction studies by Arnott *et al.* (17) have provided further evidence of variations in the phosphodiester backbone which persist as dinucleotide repeats in stretches of duplex DNA where purines and pyrimidines alternate. Most recently, base composition has also been

shown to influence DNA flexibility (18). Consequently, interest has begun focusing on the effects of alternating purine pyrimidine base sequences on the overall conformation of DNA and how changes of this nature affect DNA recognition and protein binding.

Results presented here show that polymer X, a polycation previously used as a CD probe for nucleic acids (19, 20) and chromatin (19) binds to poly(dA-dT)·poly(dA-dT) in low salt solutions and induces an inversion in the 275 nm CD band of the polynucleotide comparable to those observed in solutions of > 4 M CsF. Complex formation is accompanied by large extrinsic CD bands in the >300 nm region which are sensitive to DNA secondary structure and show some base specificity (20). Under similar salt conditions, complex formation between polymer X and poly(dG-dC)·poly(dG-dC) also results in characteristic extrinsic CD bands in the 300 nm region; however, no inversion in the intrinsic signal at 275 nm is observed. NMR spectra reveal a progressive separation in the two resonance bands of poly(dA-dT)·poly(dA-dT) with minimal line broadening as it is titrated with the polymer. Aggregation is not likely since (1) there is no apparent change in M_w as determined by static light scattering and (2) viscosities are not significantly different between treated and untreated polynucleotides.

MATERIALS AND METHODS

N-Poly{ α -[N-(4-pyridylethylene-4-pyridyl-N') α -p-xylyl] dibromide} -4-pyridylethylene-4-pyridinium bromide, "polymer X"; (see below), was synthesized, fractionated into its oligomeric components and characterized as described (19,20).

The bis-pyridyl concentration of a stock solution of octamer X in water was calculated on the basis of an extinction coefficient of $3.25 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, $\lambda_{\text{max}} = 325 \text{ nm}$ (20). For the octamer, concentration is one ninth that of bis-pyridyl units.

Calf-thymus DNA (ct-DNA), poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC), poly(dA)·poly(dT) and poly(dG)·poly(dC) were purchased from Sigma Chemical Company. Stock solutions of polynucleotides were prepared in 0.015 M NaCl, 0.0015 M Na citrate, pH = 7.0 (SSC x .1).

Octamer X-polynucleotide complexes were prepared by slowly adding octamer X to the polynucleotide with gentle agitation. Solutions of poly(dG-dC)·poly(dG-dC) were prepared according to the method of Pohl (7). The poly(dG-dC)·poly(dG-dC)-octamer X complex in ethanol was prepared under the same conditions.

CD and UV spectra were recorded at room temperature on a Cary 61 spectropolarimeter and on a Cary 14 spectrophotometer, respectively, in SSC x .1.

For ^{31}P NMR measurements, 6 mg of poly(dA-dT)·poly(dA-dT) (Sigma) were dissolved in 1.5 ml of SSC x .1, pH 7.5 and sonicated as described elsewhere (13) followed by the addition of .5 ml D_2O . Increasing amounts of the polymer ($3.1 \times 10^{-2} \text{ M}$ in SSC x .1) up to $r = .30$ were added to the undialyzed sample of poly(dA-dT)·poly(dA-dT). Unfrac-

tionated polymer was used due to insufficient amounts of the octamer available. Trimethylphosphate (TMP) in 100% D₂O was used as an external reference. NMR spectra were recorded on a Bruker WM-300 spectrometer at 121.50 Hz, sweep width 2.0 KHz, acquisition time 2s, line broadening 2.00 Hz, temperature 303°K.

Viscosimetric studies were performed with an Ostwald viscosimeter thermostatted at $28.5 \pm 0.2^\circ\text{C}$. All solutions in SSC x .1 were allowed to equilibrate to 28.5°C for 15 minutes prior to taking readings.

Light scattering was measured on a Brice-Phoenix instrument modified to allow measurements at $< 20^\circ$. Measurements from $< 20^\circ$ to 90° were taken with SSC x .1 as the solvent.

RESULTS

"Octamer X" ($n = 8$), was selected as a representative oligomer for subsequent studies with the polynucleotides poly(dA-dT).poly(dA-dT), poly(dG-dC).poly(dG-dC), poly(dA).poly(dT) and poly(dG).poly(dC). In each case, binding of octamer X resulted in a positive extrinsic CD signal consisting of maxima at 333 nm and 347 nm as well as a small shoulder at 318 nm. Extrinsic band intensities were a function of the polynucleotide base composition and r (Fig. 1). Poly(dA-dT).poly(dA-dT) (Fig. 2) and poly(dA).poly(dT) (Fig. 3) displayed the largest extrinsic molar ellipticities ($[\theta]_{333} = 3.8 \times 10^4$, $r = 0.322$; and 3.2×10^4 , $r = .246$, respectively). At equivalent ratios, poly(dG-dC).poly(dG-dC) (Fig. 4) and poly(dG).poly(dC) (Fig. 5) showed similar but much reduced signals ($[\theta]_{333} = 1.6 \times 10^4$, $r =$

Figure 1. Dependence of CD signal intensity ($[\theta]_{333}$) on r for: a) poly(dA).poly(dT); b) poly(dA-dT).poly(dA-dT); c) poly(dG-dC).poly(dG-dC); d) poly(dG).poly(dC); solvent, SSCx.1, pH = 7.0.

Figure 2. CD spectra of the poly(dA-dT)-poly(dA-dT)-octamer X complex; solvent, SSCx.1, pH = 7.0; poly(dA-dT)-poly(dA-dT) = 7.5×10^{-5} M. $r = 0$ (—), $r = .107$ (---), $r = .214$ (···), $r = .322$ (-·-·-).

.290; and 1.0×10^4 , $r = .300$, respectively). In all cases the extrinsic band intensities were directly proportional to r from $r = 0.0$ to 0.32 . (Fig. 1)

The measured intrinsic signals of poly(dG-dC)-poly(dG-dC) ($[\theta]_{275} = 0.96 \times 10^4$, $r = 0$) and poly(dA)-poly(dT) ($[\theta]_{259} = 1.0 \times 10^4$, $r = 0$) decreased linearly with increasing r and became slightly negative at ratios approaching $r = 0.30$ ($[\theta]_{275} = -0.30 \times 10^4$, $r = 0.290$ and

Figure 3. CD spectra of the poly(dA)-poly(dT)-octamer X complex; solvent, SSC/x.1, pH = 7.0; poly(dA)-poly(dT) = 8.17×10^{-5} M. $r = 0$ (—), $r = .099$ (---), $r = .197$ (···), $r = .246$ (-·-·-).

Figure 4. CD spectra of the poly(dG-dC)·poly(dG-dC)-octamer X complex; solvent, SSCx.l, pH = 7.0; poly(dG-dC)·poly(dG-dC) = 5.95×10^{-5} M. $r = 0$ (—), $r = .099$ (---), $r = .194$ (···), $r = .290$ (-·-·-).

$[\theta]_{259} = -0.24 \times 10^4$, $r = 0.296$, respectively). Poly(dG)·poly(dC) ($[\theta]_{255} = 4.0 \times 10^4$, $r = 0$) decreased in a similar fashion, but to a lesser degree ($[\theta]_{255} = 3.3 \times 10^4$, $r = .300$).

Octamer X induced inversions in the intrinsic signal of poly(dA-dT)·poly(dA-dT) were similar to those seen in CsF and LiCl solutions (8,9). As r went from 0 to .322, $[\theta]_{275}$ decreased from 0.67×10^4 to -1.8×10^4 and the intrinsic maximum shifted from 263 nm to

Figure 5. CD spectra of the poly(dG)·poly(dC)-octamer X complex; solvent, SSCx.l, pH = 7.0; poly(dG)·poly(dC) = 9.4×10^{-5} M. $r = 0$ (—), $r = .086$ (---), $r = .171$ (···), $r = .300$ (-·-·-).

Figure 6. CD spectra of Z-form poly(dG-dC)·poly(dG-dC) and the Z-form poly(dG-dC)·poly(dG-dC)-octamer X complex; solvent SSCx.001, 60% ethanol. Poly-(dG-dC)·poly(dG-dC) = 5.95×10^{-5} M (—), poly(dG-dC)·poly(dG-dC)-octamer X complex, $r = .220$ (···).

259 nm accompanied by a decrease in $[\theta]$ from $[\theta]_{263} = 1.1 \times 10^4$ to $[\theta]_{259} = 0.03 \times 10^4$.

CD results with the poly(dG-dC)·poly(dG-dC)-octamer X complex ($r = .220$) in 60% ethanol indicated the extrinsic band to be unaffected (Fig. 6). The slight reduction in $[\theta]_{333}$ has been ascribed to the dependence of the extrinsic band intensities on salt concentration below .015 M NaCl (Zarlenga, unpublished). Upon addition of octamer X, the negative intrinsic bands centered at 292 nm ($[\theta]_{292} = -1.4 \times 10^4$) and 248 nm ($[\theta]_{248} = -0.50 \times 10^4$), remained unchanged. The positive lobe at 268 nm ($[\theta]_{268} = 1.5 \times 10^4$) was red shifted to 272 nm and decreased to $[\theta]_{272} = 0.55 \times 10^4$.

All NMR spectra displayed the two characteristic ^{31}P bands for the A-T and T-A phosphodiester linkages (21) which appeared upfield from an external TMP standard. A third band appearing in the control spectra and slightly downfield (-3.90 ppm) from the poly(dA-dT)·poly(dA-dT) resonances remained unchanged throughout the addition of polymer X and was determined to be an impurity. Results indicated a gradual upfield shift of the A-T resonance (-4.33) and a concomitant downfield shift of the T-A band (-4.13) with maximum changes occurring at $r = .3$. Changes occurred with minimal line broadening. Results are summarized in Table I.

Negative ellipticities observed in the intrinsic CD band of the polynucleotide-octamer X were not the result of DNA aggregation. Viscosimetric studies ($r = .320$) to examine alterations in the tertiary structure of the complex were consistent with no change in shape or in molecular weight due to aggregation. Light scattering studies with the bulk polymer X-(ct-DNA) complex indicated no significant molecular weight or shape

TABLE I
³¹P Chemical shifts of the two phosphodiester resonances of
poly(dA-dT)-poly(dA-dT) as a function of r

	r	phosphorus atoms	chemical* shift (δ)	
poly(dA-dT)	0	P ₁ ^b	-4.13	-0.20
		P ₂ ^c	-4.33	
poly(dA-dT) + X ^a	.10	P ₁	-4.10	-0.32
		P ₂	-4.42	
poly(dA-dT) + X	.20	P ₁	-4.05	-0.41
		P ₂	-4.46	
poly(dA-dT) + X	.30	P ₁	-3.95	-0.53
		P ₂	-4.48	

* chemical shifts upfield from trimethylphosphate

a) polymer X

b) dT-P₁-dA, ref. 21

c) dA-P₂-dT, ref. 21

changes (for details, see Ref. 20). Polymer X, as with other synthetic polycations, imparted characteristic thermal stabilization upon binding nucleic acids (see Ref. 19,20,22).

DISCUSSION

Polymer X has previously been shown to associate with nucleic acids and nucleic acid analogs, to cause changes in the intrinsic CD signals of the complex and to induce a large extrinsic signal centered at 340 nm (19). The exciton splitting mechanism (23) has been invoked to explain the appearance of an extrinsic CD signal to the ordered chiral arrangement of polycation chromophores imposed by the nucleic acid secondary structure (19,20,22). The polymer itself is achiral and, therefore, possesses no optical activity in the uncomplexed state.

Nucleic acids and nucleic acid analogs were stabilized to denaturation upon binding octamer X as evidenced by increases in T_m (20). Complexation resulting in alterations of the secondary structure were indicated by reductions in [θ]₂₇₅ and were attributed in part to a transition to the C form. In the presence of octamer X, [θ]₂₇₅ for poly(dG)-poly(dC), poly(dA)-poly(dT) and poly(dG-dC)-poly(dG-dC) decreased linearly with r

up to $r = .320$. Such alterations in the intrinsic CD band are typical of salt effects observed with nucleic acids in solution and in films under conditions of limited hydration (24,25). The CD spectrum of the poly(dA-dT)·poly(dA-dT)-octamer X complex revealed an inversion in the intrinsic signal whereby the rate of reduction in $[\theta]_{275}$ was over two-fold greater than the rate of change in $[\theta]_{259}$. This phenomenon was not observed with any other polynucleotide-octamer X complexes examined (compare Figs. 2,3,4 and 5).

Unique alterations in the intrinsic band of the poly(dA-dT)·poly(dA-dT)-octamer X complex have raised several questions concerning the secondary structure of poly(dA-dT)·poly(dA-dT). Similar changes in the CD spectra of poly(dG-dC)·poly(dG-dC) in high salt (6) or 60% ethanol (7) have been attributed to a transition to the Z-form (4). Patel *et al.* (16) have verified structural anomalies in the phosphodiester backbone and reported an alternating conformation with oligo(dG-dC)·oligo(dG-dC) in high salt and regular B-type DNA conformations in low salt. In contrast, Shindo and coworkers (15) as well as Patel *et al.* (12) have presented NMR data for poly(dA-dT)·poly(dA-dT) in both low and high salt solutions respectively, as well as in the presence of a steroid diammonium ligand (12), whereby the observed chemical shifts were consistent with poly(dA-dT)·poly(dA-dT) adopting an "alternating-B" conformation under all salt conditions tested. NMR data presented here (Table I) show $\Delta\delta$ (the change in chemical shifts) of the two ^{31}P resonance bands in the poly(dA-dT)·poly(dA-dT)-polymer X complex to be similar to those reported by Patel *et al.* (12) in high salt solutions of poly(dA-dT)·poly(dA-dT). Since low salt concentrations ($\sim .015\text{M NaCl}$) were used in our work, it was concluded that the high charge to mass ratio of octamer X in conjunction with its ability to interact strongly with DNA alters the local ionic environment of the nucleic acid phosphates upon complexing. This alteration manifests itself as a gradual transition in the phosphodiester backbone to the more pronounced dinucleotide repeat reported by Patel *et al.* (12). The fact that $\Delta\delta$ is linear with respect to r and $\Delta[\theta]_{275}$ (Fig. 7) implicates a relationship between the observed inversion in the intrinsic CD band of the poly(dA-dT)·poly(dA-dT) - octamer X complex and the gradual change in the phosphate geometries between purine and pyrimidine bases. Though reductions in the positive intrinsic CD signal above 260 nm have been shown to correlate well with increases in the helix winding angle (26), to date, no theory exists which allows the calculation of nucleic acid structural parameters from CD data alone. Therefore, specific conclusions regarding the relationship between the inversion in the intrinsic CD signal and the concomitant separation in phosphate resonances cannot be made at this time.

NMR data presented in Table I ($\Delta\delta = .33$ where $r = .3$) do not fully support the classical Z-conformation for the poly(dA-dT)·poly(dA-dT) - octamer X complex since the formation of Z-DNA results in a 1.5ppm downfield shift of only one phosphate resonance band (16). Gradual increases observed in $\Delta\delta$ (Table I) together with the inversion in $[\theta]_{275}$, however, may reflect some common conformational features between the "alternating-B" form of

Figure 7a. The absolute change in ^{31}P chemical shifts ($\Delta\delta$) of poly(dA-dT)·poly(dA-dT) with respect to r .

Figure 7b. CD signal intensity ($[\theta]_{275}$) of poly(dA-dT)·poly(dA-dT) as a function of r .

poly(dA-dT)·poly(dA-dT) and the Z-form of poly(dG-dC)·poly(dG-dC), which also exhibits an alternating backbone, but in a left handed sense. This idea was supported in part by results shown in Fig. 6 where the characteristic extrinsic CD signal centered at 340 nm appeared in the Z-form poly(dG-dC)·poly(dG-dC) - octamer X complex, but was unaccompanied by additional changes in $[\theta]_{292}$. This suggested a maximally perturbed system by the 60% ethanol i.e., 60% ethanol and polymer X altered the polynucleotide in a similar manner. Similarities in backbone flexibility resulting from variations in the O-P rotational angles between purine (3'-5') pyrimidine and pyrimidine (3'-5') purine conformations in the Z DNA and the poly(dA-dT)·poly(dA-dT) octamer X complexes may account for these observations.

Recent studies by Hogan *et al.* (18) have shown that DNA base composition has a profound effect on the internal bending motions of the DNA helix, implicating A-T rich regions as being the least resistant to conformational change. As such, the unique intrinsic CD signal changes and the intensity of the extrinsic CD bands observed in the poly(dA-dT)·poly(dA-dT)-octamer X complex may be directly related to the increased torsional flexibility of the homopolymer which can more easily accommodate the induced chiral orientation of the octamer X chromophores. The lack of inverted intrinsic signal changes in the poly(dG-dC)·poly(dG-dC)-octamer X complex in low salt solutions and in the absence of alcohol, reduces the probability of that the CD changes observed in the poly(dA-dT)·poly(dA-dT)-octamer X complex can be ascribed solely to interactions between the polymer and polynucleotides of alternating base sequences.

Inasmuch as high concentrations of CsF and other salts have been shown to induce inversions in the intrinsic CD bands of both alternating (A-T) and (G-C) sequences, octamer X appears to have some specificity for poly(dA-dT)·poly(dA-dT) implicating

causes other than mere salt effects to be involved in the observed intrinsic changes. Since CD and NMR spectral changes in the poly(dA-dT)·poly(dA-dT)-polymer X complex were observed in solutions of low salt (SSCx .1), results suggest that similar conformational changes might occur in physiological conditions in the presence of basic proteins or endogenous polycations which possess regions of high charge density.

To sum up, octamer X induced a large inversion in poly(dA-dT)·poly(dA-dT) (Fig. 2) with no detectable aggregation or shape changes, and enhanced a weak minimum in poly dA·poly dT at ~ 265 nm (Fig. 3). For poly(dG-dC)·poly(dG-dC) a maximum at 270 nm and - a shoulder at 285 nm are replaced by a shoulder with $[\theta] < 0$; the minimum at 250 nm remained essentially unchanged (Fig. 4). Poly dG·poly dC showed a progressive deepening of the weak negative trough at 275 nm as it is titrated with octamer X with little diminution of the 255 nm maximum (Fig. 5). Using the ethanol induced Z-form of poly(dG-dC)·poly(dG-dC) as a reference compound (Fig. 6), it was observed that octamer X complexation is accompanied by the appearance of the extrinsic maxima at > 300 nm, a diminution and shift of the short wavelength maximum and no change in the 288 nm minimum. We conclude on the basis of these CD data that octamer X alters the secondary structure of the polydeoxynucleotides and in particular induces an inversion in the long wavelength region of the intrinsic CD signal of alternating polynucleotides presumed to be in an "alternating-B" type configuration, i.e., poly(dA-dT)·poly(dA-dT) in low salt and poly(dG-dC)·poly(dG-dC) in 60% ethanol. NMR data for the complex are consistent with the dinucleotide repeat conformation established for poly(dA-dT)·poly(dA-dT) in high salts.

Results presented here suggest the potential use of polymer X as a model for interactions between nucleic acids and larger cationic proteins which may specifically bind to and alter AT rich regions in DNA and selectively regulate DNA action.

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ABBREVIATIONS

ct-DNA, calf-thymus DNA; r, ratio of polymer positive charges to DNA phosphate; $[\theta]$, molar ellipticity, DEG-CM²/decimole, at wavelength indicated; SSC x .1 is .015 M NaCl, .0015 M Na citrate, pH = 7.0; Octamer X ("ten") structure shown where n = 8.

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