Stabilization of Z-DNA by polyarginine near physiological ionic strength

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ABSTRACT

The identification of left handed or Z-DNA in solutions of poly d(GC) in high salt suggests that left handed DNA may exist in biological systems if stabilized at lower ionic strength. In the present study we show that binding of polyarginine to the Z form of poly d(GC) results in a protein-Z-DNA complex stable near physiological ionic strength. The percentage of Z-DNA in the low salt polyarginine-poly d(GC) complex was measured from the DNA circular dichroism spectrum. The ratio of Z to B-DNA is a linear function of polyarginine concentration and is sensitive to proteolytic digestion by trypsin. These results suggest that arginine-rich proteins may stabilize Z-DNA in vivo.

INTRODUCTION

Crystallographic studies^{1,2,3} of DNA oligonucleotides with alternating dG-dC sequence have revealed a novel left-handed DNA secondary structure whose conformation differs significantly from that of B-DNA⁴. This left-handed or Z-form of DNA has also been observed in the X-ray diffraction of oriented poly d(GC) fibers⁵ and during NMR studies of the solution structure of oligo d(GC) in high salt^{6,7}. Recently, it has been demonstrated that B- and Z-DNA may coexist in plasmids containing poly d(GC) inserts⁸.

The existence of two structural isomers of the duplex DNA poly d(GC) was first demonstrated by the spectroscopic and kinetic studies of Pohl and Jovin⁹. They observed a reversible, cooperative transition for the DNA with a midpoint at 2.5 M NaCl or 0.7 M MgCl₂ which resulted in an inversion of the B-DNA circular dichroism (C.D.) spectrum and a shift in the U.V. absorption spectrum to higher wavelengths. The transition was largely entropy driven with an activation energy of approximately 22 Kcal/mole. It is now generally accepted that these changes in the optical properties of poly d(GC) are associated with the conversion from a B- to Z-helical structure. Recently, the

identification of Z-DNA as the high salt (inverted C.D.) form of poly d(GC) has been confirmed by comparison of the laser-Raman spectra of oligo d(GC)₃ in the Z-form and poly d(GC) in 4 M NaCl¹⁰.

MATERIALS & METHODS

Poly d(GC) from PL Biochemicals (S_{20,w} of 8.2) was stored in 10 mM Tris-HCl, pH 7.5 at a concentration of 2.8 mg/ml. Poly-L-arginine HCl (Sigma, degree of polymerization; 300) and Poly-L-lysine HBr (Sigma, degree of polymerization; 380) were dissolved in H₂O or 10 mM Tris-HCl, pH 7.5, at a concentration of 100 µM amino acid per ml and stored at -20°C. T7 DNA was isolated from phage that had been banded in CsCl. The DNA was extracted 5 times with distilled phenol, ethanol precipitated and dialyzed into 20 mM Tris-HCl, pH 7.5, 20 mM NaCl, 0.5 mM EDTA.

13.4 µl poly d(GC) (37.5 µg) was combined with 53.6 µl 5 M NaCl, 10 mM Tris-HCl, pH 7.5, to convert the DNA to the Z conformation. Polyarginine, 1-10 µl was combined with DNA at the desired amino acid to nucleotide ratio with rapid mixing at room temperature. After several minutes, 1.5 ml 10 mM Tris-HCl, pH 7.5, was added for a final NaCl concentration of 170 mM. All samples were incubated at room temperature for 15 minutes before optical measurements were recorded.

Circular dichroism measurements were performed using a Jasco J-10 circular dichroism instrument with rebuilt and upgraded electronic components to yield high performance CD spectra. Measurements were made at room temperature in 1 cm pathlength cuvettes. Because of the high molecular weights of the polyarginine-polyd(GC) complexes, which ranged from 18 to 60 million, a small amount of light scattering was present in these solutions which contained less than 50 µg/ml of complexes. Although light scattering may cause capricious changes in CD spectra, the degree of light scattering present in these experiments was small, and the CD spectra were highly reproducible. Moreover, the data were readily interpretable as a simple mixture of B and Z DNA forms; thus, there was no evidence that the CD measurements were significantly perturbed by light scattering.

RESULTS

The circular dichroism spectra of poly d(GC) in 10 mM Tris buffer with 0.17 M or 4.0 M NaCl is shown on Figure 1A. The normal B-DNA C.D. spectrum which occurs in 0.17 M NaCl displays a maximum ellipticity at

Figure 1: (a) Circular dichroism spectra of poly d(GC) in 10 mM Tris-HCl (pH 7.5) with 0.17 M NaCl (....) or 4.0 M NaCl (----). The DNA concentration was 0.12 μ M in phosphate. (b) Circular dichroism spectra of poly d(GC)-polyarginine at near physiological ionic strength, 10 mM Tris-0.17 M NaCl. 37.5 μ g poly d(GC) was reconstituted with polyarginine (Degree of polymerization approximately 300) and diluted to lower salt (final volume 1.5 ml). The arginine cation/DNA phosphate ratios were 0.0 (—), 0.5 (.-.-.-), 1.0 (---), 2.0 (.....), and 4.0 (-----). Spectra were recorded after 15 minutes in 0.17 M NaCl. No protein in the presence of 4.0 M NaCl (-.-.-).

270 nm and a minimum value at 250 nm. In 4.0 M NaCl, the C.D. spectrum is inverted and red shifted to that of Z-DNA⁹ with a minimum elliptical at 290 nm. The magnitude of the C.D. band at this wavelength is a measure of the relative proportion of B- and Z-DNA in solution.

The binding of DNA by the basic polypeptide polyarginine is known to occur in solution at high ionic strength. Polyarginine increases the melting temperature of DNA in 4 M NaClO₄ and the polyarginine-DNA complex is stable to banding in CsCl density gradients.¹¹ Addition of polyarginine to Z-DNA in 4.0 M NaCl results in the formation of a Z-DNA protein complex which is stable in lower salt (Figure 1B). Poly d(GC) in the Z conformation was titrated with polyarginine, diluted to a final

Figure 2: Inversion of the DNA circular dichroism spectrum ($\bar{\theta}_i$) at 290 nm as a function of amino acid residue to nucleotide ratio for the low salt polyarginine-poly d(GC) complex. Circular dichroism spectra of the poly d(GC)-polyarginine complex were recorded after 15 minutes in low salt (O) and again after 24 hours (X).

salt concentration of 0.17 M NaCl and examined by circular dichroism. In the absence of polyarginine the usual B-DNA C.D. spectrum was obtained. Samples to which polyarginine had been added exhibited C.D. profiles intermediate between those of B- and Z-DNA and at amino acid to nucleotide ratios of 2.0 and higher, the C.D. profile became indistinguishable from that of Z-DNA. The ellipticity approaches but never reaches that of the uncomplexed DNA polymer although only DNA contributes to the C.D. profile in this spectral region.

The relative proportion of DNA retained in the Z conformation in the low salt poly d(GC)-polyarginine complex may be estimated from:

$$\bar{\theta}_i = |(\Delta\epsilon_i - \Delta\epsilon_B)/(\Delta\epsilon_B - \Delta\epsilon_Z)| \quad \dots(1)$$

Where $\Delta\epsilon_i = \epsilon_L - \epsilon_R$, at an amino acid to nucleotide ratio (i), and B and Z refer to the B and Z conformations of DNA respectively. $\bar{\theta}_i$ measures the percent inversion of the DNA C.D. absorption profile at 290 nm and is unity for poly d(GC) in high salt.

In figure 2, $\bar{\theta}_i$ is plotted as a function of input amino acid per nucleotide for samples incubated 15 minutes in low salt buffer and the same samples after 24 hours at room temperature. The normalized C.D. values at 290 nm were a linear function of protein concentration and after 24 hours in 0.17 M NaCl were within 75-95% of the values obtained upon dilution. A maximum value for $\bar{\theta}_i$ of 0.7 was obtained at high amino acid per nucleotide ratios.

Treatment of the low salt Z-DNA-polyarginine complex with trypsin

resulted in a gradual inversion of the circular dichroism profile to that of B-DNA. Z-DNA (25 $\mu\text{g/ml}$) was reconstituted with a four-fold charge excess of polyarginine, and then it was incubated with 50 μg of trypsin in a volume of 1.5 ml. The transition from Z- to B-DNA due to protease treatment was monitored by a rise in DNA molar ellipticity at 290 nm (not shown). After 15 minutes a C.D. spectrum characteristic of B-DNA was obtained. Readdition of NaCl to 4.0 M returned the DNA to the Z conformation. Therefore, the retention of Z conformation in the low salt poly d(GC)-polyarginine complex is a direct consequence of the binding of polyarginine to the DNA.

The stabilization of Z-DNA near physiological ionic strength may be a unique feature of arginine binding to d(GC)-rich regions of DNA. Addition of polyarginine to purified phage T7 DNA at an amino acid to nucleotide ratio above 0.5 results in precipitation of the DNA upon dilution to low salt. In addition, CD spectra observed at lower amino acid to nucleotide ratios are characteristic of B-DNA (Figure 3). Polylysine precipitated poly d(GC) at a lysine to nucleotide ratio of 0.5 while polyarginine was effectively reconstituted to the DNA at a 5 to 10-fold higher ratio. The C.D. profile of the polylysine-poly d(GC) complex shows a reduction in ellipticity and an absorption flattening characteristic of highly turbid solutions.¹²

The polyarginine-poly d(GC) complex was characterized by measurement of U.V. scattering in the nonabsorption region of the UV absorption profile. The turbidity (τ) of the protein-DNA complex was determined as a function of polyarginine concentration at 5 nm intervals in the spectral range 360-320 nm. The weight averaged molecular weight is

Figure 3: Circular dichroism of polypeptide-DNA complexes in 0.17 M NaCl-10 mM Tris. Protein was added to DNA in 4.0 M NaCl - 10 mM Tris and diluted to low salt. The final DNA concentration was 0.115 μM in phosphate. Protein concentration is expressed as peptide cation/nucleotide ratio [+/-]. (—) poly d(GC); (·-·-·) poly d(GC)-polyarginine [+/-] = 2.0; (.....) T7 DNA-polyarginine [+/-] = 0.5; (- - -) poly d(GC)-polylysine [+/-] = 0.5.

approximated by:

$$M = 3\tau/16\pi KC \quad \dots(2)$$

Where K is an experimental constant,

$$K = 2\pi^2 n_0^2 (\partial n / \partial c)^2 / \lambda^4 \quad \dots(3)$$

n_0 is the refractive index, λ is wavelength and c is concentration of the complex in g/ml. The specific refractive index increment $(\partial n / \partial c)$ has been taken as 0.187 ml/g for both protein and DNA.¹³ The molecular weight of the poly d(GC)-polyarginine complex is a linear function of protein concentration at amino acid/nucleotide ratios lower than 0.5 and approaches a constant value of approximately 60 million daltons at higher ratios (Figure 4). In these experiments the scattered light is comparable to that which would be scattered by particles at the same concentration with molecular weights between 18 and 60 million, depending upon polyarginine concentration. Thus, the polyarginine-DNA complex is crosslinked to a large molecular species whose size may be a function of the degree of polymerization of both the protein and DNA.

DISCUSSION

Binding of polyarginine to the Z-form of DNA results in a protein-Z-DNA complex stable near physiological ionic strength. The percent DNA retained in the Z conformation at several protein concentrations was estimated from the C.D. profile of the polyarginine-poly d(GC) complex in low salt. C.D. spectra at amino acid to nucleotide ratios above 0.5 exhibit negative minimum ellipticities at 290 nm and a positive C.D.

Figure 4: Molecular weight of the polyarginine-poly d(GC) complex determined from turbidity measurements in low salt. Error bars represent 90% confidence limits determined from 9 measurements in the spectral range of 360 to 320 nm.

band centered at 260-270 m μ , in qualitative agreement with the C.D. profile of Z-DNA (Figure 1A). The ellipticity at 290 m μ of the poly-arginine-poly d(GC) complex in low salt approaches to within 70% of the value for Z-DNA in 4.0 M NaCl at the same concentration. Similar results have been observed for poly d(GC) with divalent ions or dehydrating agents where the B-Z transition in DNA was measured by inversion of the C.D. profile.¹⁴ The observed variation in position and amplitude of the C.D. bands was attributed to conformational flexibility in the DNA helix giving rise to a family of left-handed DNA structures. The DNA in the polyarginine-poly d(GC) complex may adopt a left handed DNA conformation which differs in helical parameters such as base twist or tilt from that of Z-DNA in 4.0 M salt. The low salt polyarginine-poly d(GC) complex may, therefore, exhibit a different molar ellipticity than that of the high salt Z-DNA polymer. The circular dichroism data is consistent with a linear correlation of % Z-DNA with amino acid to nucleotide ratio determined from the apparent red shift in the U.V. absorption profile⁹ corrected for Rayleigh scattering (unpublished results).

The spectral changes accompanying polyarginine binding to poly d(GC) do not result from light scattering or precipitation of the protein-DNA complex in low salt. Turbidity measurements indicate little or no increase in light scattering at amino acid to nucleotide ratios above 0.4 (Figure 4) while the percent inversion of the C.D. absorption profile at 290 m μ (θ_1) is linear at much higher ratios. Also, the C.D. profiles of the polyarginine-poly d(GC) complex remain unchanged after 24 hours at room temperature and exhibit no apparent flattening or loss of intensity evident of increased turbidity.¹²

Treatment of the low salt complex with trypsin leads to inversion of the C.D. spectra to that of B-DNA and a decrease in solution turbidity to that of uncomplexed DNA. The molecular weight of the polyarginine-poly d(GC) complex is roughly equal to that of intact T₄ phage of 120 million daltons. If association into 60-100 million molecular weight particles is being formed at all protein concentrations, the binding of polyarginine to poly d(GC) may be highly cooperative with a mixture of free DNA molecules in the B form and associated molecules in the Z form. Van deSande and Jovin¹⁵ have identified a left-handed form of polyd(GC) in low salt MgCl₂-Ethanol solutions which readily associates or aggregates into higher molecular weight species. This Z* DNA is a

substrate for several intercalating and nonintercalating drugs and acts as a template for RNA polymerase. Association of polyd(GC)-polyarginine into stable Z-DNA-protein complexes may arise from a combination of polyarginine binding and Z-DNA aggregation or perhaps from association into Z*-polyarginine complexes of approximately 60-100 million daltons.

Polyarginine has been shown to preferentially interact with d(GC)-rich regions of DNA¹¹, an observation explained by specific amino acid-DNA side chain interactions involving the O6 and N7 positions of guanine¹⁶. NMR¹⁷, laser Raman¹⁸ and theoretical¹⁹ studies of arginine and arginine-rich histone binding to DNA have indicated a specificity of arginine for d(GC)-rich regions of DNA with participation of the guanine N7 in the hydrogen-bonding interaction. The B to Z transition in poly d(GC) is associated with rotation of guanine from an anti to syn conformation¹ in which the guanine N7 is more accessible for charge interactions. For example, X-ray analysis of the hexamer d(GC)₃ has revealed a Z-DNA conformation in which the guanine N7 and phosphodiester linkage is bridged by a hydrated magnesium ion². Recent NMR studies²⁰ of oligoarginines suggest that hydrogen-bonding of the arginine N1 proton and its cis-guanidinium group leads to particularly favorable ionic interactions which may preferentially stabilize the Z-form of DNA. The retention of Z-DNA in the poly d(GC)-polyarginine complex upon dilution to lower salt may result from either thermodynamic or kinetic stabilization of the Z-DNA conformation.

The isolation of a stable Z-DNA-polyarginine complex in 0.17 M NaCl suggests that arginine rich proteins might stabilize Z-DNA in vivo. Behe and Felsenfeld²¹ have observed the B- to Z-transition in poly d(G-M⁵C) with a midpoint at 0.7 M NaCl or 0.6 mM MgCl₂ in the presence of 50 mM NaCl. Other factors which may influence the stability of Z-DNA or B-Z DNA hybrids near physiological ionic strength by lowering the activation energy for the B-Z transition may include the binding of arginine-rich DNA regulatory proteins and removal of negative superhelical turns in DNA with a left-handed helical conformation. The results of this study suggest that Z-DNA may be stabilized by specific protein interactions and offers an experimental approach to the isolation of naturally occurring Z-DNA-protein complexes.

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