Volume 17 Number 10 1989 Nucleic Acids Research

Direct evidence for the presence of left-handed conformation in a supramolecular assembly of polynucleotides

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Received April 10, 1989; Revised and Accepted April 20, 1989

ABSTRACT

Hexammine cobalt(III) chloride ($Co(NH_3)_6^{3+}$) provokes a B-DNA → Z-DNA → Ψ-DNA conformational transition in poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC). The circular dichroism spectrum of Ψ-DNA is characterized by a manyfold increase of positive ellipticity in the range of 300-225 nm and the complete absence of a negative peak. In order to ascertain the helical handedness of Ψ-DNA, we used a recently developed enzyme immunoassay technique. This method consisted of treating the polynucleotides with $Co(NH_3)_6^{3+}$ to convert them to the Z- or Ψ-DNA forms and immobilizing these conformations on a microtiter plate. The plates were subsequently treated with a monoclonal anti-Z-DNA antibody Z22, alkaline phosphatase conjugated, affinity purified immunoglobulins, and the phosphatase substrate. The enzyme-substrate reaction was monitored by reading the absorbance at 405 nm with a microplate autoreader. The monoclonal anti-Z-DNA antibody had no reactivity to the B-DNA form, but bound strongly to both the Z- and Ψ-DNA forms, showing that $Co(NH_3)_6^{3+}$-induced Ψ-DNA form of the polynucleotides exists in the left-handed Z-DNA conformation.

INTRODUCTION

Multivalent ions like $Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$ are excellent promoters of the transition of poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC) from their usual right-handed B-DNA form to the left-handed Z-DNA conformation at micromolar concentrations of these counterions (1-4). Increasing concentrations of $Co(NH_3)_6^{3+}$ are also known to provoke a supramolecular organization of the polynucleotides from the Z-DNA form to a Ψ-DNA form (5-6). The Ψ-DNA form is a believed to be a twisted, tightly packaged assembly of DNA. Several agents, including polylysine, spermine, ethylene glycol, and salt are known to convert native DNAs to the Ψ-DNA form (7-9). The formation of this structural form of DNA is characterized by a large (10- to 100-fold) increase in the intensity of the molar ellipticity of DNA in circular dichroism (CD) spectroscopic measurements. This property has presented a basic problem in interpreting the CD spectrum of Ψ-DNA and assigning its helical handedness. This uncertainty has recently led to the conclusion that Ψ-DNA is the end product in the

sequential transitions of B-DNA to an organized form of the polynucleotides and that Z-DNA is an intermediate in B-DNA to Ψ-DNA transition (10).

In the case of poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC), the Ψ-DNA form is induced and stabilized at counterion concentrations that are far higher than that required to stabilize the Z-DNA form. It is therefore possible that Ψ-DNA is a supramolecular organization of Z-DNA conformation. In order to characterize the $Co(NH_3)_6^{3+}$-induced Ψ-DNA form of poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC), we treated the polynucleotides with different concentrations of $Co(NH_3)_6^{3+}$ and titrated their conformational status on a microtiter plate with a monoclonal anti-Z-DNA antibody (11,12). Our results show that the Z-DNA form induced by $Co(NH_3)_6^{3+}$ retains its left-handedness, as evidenced by its binding to a monoclonal anti-Z-DNA antibody Z22 and a polyclonal rabbit anti-Z-DNA antibody, even though the CD spectra of the polynucleotides underwent several changes depending on the concentration of the counterion. In contrast, another trivalent cobalt complex, $Co(en)_3^{3+}$ induced a Z-DNA conformation which at higher concentrations of the counterion assumed a Ψ-DNA-like form and then aggregated to a non-Z-DNA conformation as detected by the reactivity of these forms to the monoclonal anti-Z-DNA antibody.

MATERIALS AND METHODS

Polynucleotides

Poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC) were purchased from Pharmacia, Inc. (Piscataway, N.J.). The polynucleotides were dissolved in a buffer containing 50 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4, and dialyzed extensively from the same buffer using a Spectrapor membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) with a molecular weight cut off of 6,000-8,000. Stock solutions of the polynucleotides were prepared at 750 µg/ml and stored at 4°C. These solutions were diluted to 35 µg/ml and 10 µg/ml, respectively for the CD spectroscopic and enzyme immunoassay experiments. The optical density of the polynucleotide solutions was measured with a Beckman DU-8B spectrophotometer. Molar concentrations were calculated using the molar extinction coefficients of 8,400 and 6,800 for poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC), respectively. Brominated poly(dG-dC).poly(dG-dC) was prepared by the method described by Lafer et al. (13).

Hexammine Cobalt(III) Chloride and Tris(ethylenediamine) Cobalt(III) Chloride

$Co(NH_3)_6Cl_3$ was synthesized from $CoCl_2$ and NH_4OH according to the procedure of Work (14) and purified by three-fold recrystallization from ethanol. A stock solution of this compound was prepared in double distilled, deionized water at a high concentration to keep the

volume of the counterion solution added to the polynucleotide at less than 3% of the total volume of solution. The concentration of $Co(NH_3)_6^{3+}$ was determined by measuring the optical density at 475 nm and using the molar extinction coefficient of 56 (15). $Co(en)_3^{3+}$ was purchased from Aldrich Chemical Co. (Milwaukee, WI), recrystallized from ethanol, and used as a solution in double distilled, deionized water. The extinction coefficient of $Co(en)_3^{3+}$ is 77 at 466 nm.

Monoclonal and Polyclonal Anti-Z-DNA Antibodies

The monoclonal antibody Z22 used in this study was a kind gift from B. David Stollar, M.D., Professor and Chairman, Department of Biochemistry, Tufts University, Boston, MA. This antibody was prepared by the hybridoma technique and extensively characterized by Dr. Stollar and his associates (16-18). We received Z22 at a concentration of 350 µg/ml in phosphate buffered saline (PBS: 0.01M phosphate, 0.15 M NaCl, pH 7.4) and stored it at -70°C in aliquots of 10 to 100 µl. This stock solution was thawed and diluted in PBS containing 0.05% Tween 20 (polyoxyethylene sorbitan monolaurate) and 0.02% NaN_3 for the enzyme immunoassays. We prepared Z-DNA specific polyclonal antibodies in rabbits by immunizing with brominated poly(dG-dC).poly(dG-dC) (19). The induced antibodies reacted with high affinity to the immunizing antigen as well as different forms of Z-DNA, but failed to react with calf thymus DNA or synthetic polynucleotides in the double-stranded B-DNA form. Therefore, we used this rabbit serum as a second antibody reagent to assess the conformational status of poly(dG-dC).poly(dG-dC) and poly(dG-m^5dC).poly(dG-m^5dC) in the presence of various concentrations of $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$.

Immunochemicals

Protamine sulfate, alkaline phosphatase conjugated, affinity purified polyvalent goat anti-mouse immunoglobulins, and phosphatase substrate (p-nitrophenyl phosphate) were purchased from Sigma Chemical Company (St. Louis, MO). Alkaline phosphatase conjugated affinity purified goat anti-rabbit IgG (heavy and light chain specific) was obtained from Organon Teknika Corp., West Chester, PA.

Spectroscopic Studies

Ultraviolet spectra of the polynucleotides in the presence of various concentrations of $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ were recorded with a Beckman DU-8B spectrophotometer. The CD spectra of these complexes were recorded with a Jasco J41 spectropolarimeter. The molar ellipticity was calculated from the equation, $[\theta] = \theta/cl$, where $[\theta]$ is the molar ellipticity, θ is the relative intensity, c is the molar concentration of the polynucleotides, and l is the path length of the cell in centimeters. In CD spectroscopy, the inversion of the spectrum of the polynucleotide was taken as an indication of the B-DNA to Z-DNA transition (20). The intensity of the peak at 292 nm was used as a marker of the conformational transitions of the

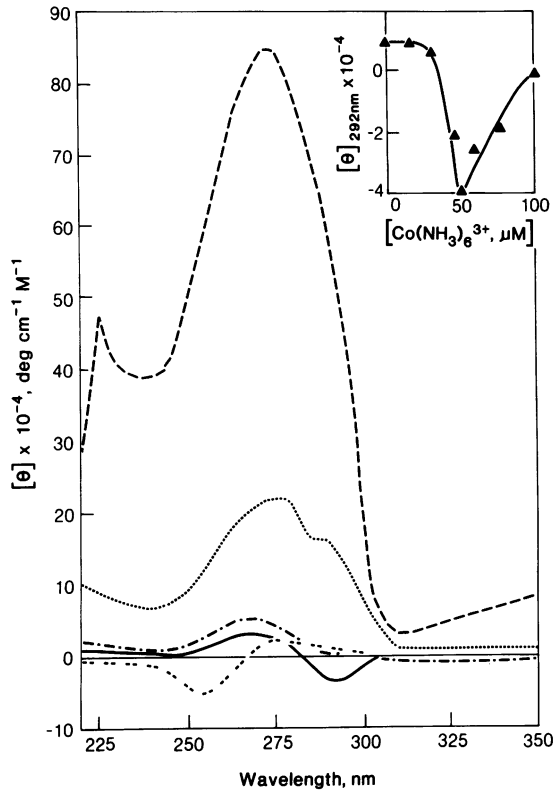

Figure 1. Circular dichroism spectra of poly(dG-dC).poly(dG-dC) in the presence of 0 (-- --); 50 (———); 75 (— · —); 100 (·····) and 1000 (– – – –) μM $Co(NH_3)_6^{3+}$ in 50 mM NaCl, 1 mM Na cacodylate and 0.15 mM EDTA (pH 7.4 at 22°C). The inset shows a plot of $[\theta]_{292\ nm}$ versus the concentration of $Co(NH_3)_6^{3+}$ in the range of 0 to 100 μM.

polynucleotides. On B-DNA to Z-DNA transition, this peak changed from a positive value to a strong negative value. In all spectroscopic measurements, the polynucleotides were mixed with the necessary concentrations of metal complexes and incubated fo: 1 h to attain equilibrium. The spectra were recorded in the wavelength region of 350 to 220 nm. The concentration of metal complexes at the midpoint of B-DNA to Z-DNA transition was determined by plotting $[\theta]_{292nm}$ against the concentration of $Co(NH_3)_6^{3+}$ or $Co(en)_3^{3+}$.

Enzyme Immunoassay Protocol

We coated microtiter plates (Cat #3590 from Costar, Cambridge, MA) with 300 μl/well of a 0.0001% solution of protamine sulfate to facilitate the adsorption of the polynucleotides to the polystyrene surface (11,12,21). The microtiter plates were incubated for 90 min at room temperature and washed 3 times with double distilled, deionized water using a Perkin Elmer-Cetus PRO/PETTE instrument with a PRO/WASH head. The plates were then treated with

0.2 ml/well of poly(dG-dC).poly(dG-dC) or poly(dG-m^5dC).poly(dG-m^5dC) that was previously incubated for 2 h with the necessary concentration of $Co(NH_3)_6^{3+}$. Polynucleotide solutions used in this study had an absorbance of 0.15 at 260 nm. In order to achieve a monolayer coating of the polynucleotides on the surface of the microtiter wells and to attain equilibrium conditions, we incubated the microtiter plates with the polynucleotide solutions for 16 h at 4°C. The plates were then washed 3 times with PBS-Tween 20-NaN_3 (washing buffer). After the washings, the plates were treated with 0.2 ml/well of the monoclonal antibody Z22 at a concentration of 0.35 µg/ml in the washing buffer and incubated for 1 h at 37°C. The plates were washed 3 times and treated with 200 µl/well of alkaline phosphatase conjugated goat anti-mouse polyvalent immunoglobulins (Sigma) diluted 1:350 in the washing buffer. The plates were incubated for 1 h at 37°C, washed 3 times and treated with a freshly prepared solution of phosphatase substrate (p-nitrophenyl phosphate) at a concentration of 1 mg/ml in 0.05 M sodium bicarbonate, 0.001 M $MgCl_2$ and 0.02% NaN_3, pH 9.0. The plates were incubated at room temperature in the dark for 30 min to complete the enzyme-substrate reaction. This reaction was stopped by the addition of 50 µl/well of NaOH. The optical density was then read with a Biotek (Winooski, VT) EL309 Microplate Autoreader. This instrument has a useful range of 0 to 2.99 optical density (O.D.) units.

We also conducted experiments using a polyclonal anti-Z-DNA antibody raised in rabbits. The experimental procedure using this antibody was essentially the same as described above with two exceptions: (1) Rabbit serum (1:400) was used in place of the monoclonal anti-Z-DNA antibody; (2) Alkaline phosphatase conjugated affinity purified goat anti-rabbit IgG was used instead of alkaline phosphatase conjugated affinity purified anti-mouse polyvalent immunoglobulins.

RESULTS

Spectroscopic Studies

Figure 1 shows the circular dichroism spectra of poly(dG-dC).poly(dG-dC) in the presence of different concentrations of $Co(NH_3)_6^{3+}$. At low concentrations (<25 µM) of this counterion, the CD spectra of the polynucleotide remained in the B-DNA form with a negative peak at 255 nm and a positive peak at 276 nm. With increasing concentrations of the metal complex, the CD spectrum changed to that of the Z-DNA form. The CD spectrum of Z-DNA was characterized by a positive peak at 265 nm and a strong negative peak at 292 nm. The concentration of $Co(NH_3)_6^{3+}$ at the midpoint of B-DNA to Z-DNA transition, determined from a plot of $[\theta]_{292nm}$ versus concentration of $Co(NH_3)_6^{3+}$, was 35 µM in 50 mM NaCl buffer (Table I). With $Co(NH_3)_6^{3+}$, the Z-DNA form of poly(dG-dC).poly(dG-dC) was stabilized over the concentration

Table I. Concentrations of cobalt complexes stabilizing different conformations of poly(dG-dC).poly(dG-dC) and poly(dG-m^{5}dC).poly(dG-m^{5}dC).

Counterion	Polynucleotide	Counterion concentration (μM)*				
		B-DNA	B → $Z_{midpoint}$	Z-DNA	Ψ-DNA	non-Z-DNA
$Co(NH_3)_6^{3+}$	Poly(dG-dC)	0-25	35	50-75	75-1000	ND**
	Poly(dG-m^{5}dC)	0-2	4.1	10-400	400-1000	N.D.**
$Co(en)_3^{3+}$	Poly(dG-dC)	0-200	250	300-400	400-600	600-1000
	Poly(dG-m^{5}dC)	0-5	7.5	10-250	250-1000	ND**

*All measurements were done in a buffer of 50 mM NaCl, 1 mM Na cacodylate, and 0.15 mM EDTA (pH 7.4).
**Not detected up to 1000 μM of counterion.

range of 50 to 75 μM only. Above this concentration of $Co(NH_3)_6^{3+}$, there was a dramatic shift in the CD spectrum of the polynucleotide with a wide positive band centered at 275 nm. The negative peak at 292 nm also disappeared (Figure 1 inset). At 500 μM $Co(NH_3)_6^{3+}$, the intensity of this band was 45-fold higher than that of the B-DNA form. Such an increase in the molar ellipticity of DNA is a hallmark of the Ψ-DNA conformation (7-10).

We also examined the effects of $Co(NH_3)_6^{3+}$ on the conformation of poly(dG-m^{5}dC).poly(dG-m^{5}dC) using CD spectroscopy. In this case, the CD spectrum of the polynucleotide underwent a conformational transition from B-DNA to Z-DNA form between 2 and 10 μM concentrations, with a midpoint concentration of 4 μM. The Z-DNA form was stabilized in the concentration range of 10 to 100 μM $Co(NH_3)_6^{3+}$. Above this concentration of the counterion, the CD spectrum of the polynucleotide changed to that of the Ψ-DNA form. The intensity of the 275 nm peak of poly(dG-m^{5}dC).poly(dG-m^{5}dC) was 10-fold higher than that of the B-DNA form. The Ψ-DNA form was retained up to a concentration of 1000 μM $Co(NH_3)_6^{3+}$. Above this concentration, extensive aggregation of the polynucleotide occurred as evidenced by a turbid solution.

In order to further understand the role of the ligand in the ability of metal complexes to provoke Z- and Ψ-DNA conformational transitions of poly(dG-dC).poly(dG-dC), we recorded the CD spectra of this polynucleotide in the presence of $Co(en)_3^{3+}$ in 50 mM NaCl, 1 mM Na cacodylate, 0.15 mM EDTA, pH 7.4. Figure 2 shows the CD spectra of poly(dG-dC).poly(dG-dC) in the presence of various concentrations of this counterion. At $Co(en)_3^{3+}$ concentrations up to 200 μM, the CD spectrum of the polynucleotide remained in the B-DNA form. At 275 μM $Co(en)_3^{3+}$, the CD spectrum of poly(dG-dC).poly(dG-dC) showed a negative band centered at

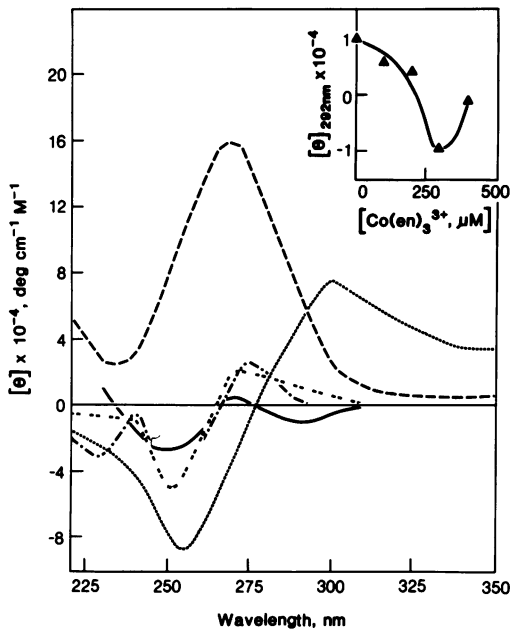

Figure 2. Circular dichroism spectra of poly(dG-dC).poly(dG-dC) in the presence of 0 (— · —); 100 (-- --); 275 (———); 400 (– – – –) and 750 (·····) μM $Co(en)_3^{3+}$ in 50 mM NaCl, 1 mM Na cacodylate and 0.15 mM EDTA (pH 7.4 at 22°C). The inset shows a plot of $[\theta]_{292nm}$ versus the concentration of $Co(en)_3^{3+}$ in the range of 0 to 100 μM.

255 nm, with a simultaneous appearance of a negative band at 292 nm. The formation of this band and the conversion of the polynucleotide to the Z-DNA form was, however, partial in this case compared to the intensitiy of Z-DNA formed with $Co(NH_3)_6^{3+}$ and poly(dG-dC).poly(dG-dC). At 400 μM $Co(en)_3^{3+}$, the CD spectrum of the polynucleotide changed to a Ψ-DNA-like form with an 8-fold increase in the molar ellipticity of the positive peak at 270 nm. At 750 μM $Co(en)_3^{3+}$, there was a dramatic change in the CD spectrum of the polynucleotide with a negative peak at 255 nm and a positive peak at 298 nm. In addition, a significant spectral shift was observed even at 350 nm, indicating the aggregation of the polynucleotide. Taken together, our CD spectral results showed that $Co(en)_3^{3+}$ provoked B-DNA → Z-DNA → Ψ-DNA → B-DNA (aggregated) transitions in poly(dG-dC).poly(dG-dC). Similar experiments with poly(dG-m^5dC).poly(dG-m^5dC) showed that $Co(en)_3^{3+}$ could induce the B-DNA → Z-DNA → Ψ-DNA transitions in this polynucleotide. But, no change in the conformation of the Ψ-DNA form to a B-DNA-like aggregated form was observed up to a concentration of 1000 μM $Co(en)_3^{3+}$ (Table I).

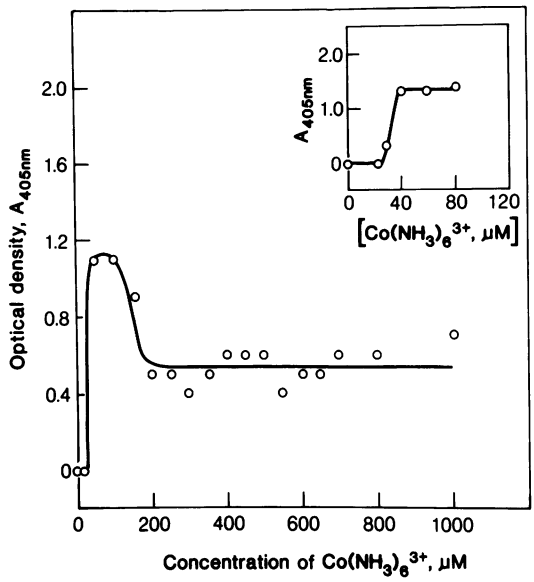

Figure 3. Enzyme immunoassay results of poly(dG-dC).poly(dG-dC) treated with different concentrations of $Co(NH_3)_6^{3+}$. The inset shows the data at low concentrations of $Co(NH_3)_6^{3+}$. Monoclonal anti-Z-DNA antibody Z22 was used in this study. The buffer conditions were the same as in Figure 1.

Enzyme immunoassay

In order to examine the conformational status of the polynucleotides under various concentrations of $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$, we used the enzyme immunoassay technique with a highly specific monoclonal anti-Z-DNA antibody, Z22. This antibody has a strong reactivity toward the Z-DNA form of poly(dG-dC).poly(dG-dC), poly(dG-m^5dC).poly(dG-m^5dC), and brominated poly(dG-dC).poly(dG-dC) (11,12,16-18). In our assay, the polynucleotides were mixed with different concentrations of $Co(NH_3)_6^{3+}$, incubated for 1 h at room temperature, and added to the microtiter wells to immobilize the conformational state of the polynucleotide (11). The plates were then treated with Z22, enzyme conjugated immunoglobulins, and the enzyme substrate, p-nitrophenyl phosphate. The optical density measured at 405 nm was a measure of the amount of Z-DNA on the microtiter plate. Figure 3 shows the results of the enzyme immunoassay of poly(dG-dC).poly(dG-dC) complexed with different concentrations of $Co(NH_3)_6^{3+}$. At low concentrations of $Co(NH_3)_6^{3+}$, the optical density is almost zero, showing that Z22 had no binding to the polynucleotide in the B-DNA form. As the concentration of $Co(NH_3)_6^{3+}$ increased, Z22 showed increasing levels of binding to poly(dG-dC).poly(dG-dC) on

the microtiter plate. In the concentration range of 30 to 40 μM $Co(NH_3)_6^{3+}$, there was a smooth transition region where the optical density increased from 0 to 1.15 O.D. The midpoint concentration of $Co(NH_3)_6^{3+}$ determined from the transition curve of the enzyme immunoassay was 35 μM in 50 mM NaCl buffer, a value similar to that determined from the CD spectroscopy. The optical density values peaked between 40 and 100 μM $Co(NH_3)_6^{3+}$. At higher concentrations, the optical density values decreased slightly and stabilized at about 45% of the peak value. These results suggest that the Ψ-DNA form of poly(dG-dC).poly(dG-dC) provoked by $Co(NH_3)_6^{3+}$ at concentrations higher than that required to stabilize the Z-DNA form still retained the left-handed conformation.

In our enzyme immunoassays, we used several controls to ascertain that the monoclonal anti-Z-DNA antibody recognized the Z-DNA form of the polynucleotide. In all microtiter plates, we used brominated poly(dG-dC).poly(dG-dC) in duplicate wells as a positive control because this polynucleotide is known to exist in the Z-DNA conformation at a physiologically relevant cationic concentration of 0.15 M NaCl (13). In all our assays using the monoclonal anti-Z-DNA antibody, there was a positive color development in wells coated with this polynucleotide and the mean optical density value was 2.1±0.2. In contrast, we observed no color reaction in wells coated with double stranded or denatured calf thymus DNA. These results demonstrate that Z22 is highly specific for the Z-DNA conformation, as reported previously (11,12,16-19).

We also used a polyclonal rabbit serum in our enzyme immunoassays. This serum was produced by immunization of a New Zealand White rabbit with brominated poly(dG-dC).poly(dG-dC) as described by Lafer et al. (13). The serum thus produced bound strongly with brominated poly(dG-dC).poly(dG-dC) as well as polyamine-treated poly(dG-m^5dC).poly(dG-m^5dC) that showed Z-DNA CD spectrum. In our enzyme immunoassays using this rabbit serum, there was strong reactivity in wells coated with complexes of poly(dG-dC).poly(dG-dC) and $Co(NH_3)_6^{3+}$ up to a $Co(NH_3)_6^{3+}$ concentration of 1000 μM. The optical density values at 405 nm were 1.5 and 1.2 in wells containing poly(dG-dC).poly(dG-dC) with 50 and 200 μM $Co(NH_3)_6^{3+}$, respectively. This result further confirms that the Ψ-DNA form of the polynucleotide is indeed a left-handed Ψ conformation.

In the next set of experiments, we examined the conformational alterations of poly(dG-m^5dC).poly(dG-m^5dC) in the presence of various concentrations of $Co(NH_3)_6^{3+}$ using the enzyme immunoassay technique. Figure 4 shows the optical density values plotted against the concentration of the counterion. There was no reaction of Z22 to microtiter wells coated with the polynucleotide alone or that complexed with up to 2 μM of $Co(NH_3)_6^{3+}$. Increasing concentrations of the counterion showed a concomitant increase in the binding of Z22 to the

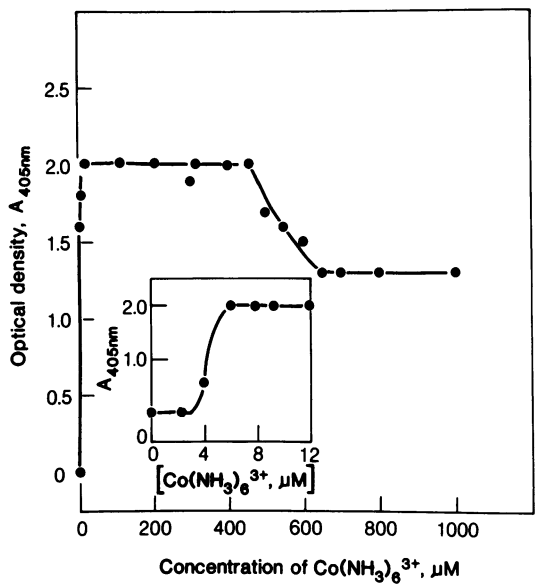

Figure 4. Enzyme immunoassay results of poly(dG-m^{5}dC).poly(dG-m^{5}dC) treated with different concentrations of $Co(NH_3)_6^{3+}$. The inset shows the data at low concentrations of $Co(NH_3)_6^{3+}$. Monoclonal anti-Z-DNA antibody Z22 was used in this study. The buffer conditions were the same as in Figure 1.

polynucleotide. There was a leveling off of the O.D. values between 6 and 400 μM $Co(NH_3)_6^{3+}$. The midpoint value of the counterion determined from a plot of A_{450nm} versus the concentration of $Co(NH_3)_6^{3+}$ (inset to Figure 4) was 4.2 μM. This value is similar to that determined from the CD spectral measurements (Table I). As in the case of poly(dG-dC).poly(dG-dC), there was a significant reduction in optical density values at $Co(NH_3)_6^{3+}$ concentrations above 500 μM.

We next carried out a set of enzyme immunoassay experiments to examine the conformational status of poly(dG-dC).poly(dG-dC) treated with different concentrations of $Co(en)_3^{3+}$ using the monoclonal anti-Z-DNA antibody. The results of our enzyme immunoassay are shown in Figure 5. There was no reaction between the polynucleotide and Z22 up to 200 μM $Co(en)_3^{3+}$ as detected by the optical density values. At 200 μM $Co(en)_3^{3+}$, Z22 showed a positive reaction. The optical density showed a concomitant increase up to 400 μM $Co(en)_3^{3+}$. Between 400 and 600 μM $Co(en)_3^{3+}$, there was a sharp decrease in the optical density values. There was no reaction between poly(dG-dC).poly(dG-dC) and Z22 above 600 μM $Co(en)_3^{3+}$.

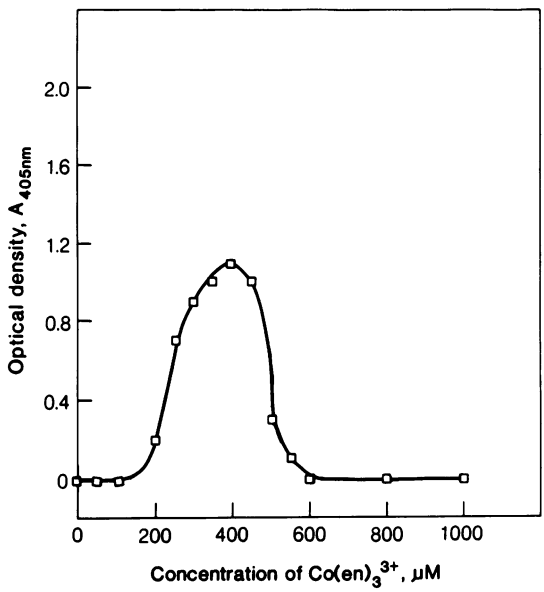

Figure 5. Enzyme immunoassay results of poly(dG-dC).poly(dG-dC) treated with different concentrations of $Co(en)_3^{3+}$. The buffer conditions were the same as in Figure 2.

This result is consistent with our CD spectral studies in which we found that the polynucleotide assumed an aggregated B-DNA-like conformation at high concentrations of this counterion. In this case, the polynucleotide underwent a conformational transition from Z-DNA to a non-Z-DNA conformation. Since the non-Z-DNA conformation had no reactivity with Z22, sequential changes in the conformation of the polynucleotide appear to produce almost 100% transitions without any contamination by the preceding forms.

We also conducted enzyme immunoassay experiments with poly(dG-m^5dC).poly(dG-m^5dC) treated with different concentrations of $Co(en)_3^{3+}$ (results not shown). Our result showed that Z22 bound to the polynucleotide in the presence of 10 to 1000 μM $Co(en)_3^{3+}$ (Table I). In contrast to the ability of poly(dG-dC).poly(dG-dC) to undergo a change from the left-handed Ψ-DNA conformation to a non-left-handed form at high concentrations of $Co(en)_3^{3+}$, there was no change in the handedness of Ψ-DNA form of poly(dG-m^5dC).poly(dG-m^5dC) up to 1000 μM of this counterion. This result is consistent with the facile nature of poly(dG-m^5dC).poly(dG-m^5dC) to assume the Z-DNA form under mild conditions in the presence of multivalent cations including $Co(NH_3)_6^{3+}$ and polyamines (1).

DISCUSSION

Results of our CD spectroscopic studies demonstrate that $Co(NH_3)_6^{3+}$ is capable of inducing and stabilizing the Z- and Ψ-DNA structures in poly(dG-dC).poly(dG-dC) and poly(dG-m^5dC).-poly(dG-m^5dC) depending on the concentration of the counterion. In addition, results of the enzyme immunoassay with monoclonal and polyclonal anti-Z-DNA antibodies provide evidence for the first time that the supramolecular organization of these polynucleotides in the Ψ-DNA form is in the left-handed conformation. The formation of Ψ-DNA form of poly(dG-dC).poly-(dG-dC) found in this study is comparable to that reported by Zacharias *et al* (22) in the presence of Na acetate and that reported by Shin et al (10) in the presence of $Co(NH_3)_6^{3+}$. With Na acetate, the Ψ-DNA form was observed in CD spectroscopy at the midpoint of B-DNA to Z-DNA transition (22). The report of Shin *et al* (10) suggested that the polynucleotide underwent a series of conformational transitions, B → Z → U → Ψ, where U stands for an unknown conformation. These investigators concluded that Z and U are intermediate conformations of the polynucleotide in its sequential transition from the B-DNA to the Ψ-DNA form. In either case, however, the helical handedness of the polynucleotide was not known unequivocally because the interpretation of the CD spectra was obscured by the 10- to 50-fold increase in the positive peak of the molar ellipticity of Ψ-DNA. In the enzyme immunoassay, this difficulty is circumvented by the use of a highly specific monoclonal anti-Z-DNA antibody (11). In several recent studies, it has been shown that this antibody had no reactivity to the polynucleotides under conditions at which they existed in the B-DNA form (11,12,16,18).

The use of the enzyme immunoassay technique to study the conformational dynamics of polynucleotides has several advantages over conventional spectroscopic techniques. $Co(NH_3)_6^{3+}$ is a good promoter of Z-DNA conformation as well as the condensation and aggregation of DNA (2,15,23). Collapsed DNA structures of high degree of organization, including the $Co(NH_3)_6^{3+}$-induced toroidal condensation of DNA are important to understand the compaction of DNA in virus heads and chromatin (24-26). Ψ-DNA is believed to be a twisted, tightly packaged supramolecular assembly of DNA (5-8,21). The aggregation of DNA is a serious impediment in the use of CD spectroscopy to study the conformational transitions of DNA because the aggregated particles scatter circularly polarized light (27). Maestre *et al* (28) showed that this scattering can lead to erroneous interpretation of CD spectral results of DNA. In this respect, the enzyme immunoassay has the advantage of recognizing antigenic determinants on the DNA structure irrespective of its aggregation status. In earlier studies, we showed that a pre-existing conformation of DNA can be immobilized on a microtiter plate without further structural alterations (11,12). In the present study, we demonstrate that the supramolecular assembly of poly(dG-dC).poly(dG-dC) and poly(dG-m^5dC).poly(dG-m^5dC) formed in the

presence of $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ is capable of binding to a monoclonal anti-Z-DNA antibody and a polyclonal rabbit serum containing antibodies raised against an authentic form of Z-DNA. With these antibodies, however, there is a significant reduction in the optical density values at high concentrations of the counterion. A possible reason for this might be the inaccessibility of a fraction of the polynucleotides to anti-Z-DNA antibody due to the tight packaging of the polynucleotide in the Ψ-DNA form (6,7,22). Despite this difficulty, the present study provides direct evidence for the existence of poly(dG-dC).poly(dG-dC) and poly(dG-m^5dC).poly(dG-m^5dC) in left-handed Ψ-DNA form.

Since Z-DNA has a high tendency to aggregate, it is possible that the monoclonal and polyclonal antibodies are binding to an aggregated form of the polynucleotide such as the Z* form (29-31) that is formed in the presence of divalent cations after increasing the temperature of the solution to about 55°C. We discard this possibility for two reasons: (1) The Z* form is formed by no significant perturbation of optical properties including the CD spectrum of the polynucleotide (29,30). In the case of $Co(NH_3)_6^{3+}$- and $Co(en)_3^{3+}$- induced Ψ-DNA formation, a manyfold increase in the molar ellipticity of the polynucleotides is observed. (2) All conformational alterations reported in this paper are produced at 22°C. In contrast, aggregated Z* form is formed only after heating of the polynucleotides (29,30).

Results presented in this study also provide evidence for differential effects of counterions on the conformation of polynucleotides. Even though both $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ are trivalent cations with the same central metal atom, only the former is capable of stabilizing poly(dG-dC).poly(dG-dC) in the left-handed Z- and Ψ-DNA conformations up to 1000 μM concentration of the counterion. In the case of $Co(en)_3^{3+}$ and poly(dG-dC).poly(dG-dC), the Ψ-DNA form assumes an aggregated non-left-handed conformation at high concentrations of the counterion. A possible reason for this might be the differences in the mode of interaction of the two cations with the polynucleotide. In the case of $Co(NH_3)_6^{3+}$, Gessner *et al* (32) showed that the interaction of this cation with $(dC\text{-}dG)_3$ is stabilized by five hydrogen bonds formed between the ammonia molecules of $Co(NH_3)_6^{3+}$ and the phosphate groups of the oligonucleotide as well as the N7 and O6 positions on guanine. A similar hydrogen bonding pattern has been observed in $Co(NH_3)_6^{3+}$-induced stabilization of the tertiary structure of yeast phenylalanine tRNA (33). Substitution of NH_3 by $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$ in $Co(en)_3^{3+}$ appears to introduce a steric hindrance for the formation of this type of hydrogen bonding. Therefore, the ethylene diamine complex is less efficient to form a high affinity complex with DNA under conditions such as the twisted tight packaging that is characteristic of the Ψ-DNA form. Our CD results of the interaction of poly(dG-dC).poly(dG-dC) show that the polynucleotide reverted to a non-Z-DNA form

immediately after its assuming the left-handed Ψ-DNA organization. This result provides further support to the conclusion that the conformational alterations in these polynucleotides induced by $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ are cooperative transitions and that each conformation is not contaminated by the preceding form.

The ability of Z-DNA to undergo further structural organizations including the Ψ-DNA form in the presence of $Co(NH_3)_6^{3+}$ has several important biological implications. With the discovery of physiologically compatible conditions, including the presence of natural polyamines (1,34-36) and negative supercoiling (37-39) in physiologic cation concentrations, it has become evident that Z-DNA has a potential biological function if the right combination of DNA sequence distribution and the microenvironment to provoke the Z-DNA conformation is available. The reported inability of Z-DNA to form nucleosomes under reconstitution conditions (40), however, posed a problem in realizing this potential because the DNA in the cell is tightly packaged in the form of chromatin. It is known from earlier studies that Z-DNA has a tendency to aggregate (2,41) and to form compacted structures like toroids and rods (2). The conformational status of these aggregated or organized forms of DNA was not known with any precision because CD spectroscopy, the most commonly used technique in the study of DNA conformational transitions, failed to provide any information on the handedness of these supramolecular structures. The results presented in this report clearly show that in the case of the polynucleotides, the left-handed conformation can be maintained in highly organized structures like the Ψ-DNA form. This form is considered to be important in the packaging of DNA in the cell.

In conclusion, results presented in this paper show that $Co(NH_3)_6^{3+}$ is capable of converting poly(dG-dC).poly(dG-dC) and poly(dG-m^5dC).poly(dG-m^5dC) to the left-handed Z- and Ψ-DNA structures. Using an enzyme immunoassay with a highly specific monoclonal anti-Z-DNA antibody, we demonstrate that the Ψ-DNA structure of these polynucleotides exists in the left-handed Z-DNA form.

ACKNOWLEDGEMENTS

We thank B. David Stollar, M.D., Professor and Chairman, Department of Biochemistry, Tufts University School of Medicine for the monoclonal anti-Z-DNA antibody and for a critical reading of an earlier form of this manuscript. We also thank Victor A. Bloomfield, Ph.D., Professor and Head, Department of Biochemistry, University of Minnesota and Ronald P. Messner, M.D., Professor and Director, Section of Rheumatology, Department of Medicine, University of Minnesota for their support during the performance of a part of this study in their laboratories.

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