# Terbium(III)-induced conformational transition in poly(dG-dC) fluorescence and circular dichroic studies

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Poly(dG-dC) in 60% aqueous alcohol exhibits the characteristic inversion of the circular dichroism spectrum associated with the formation of left-handed helix. Upon complexation with $Tb^{3+}$, poly(dG-dC) in this medium induces marked enhancement of the Tb(III) fluorescence emission at 488 and 545 nm, when excited at 290 nm. The degree of fluorescence enhancement is dependent on the concentration of Tb(III) at a fixed poly(dG-dC) concentration. Neither poly(dG-dC) in water nor poly(dA-dT) in water or 60% alcohol, causes any significant fluorescence spectral changes of $Tb^{3+}$. Tb(III)-poly(dG-dC) in 60% alcohol shows circular dichroic spectra associated with a broad positive molar ellipticity ranging from 6000 to 10 000 degree · $cm^2 \cdot dmol^{-1}$ between 270 and 280 nm, and a small negative band around 240 nm.

*Poly(dG-dC)* *Fluorescence* *CD* *$Tb^{3+}$*

## 1. INTRODUCTION

The use of trivalent lanthanide cations, particularly $Tb^{3+}$ and $Eu^{3+}$, as fluorescent probes for determining the structures of nucleic acids and proteins has markedly increased in the recent past. This is so, as the resonance energy levels of these ions fortuitously overlap with the triplet energy states of protein and nucleic acid ligands, irradiated with ultraviolet light [1]. As a result, an intermolecular energy transfer from the organic ligand to the central metal atom takes place along with the radiation-less deactivation of the ions themselves [2]. This property has been utilized extensively to mimic the alkaline earth metal ion-binding sites in nucleic acids or in nucleic acid-protein complexes [3–9]. Appropriate irradiation of the Tb(III)-organic ligand complex results in an enhanced emission signal unlike Tb(III) alone in the buffer and therefore is taken as a parameter to follow the degree and nature of the Tb(III)-ligand interaction. Recently, Gross and Simpkins [8] have analyzed in detail the nature of interaction between Tb(III) and different nucleic acid ligands. The general picture that emerges from this study, in conformation of earlier reports [6,7], indicates: (i) two-site binding of Tb(III) with nucleic acids, i.e. at the phosphate linkage along with the coordination with an electron donor group, (ii) large enhancement of the Tb(III) fluorescence upon binding to either xanthine or guanine residues, (iii) and an effect of secondary and tertiary structure of nucleic acids on the energy transfer mechanism.

In the light of the above, it is of interest to monitor the changes in the Tb(III) fluorescence associated with any change in the conformation of the bound ligand. This is particularly important as the first requirement of a fluorescent probe is that it should not disturb the conformation of the studied macromolecule. I have used here poly(dG-dC) as the macromolecular ligand for Tb(III) and analyzed the conformational alteration of the ligand upon (Tb(III) binding by fluorescence and circular dichroic (CD) spectral studies. The choice of the ligand here becomes obvious as Tb(III) shows appreciable fluorescence emission with guanine residue and poly(dG-dC) has been very well studied with respect to their right-handed to



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left-handed helical transition (B→Z) under the influence of various cations [10–12] or with change in solvent dielectric constant [13].

## 2. MATERIALS AND METHODS

The double helical alternating copolynucleotides, poly(dG-dC) and poly(dA-dT) were products of Pharmacia/PL Biochemicals (lot no. 782–73 and 782–38, respectively). They were subsequently dialyzed against EDTA, treated with Bio-Rad Chelex-100 and desalted over a column containing Biogel P4 (mesh size 100–200) previously equilibrated with double-distilled water. The elution of the column was carried out by double-distilled water so as to make the nucleic acid salt-free. Similarly, twice-distilled absolute ethanol (Merck) was also Chelex-treated before use. The concentration of the nucleic acids were determined spectrophotometrically using molar extinction coefficients of $6.8\times10^3$ at 260 nm for poly(dA-dT) and $8.4\times10^3$ at 254 nm for poly(dG-dC) [11]. $TbCl_3\cdot 6H_2O$ was a product of Aldrich and a stock solution of 10 mM in distilled water was prepared and titrated against EDTA [14]. $S_1$ nuclease was a gift from C.W. Wu, Stony Brook, USA.

Fluorescence spectroscopic measurements have been carried out in a 650-10S Hitachi spectrofluorimeter. Solutions containing terbium were routinely excited at 290 nm and the absorbances at the excitation wavelength were always maintained less than 0.1 to avoid the inner filter effect. The intense second harmonic band at 580 nm has no effect on the emission peaks of Tb(III). The emission spectra thus recorded are all uncorrected. Circular dichroic measurements were done in a Jovin-Yvon mark V dichrograph at 25°C. Each reported spectrum is the average of at least 3 scans.

Complexes between Tb(III) and nucleic acids were prepared by adding different molar ratios of $TbCl_3$ to the nucleic acid solution. The ratio of the molar concentration of the metal to the nucleic acid is denoted here by $r$ of Tb(III) in the medium compared to poly(dG-dC). Typically, a 100 $\mu$M solution of poly(dG-dC) was used for CD studies. For fluorescence measurements, either the same solution was diluted 10-fold [i.e. 10 $\mu$M nucleic acid with 20–100 $\mu$M Tb(III)] or a fresh complex was prepared. Both methods of preparation gave identical results within experimental error.

## 3. RESULTS

### 3.1. *Tb(III)-poly(dG-dC)*

All fluorescence measurements reported here have been carried out at a constant temperature of 24°C. Various combinations of conditions previously revealed that in the case of poly(dG-dC) a strongly dehydrating medium such as high salt concentration or the addition of alcohol resulted in the B→Z transition [10–13]. Since a high ionic strength in the medium gives rise to additional problems such as $Tb(OH)_3$ precipitation and competition of the medium with the phosphate groups of the macromolecule for Tb(III) binding, it was decided to follow the conformational transition of nucleic acids in 60% alcohol.

Fluorescence emission spectra of Tb(III)-poly (dG-dC) and Tb(III)-poly(dA-dT) in 60% aqueous ethanol and pure water are reported in fig.1.

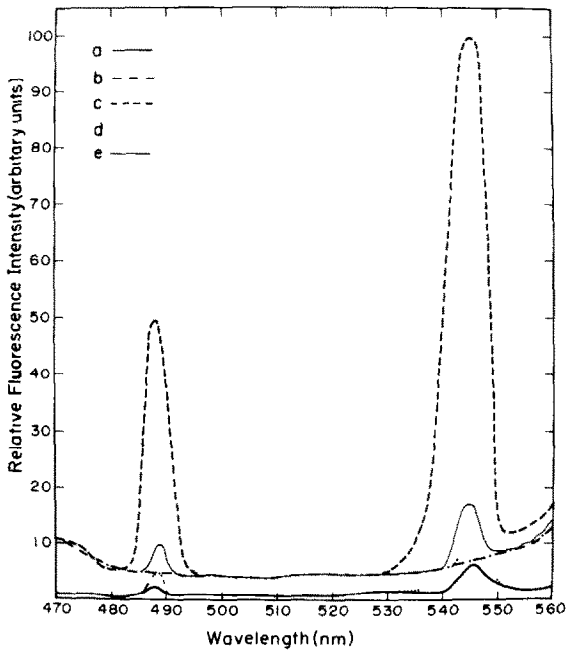


Fig.1. Fluorescence emission spectra of $Tb^{3+}$ and its complex with poly(dA-dT) or poly(dG-dC) in water and in 60% alcohol, when excited at 290 nm at 24°C. (a) Tb(III)-poly(dG-dC) ($r=10$) in water. (b) Tb(III) (0.1 mM) in 60% alcohol. (c) Tb(III)-poly(dG-dC) ($r=10$) in 60% alcohol. (d) Tb(III)-poly(dA-dT) ($r=10$) in water. (e) Tb(III)-poly(dA-dT) ($r=10$) in 60% alcohol. $r$, ratio of molar concentration of $Tb^{3+}$ to nucleic acid, where the concentration of poly(dG-dC) or poly(dA-dT) is kept at 10 $\mu$M.

Tb(III) complexes have been reported earlier to show two sharp peaks at 488 and 545 nm upon energy transfer [1-9]. Both the Tb(III)-poly(dG-dC) and Tb(III)-poly(dA-dT) complexes in water at 10-fold molar excess of $Tb^{3+}$ exhibit no appreciable fluorescence emission signals. It may also be noted from fig.1b that Tb(III) alone in 60% alcohol does not show any emission spectrum, but upon addition of poly(dG-dC), two sharp Tb(III) peaks appear as shown in fig.1c. This enhancement of the fluorescence signals for Tb(III)-poly(dG-dC) in 60% alcohol is remarkable when compared to that observed for the same complex in water or for the Tb(III)-poly(dA-dT) system in 60% alcohol (see fig.1e). The enhancement of the fluorescence intensity at both 488 and 545 nm is found to be dependent on the molar ratio of Tb(III):poly(dG-dC) as expected and is depicted in fig.2.

What is the conformation of poly(dG-dC) in 60% alcohol in the presence of various concentration of Tb(III)? This question is answered by an analysis of the CD spectra of the different Tb(III)-poly(dG-dC) complexes, as shown in fig.3. Poly(dG-dC) alone shows a typical Z-DNA CD profile in 60% alcohol (fig.3a) with an intense negative band at 290 nm and a positive maximum at 265 nm. Upon addition of various mole ratios of Tb(III), a series of dichroic spectra of the complexes are generated and shown in fig.3b-d, where fig.3b is due to the complexes of Tb(III)-poly(dG-dC) having $r$ between 2 and 6 ($r$=[Tb(III)]/poly(dG-dC)) and fig.3c and d denote $r=8$ and $r=10$, respectively. $Tb^{3+}$ appears to induce a major conformational change in the left-handed form of poly(dG-dC). The large negative band at 290 nm vanishes whereas the positive maximum at 265 nm shifts to longer wavelengths by more than 10 nm, with an initial marginal increase in molar ellipticity value, which subsequently decreases with increase in concentration of Tb(III). In the lower wavelength region, the intensity of the positive band at 225 nm decreases with $Tb^{3+}$ concentration and at $r=8$ or 10, a small negative band appears around 240 nm. It is interesting to note that the conformational alteration of the left-handed form of poly (dG-dC) by Tb(III) is also associated with an intense enhancement of the emission signal of $Tb^{3+}$. However, by comparing figs 2 and 3, it is apparent that the major conformational change in left-

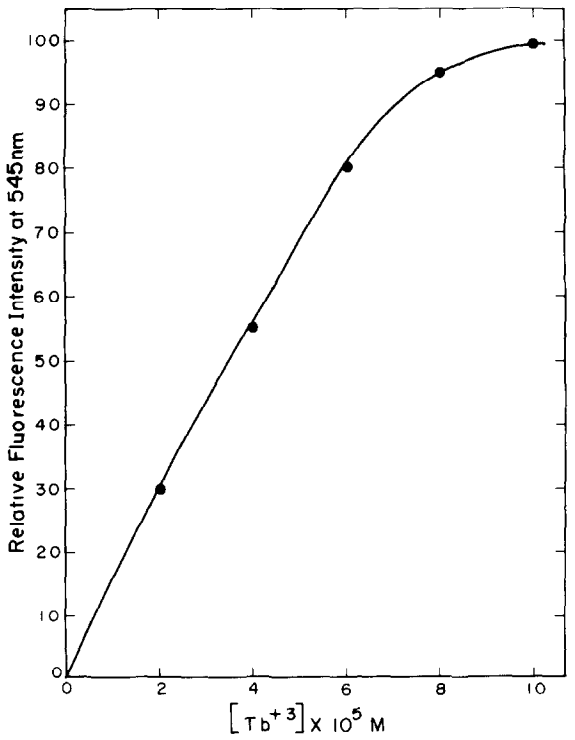


Fig.2. Variation of the fluorescence intensity of Tb(III)-poly(dG-dC) at 545 nm with concentration of Tb(III), when excited at 290 nm in 60% alcohol Concentration of poly(dG-dC), 10 $\mu$M.

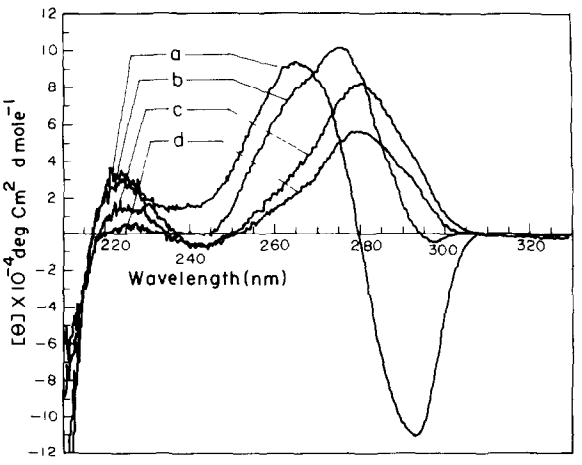


Fig.3. CD spectra of TB(III)-poly(dG-dC) in 60% alcohol taken at 25°C. (a) Poly(dG-dC). (b) Tb(III)-poly (dG-dC) ($r$=2-6). (c) Tb(III)-poly(dG-dC) ($r$=8). (d) Tb(III)-poly(dG-dC) ($r$=10). $r$, ratio of the molar concentration of $Tb^{3+}$ to nucleic acid. The concentrations of the nucleic acids are kept at 100 $\mu$M.

handed poly(dG-dC) takes place at a lower metal-to-polynucleotide ratio, which is otherwise associated with a lesser degree of fluorescence enhancement.

## 4. DISCUSSION

Poly(dA-dT), either in the presence of 60% alcohol, or under the influence of high ionic strength, stays only in the right-handed helical structure, and no major change is noticeable for poly(dA-dT) in the presence of Tb(III). I show here that the Tb(III)-poly(dA-dT) complex also exhibits only very weak fluorescence emission. Left-handed poly(dG-dC) induces, on the other hand, a significant enhancement of Tb(III) fluorescence, having changed itself in the process, into a stable altered conformational state. It should also be mentioned that the Tb(III) fluorescence enhancement is a sensitive probe of single-stranded, non-base-paired regions of highly structured nucleic acids. Therefore, different batches of poly(dG-dC) were extensively digested with $S_1$ nuclease, ethanol precipitated and dialyzed to remove the nicks, single-stranded tails from the nucleic acid samples before subjecting them to the fluorescence measurements.

Haertle et al. [15] have shown recently that in low salt aqueous medium (pH 6) Tb(III) induces the B→Z transition in poly(dG-dC), with enhancement of the fluorescence intensity. However, no further analysis of the left-handed CD pattern in the presence of Tb(III) has been attempted here. The present work deals with the complementary process, namely the addition of $Tb^{3+}$ to DNA which is already in the left-handed configuration. The increase in the induced fluorescence intensity of Tb(III) in the later case, is dramatic, and an order of magnitude higher than that of the Tb(III)-right handed DNA complex. By analyzing the CD spectral change induced by Tb(III) on poly(dG-dC) in aqueous medium [15] i.e. from the ratio of the positive to negative ellipticities, it appears that Tb(III)-poly(dG-dC) may not be in the exact left-handed DNA configuration, but rather is represented by some intermediate form. However, the stoichiometry of binding of Tb(III) to the phosphates is comparable in both cases over the same concentration range.

What is the conformation of the Tb(III)-poly (dG-dC) complex in 60% alcohol which is responsible for the enhanced fluorescence emission spectrum? Gersanovski et al. [9] have reported that the accessibility of the N-7 of guanine of Tb(III) is required for structural alterations in the aggregated state to occur. It has also been well established [16] that the imidazole ring containing N-7 of the guanine protrudes outward from the helix axis in Z-DNA rendering it accessible to the environment. Although the CD profile of Tb(III)-poly(dG-dC) resembles the aggregate $\psi(+)$-DNA form [17], its molar ellipticity values are an order of magnitude less than expected. However, Eichhorn et al. [18] have noted an intermediate structure of DNA (obtained under the influence of metal ions) having the same CD spectrum as shown here for Tb(III)-poly(dG-dC) ($r = 2$–$6$) and have named it a X-DNA (spectra 3b and c also resemble the CD pattern of A-DNA). I have noted in the course of this study that the structure is stable, does not produce the $\psi(+)$-form even during long time periods, and is associated with intense fluorescence emission, probably due to the exposure of the electron-donating group of the guanine moiety to $Tb^{3+}$.

The enhancement of the fluorescence emission of Tb(III) by left-handed poly(dG-dC) is not due to the Z-helical structure but rather due to an intermediate form of alternating purine-pyrmidine sequence that is stabilized upon complexation. It would be interesting to see whether such phenomena occur within a native DNA or circular plasmids having a stretch of dG-dC sequence, and if so whether it would be possible to identify them with the help of lanthanide fluorescence.

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