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Interactions of the human telomeric DNA with terbium–amino acid complexes

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Abstract

The human telomeric DNA can form four-stranded structures: the G-rich strand adopts a G-quadruplex conformation stabilized by G-quartets and the C-rich strand may fold into an I-motif based on intercalated $C \cdot C^+$ base pairs. There is intense interests in the design and synthesis of compounds which can target telomeric DNA and inhibit the telomerase activity. Here we report the thermodynamic studies of the two newly synthesized terbium–amino acid complexes bound to the human telomeric G-quadruplex and I-motif DNA which were studied by means of UV–Visible, DNA meltings, fluorescence and circular dichroism. These two complexes can bind to the human telomeric DNA and have shown different features on DNA stability, binding stoichiometry, and sequence-dependent fluorescence enhancement. To our knowledge, this is the first report to show terbium–amino acid complexes can interact with the human telomeric DNA.

Keywords: Human telomeric DNA; Spectral titration; Ligand–DNA interactions; DNA recognition; Circular dichroism

1. Introduction

Telomeres are specific nucleoprotein structure at the end of all eukaryotic chromosomes, and they are essential for genome integrity and appear to play an important role in cellular aging and cancer. Most telomeric DNA consists of simple repetitive sequences with G-rich termini [1,2]. The telomeric DNA has a unique mode of replication based on a special reverse transcriptase-like enzyme called telomerase, and cancer cells often express high levels of telomerase while the somatic cells express low [3]. Human telomeric DNA composed of $(TTAGGG/CCCTAA)_n$ repeats may form a classical Watson–Crick duplex, while each individual strand has the propensity to form four-stranded structures: the G-rich strand adopts a G-quadruplex conformation stabilized by G-quartets and the C-rich strand may fold into an I-motif based on intercalated $C \cdot C^+$ base pairs [1,2,4–6] (Scheme 1). There is intense interests in the design and synthesis of compounds which can target telomeric DNA and inhibit the telomerase activity [4,7,8].

Lanthanide complexes have been widely used as probes in luminescent resonance energy transfer (LRET) for bioassays and as reagents for diagnosis in magnetic resonance imaging (MRI) [9,10]. Herein, we report two terbium–amino acid complexes which were synthesized under near physiological conditions can bind to human telomeric G-quadruplex and I-motif DNA and show different features on DNA stability, binding stoichiometry, and sequence-dependent fluorescence enhancement.

2. Materials and methods

2.1. Synthesis and crystallization of $[Tb_2(DL\text{-}Cys)_4(H_2O)_8]Cl_2$ (complex **1**) and $[Tb_2(DL\text{-}HVal)_4(H_2O)_8]Cl_6 \cdot 2H_2O$ (complex **2**)

DL-Cysteine(0.24 g, about 2 mmol) or DL-valine(0.23 g, about 2 mmol) was added as solid in an aqueous solution

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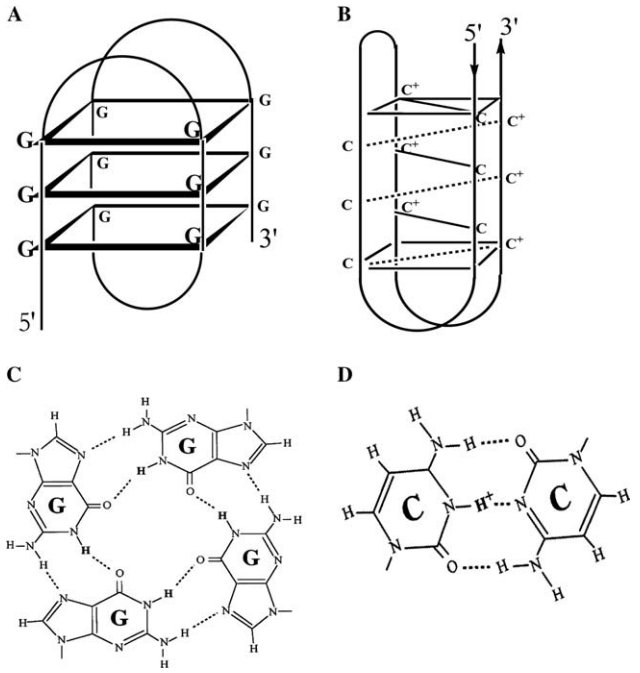

Scheme 1. Fold structure of the G-rich strand (A) and C-rich strand (B) of human telomeric DNA; C: the G-quartet; D: the C.C^{+} hemiprotonated base pair of the "Building blocks" for quadruplex formation.

of $TbCl_3$ (0.1 M, 20 ml). The amount of DL-cysteine (or DL-valine) was slightly less than that of $TbCl_3$. With stirring, an aqueous NaOH solution (0.5 M) was added dropwise to above solution until pH $\approx$ 7.0. After stirred continuously in a thermostat (333 K) for 6 h, the mixture was filtered and the filtrate was allowed to stand at room temperature. Colorless crystals appeared in about eight weeks. The crystalline product was collected by filtration, washed with mixture of tetrahydrofuran/ether (1:1 v/v), and dried in a desiccator charged with silica gel.

Element analysis of the complex was carried out on Elementar Analysensysteme GmbH Vario EL (HAMAU, Germany). Element analysis for complex **1**: Calc. (%) for $C_{12}H_{38.8}Cl_2Tb_2N_4O_{16}S_4$: Tb, 31.40; C, 14.24; H, 3.86; N, 5.53. Found: Tb, 31.62; C, 14.25; H, 3.75; N, 5.50.

Crystal data for complex **1**: $M = 1012.25$; orthorhombic; space group: Fddd; $a = 11.462(7)$ Å, $b = 21.214(8)$ Å, $c = 35.636(16)$ Å, $V = 8665(7)$ Å^3; $T = 293$ K, $Z = 8$, absorption coefficient: 3.605 mm^{-1}; reflections collected: 2483; independent reflections: 2047 ($R_{int} = 0.0336$); data/restraints/parameters: 2047/0/103; final R induces [$I > 2\sigma(I)$]: $R_1 = 0.0965$, $wR_2 = 0.2197$. Intensity data were collected on a Siemens SMART CCD diffractometer with graphite-monochromatic Mo Kα($\lambda = 0.710\,73$ Å) radiation at a temperature of 298 $\pm$ 2 K. The structure was solved by direct methods using the SHELXTL-97 crystallographic software package and refined by full matrix least-squares on F^2.

Element analysis for complex **2**: Calc. (%) for $C_{20}H_{64}Cl_6Tb_2N_4O_{18}$: Tb, 26.95; C, 20.37; H, 5.47; N, 4.75. Found: Tb, 27.16; C, 20.32; H, 5.48; N, 4.77.

Crystal data for complex **2**: $M = 1179.29$; monoclinic; space group: $C2/c$; $a = 26.438(3)$ Å, $b = 11.3555(14)$ Å, $c = 16.310(2)$ Å, $V = 5680(4)$ Å^3; $T = 293$ K, $Z = 4$, absorption coefficient: 3.600 mm^{-1}; reflections collected: 12,084; independent reflections: 4327 ($R_{int} = 0.0186$); data/restraints/parameters: 4327/4/248; final R induces [$I > 2\sigma(I)$]: $R_1 = 0.0261$, $wR_2 = 0.0755$.

The details of the crystal data, refinement and final statistics are summarized in Table 1.

2.2. Bioassay

UV–Visible absorbance measurements and DNA UV melting studies were made on a Cary 300 C (Varian USA) UV–Visible spectrophotometer, equipped with Peltier temperature control accessory [11]. The DNA oligonucleotides were synthesized by Sangon and were used without further purification. Tris–HCl buffer (50 mM Tris base, 100 mM NaCl, pH 7.1) was used in the case of G-quadruplex. The buffer solution containing 150 mM NaCl pH 5.5 was used for 22-mer C-rich single-stranded DNA. The concentrations of DNA were determined by measuring the absorbance at 260 nm after melting, and the extinction coefficients were estimated by the nearest-neighbor model [11]. Fluorescence titrations were conducted on a JASCO FP-6500 spectrofluorometer [12]. Excitation wavelength was 260 nm. The emission spectrum was collected from 350 nm to 650 nm. Circular dichroism spectra were measured at 20 °C on a JASCO J-810 spectropolarimeter with a computer-controlled water bath as previously described [13]. Three scans were accumulated and automatically averaged.

2.3. Determination of binding constants

DNA binding constants were determined by fluorescence titration as described previously [14]. Titration data were fit directly by nonlinear least-squares methods to get binding constants, using a fitting function incorporated into the program FitAll (MTR Software, Toronto). Errors were evaluated by a Monte Carlo analysis, using a routine that has been added to the FitAll package (MTR Software, Toronto).

The observed fluorescence is assumed to be a sum of the weighted concentrations of free and bound ligand:

$$F = F^0(C_t - C_b) + F^b C_b \quad (1)$$

where F is the apparent fluorescence at each DNA concentration, F^0 is the fluorescence intensity of free ligand, and F^b is the fluorescence intensity of the bound species. C_t and C_b are the molar concentrations or total and bound ligand, respectively. For the interaction of a ligand D with a DNA site S, it can be easily shown that:

$$Kx^2 - x(KS_0 + KD_0 + 1) + KS_0D_0 = 0 \quad (2)$$

where $x = C_b$, K is the association constant, S_0 is the total site concentration and D_0 is the total ligand concentration.