Biophysical Chemistry 219 (2016) 49–58

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Binding of the alkaloid coralyne to parallel G-quadruplex DNA $[d(TTGGGGT)]_4$ studied by multi-spectroscopic techniques

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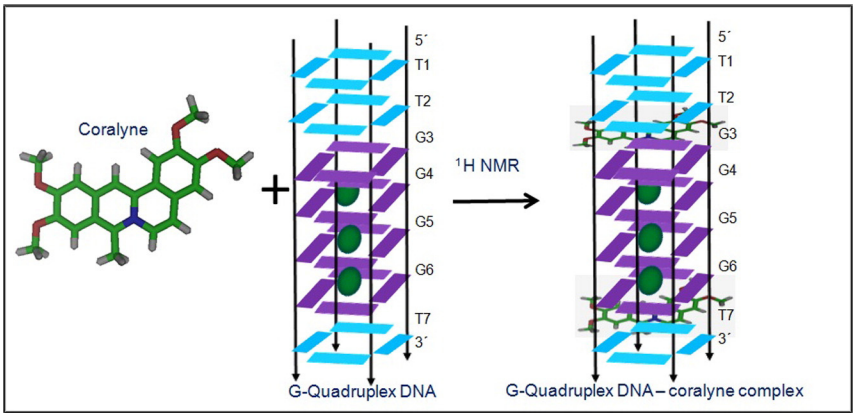
HIGHLIGHTS

- Binding induces negative circular dichroism band of coralyne at 315 nm.
- Absorbance/fluorescence change accompanied by 10–12 nm red/blue shift.
- Fluorescence lifetime of 6 ns and 12 ns indicate two conformations of coralyne.
- ^{1}H NMR spectra shows broadening and upfield shift of G3NH, G6NH and G3H8 protons.
- Non-intercalative sequence specific binding at two sites of TTGGGGT and TTAGGGT.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form 17 August 2016
Accepted 17 September 2016
Available online 01 October 2016

Keywords:
Parallel G-quadruplex DNA
Alkaloid coralyne
Circular dichroism
Absorbance and fluorescence
Nuclear magnetic resonance
Non-intercalative binding

ABSTRACT

Binding of coralyne to tetra-molecular parallel G-quadruplex DNA $[d(TTGGGGT)]_4$ was evaluated for the first time using biophysical techniques. Absorbance titrations show hypo/hyper-chromism accompanied by 12 nm red shift with binding constant $K_b = 0.2–4.0 \times 10^6\ M^{-1}$. Binding induces a negative circular dichroism band of coralyne at 315 nm. Quenching of fluorescence (~64%) along with 10 nm blue shift in emission maxima indicates proximity of coralyne to guanine bases. Job plot indicates existence of multiple complexes. The observed two fluorescence life times, 6 and 12 ns, with relative abundance 33% and 63%, respectively suggest two binding sites/conformations in complex. 1D ^{1}H NMR spectra reveal significant broadening and upfield shift of G3NH, G6NH and G3H8 proton signals upon binding. The NOESY spectra reveal sequence specific non-intercalative interaction of coralyne in monomeric form at two sites close to A3/G3 and G6 bases of $[d(TTGGGGT)]_4$ and $[d(TTAGGGT)]_4$, which has implications in anti-cancer drug action.

1. Introduction

Guanine-rich sequences in telomeric region of human chromosomes form G-quadruplex DNA structure through Hoogsteen hydrogen bonding of guanine tetrads, which in turn inhibit the telomere elongation carried out by telomerase enzyme [1–4]. In cancerous cells, higher telomerase activity maintains the length of telomere thus prolonging cell life. Telomerase inhibition in cancer cells has been found to shorten telomere length in it over successive cell divisions which subsequently causes cell death [5]. Induction or stabilization of G-quadruplex structure in telomeric DNA upon ligand binding inhibits telomerase

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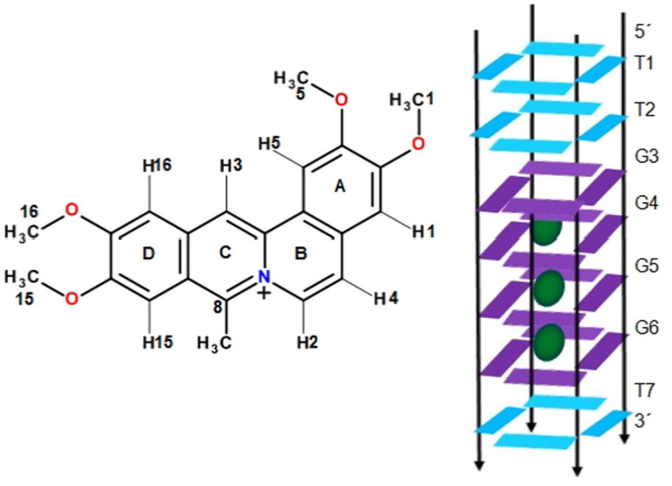
http://dx.doi.org/10.1016/j.bpc.2016.09.006
0301-4622/

elongation and is a powerful approach to target cancer cells [6]. Besides the telomeric region, G-quadruplex DNA forming guanine repeats are prevalent in promoter regions of various proto-oncogenes like *c-kit, K-Ras, c-Myc*, VEGF, *Bcl-2* and the regulation of their expression using G-quadruplex DNA binding ligands provides checkpoint for malignancy [7]. Compounds having a delocalized π aromatic ring system and planarity with a cationic charge are identified as better G-quadruplex DNA binders, such as telomestatin, acridines like RHPS4, PIPER and BRACO-19, cationic porphyrins like TMPyP4 [8]. The pharmacological and pharmacokinetic properties of these compounds are a major concern particularly for typically hydrophilic and positively charged molecules. As a result, in spite of knowledge of several G-quadruplex DNA binders, only quarfloxin has had some clinical success [6]. More and more G-rich regions in human genome are mapped with time and the hunt for better G-quadruplex DNA binding ligands therefore continues.

Coralyne (Scheme 1) is a synthetic planar protoberberine alkaloid structurally related to berberine class of compounds and exhibits efficient anti-leukemic activity [9,10]. It interacts with all forms of DNA but shows better affinity towards G-quadruplex and triplex DNA than duplex DNA [11,12]. Besides, it exhibits human topoisomerase I poisoning activity and photo induced damage of DNA, which could provide multiple targeting strategies for anti-cancer therapy [10,13]. Coralyne is also found to form a stronger inclusion complex with cyclodextrins thereby yielding chances of greater bioavailability for drug delivery [14].

Coralyne binds and stabilizes monomeric anti-parallel human telomeric G-quadruplex DNA and consequently inhibits human telomerase activity with a greater affinity than its related alkaloids e.g. berberine, palmatine and sanguinarine [15,16]. It binds and stabilizes anti-parallel G-quadruplex DNA formed by insulin-linked polymorphic region (ILPR) [17]. It induces formation of dimeric and tetrameric G-quadruplex DNA [16] but not the monomeric form. Its mode of binding to G-quadruplex DNA is expected to be different in the presence [15] and absence of loops. We have chosen the parallel tetra-molecular G-quadruplex DNA forming sequence $[d(TTGGGGT)]_4$ (Scheme 1) as a model for studying interaction of coralyne with G-quadruplex DNA in the absence of loops. Absence of the loop regions in parallel G-quadruplex DNA could throw light on the mode of ligand binding like end stacking, groove binding or intercalation between G-tetrads along sites apart from loops. Studies on interaction of coralyne with parallel form of G-quadruplex have not been reported so far in literature, although it is found to be more prevalent and several ligands [18,19] including peptides [20] are known to induce formation of parallel G-quadruplex. The TTGGGG repeats are the G-quadruplex DNA forming sequence found in the telomeres of the ciliate *Tetrahymena thermophila*, which is used as model organism to understand telomere and telomerase biology [1,2]. Biophysical and structural studies on binding of some ligands namely distamycin, cyanine, mitoxantrone etc. with this model sequence have since been reported [21–23]. The interactions at molecular level can only be directly ascertained by NMR techniques. However, there is not a single investigation on binding of coralyne to any form of G-quadruplex DNA by NMR techniques so far. The present study focuses on understanding the binding of coralyne to $[d(TTGGGGT)]_4$ by absorbance, steady state fluorescence, fluorescence lifetime, Circular Dichroism (CD) and ^{1}H proton NMR spectroscopic techniques. We have also used $[d(TTAGGGT)]_4$, the human telomeric repeat sequence, in present studies for comparison.



Scheme 1. Chemical structure of coralyne and the schematic representation of $[d(TTGGGGT)]_4$.

2. Materials and methods

2.1. Chemicals

Coralyne chloride hydrate, the desalted oligonucleotide sequence d(TTGGGGT), d(TTAGGGT) and d(CCAATTGG) were purchased from Sigma Aldrich Co. LLC, USA. Potassium chloride, Potassium phosphate, EDTA, etc. were obtained from Merck KGaA, Darmstadt, Germany. Deionized water used for preparing buffers and ligand stock solution was obtained from Milli-Q™ water purification system with 0.22 μm filter, Millipore make. Coralyne stock solution was prepared freshly in deionized water. Since it is light sensitive, it was kept in dark before taking measurements. The concentration of coralyne was estimated from absorbance measurements using molar absorption coefficient, $\varepsilon = 14{,}500\ M^{-1}\ cm^{-1}$ at λ_{max} of 420 nm [15]. All coralyne-DNA interaction studies were conducted in 20 mM potassium phosphate buffer containing 10 mM K_2HPO_4, 1 mM EDTA and 100 mM KCl (pH 7.0). The oligomer d(TTGGGGT) and d(TTAGGGT) were dissolved in buffer and heated at 95 °C for 10 min and allowed to cool slowly overnight to room temperature to enable proper formation of G-quadruplex structure and stored at 4 °C after equilibration. The self-complementary DNA oligomer d(CCAATTGG) was dissolved in 10 mM potassium phosphate buffer. The concentration of tetra-molecular parallel G-quadruplex DNA $[d(TTGGGGT)]_4$, $[d(TTAGGGT)]_4$ and duplex DNA, $[d(CCAATTGG)]_2$, was calculated using $\varepsilon = 65{,}900$, 69,800 and 76,300 $M^{-1}\ cm^{-1}$, respectively per strand based on nearest neighbor method as specified by the manufacturer. The concentration of DNA (N) is reported per tetra-molecular parallel G-quadruplex $[d(TTGGGGT)]_4$, $[d(TTAGGGT)]_4$ and duplex $[d(CCAATTGG)]_2$ DNA molecule throughout the study.

2.2. UV–visible absorbance experiments

The absorbance experiments were carried out in CARY-100 Bio UV–visible spectrophotometer (Varian, USA) equipped with Peltier controlled thermostatic cell holder using quartz cell of 1 cm optical pathlength in the wavelength range of 200–600 nm at 25 °C. Increasing concentration of $[d(TTGGGGT)]_4$ was added to coralyne solution (3.1 μM) of fixed concentration in buffer to obtain coralyne (L) to DNA (N) mole equivalent ratios (L/N) of 0.13 to 18.96. Five minute incubation time was provided after each titration before recording spectrum to enable proper equilibration. The equilibrium binding constant (K_b) was obtained from absorbance titration data using half-reciprocal plot according to the following equation [24]:

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad (1)$$

where [DNA] = [N] is the concentration of G-quadruplex DNA $[d(TTGGGGT)]_4$, ε_a is the apparent molar absorption coefficient obtained by calculating ratio of observed absorbance of coralyne-DNA complex to the fixed coralyne concentration (A_{obs}/[L]), ε_f and ε_b correspond to the molar absorption coefficient of coralyne in its free and bound form, respectively.