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Studying the interaction between four-stranded nucleic acid structures and Actinomycin-D and Berberine

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Abstract

The binding phenomenon between non-canonical nucleic acid structures, specially four-stranded structures such as G-quadruplexes and i-motifs, with small molecules has garnered significant interest in recent years since it can result in the design of therapeutic systems which target unusual nucleic acids involved in disease pathogenesis. This study investigates the binding interaction between Actinomycin D, a chemotherapeutic agent, and Berberine, a natural alkaloid found in plants, with G-quadruplexes and i-motifs fomed by four repeats of the *C9orf72* repeat whose expansion is known to cause ALS/FTD. The binding phenomenon has been monitored via UV spectroscopy where we have shown that both Actinomycin D and Berberine exhibit binding with G-quadruplexes and i-motifs reflected in the change in absorbance. Based on the observed hyperchromocity and the dissociation constants calculated, Actinomycin D and Berberine are shown to have slightly higher affinity for the i-motifs formed under acidic conditions.

Key words: G-quadruplexes, i-motifs, Berberine, Actinomycin D, Drug-DNA binding

1. Introduction

Nucleic acids can adopt multiple folding patterns aside from the standard double stranded B-DNA. Four-stranded structures also known as G-quadruplexes and i-motifs are considered non-canonical nucleic acids and have been explored extensively in literature due to their possibe roles in various biological proecesses including gene expression, maintenance of genomic stability and protection of chromosomes [1]. Furthermore, they have been implicated in multiple diseases including cancer and neurodegenerative diseases such as ALS /FTD and CANVAS [2][3]. Nucleic acids (DNA and RNA) which have sequences rich in guanine are prone to G-quadruplex formation. Their structural basis is the formation of planar strcutures known as G-tetrads which arise due to the ability of guanine molecules to self-associate via Hoogsteen hydrogen bonds (Fig. 1A). The G-tetrads then stack on top of eachother to form the four-stranded higher order structures (Fig. 1B). The presence of a monovalent cation such as sodium or potassium is necessary for G-quadruplex formation. I-motifs on the other hand, are also four-stranded structures but airse from sequences rich in cytosine molecules (Fig. 1C). Cytosine protonation is necessary for formation and stability of i-motifs since the presence of cytosine base pairs where one cytosine is protonated is the basis for structure formation. In order for cytosine protonation to occur, acidic environmental pH is required [4]. Due to the involvement of non-canonical nucleic acid structures in multiple biological processses and diseases, both G-quadruplexes and i-motifs have been explored as therapeutic targets. Small molecules that exhibit binding to either of these structures with the goal of structure stabilization or destabilization, depending on the sequence and disease, have been the subject of investigation [5].

Multiple small molecules have been studied in terms of their binding properties to non-B DNA structures including G-quadruplexes and i-motifs[5]. Consdering the extremely polymorphic behaviour of these strcutures, small molecules which exhibit specificity in binding to a certain folding pattern are needed. Actinomycin D is a chemotherapeutic agent that treats various types of cancer (Fig. 1D). Its' mechanism of action is through binding to DNA and inhibition of RNA synthesis. Actinomycin D has been shown to bind to G-quadruplexes with high affinity. The siginificance of the interaction between actinomycin D and G-quadruplex structures lies in their ability to influence the stability and function of G-quadruplexes in genomic regions potentially affecting gene regulation [6][7]. The interaction between Actinomycin D and i-motifs has also

been studied however binding affinity was higher for G-quadruplexes and double-stranded DNA [8] .Berberines are naturally occurring alkaloids found in several plants (Fig. 1E). They have also been shown to bind to G-quadruplexes with significant affinity through van der Waals interactions and hydrogen bonding which resulted in stabilization of the G-quadruplex structures [9]. As for i-motifs, berberine was found to not only interact with them resulting in structure stabilization, but also result in strong fluorescence activity[10][11] .

Here, we have aimed to invesgtigate whether Actinomycin D and Berberine interact with G-quadruplexes and i-motifs formed by $d(GGGGCC)_4$ and $d(CCCCGG)_4$ oligonucleotides using UV-spectroscopy. The sequence chosen is four repeats of the *C9orf72* repeat associated with ALS/FTD pathogenesis through repeat expansion[12] .Both G-rich and C-rich *C9orf72* repeats have been previously confirmed to form G-quadruplexes and i-motifs respectively [13][2].

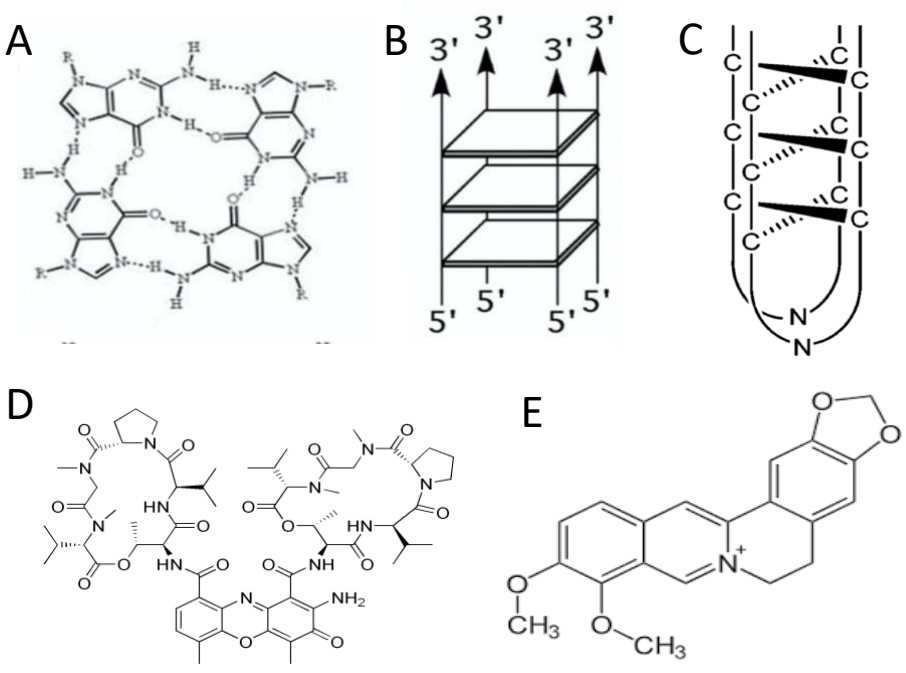

Figure 1. (A) G-tetrad (B) An intermolecular parallel-stranded G-quadruplex structure (C) An i-motif(E) Actinomycin D molecule (F) Berberin molecule

2. Materials and Methods

Sample Preparation

Oligonucleotides listed in Table 1 were purchased from ACGT Corp (Toronto, ON, Canada). Berberine and Actinomycin D were purchased from ……Concentrations of the oligonucleotide were determined by recording the absorbance at 260 nm with molar extinction coefficients (Fig. 1B). G-rich oligonucleotide was dissolved in buffer containing 10 mM Tris, 100 mM KCl, 0.1 mM EDTA (pH 7.4). C-rich oligonucleotide was dissolved in 10 mM sodium phosphate buffer, 150 mM NaCl, 0.1 mM EDTA (pH 5.2).Samples were heated to 95 °C and allowed to cool to room temperature overnight. Actinomycin D and Berberine chloride were purchased from Sigma-Aldrich.

Table 1: The oligonucleotides used in this study

Studied oligonucleotides 5' to 3'	Extinction coefficient (Ɛ) ($M^{-1}.cm^{-1}$)
$d(GGGGCC)_4$	248560
$d(CCCCGG)_4$	208880

UV-visible spectroscopy

Prefolded G-quadruplexes and i-Motfs with 320 μM concentration were titrated into a cuvette containing 3.4 µM of Berberine and Actinomycin D. Absorption spectra were collected with a Varian Cary 100 UV–Vis spectrophotometer from 300 nm to 600 nm using cuvettes with an optical path length of 1 cm. The concentration of free Berberine and Actinomycin D were determined using the extinction coefficients of 12400 $M^{-1}\,cm^{-1}$ and 24500 and at absorbance values of 425 nm and 475 nm respectively. The titration was stopped when three successive additions of the sample resulted in no further shift of the peak with the maximum absorption.

All values were corrected for the dilution effect. The fraction (α) of bound drug was determined as follows:

$$\alpha = \left(Abs\ Drug_{free} - Abs_{mixture}\right)/\left(Abs\ Drug_{free} - Abs_{bond}\right) \quad I$$

where Abs Drug $_{free}$ is the absorbance of free Berberine and Actinomycin D in the absence of any added DNA, $Abs_{mixture}$ is the absorbance at any point after the beginning of the addition of the DNA and Abs_{bond} is the absorbance of fully bonded Berberine and Actinomycin D measured at 425 nm and 473 nm.

The percentage of hyperchromicity of the is calculated as follows:

$$\%\text{hyperchromicity} = \left(\frac{\varepsilon b - \varepsilon f}{\varepsilon b}\right) \times 100, \quad III$$

$$Where\ \varepsilon_{bond} = Abs\ Drug_{bond}/[Drug]_{bond}$$

Also, the dissocation constant can be extracted from the α vs. concentration curve with KD being the concentration of drug at which half the DNA is bound.

Results and Discussion

The interaction between Berberine and Actinomycin D and the four-stranded structures formed by the G-rich d(GGGGCC)4 strands (G-quadruplexes) and C-rich d(CCCCGG)4 strands (i-Motifs) was studied by measuring the visible absorption spectra of the small molecules in the absence and presence of DNA (Fig. 2 and Fig. 3). For Berberine (Fig 2A and 2B), upon titration with both G-quadruplexes and i-Motifs, a hyperchromic effect is seen indicative of a binding. The fraction of bound berberine (α) is also shown vs. DNA concentration (shown in the inset) was used to calculate the dissociation constants. The percent hyperchromicity for Berberine/G-quadruplex structures and Berberine/i-Motif structures were 16% and 5% respectively with negligible bathochromic shift. The dissocation constants (k_D) were 3.9 µM and 1.7 µM respectively. Therefore, the affinity of Berberine is higher for i-motif structures relative to the G-quadruplexes.

As for Actinomycin D (Fig 3A and 3B), upon titration with both G-quadruplexes and i-Motifs, a hyperchromic effect is also seen indicative of a the presence of a binding phenomenon. The fraction of bound actinomycin D (α) is also shown vs. DNA concentration (shown in the inset) was used to calculate the dissociation constants. The percent hyperchromicity for Actinomycin/G-quadruplex structures and Actinomycin/i-Motif structures were 9% an d 10% respectively. The dissocation constants (k_D) were 2.3 µM and 1.8 µM respectively showing higher affinity of Actinomycin D for i-motifs. Previously similar results for the interaction of Actinomycin D and G-quadruplexes and i-motifs has been reported where they conclude that affinity of Actinomycin D for double stranded DNA is higher than of G-quadruplexes and i-motifs[14][15].

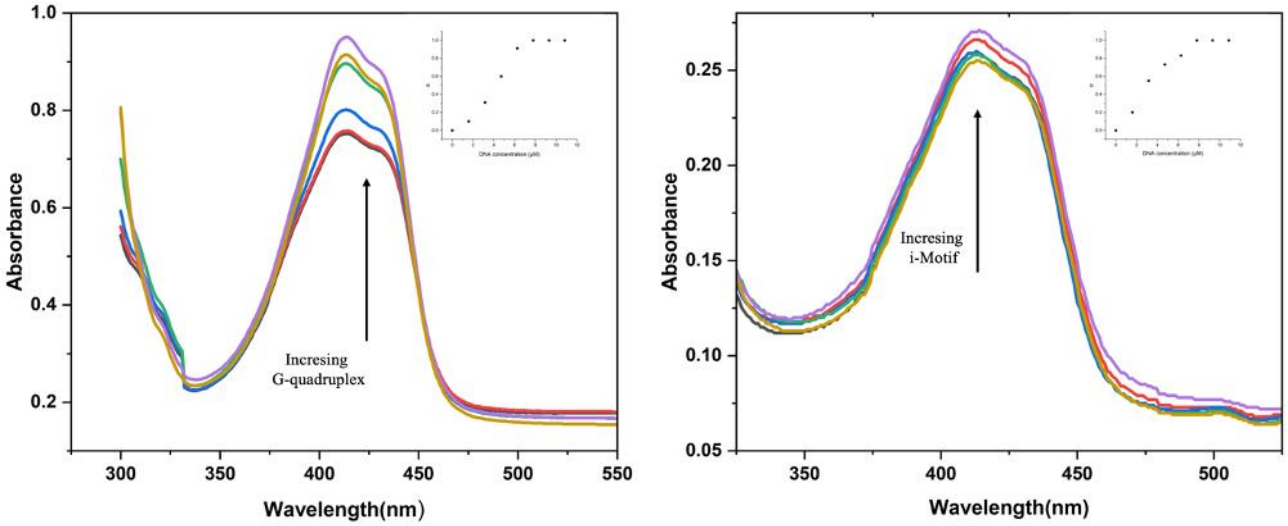

Figure 2. Visible absorption spectra of Berberine in the absence and presence of an increasing concentration of pre-folded (A) d(GGGGCC)4. and (B) d(CCCCGG)4. Inset: Alpha vs. DNA concentration

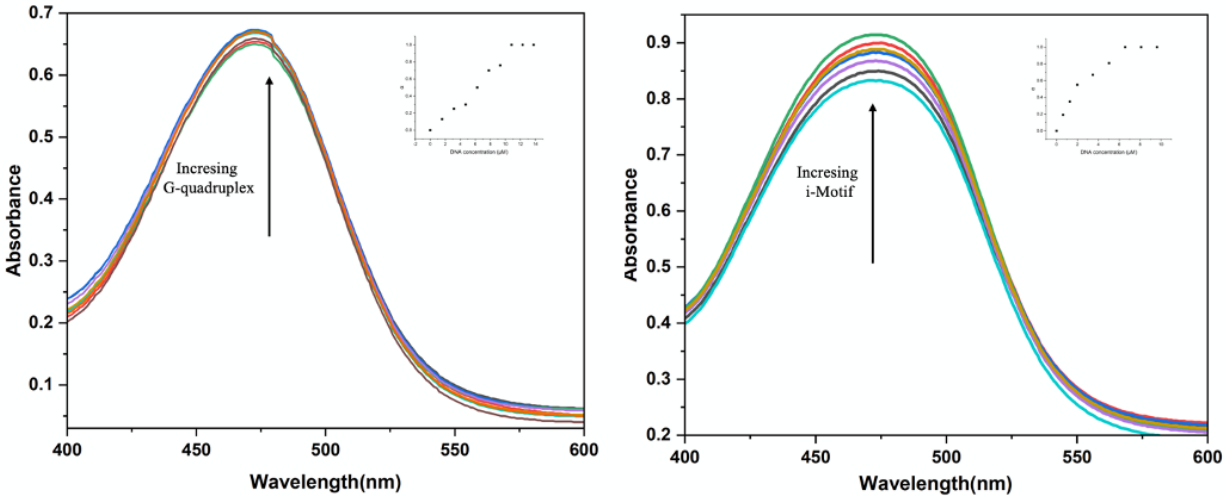

Figure 3. Visible absorption spectra of Actinomycin D in the absence and presence of an increasing concentration of pre-folded (A) d(GGGGCC)4. and (B) d(CCCCGG)4. Inset: Alpha vs. DNA concentration

Conclusion

All in all, the understanding of how small molecules bind to non-canonical nucleic acid structures involved in disease pathogenesis can help design new therapeutic strategies, particularly in gene targeting in various diseases. In this study, we used UV-spectroscopy to investiage whether Actinomycin D and Berberine bind to G-quadruplexes and i-motifs formed by the *C9orf72* repeat. We have shown that upon binding, a hyperchromic effect is seen in all cases. Also, based on the dissociation constants derived from the binding plots, both Actinomycin D and Berberine exhibit higher affinity for the i-motfs formed under acidic pH.

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