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Binding Studies of Natural Product Berberine with DNA G-Quadruplex

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Abstract: Problem statement: The ends of chromosome had highly repetitive short G and C-rich sequences of DNA. These sequences were known to form stable tetraplex type of secondary structures which help to maintain gene integratity after cell divison. **Approach:** Any reagent which controls the random cell division would be useful to design anticancer drugs. Therefore a many natural and synthesized molecules which stabilized tetraplex structures are targeted as anticancer drug entities. **Results:** Among them, Berberine hydrochloride natural product and its analogues are well studies as G-quadruplex stabilizing agent. In this report, DNA sequence 5'-G_3-C_5-G_3-3' has been designed which has probability to form i-motif and G-qua druplex types of secondary structures. Herein we studied the interaction between this DNA strands and Berberine hydrochloride by ^{1}H-NMR techniques and UV in two different PH (4.7 and 7.4) conditions. **Conclusion/Recommendations:** Our preliminary results showed that Berberine bind with this DNA strand in both pH conditions which is further supported by UV melting experiments. In future this sequence can be used as probe to screen out tetraplex binding natural products which help to generate new anticancer drugs.

Key words: Secondary structures, melting experiments, anticancer drugs, eukaryotic chromosomes, telomerase enzyme, genome integrity, hydrogen bonded, enzyme activities, i-motif tetrplex, preliminary results, berberine bind, hydrogen bonding

INTRODUCTION

Eukaryotic chromosomes have telomeres at their ends with specific DNA sequence and protein. Telomeres are very important for genome integrity in eukaryotes which have important role in cellular aging and cancer (Greider, 1998). The mode of replication in telomeric DNA is very exceptional which depends upon telomerase enzyme (Gomez *et al*., 2004). The activity of this enzyme has been tightly regulated in somatic cells while level of that enzyme activities are very high in cancerous cells (Ma *et al*., 2009; Mendez-Bermudez *et al*., 2009). In many organisms, DNA sequences of telomeric region have highly repetitive short G and C-rich repeated units. For an instant, the human telomric DNA has (5'-TTAGGG-3'): (5'-CCCTAA-3') repeated motif (Balasubramanian and Neidle, 2009). In Fig. 1, it has been established that repetitive G-rich DNA strand adopts a four stranded G-quadruplex type of tetraplex structure, while C-rich strand adopt another tetraplex structure as i-motif (Hurley, 2001; Burge *et al*., 2006) Fig 1. The structure G-quadruplexes formed by hydrogen bonding and their stability depend on monocations (Na+/K+) (Fig. 1-2) (Mergny *et al*., 2005; Haider *et al*., 2002). Whereas the formation of i-motif by intercalation of two semiprotonated duplexes. These duplexes are complex of neutral and N5 protonated cytocine (C) DNA strands (Bucek *et al*., 2009; Peng *et al*., 2009). Since the G-quadruplex structure has a unique role in cell division process and hence it was targeted to design anticancer drugs (Mendez-Bermudez *et al*., 2009; Neidle and Read, 2000). Recently the application of G-quadruplex as in development of functional non-covalent assemblies such as G-wire, (Vesenka *et al*., 2007) nanopore, (Shim *et al*., 2009) ion-channels (Sket and Plavec, 2007) and self-assembled ionophores. The formation of G-quadruplex and i-motif structure from their respective DNA sequences has been studied by X-Ray studied and spectroscopic techniques: UV (Mergny *et al*., 1998) CD (Gray *et al*., 2002; Ito *et al*., 2002) and NMR (Wang *et al*., 1991; Feigon *et al*., 1995, Furey *et al*., 1998).

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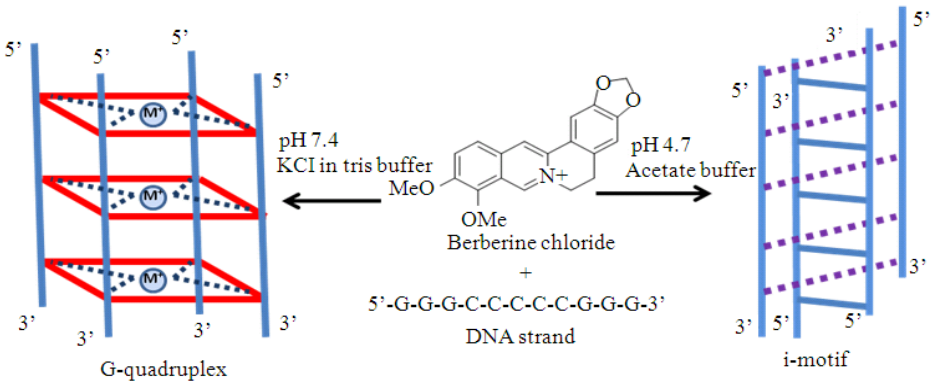


Fig. 1: Pictorial representation of DNA tetraplex-G-quadruplex and i-motif

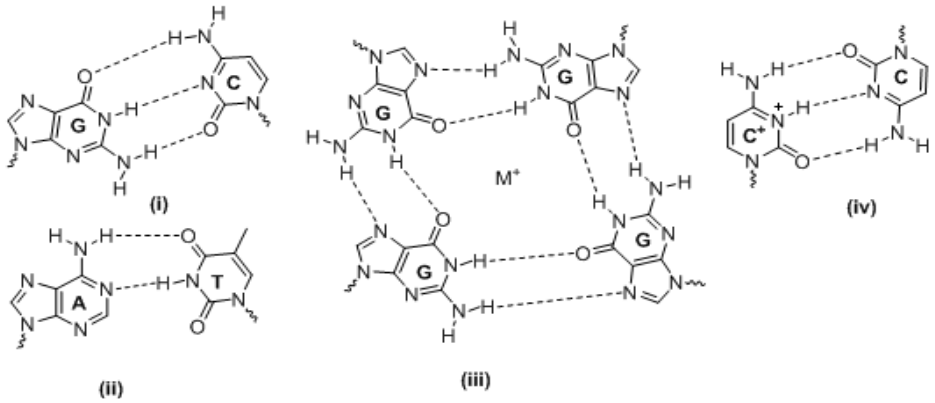


Fig. 2: (i) and (ii) Watson Crick A.T and G.C base pairs (iii) Hydrogen bonding G-quadruplex with monovalent metal ion (iv) C.C+ base pairs in imotif tetrplex

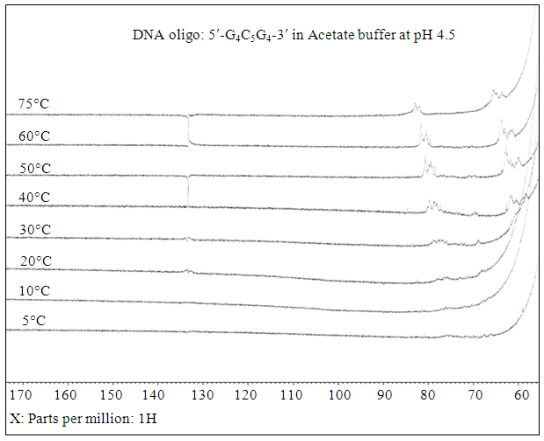
In NMR studies, the G-quadruplex, i-motif and Watson-Crick double helix have distinct ^{1}H-NMR signature of hydrogen bonded imino protons (Wang *et al*., 1991). In ^{1}H-NMR of DNA/RNA, the chemical shift (δ, in ppm) of imino protons have characteristic signatures such as δ10-12 for G-quadruplexes, δ15-16 for C.C+ base pairs or i-motif and δ12-14 for Watson-Crick duplex (Fig. 2) (Phan and Mergny, 2002). Due to immense demand to increase stability of DNA G-quardruplex Structures, many sincere efforts have been made including studies of G-quadruplex with modified nucleic acid analogues. (Searle *et al*., 2004 Sharma and Ganesh, 2005) Many natural products have been also identified which stabilize DNA G-quadruplex. Among them berberine hydrochloride is well established as G-quadruplex binding agent (Sun *et al*., 2009; Zhou *et al*., 2009). Herein we are reporting the binding affinity of berberine hydrochloride with designed DNA strand under different PH conditions with ^{1}H-NMR techniques and UV-thermal melting experiment.

MATERIALS AND METHODS

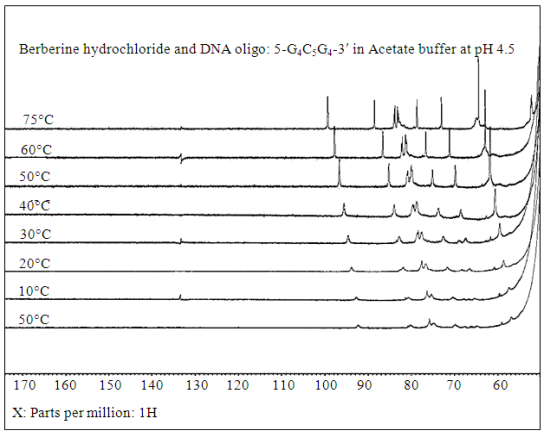
A DNA strand d (5'-G_3-C_5-G_3-3') of concentration 500 µM was taken in buffer (50% D_2O) at two different pHs. Acetate Buffer for acidic pH 4.7 and Phosphate buffer for pH 7.4 were used to prepare sample for ^{1}H-NMR (400 MHz). Studies before and after annealing at different temperature. The similar experiments were repeated with DNA binding natural product Berberine hydrochloride (250 µM) and ^{1}H-NMR spectra were recorded at different temperature.

RESULT AND DISCUSSION

A DNA strand d (5'-G_3-C_5-G_3-3') (1) was designed to study the binding affinity with Berberine hydrochloride in different PH conditions. This DNA strand has potential to form duplex, hairpin and tetraplex (G-quadruplex and i-motif) type of complexes under different buffers conditions.



(A)



(B)

Fig. 3: (A). ^{1}H-NMR of DNA in acetate buffer of PH 4.5 (B) ^{1}H-NMR of DNA: Berberine in acetate buffer of PH 4.5 at different temperature range (5-75^{o}C) in temperature

^{1}H-NMR and ^{1}H-^{1}H-COSY/NOSEY NMR spectra of HPLC purified DNA (1) (500 µM) were recorded in D_2O to check strand purity. All spectra are given in Supplementary Information (SI). These NMR data was also used as control studies for ^{1}HNMR studies. For studies of DNA and berberine interaction, DNA 1 was treated with Berberine hydrochloride (250 uM) in two different buffers of PHs 4.5 and 7.4 with D_2O (50%).

Acidic buffer condition: Since acidic pH stabilized i-motif type of secondary structure in C-rich DNA strand. DNA 1 was therefore annealed in acetate buffer(10mM) of PH 4.7 without salt by following given procedure.This annealed samples were subject to temperature dependent ^{1}H-NMR experiments. In this experiment, ^{1}H-NMR spectra of DNA 1 were recorded at different temperatures 5, 10, 20, 30, 40, 50, 60 and 75°C. The horizontally stacked ^{1}H-NMR spectra of δ5-17 region at different temperatures are given in Fig. 2A. These spectra show a clear upfield shift in chemical shift of DNA protons at δ (ppm) 8.0, 8.0, 7.6, 7.5, 7.3 and 6.6. The similar sets of experiments were repeated with equamolar concentration of berberine hydrochloride and DNA 1 under similar PH condition. Their horizontally stacked ^{1}H-NMR spectra at different temperature are also given Fig. 2B where DNA proton ^{1}H-NMR signal were masked with protons signal of berberine. Apparently these spectra show temperature dependent upfield shift in chemical shift of Berberine protons δ (ppm) 9.2, 8.0 and 7.5 in DNA: Berberine complex. In both experiments, we were unable to find NMR signal of hydrogen bonded NH protons of DNA nucleobase in region of δ10-17 ppm. While the appearance of a broad signals different temperature under acedic PH condition was not steady (Fig. 3A-B). These could be possible due to deuterated exchange for imino protons in excess D_2O in buffer. From literature, the binding affinity in DNA-Drug interaction has been studied by using temperature dependent ^{1}H-NMR techniques from chemical shift Vs temperature profiles (Sarma, 1980). Here we have also extracted temperature Vs chemical shift profiles from Fig. 2 for DNA or Berberine. In Fig. 4A, the profile (A-I) of Fig. 4 is chemical shift Vs temperature are depicted for affected ^{1}H-NMR signal of DNA 1 at δ 8.0 (ppm) under acidic buffer (PH 4.5) condition while similar profile at δ 7.6, 7.5, 7.3 and 6.6 of DNA protons are provided in SI. This profile (A-I) suggests the formation of regular secondary structure by DNA 1 at low temperature under acidic condition which further melted with increase of temperature. Similar temperature profiles are also extracted from Fig. 2B for DNA: berberine complex with upfiled shift protons (Fig. 4A-II). In A-II is proton signal at δ (ppm) 9.2 of Berberine in DNA: Berberine complex at acidic PH while at δ (ppm) 8.0 and 7.5 are provided in SI. This profile (A-II) also suggests the formation of secondary structures by DNA 1 in presence berberine at acidic PH. It could be possible by the formation i-motif or semiprotonated duplex which bind with berberine.

Physiological buffer condition: Since berberine is G-quadruplexing agents and DNA 1 has G-quadruplexing G-rich strand. DNA 1 (250µM) was therefore annealed in presence of Berberine/without berberine under Tris buffer (10mM, PH 7.4) having KCl (100mM) salt by following procedure. These annealed DNA samples were also subjected to time dependent ^{1}H-NMR experiments with berberine and without berberine.

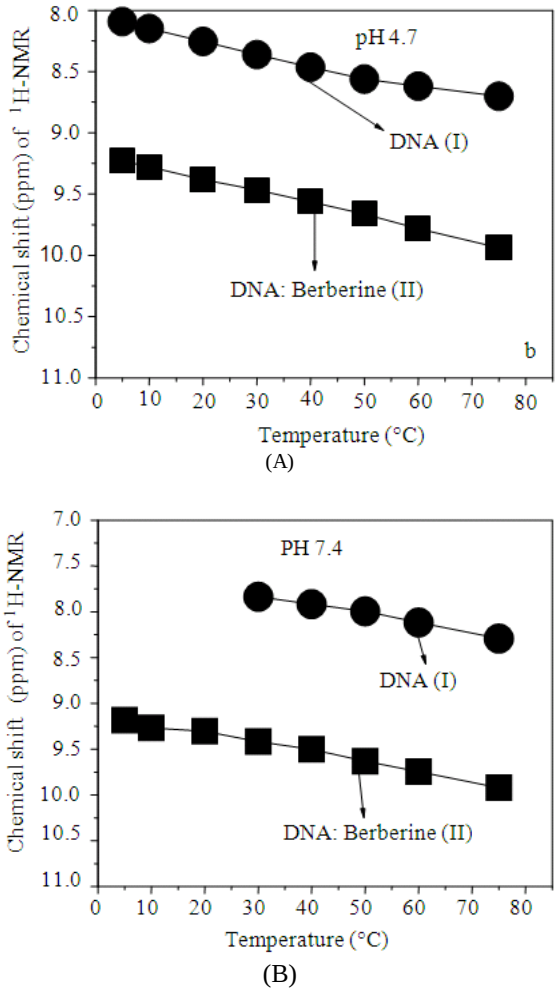


Fig. 4: ^{1}H-NMR Chemical Shift (ppm) Vs Temperature (^{o}C) profile: (A) Acidic pH 4.5; (I) chemical shift (δ) of DNA at 8.0 (ppm) with respect to temperature and δ of berberine at 9.2 (ppm) with respect to temperature (5-75°C) in DNA: Berberine complex (II). (B) Physiological pH: (I) δ of DNA at 7.80 (ppm) with respect to temperature (5-75°C) and (II) chemical shift (δ) of berberine at 9.2 (ppm) with respect to temperature (5-75°C) in DNA: Berberine complex under acidic pH 7.4

In these experiments ^{1}H-NMR spectra were recorded at different temperatures 5, 10, 20, 30, 40, 50, 60 and 75°C with berberine and then without berberine.Their horizontally stacked spectra with respect to temperature for DNA and DNA: Berberine samples are depicted SI. Here we were also unable to find NMR signals of hydrogen bonded NH protons of DNA nucleobase in any experiment. But we observed temperature dependent upfield shift in DNA nucleobase or berberine protons signals. Similarly we also created temperature Vs chemical shift profile for DNA and berberine under physiological PH 7.4 from their spectra (Fig. 3B). B-I profile for DNA while B-II for berberine are given. These profiles also show temperature dependent upfield change in proton of DNA at δ 7.8 (ppm) and berberine δ 9.2 (ppm). Up field chemical shift of DNA protons at 7.6, 8.0 were observed for DNA while that of berberine at δ 7.9 9.2, 8.0, 7.5 and 7.4 in DNA and Berberine under buffer PH 7.4 (see SI). These results also support the formation of secondary structure by DNA 1 at physiological PH which may bind with berberine hydrochloride. Since berberine hydrochloride is G-quadruplexing agent so it could be possible the formation of G-quaduplex under physiological PH.

UV-Tm experiment: Thermal stabilities of complexes formed by DNA 1 with berberine and without berberine were studied by UV melting experiments for both pH condition at two wavelengths 260 nm and 295 nm by following our reported procedure (Phan and Mergny, 2002). Their UV melting profiles are given SI. Since UV-melting experiment wavelength 295 nm and 260 nm have been used to determine Tm of duplex and tetraplex respectively (Phan and Mergny, 2002; Sharma and Ganesh, 2005). These melting profiles further support the formation of stable secondary structures. Due to lack of clear transition, we were unable to extract accurate melting temperature ($T_{1/2}$) from these UV-Tm profiles.

CONCLUSION

We have performed temperature dependent ^{1}H-NMR experiments on DNA 1 (5'-G_3-C_5-G_3-3') in presence of berberine hydrochloride under two pH conditions PH's 4.5 (no salt) and 7.4 (with 100 mM KCl). For control studied we have also performed similar set of experiments in absence of Berberine under similar conditions. Results of these experiments have suggested the formation secondary structures by DNA 1 under both pH conditions. These structures bind strongly with G-quadruplex stabilizing agent Berberine hydrochloride under both pH conditions. These binding results were also supported by UV thermal melting experiments. These binding affinities of berberine with DNA could suggest the formation i-motif and G-quadruplex types of secondary structures at PH 4.5 and 7.4 respectily. At this stage we do not enough evidence about DNA i-motif and G-quadruplex formation but our future works are in progress to evaluate the interaction

of berbarine hydrochloride with DNA 1. In future this type of DNA strand can be used to design a probe to screen out tetraplex binding natural products which help to generate verities of new anticancer drugs.

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