Research Paper

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Biocalorimetric Analysis of the Binding of Planar And Non Planar Molecule with G-Quadruplex Human Telomeric DNA.

Biosciences

KEYWORDS : human telomeric G- Quadruplex; ethidium; palmatine; ITC.

Dr. Kakali Bhadra Assistant Professor University of Kalyani Department of Zoology

ABSTRACT

Binding and stabilization of G-quadruplex with small molecules show strong relevance to control cancer. Thermal techniques, viz., isothermal calorimetry, differential scanning calorimetry and optical thermal melting studies, are applied for the characterization of the human telomeric G-quadruplex structure with particular emphasis on the binding with palmatine and ethidium, as non planar and planar molecule, respectively. Binding affinity of 8.62×10^5 and 2.0×10^5 M^{-1} was reported at 25±0.5 °C, with the planar and non planar molecule, respectively. The thermodynamic profile of the interaction indicated an enthalpy driven exothermic binding in case of ethidium where as with palmatine it indicated entropy driven exothermic binding. Heat capacity values of -188 and -95 cal/mol K, respectively, for ethidium and palmatine, suggesting significant differences in the hydrophobic contribution to the binding. This presents a complete thermodynamic profile of two structurally different molecules binding to G-quadruplex structure for the future targeted better therapeutic agents in anticancer treatment.

INTRODUCTION:

There is a strong evidence for the existence of quadruplex structure *in vivo* in several biologically significant regions of the genome that have regulatory function during transcription and replication (Granotier et al., 2005). The key structural feature of the quadruplex is a series of stacked guanine tetrads held together in a coplanar cyclic array by both Hoogsteen and Watson-Crick hydrogen bonds (Fig.1A). In the presence of monovalent cations such as potassium or sodium ions, this single G-rich strand can form the unique four-stranded G-quadruplex conformations (Prtraccone et al., 2004 ; Chaires, 2010). Depending on the incubation conditions, the human telomeric sequence is known to form different types of G-quadruplex structure. For example, antiparallel G-quadruplexes were formed in Na^+ solution, whereas a mixture of parallel and antiparallel G-quadruplexes were formed in K^+ solution (Rezler et al., 2005) (Fig.1B). Folding of telomeric G rich single strand $(GGGTTA)_n$ into G4 DNA has been found to inhibit telomerase activity (Zahaler et al., 1991) hence any molecule that favors G4 formation by stabilizing the structure, will lock the telomeric substrate into an inactive conformation that is no longer recognized nor extended by the enzyme (Mergny and Helene, 1998). Thus stabilization of G-quadruplexes can be considered as an original strategy to achieve antitumor activity.

The binding of G-quadruplexes with some planar and non planar aromatic molecules has been reported earlier also (Seenisamy et al., 2005; Zhou et al., 2005; Gunaratnam et al., 2005) but the molecular aspects of their interaction and energetics remained almost obscure. In order to understand quadruplex-small molecule interaction, this paper deals with the study of the human telomeric quadruplex DNA stabilization through binding with natural alkaloid palmatine and it has been further compared with the classical planar double stranded DNA intercalator, ethidium bromide. Hence, the main objective of this study is to elucidate the structural basis, folding and stability of the quadruplex conformation in the model oligonucleotide, stabilization through binding with ethidium and palmatine as planar and non planar molecule, respectively, (Fig. 1) and the thermodynamic characterization of their binding.

MATERIALS AND METHODS:

Materials:

Ethidium and palmatine was the product of Sigma-Aldrich. The concentration was determined using molar extinction coefficient of ε_{480} = 5680 M^{-1} cm^1 for ethidium and ε_{344} =22500 M^{-1} cm^1 for palmatine. The 22-mer oligonucleotide sequence, d(AGGGTTAGGGTTAGGGTTAGGG), was obtained from Integrated DNA Technology Inc. USA. Concentration of the oligonucleotide solution was determined using procedures reported earlier (Marky et al., 1983). The experiments were performed in 50 mM MOPS (4-morpholinepropanesulfonic acid) buffer, pH 6.81, 25 °C containing 0.1 M KCl).

UV optical melting study:

Absorbance versus temperature profiles (melting curves) of the oligo (20 μM) was recorded on a Shimadzu Pharmaspec 1700 unit equipped with the peltier controlled TMSPC-8 model accessory.

Circular dichroism studies:

Circular dichroism (CD) spectra were recorded on a Jasco J715 spectropolarimeter (Jasco International Co. Ltd., Tokyo, Japan) attached with a temperature controller and thermal programmer model PTC 343 interfaced to a PC.

Isothermal titration calorimetry (ITC) and its analysis of binding:

ITC experiments were performed on a Microcal VP-ITC microcalorimeter (MicroCal, Inc., Northampton, MA, USA) . Origin 7.0 software, was used for data acquisition and manipulation.

Differential scanning calorimetry (DSC):

To investigate the helix-coil transition, excess heat capacities as a function of temperature were measured on a Microcal VP-differential scanning calorimeter (MicroCal, Inc., Northampton, MA, USA) as reported earlier.

RESULTS AND DISCUSSION:

Spectropolarimetric and calorimetric characterization of the quadruplex structure

CD spectra are sensitive to DNA base stacking, eg., guanine base stacking in G-quadruplexes, which is related to the orientations of G-strands and can be used to effectively distinguish between parallel and antiparallel G-quartet structures (Mergny and Maurizot, 2001). CD spectrum of the $d[AG_3(T_2AG_3)_3]$ quadruplex is presented in Fig. 2A. The spectrum has a major positive band with maximum at 290 nm and a minor negative band at 238 nm. This type of CD spectrum is characteristic of mixture of typical parallel- antiparallel quadruplex structure (Shi et al., 2010). It was also suggested that the 295 nm positive band arises due to a folded back hairpin G-quartet structure (Balagurumoorthy et al., 1992). The CD melting profile of the quadruplex structure monitored at 240 and 290 nm is presented in the inset of Fig. 2A that revealed T_m values of 64 and 63 °C respectively. The optical melting profile (inset of Fig.2B) of the quadruplex monitored at 295 nm revealed a hyperchromicity of 36 % with a T_m of 64.8 °C. Further DSC thermoprofile (Fig. 2B) of the oligonucleotide revealed a change in enthalpy of helix denaturation (ΔH_{cal}) of 3.57×10^4 kcal/mol and the vant'Hoff enthalpy (ΔH_v) of 3.94×10^4 kcal/mol for the free polymer. Both UV and CD melting profiles showed more or less cooperative behavior. DSC) profile of the quadruplex also showed a complete cooperative thermal curve (inset of Fig. 2B) revealing a strong transition at 65.78 ± 0.04 °C. The ratio of ΔH-

$_{cal}$ to ΔH_v reveals near unity indicating a cooperative and reversible transition for the thermal unfolding of the quadruplex structure (Fig. 2B).

Energetics of the interaction

DNA-targeted drug design requires accurate, reliable and rapid methods to directly obtain the thermodynamic parameters. Calorimetry is a technique that fulfils all these criteria (Bhadra and Kumar, 2010). Fig. 3 A, B (upper panel) represents the raw ITC profile, resulting from the titration of ethidium and palmatine, respectively, to the G-quadruplex polymer. Each of the heat burst curve in the figure corresponds to a single injection. The areas under these heat burst curves are determined by integration to yield the associated injection heats. These injection heats are corrected by subtracting the corresponding dilution heats derived from the injection of identical amounts of the injectants (ligand) into buffer alone. In the lower panel of the figure (Fig. 3C,D), the resulting corrected injection heats are plotted against the respective molar ratios at four different temperatures *viz*. 10, 15, 25 and 30 °C, respectively. In this panel the data points reflect the experimental injection heat while the solid lines reflect calculated fits of data.

The corrected isotherms showed single site binding, both with palmatine and ethidium, indicating one type of complexation. In both the cases, the binding is exothermic and the stoichiometry is found to be one mole of ligand binding per mole of quadruplex. The results of temperature dependence of the thermodynamic parameters compared in the range of 10-30 °C, using the equation $\Delta C_p^o = \delta(\Delta H^o)/\delta t$. All the binding parameters are presented in Table 1. The temperature dependence of the enthalpy yielded an estimate for heat capacity (ΔC_p^o). The slope of the line revealed value of -188 and -95 cal/mol K, respectively with ethidium and palmatine. Further, the variation of ΔH^o and ΔG^o with $T\Delta S^o$ for the binding are also determined (figure not shown). The value of the slope, that is $\delta\Delta H^o$ / $\delta(T\Delta S^o)$ is found to be 0.95 and 0.57, respectively with ethidium and palmatine for binding to the model quadruplex. Thus the thermodynamic parameters regarding the ethidium and palmatine binding to quadruplex revealed that the binding enthalpy (ΔH^o) increased and the entropy term ($T\Delta S^o$) (a favorable term in the ΔG^o) decreased with increasing temperature keeping the ΔG^o almost constant (exhibits only small changes). Linear relationship of enthalpy change with $T\Delta S^o$ with slope unity is an indication of complete compensation and this occurs in systems with ΔC_p^o not equal to zero and $\Delta C_p^o > \Delta S^o$. This type of compensation was observed for many biomolecular interactions (Chaires, 2006) and suggested a significant hydrophobic component to the binding energies and the results revealed a complete compensation to the binding of the small molecules with the quadruplex DNA.

CONCLUSIONS:

The biocalorimetric analysis of the interaction of ethidium and palmatine as planar and non planar molecule, respectively, with the model quadruplex structure, showed specific differences in binding. The planar molecule, bound favorably to the quadruplex structure compared to the buckled one, as revealed by higher affinity, higher hydrophobic contribution and better enthalpy -entropy compensation behavior. Thus, the results from the thermodynamic parameters highlighted the importance of planarity in small molecule in stabilizing the quadruplex DNA structure for better development of quadruplex targeted therapeutic agents.

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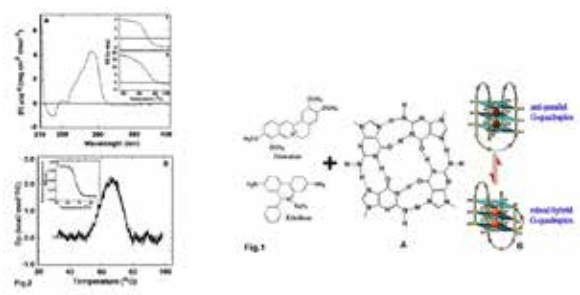
Figure legends:

Fig.1 Chemical structures of palmatine and ethidium binding with (A) a G tetrad having (B) antiparallel and mixed hybrid G-quadruplex structure.

Fig.2 (A) CD spectra of the quadruplex DNA (6 Δ M) showing a positive peak at 290 nm and a small negative peak at 238 nm. Inset: CD melting profiles of the oligomer (B) Representative DSC thermogram of the oligomer. Inset: optical thermal melting profile of the quadruplex DNA.

Fig.3 ITC profile for the titration of (A) ethidium and (B) palmatine (200 ΔM each) into a 10 ΔM solution of quadruplex DNA. Each heat burst curve (in the bottom part of upper panel) is the result of a 7ΔL injection of the ligands into the oligomer. (C and D) Plots of enthalpy changes against molar ratio to show the integrated heat results of the titration at 10 °C (▪), 15 °C (Δ), 25 °C (Δ) and 30 °C (°), respectively.



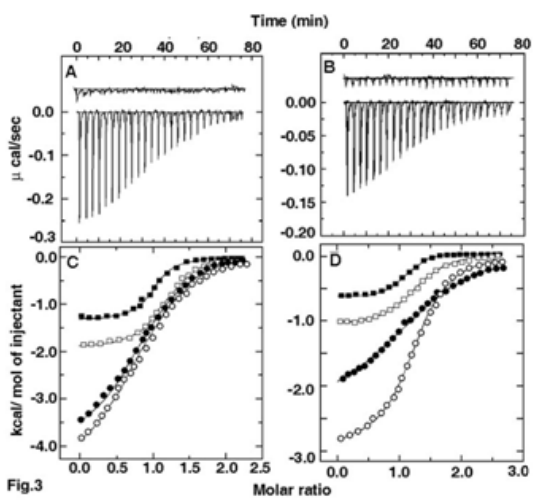


Fig.3

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