Biochemical and Biophysical Research Communications 459 (2015) 75–80

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Quadruplex forming promoter region of c-myc oncogene as a potential target for a telomerase inhibitory plant alkaloid, chelerythrine

Saptaparni Ghosh, Dipak Dasgupta*

Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Block-AF, Sector-I, Bidhan Nagar, Kolkata, 700064, India

ARTICLE INFO

Article history:
Received 2 February 2015
Available online 21 February 2015

Keywords:
Plant alkaloid
Chelerythrine
G-quadruplex
Promoter region of c-myc oncogene
Calorimetry

ABSTRACT

Guanine rich sequences present in the promoter region of oncogenes could fold into G-quadruplexes and modulate transcription. Equilibrium between folding and unfolding of the quadruplexes in these regions play important role in disease processes. We have studied the effect of a putative anticancer agent chelerythrine on G-rich NHE III_1 present in the promoter region of c-myc oncogene. We have demonstrated the ability of chelerythrine, a telomerase inhibitor, to block the hybridization of Pu27 with its complementary strand via folding it into a quadruplex structure. Calorimetry shows that the association of Pu27 with chelerythrine is primarily enthalpy driven with high binding affinity (~10^5 M^{-1}). The association does not lead to any major structural perturbation of Pu27. The resulting 2:1 complex has enhanced stability as compared to free Pu27. Another notable feature is that the presence of molecular crowding agent like ficoll 70 does not change the mode of recognition though the binding affinity decreases. We suggest that the anticancer activity of chelerythrine could be ascribed to its ability to stabilize the quadruplex structure in the c-myc promoter region thereby downregulating its transcription.

1. Introduction

The human oncogene, c-myc, plays an important role in many cellular events and its overexpression is related to an increase of cellular proliferation in a variety of malignant tumors. Guanine-rich nuclear hypersensitive element III_1, present in its promoter region controls 80–90% of the transcription activity of c-myc [1–5]. Previous reports have shown that this 27 nucleotide guanine-rich sequence (Pu27, Table T1), downregulates c-myc transcription upon folding into a quadruplex structure which acts as a transcriptional repressor element [3,6–12]. Several small molecules which can induce and stabilize this quadruplex structure are, therefore, putative agents to downregulate c-myc expression [13].

A large number of plant alkaloids and their derivatives are known to possess anticancer activity and recent studies have shown that association with quadruplex DNA might be a possible mechanism [7,8,14–20]. Earlier reports from our laboratory have attributed the quadruplex binding potential of the plant alkaloids sanguinarine (SGR, Fig. 1A(i)) and ellipticine as one of the plausible mechanisms of their potentials as anticancer agents [16,17]. Among these two small molecules, SGR is structurally similar to chelerythrine (CHL) (Fig. 1A(ii)), the molecule studied in the current report. Here we have reported that association of CHL with Pu27 blocks its hybridization with the complementary strand leading to an inhibition of its extension by polymerase. We have studied the association of CHL with Pu27 and the subsequent structural alteration, if any, of the quadruplex. The ability of CHL to specifically inhibit protein kinase C is currently the proposed mechanism put forward for its anticancer activity [21]. Our results suggest that targeting quadruplex DNA formed by the sequence upstream of c-myc oncogene is an additional mechanism by which CHL might impart its anticancer activity.

A variety of biomolecules are present inside the living cells leading to a molecular crowded environment optimized for biomolecular functions [22–24]. The structure and stability of some types of G-quadruplexes are also altered in the presence of molecular crowding agents. *A priori* one can anticipate that it might affect their molecular recognition by ligands. Therefore, we have also reported here the effect of molecular crowding with ficoll as the molecular crowding agent on the association between CHL and Pu27.

* Corresponding author. Fax: +91 33 23374637.
E-mail address: dipak.dasgupta@saha.ac.in (D. Dasgupta).

http://dx.doi.org/10.1016/j.bbrc.2015.02.072
0006-291X/

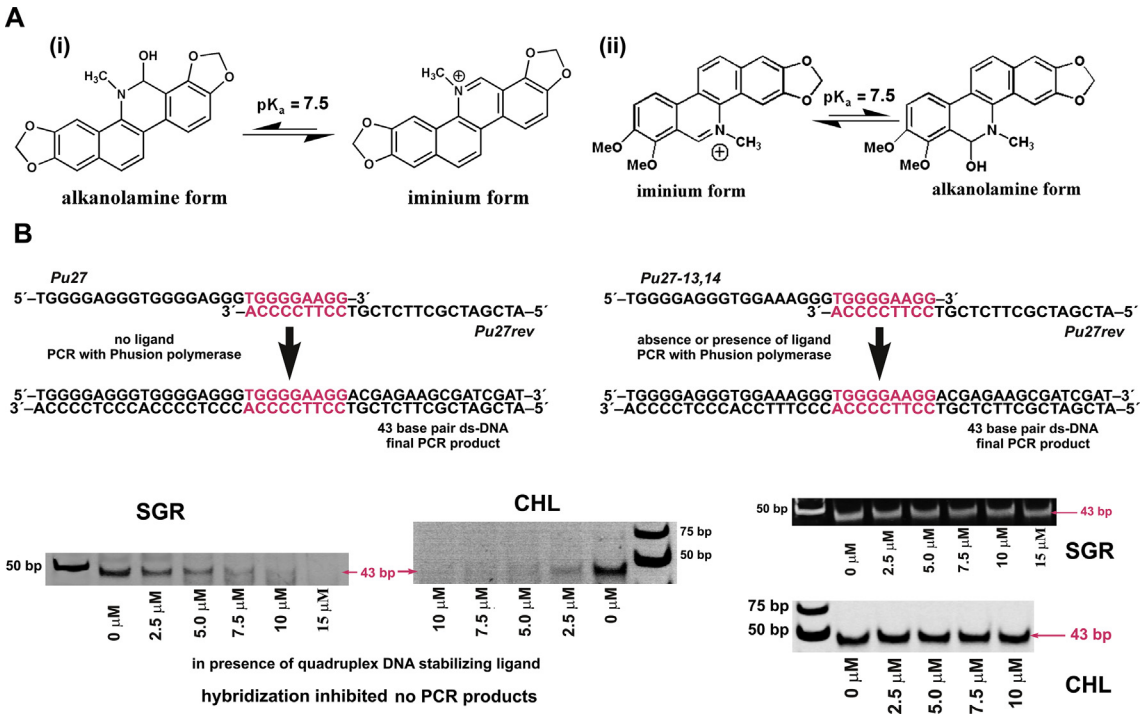

Fig. 1. (A) Chemical structure of (i) sanguinarine (SGR) and (ii) chelerythrine (CHL). The protonated form intercalates into double stranded DNA. (B) Schematic representation of PCR-stop assay. The experiments were run in triplicate with indicated amounts of the molecule.

2. Materials and methods

2.1. Materials and preparation of stock solutions

CHL, SGR, potassium phosphate monobasic, potassium phosphate dibasic, potassium chloride, sodium acetate, acetic acid, ficoll 70, acrylamide/bisacrylamide solution and desalted and HPLC purified oligomers (Table T1) were purchased from Sigma Chemical Corporation, USA. Phusion high-fidelity DNA polymerase was obtained from Thermo Scientific. SYBR Green I nucleic acid gel stain was obtained from Invitrogen, USA. Ultra low range DNA ladder was obtained from Fermentas. All buffers were prepared in MilliQ water from Millipore Water System, Millipore, USA and filtered through 0.1 μm filters from Millipore, USA prior to use.

CHL, SGR, quadruplex and duplex DNA were prepared using standard protocol [16,17,25]. The concentrations of the oligomers were determined by absorbance measurement using molar extinction values (ε) as listed in Table T1. To study the effects of molecular crowding on Pu27-CHL interactions, Pu27 was dissolved in 10 mM potassium phosphate buffer, pH 6.8, containing 150 mM KCl in presence of 40% (w/v) ficoll 70. All experiments were carried out in 10 mM potassium phosphate buffer, pH 6.8, containing 150 mM KCl either in the presence or absence of 40% (w/v) ficoll 70.

Table 1
Dissociation constant (determined from absorbance studies and ITC) and stoichiometry for the interaction of chelerythrine and Pu27 in the absence and presence of ficoll in 10 mM potassium phosphate buffer pH 6.8 containing 150 mM KCl.

System	Dissociation constant, K_d (μM) at 25 °C		Stoichiometry
	Absorption	ITC	
Pu27-CHL (dilute)	2.54 ± 0.20	3.58 ± 0.65	1:2 (Job Plot)
Pu27-CHL (MC)[a]	4.11 ± 0.24	ND	1:2

[a] MC-molecular crowding with 40% (w/v) ficoll 70, ND-not determined.

2.2. Methods

2.2.1. PCR-stop assay

PCR-stop assay was performed with a modified protocol of the previous studies [7–9,26]. Pu27, Pu27-13,14 and Pu27rev (Table T1) were used in the current study. The reactions were performed in 1 × PCR buffer, containing 4 μM of each oligonucleotide, 0.16 mM dNTP, 0.04 U phusion high-fidelity DNA polymerase and indicated amount of CHL or SGR. Reaction mixtures were incubated in a Veriti 96 well thermal cycler (Applied BioSystem) with the following cycling conditions: 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. The amplified products were resolved on 18% non-denaturing polyacrylamide gel in 1 × TBE, followed by staining with SYBR Green I.

2.2.2. Absorption spectroscopy

Absorption spectra were measured in CECIL spectrophotometer. 10 μM of CHL was titrated against Pu27 in absence and presence of 40% (w/v) ficoll 70. The dissociation constant and stoichiometry was determined from the plot of $\Delta A/\Delta A_{max}$ at 316 nm versus quadruplex concentration [16,17,25]. The method of continuous variation (Job Plot) was also employed to determine the binding stoichiometry [27].

2.2.3. Isothermal titration calorimetry

ITC experiments were done in iTC200 Microcalorimeter, Microcal Inc., USA at different temperatures between 10 °C and 35 °C. 25 μM CHL was titrated against Pu27.

25 μM CHL was titrated against Pu27 duplex to determine the association constant for the interaction at 25 °C. To maintain the

ionic strength of the solution, 150 mM KCl was added to the buffer prior titration.

The isotherms were analyzed using the in-built Microcal LLC ITC software. 'One set of sites' model yield the best fitted curve for the obtained data points. Equilibrium association constant (K_a), enthalpy (ΔH) and entropy (ΔS) of association were obtained after fitting each isotherm. Change in heat capacity (ΔC_p) and extent of enthalpy-entropy compensation for the association were calculated [16,17].

2.2.4. Circular dichroism

CD spectra were recorded on a JASCO J715 spectropolarimeter (Jasco Cooperation, Tokyo, Japan) at 25 °C in absence and presence of 40% (w/v) ficoll 70. The CD scans were recorded within the wavelength range of 220–420 nm in a reaction volume of 300 μl (scan speed: 200 nm per minute, step size: 0.1 nm, time constant: 2 s, bandwidth: 1 nm, path length of cuvette: 0.1 cm). 20 μM Pu27 was titrated with increasing concentration of CHL. Convex constraint analysis (CCA) was performed on the spectral set in order to extract the basis spectra and their associated coefficients [28].

2.2.5. Differential scanning calorimetry

The heat capacity of the quadruplex alone (100 μM) and in presence of CHL (70 μM of Pu27 and 190 μM CHL incubated for 1 h) were measured as a function of temperature (10 °C–130 °C) in a VP DSC Microcalorimeter (Microcal, LLC, Northampton, MA). The thermograms obtained were analyzed using the in-built VPViewer software with Origin 7.0. The non-2-state (cursor initiation) model of curve fitting was used to fit the raw thermograms.

3. Results and discussion

TRAP (Telomerase Repeat Amplification Protocol) assay demonstrates the dose dependent decrease in telomerase activity by CHL in extracts from breast cancer cell line, MDAMB-231 (Fig. S1). Telomerase activity decreased by 1.2 fold on addition of 2 μM CHL and by 2.1 fold on addition of 10 μM CHL to the extract. We have reported similar effect in HeLa cell line [25]. PCR-stop assay indicates that CHL induces a structure in Pu27 that can block the 5′ to 3′ extension by a polymerase (Fig. 1B, Fig. S2). This is due to CHL induced stabilization of the quadruplex structure of Pu27. The positive control with Pu27-13,14, which does not fold into a quadruplex structure, shows no such dose dependent decrease of the final product (Fig. 1B). These results indicate that CHL has the potential to interact with quadruplex structures and modulate both telomerase activity and extension of duplex DNA by polymerase.

Pu27 folds into a mixture of two all-parallel quadruplexes [29,30]: 1:2:1 conformer (major) and 1:6:1 conformer (minor) [31,32]. Previous NMR reports have shown that this sequence display broad envelopes arising from dynamic equilibrium between the conformers [29,33]. This is a major obstacle to study the association by NMR. Hence, we have probed the structural basis of the association by alternate physico-chemical techniques.

Absorbance of CHL at 316 nm decreases on binding to Pu27. The 12 nm red shift of this peak (Fig. S3A *Inset*) can be attributed to the

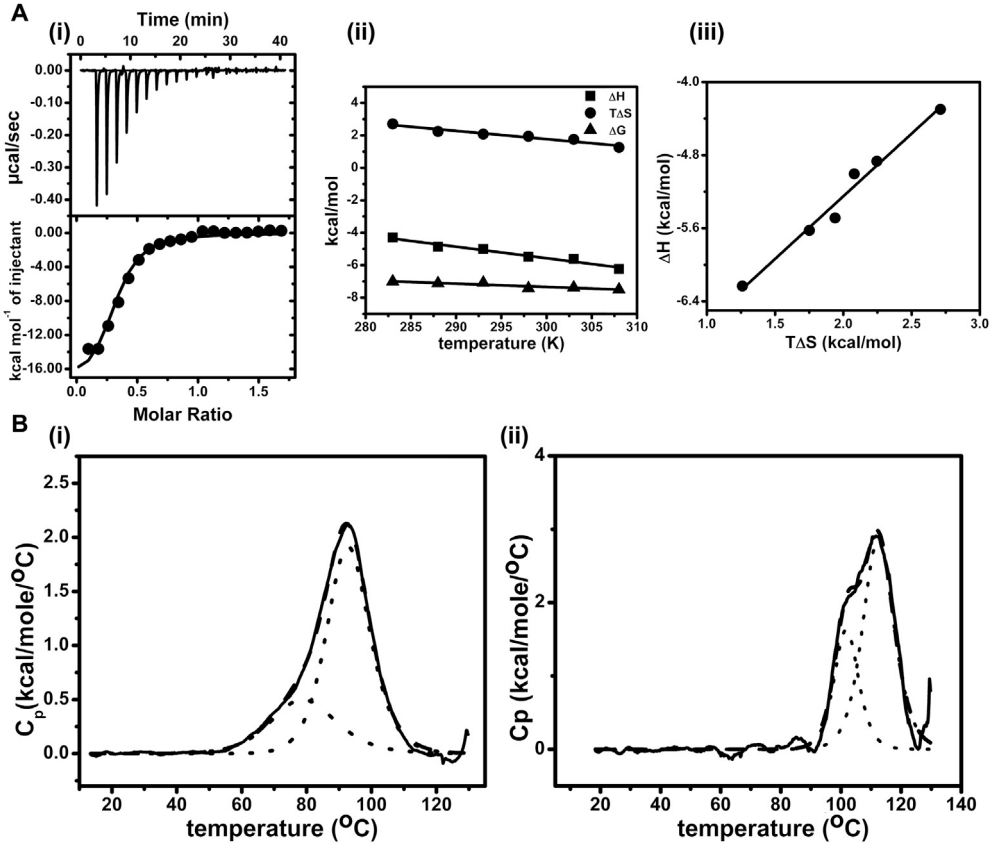

Fig. 2. Panel A: (i) Representative ITC profile for the titration of Pu27 against chelerythrine at 25 °C. (ii) Variation of thermodynamic parameters for the association of chelerythrine with Pu27 as a function of temperature. (iii) Enthalpy–entropy compensation plot for the interaction of chelerythrine with Pu27. **Panel B**: Heat capacity profile for Pu27 in (i) absence and (ii) presence of saturating chelerythrine concentrations at a scan speed of 60 °C/hour at approximately 35 psi pressure. The observed data (solid line) were deconvoluted (dotted lines) using the inbuilt "2-state" model for curve fitting software. The dashed curve denotes the overall fit of the data.

stabilization of π^* orbital of CHL [34]. The binding parameters are summarized in Table 1. The two CHL molecules (Fig. S3C) might either end stack on or intercalate between the quartet planes. However, presence of three double chain reversal loops, that spans the quadruplex groove and potassium ions intercalated between the quartets precludes the later possibility. It appears more likely that CHL will end stack on the quartets.

The thermodynamic parameters (Table T2) obtained from ITC (Fig. 2A (i)) indicates that both enthalpy and entropy contribute favorably towards the association, though the interaction is mostly enthalpy driven. A slope of 1.37 ± 0.10 (Fig. 2A (iii)) for ΔH versus TΔS supports this observation. The relatively low magnitude of ΔC_p (-71.0 ± 6.27 cal/mol/K, Fig. 2A(ii)) along with results obtained from DSC and CD studies indicates that the association does not lead to a major structural alteration of Pu27. The negative enthalpic contribution originates from non-covalent interaction like stacking [35,36]. The positive entropic contribution, which further reinforces the negative free energy change, arises from the release of bound water molecules [35,36]. Negative sign of ΔC_p further corroborates this observation [35,36]. The higher affinity of CHL for Pu27 quadruplex as compared to Pu27 duplex (Fig. S4) supports our hypothesis that under *in vivo* conditions CHL will bind and stabilize the quadruplex structure adopted transiently by this sequence. Decrease of c-myc transcription may be the downstream event.

CHL binding stabilizes Pu27 and increases the total enthalpy of transition (Fig. 2B and Table T3). Its melting is characterized by two major thermal transitions in the free and ligand bound forms. Comparison of these melting temperatures with those of mutant Pu27 sequences (Pu121 and Pu161) in absence and presence of CHL indicates that the lower and the higher melting temperatures correspond to the melting of the 1:6:1 and 1:2:1 conformer, respectively (Fig. S5 and Table T4). Absence of a significant change in the enthalpy of melting (Table T3) of the two conformers upon CHL addition indicates that ligand binding does not perturb the equilibrium between the conformers.

CD spectrum of Pu27 (Fig. 3A, Spectrum in black line) is characteristic of a parallel quadruplex. Molar ellipticity values of the two signature peaks decreases upon complex formation with CHL (Fig. 3A and B) but no structural alteration of Pu27 occurs. This can be ascribed to the interaction between the chromophoric group of CHL and Pu27 quartets [17,37] rather than disruption of the quadruplex structure on ligand binding [38,39]. This is further corroborated from the enhanced melting temperature of Pu27-CHL complex. CCA analysis could best deconvolute the CD spectra for the interaction into two basis spectra (Fig. 3C). Components 1 and 2 characterize the CD spectrum of unbound and ligand bound Pu27 respectively. The variation of the associated coefficients of each basis spectra with CHL concentration (Fig. 3D) shows (a) a decrease in the percent population of component 1 and (b) a concomitant increase in the percent population of component 2.

Comparative analysis of the absorbance (Fig. S3B & S3D) and CD (Fig. 3 and S6) studies in absence and presence of molecular crowding agent, ficoll, leads us to propose that the molecular recognition process between Pu27 and CHL is not perturbed to any significant degree under crowding condition. Also, ficoll does not cause any significant structural alteration of Pu27. However, the binding affinity and change in free energy of association is reduced under the crowding condition.

We have earlier reported the interaction of Pu27 with SGR, a plant alkaloid containing the same planar aromatic moiety as CHL

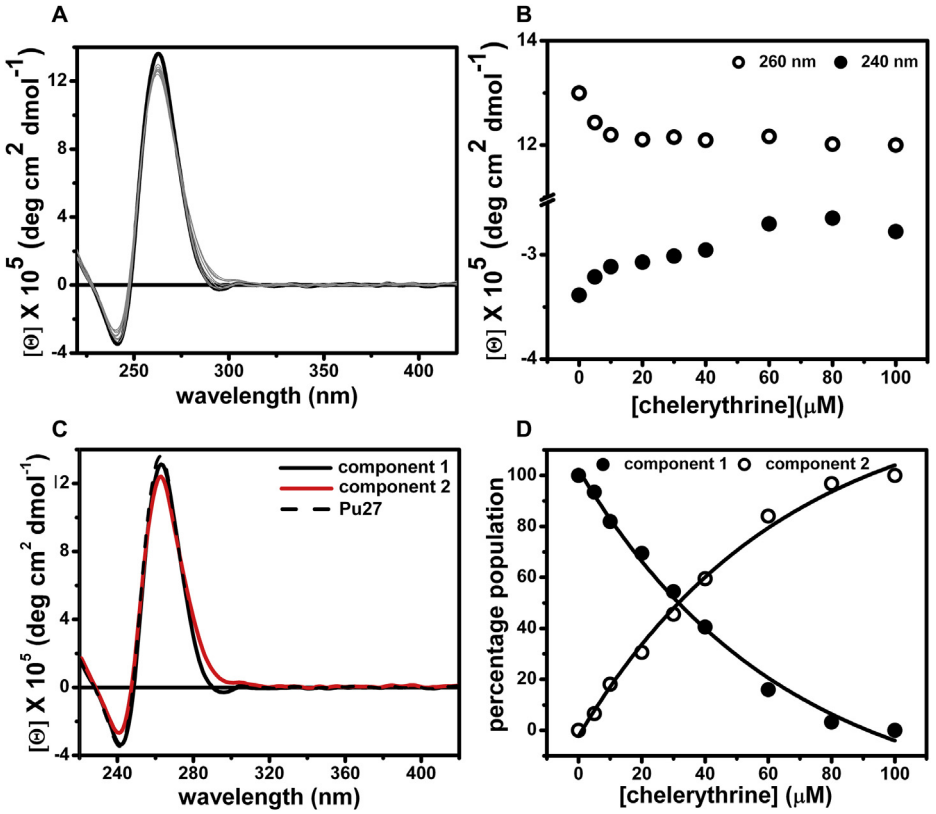

Fig. 3. (A) CD spectra resulting from the interaction of Pu27 with increasing chelerythrine concentration at 25 °C. (B) Variation in the molar ellipticity value at two selected wavelengths for Pu27 chelerythrine interaction. (C) Basis spectra obtained from CCA analysis of CD spectra shown in A and (D) Contribution of CCA components as a function of chelerythrine concentration.

[17]. PCR stop assay results indicate that CHL is a more potent inhibitor of Pu27 hybridization with its complementary strand than SGR at identical concentration (Fig. 1B). Binding affinity of CHL and SGR for Pu27 is comparable; the binding stoichiometry is different. Two CHL molecules bind to one molecule of Pu27. In contrast three SGR molecules bind to one molecule of Pu27. The first two binding sites are same. While, the third SGR molecule binds to the second loop of Pu27 thereby perturbing the equilibrium between 1:2:1 and 1:6:1 conformers. This is reflected in the high ΔC_p value for SGR-Pu27 interaction (-151.54 ± 25.07 cal mol^{-1} K^{-1}). [17] The deconvolution of the CD spectra into three components in case of SGR in contrast to two components in case of CHL also corroborates this result. From the thermodynamic perspective, Pu27-CHL association is primarily enthalpy driven while both enthalpy and entropy contribute equally in case of SGR-Pu27 interaction. Additionally, DSC results show that the bound state 1:2:1 conformer contributes to a greater extent to the total enthalpy of melting than the 1:6:1 bound conformer when CHL is the ligand. In case of SGR the scenario is reversed. The difference in the molecular recognition process of Pu27 by the two ligands, CHL and SGR, may be attributed to the difference in their shapes. A comparison of the space filling model of the energy minimized structure of the two molecules (Fig. S7) shows that SGR has a greater curvature than CHL due to presence of two -OCH_3 instead of a dioxole ring at one end of CHL. This structural difference between SGR and CHL, favors facile Pu27 loop binding in case of SGR.

Conflict of interest

There are no conflicts of interest to declare.

Acknowledgments

This work was supported by the intramural grant Biomolecular Assembly, Recognition and Dynamics (BARD) project [Grant Number: 12-R&D–SIN–5.04-0103] from Department of Atomic Energy (DAE), Govt. of India. The authors thank Chemical Science Division, SINP for the CD experiments. SG thanks Ambarnil Ghosh, Manindra Bera and Manas Mandal of SINP for fruitful discussion. The authors would also like to thank Dr. Shantanu Chowdhury and Dr.Anirban Kar of IGIB for help with TRAP experiments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.072.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.072.

References

[1] T.A. Brooks, L.H. Hurley, The role of supercoiling in transcriptional control of MYC and its importance in molecular therapeutics, Nat. Rev. Cancer 9 (2009) 849–861.
[2] V. Gonzalez, L.H. Hurley, The c-MYC NHE III(1): function and regulation, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 111–129.
[3] A. Siddiqui-Jain, C.L. Grand, D.J. Bearss, L.H. Hurley, Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11593–11598.
[4] T. Simonsson, P. Pecinka, M. Kubista, DNA tetraplex formation in the control region of c-myc, Nucleic Acids Res. 26 (1998) 1167–1172.
[5] T. Tomonaga, D. Levens, Activating transcription from single stranded DNA, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 5830–5835.
[6] H.J. Kang, H.J. Park, Novel molecular mechanism for actinomycin D activity as an oncogenic promoter G-quadruplex binder, Biochemistry 48 (2009) 7392–7398.
[7] Y. Ma, T.M. Ou, J.Q. Hou, Y.J. Lu, J.H. Tan, L.Q. Gu, Z.S. Huang, 9-N-Substituted berberine derivatives: stabilization of G-quadruplex DNA and down-regulation of oncogene c-myc, Bioorg. Med. Chem. 16 (2008) 7582–7591.
[8] Y. Ma, T.M. Ou, J.H. Tan, J.Q. Hou, S.L. Huang, L.Q. Gu, Z.S. Huang, Quinolino-benzo-[5, 6]-dihydroisoquindolium compounds derived from berberine: a new class of highly selective ligands for G-quadruplex DNA in c-myc oncogene, Eur. J. Med. Chem. 46 (2011) 1906–1913.
[9] T.M. Ou, Y.J. Lu, C. Zhang, Z.S. Huang, X.D. Wang, J.H. Tan, Y. Chen, D.L. Ma, K.Y. Wong, J.C. Tang, A.S. Chan, L.Q. Gu, Stabilization of G-quadruplex DNA and down-regulation of oncogene c-myc by quindoline derivatives, J. Med. Chem. 50 (2007) 1465–1474.
[10] S. Balasubramanian, L.H. Hurley, S. Neidle, Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat. Rev. Drug Discov. 10 (2011) 261–275.
[11] R.V. Brown, F.L. Danford, V. Gokhale, L.H. Hurley, T.A. Brooks, Demonstration that drug-targeted down-regulation of MYC in non-Hodgkins lymphoma is directly mediated through the promoter G-quadruplex, J. Biol. Chem. 286 (2011) 41018–41027.
[12] L.H. Hurley, D.D. Von Hoff, A. Siddiqui-Jain, D. Yang, Drug targeting of the c-MYC promoter to repress gene expression via a G-quadruplex silencer element, Sem. Oncol. 33 (2006) 498–512.
[13] S. Mansilla, J. Portugal, Occurrence of DNA sequences specifically recognized by drugs in human promoters, J. Biomol. Struct. Dyn. 19 (2002) 669–679.
[14] K. Bhadra, G.S. Kumar, Interaction of berberine, palmatine, coralyne, and sanguinarine to quadruplex DNA: a comparative spectroscopic and calorimetric study, Biochim. Biophys. Acta 1810 (2011) 485–496.
[15] M. Franceschin, L. Rossetti, A. D'Ambrosio, S. Schirripa, A. Bianco, G. Ortaggi, M. Savino, C. Schultes, S. Neidle, Natural and synthetic G-quadruplex interactive berberine derivatives, Bioorg. Med. Chem. Lett. 16 (2006) 1707–1711.
[16] S. Ghosh, A. Kar, S. Chowdhury, D. Dasgupta, Ellipticine binds to a human telomere sequence: an additional mode of action as a putative anticancer agent? Biochemistry 52 (2013) 4127–4137.
[17] S. Ghosh, S.K. Pradhan, A. Kar, S. Chowdhury, D. Dasgupta, Molecular basis of recognition of quadruplexes human telomere and c-myc promoter by the putative anticancer agent sanguinarine, Biochim. Biophys. Acta 1830 (2013) 4189–4201.
[18] W.J. Zhang, T.M. Ou, Y.J. Lu, Y.Y. Huang, W.B. Wu, Z.S. Huang, J.L. Zhou, K.Y. Wong, L.Q. Gu, 9-Substituted berberine derivatives as G-quadruplex stabilizing ligands in telomeric DNA, Bioorg. Med. Chem. 15 (2007) 5493–5501.
[19] I. Bessi, C. Bazzicalupi, C. Richter, H.R. Jonker, K. Saxena, C. Sissi, M. Chioccioli, S. Bianco, A.R. Bilia, H. Schwalbe, P. Gratteri, Spectroscopic, molecular modeling, and NMR-spectroscopic investigation of the binding mode of the natural alkaloids berberine and sanguinarine to human telomeric G-quadruplex DNA, ACS Chem. Biol. 7 (2012) 1109–1119.
[20] S.K. Pradhan, D. Dasgupta, G. Basu, Human telomere d[(TTAGGG)4] undergoes a conformational transition to the Na+-form upon binding with sanguinarine in presence of K+, Biochem. Biophys. Res. Commun. 404 (2011) 139–142.
[21] J.M. Herbert, J.M. Augereau, J. Gleye, J.P. Maffrand, Chelerythrine is a potent and specific inhibitor of protein kinase C, Biochem. Biophys. Res. Commun. 172 (1990) 993–999.
[22] K. Luby-Phelps, Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area, Int. Rev. Cytol. 192 (2000) 189–221.
[23] A.P. Minton, The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media, J. Biol. Chem. 276 (2001) 10577–10580.
[24] S.B. Zimmerman, S.O. Trach, Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of Escherichia coli, J. Mol. Biol. 222 (1991) 599–620.
[25] S. Ghosh, J. Jana, R.K. Kar, S. Chatterjee, D. Dasgupta, Plant alkaloid chelerythrine induced aggregation of human telomere sequence- a unique mode of association between a small molecule and a quadruplex, Biochemistry (2015).
[26] D. Peng, J.H. Tan, S.B. Chen, T.M. Ou, L.Q. Gu, Z.S. Huang, Bisaryldiketene derivatives: a new class of selective ligands for c-myc G-quadruplex DNA, Bioorg. Med. Chem. 18 (2010) 8235–8242.
[27] C.Y. Huang, Determination of binding stoichiometry by the continuous variation method: the job plot, Methods Enzymol. 87 (1982) 509–525.
[28] A. Perczel, M. Hollosi, G. Tusnady, G.D. Fasman, Convex constraint analysis: a natural deconvolution of circular dichroism curves of proteins, Protein Eng. 4 (1991) 669–679.
[29] A. Ambrus, D. Chen, J. Dai, R.A. Jones, D. Yang, Solution structure of the biologically relevant G-quadruplex element in the human c-MYC promoter. Implications for G-quadruplex stabilization, Biochemistry 44 (2005) 2048–2058.
[30] R.I. Mathad, E. Hatzakis, J. Dai, D. Yang, c-MYC promoter G-quadruplex formed at the 5'-end of NHE III1 element: insights into biological relevance and parallel-stranded G-quadruplex stability, Nucleic Acids Res. 39 (2011) 9023–9033.
[31] J.M. Dettler, R. Buscaglia, V.H. Le, E.A. Lewis, DSC deconvolution of the structural complexity of c-MYC P1 promoter G-quadruplexes, Biophys. J. 100 (2011) 1517–1525.

[32] M.W. Freyer, R. Buscaglia, K. Kaplan, D. Cashman, L.H. Hurley, E.A. Lewis, Biophysical studies of the c-MYC NHE III1 promoter: model quadruplex interactions with a cationic porphyrin, Biophys. J. 92 (2007) 2007–2015.
[33] A.T. Phan, Y.S. Modi, D.J. Patel, Propeller-type parallel-stranded G-quadruplexes in the human c-myc promoter, J. Am. Chem. Soc. 126 (2004) 8710–8716.
[34] M. Janovska, M. Kubala, V. Simanek, J. Ulrichova, Fluorescence of sanguinarine: spectral changes on interaction with amino acids, Phys. Chem. Chem. Phys. 12 (2010) 11335–11341.
[35] I. Haq, Thermodynamics of drug-DNA interactions, Arch. Biochem. Biophys. 403 (2002) 1–15.
[36] J. Ren, T.C. Jenkins, J.B. Chaires, Energetics of DNA intercalation reactions, Biochemistry 39 (2000) 8439–8447.
[37] J.H. Fan, E. Bochkareva, A. Bochkarev, D.M. Gray, Circular dichroism spectra and electrophoretic mobility shift assays show that human replication protein A binds and melts intramolecular G-quadruplex structures, Biochemistry 48 (2009) 1099–1111.
[38] M.J. Morris, K.L. Wingate, J. Silwal, T.C. Leeper, S. Basu, The porphyrin TmPyP4 unfolds the extremely stable G-quadruplex in MT3-MMP mRNA and alleviates its repressive effect to enhance translation in eukaryotic cells, Nucleic Acids Res. 40 (2012) 4137–4145.
[39] Z.A. Waller, S.A. Sewitz, S.T. Hsu, S. Balasubramanian, A small molecule that disrupts G-quadruplex DNA structure and enhances gene expression, J. Am. Chem. Soc. 131 (2009) 12628–12633.