1-1-2010

A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives

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Abstract

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Keywords

selective, berberine, derivatives, spectrometric, investigation, mass, novel, quadruplex, dna, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Gornall, K., Samosorn, S., Tanwirat, B., Suksamrarn, A., Bremner, J. B., Kelso, M. J. & Beck, J. L. (2010). A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives. Chemical Communications, 46 (35), 6602-6604.

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A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives†

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Received 17th June 2010, Accepted 16th July 2010
DOI: 10.1039/c0cc01933j

ESI mass spectrometry was used to assess the binding of 13-substituted, 5-nitro-2-phenylindolyl- and 2-naphthalenyl-based berberine derivatives to inter- and intramolecular G-quadruplex DNA molecules. In contrast with the parent berberine, the compounds showed selectivity for quadruplex over duplex DNA and stabilised the quadruplex structure. They represent a new class of quadruplex DNA-selective ligands.

Sequences of DNA containing tracts of contiguous guanosine residues can fold to form quadruplex structures (qDNA) in which four guanosine residues are positioned in a planar arrangement stabilised by Hoogsten base pairing. The qDNA structures are further stabilised by the presence of monovalent cations in the central cavity.[1] Such sequences are found in the single-stranded overhangs (telomeres) at the ends of chromosomes. As a consequence of the "end-replication effect", telomeres in normal somatic cells shorten with each round of replication, eventually signalling cell senescence and apoptosis.[2] In ~85% of cancer cells, telomeres are extended by the addition of TTAGGG repeats by the enzyme telomerase. The maintenance of telomere length is one factor that is linked to tumour growth.[3,4] It has been suggested that the stabilisation of qDNA structures inhibits telomere extension.[4] Consequently, small molecule ligands that selectively bind and stabilise qDNA structures over duplex DNA, including those implicated in oncogene promoters, have exciting potential for development as new anticancer leads.[4]

There is a relatively limited number of ligand classes that selectively bind qDNA. These include, amongst others, telomestatin, 9-anilino proflavine derivatives and acridine derivatives (*e.g.* BRACO-19), and bis-indole carboxamides.[5] The alkaloid berberine (Berb, **1**, Fig. 1) and its 13-piperidino derivative show moderate and approximately equal abilities to stabilise an intramolecular qDNA as judged by thermal melting experiments.[6] Coralyne, a synthetic derivative of berberine with a fully aromatic core, shows a much greater stabilising effect against melting.[6] Recently we showed using

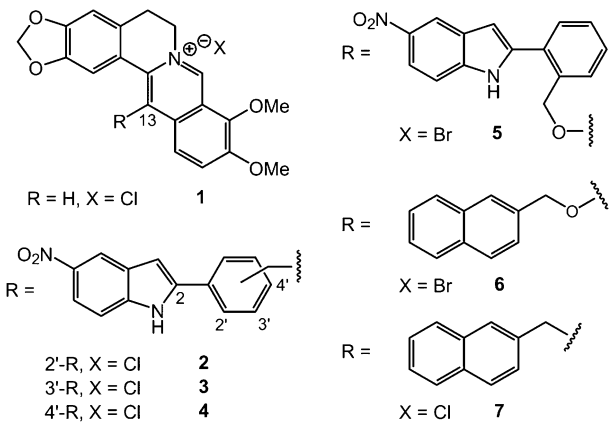

Fig. 1 Structures of berberine (**1**) and 13-substituted derivatives.

electrospray ionisation mass spectrometry (ESI-MS) that a berberine derivative substituted at its 13-position with a 5-nitro-2-phenylindolylmethyl group (**2**) had modest binding affinity for a tetrameric qDNA, and significant selectivity over duplex DNA.[7]

Compound **2** and its *meta* and *para* isomers, **3** and **4** (Fig. 1), were originally designed and synthesised as dual action hybrid antibacterials. One action targets inhibition of the bacterial NorA efflux pump through inclusion of the NorA pump inhibitor 5-nitro-2-phenylindole (INF55).[8] The pump inhibitor is designed to potentiate the antibacterial effects of berberine.[9–11] Analogues bearing a methylene ether linkage at the berberine 13-position, **5**, and a 2-naphthalenyl congener, **6**, were similarly synthesised and studied as dual action antibacterials.[12] As berberine is known to interact with DNA[7] and to have effects on telomerase activity,[13] it was of interest to characterise and compare the qDNA binding properties of these 13-substituted berberines. In the current work, compounds **2–6** were investigated using ESI-MS alongside berberine and daunomycin (Dn, a DNA-intercalating anticancer anthracycline) for their abilities to bind three different qDNA (Q4, Q1, Q2) and duplex DNA (D1, D2, F10) sequences.‡ A 2-naphthalenyl analogue **7** was also studied to explore how changing the heteroaryl substituent affects DNA binding.

Detailed characterisation of the qDNA by CD spectroscopy and ESI ion mobility mass spectrometry is reported elsewhere.[14] Briefly, the CD and mass spectra showed that in 150 mM ammonium acetate, Q4 was tetrameric with a parallel strand orientation, while Q1 and Q2 were both present as anti-parallel qDNA consisting of one and two strands, respectively. This is in agreement with other work.[14–16] Maintenance of the folded qDNA structures *in vacuo* was supported by

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† Electronic supplementary information (ESI) available: (a) Synthesis and characterisation of **7**. (b) Abundances of each complex in mixtures containing ligands and D2, Q4, Q1 and Q2; Fig. S1–S4. (c) Number of ammonium ions associated with qDNA in the presence of **1** or **7** (Fig. S5). See DOI: 10.1039/c0cc01933j

observations that Q4 bound to four ammonium ions (NH_4^+) and Q2 bound up to three NH_4^+. No NH_4^+ was associated with Q1 as has been observed in other work,[16] but ions corresponding to adduction of Q1 with adventitious Na^+ and K^+ were present.§

Each DNA sequence was treated with each ligand in 1 : 1, 1 : 3, 1 : 6 and 1 : 9 (DNA : ligand) molar ratios and ESI mass spectra of each mixture acquired as previously described.[7]

Fig. 2 shows representative examples of the mass spectra obtained. Spectra shown are 1 : 9 mixtures of Q4 with **1** or **7**, Q2 with **1** or **6** and Q1 with **7**. Fig. 2(A) and (B) show that the 5− ions of Q4 ($[Q4 + 4NH_4^+ - 9H]^{5-}$) were the most abundant under the conditions. Berb (**1**) bound extensively to Q4 as previously observed,[7] and to every sequence tested with the exception of Q2. Fig. 2(C) and (D) show the spectra of Q2 with **1** and **6**, respectively. Ligand **6**[12] bound to this sequence with a strong preference for the binding of two molecules to Q2. Ligand **7** also bound appreciably to Q1 (Fig. 2(E)). Fig. 3 provides a summary of the binding data for all mixtures. The intensities of all ions from DNA–ligand complexes were summed and expressed as a percentage of the sum of the intensities of all DNA (free + bound) in the ESI mass spectra. Both **1** and Dn showed no selectivity for qDNA over duplex DNA. In contrast, **7**, **6** and all the other berberine derivatives did not bind appreciably to duplex DNA. The

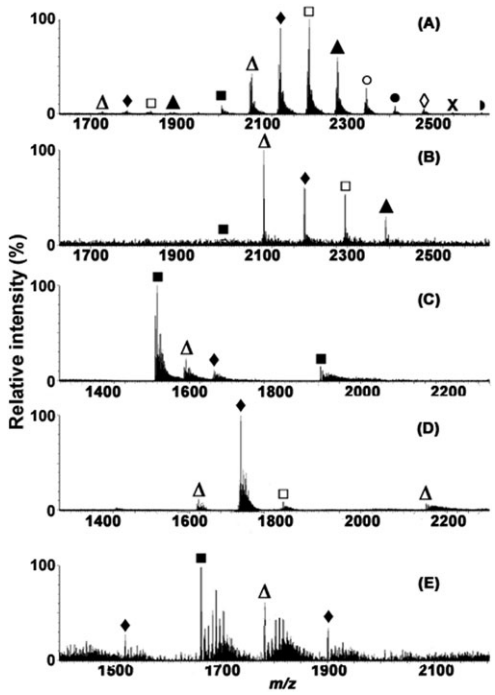

Fig. 2 Negative ion ESI mass spectra of 1 : 9 qDNA–ligand mixtures. (A) Q4/**1**. (B) Q4/**7**. (C) Q2/**1**. (D) Q2/**6**. (E) Q1/**7**. The most abundant ion in each spectrum was: $[Q4 + 4NH_4^+ + 3(\mathbf{1}) - 9H]^{5-}$, *m/z* 2211.8; $[Q4 + 4NH_4^+ + 1(\mathbf{7}) - 9H]^{5-}$, *m/z* 2105.3; $[Q2 + 2NH_4^+ - 7H]^{5-}$, *m/z* 1521.9; $[Q2 + 3NH_4^+ + 2(\mathbf{6}) - 8H]^{5-}$, *m/z* 1721.5; and $[Q1 - 4H]^{4-}$, *m/z* 1662.3, respectively. ■ qDNA alone. Δ qDNA + 1 ligand. ◆ qDNA + 2 ligands. □ qDNA + 3 ligands. ▲ qDNA + 4 ligands. ○ qDNA + 5 ligands. ● qDNA + 6 ligands. ◇ qDNA + 7 ligands. × qDNA + 8 ligands.

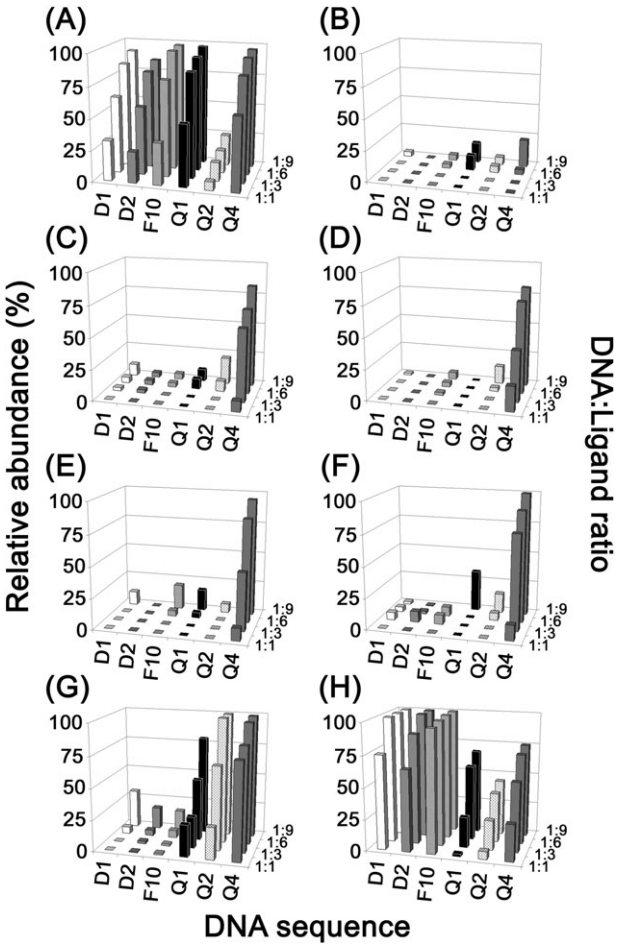

Fig. 3 Relative abundances of DNA : ligand complexes for each ligand in 1 : 1–1 : 9 mixtures determined from negative ion ESI mass spectra. (A) **1**, (B) **2**, (C) **3**, (D) **4**, (E) **5**, (F) **7**, (G) **6**, and (H) Dn.

modest binding to Q4 previously observed for **2** was enhanced in its *m*- and *p*-isomers while the selectivity against duplex DNA was maintained. A similar binding profile was observed for **5**. Ligands **7** and **6** also showed a substantial preference for qDNA. Ligand **6** bound to all the qDNAs while **7** exhibited a preference for Q4. This representation of the data does not reveal any preferred stoichiometries of binding. The abundances of each ligand–DNA complex of a particular stoichiometry relative to all DNA as judged by the ESI mass spectra are shown in the ESI† (Fig. S1–S4 for D2, Q4, Q1 and Q2, respectively). Berb (**1**) bound extensively (up to five or more Berb bound) to all the duplex DNAs and to Q4 and Q1. This extensive binding to Q4 and lack of selectivity for DNA structure was observed in our earlier experiments and may suggest multiple binding modes.[7] The binding stoichiometry of the duplex intercalator, Dn, was similar. In mixtures containing the ligands with a preference for Q4 (**2–4**, **5** and **7**) no clear preference for a particular binding stoichiometry was observed (Fig. S2, ESI†). Q2 showed a preference for binding two molecules of **6** (Fig. S4, ESI†) and Q1 also approached saturation when two molecules of **6** were bound (Fig. S3, ESI†). The observations made using ESI-MS are significant because other methods that demonstrate binding to qDNA such as gel electrophoresis[6] reveal the number of strands present in the

qDNA, but do not yield precise information about the number of ligand molecules that are bound.

QDNA is stabilised by mononvalent cations that sit between the layers of G-quartets.[15] In the ammonium acetate solutions necessary for ESI-MS, NH_4^+ is retained in the structure.[7,16,17] Q4 has five G-tetrads and the predominant ions in the ESI mass spectra were from Q4 + $4NH_4^+$ with Q4 + $3NH_4^+$ at lower abundance.[7] In ESI mass spectra of Q2 alone, Q2 + $2NH_4^+$ represented ~60% of the DNA with Q2 + $3NH_4^+$ present at ≥ 30%. It is not clear whether the presence of various species with different numbers of bound NH_4^+ reflect the structures that are present in solution (including slipped structures where, for example, the first G in one strand aligns with the second G in another strand),[18] or whether NH_4^+ may be lost in the mass spectrometer even under gentle conditions. It was possible to subject Q2 and Q4 to collision-induced dissociation (select an ion and increase the potential in the collision cell) and to observe the loss of NH_4^+ without dissociation to single strands. The binding of the qDNA-selective ligands, but not berberine (**1**), stabilised qDNA against dissociation of NH_4^+. For example, the ratio of the abundances of ions Q4 + $4NH_4^+$/Q4 + $3\ NH_4^+$ for Q4 alone was the same irrespective of whether **1** was bound to Q4. This suggests that Berb had little influence on the stability of Q4 + $4NH_4^+$ over Q4 + $3NH_4^+$ in the gas phase or that it bound equally well to these species if they were present in solution. In contrast, when **7** was present, only ions from Q4 + $4NH_4^+$ + *n* (**7**) were observed consistent with stabilisation of Q4 + $4NH_4^+$ and supporting that this compound exhibits structural elements that favour binding to this qDNA (Fig. S5, ESI†). Stabilisation was observed in this way to varying extents for all the berberine derivatives bound to Q4 or Q2. When **6** was bound to Q1, structures that bound three adventitious K^+ ions were stabilised. Previously, a 13-piperidino berberine induced formation of a dimeric qDNA (*cf*. Q2), albeit more weakly than the parent berberine, but in common with berberine, did not induce folding of an intramolecular qDNA.[6]

These experiments and others[19] highlight the utility of ESI-MS for screening ligand-binding to qDNA. The 13-substituted berberine derivatives (**2–4**, **5** and **7**) have a substantial binding preference for the tetrameric intermolecular qDNA, Q4. Recently, minimum energy conformational analyses of **2–4** showed that the molecules are not planar,[10] and are therefore unlikely to bind to duplex DNA as classic minor groove binding or intercalating ligands. A proposed mode for binding to Q4 is stacking on the ends as has previously been observed for Dn, where three Dn molecules were stacked on the 5′ end of a tetrameric qDNA similar to Q4[20] with either the berberine or 5-nitro-2-phenylindole moiety interacting with the grooves of the qDNA. Replacement of the 5-nitro-2-phenylindole moiety with a 2-naphthalenyl-based substituent in **6** enhanced binding affinity (less free DNA in mixtures), to all types of qDNA, including the intramolecular Q1 DNA. These ligands may thus serve as useful probes of different qDNA structures and as new leads for anticancer drugs. To begin to define structural features common to ligands that are selective for qDNA, the structures of the complexes will need to be determined. The screening by ESI-MS here has shown which of the berberine derivatives form the most stable complexes and has informed our starting points for crystallisation trials.

The authors acknowledge the Australian Research Council, U. Wollongong, Srinakharinwirot University, the Thailand Research Fund (MRG 4980158), and the Thai Commission on Higher Education. KCG acknowledges an APA scholarship.

Notes and references

‡ The qDNA here is named for the number of strands that associate to form the qDNA structure: Q4, $(T_2G_5T)_4$; Q1, $G_3(T_2AG_3)_3$ is used as a model for the human telomeric sequence; Q2, $(G_4T_4G_4)_2$. The structures of these or similar qDNA sequences have been determined.[15] The duplex DNA (one strand only shown) was: D1, CCTCTCTGGACCTTCC, and D2, GCTGCCAAATACCTCC. Forked DNA, F10, contained a 10-base pair sequence available for Watson–Crick H-bonding with its complementary strand with a stretch of 10 adenines (A) at the 3′ end of the template strand and at the 5′ end of the complementary strand, template strand: TGCTCGGACGAAAAAAAAAA.

§ Q1 was associated with adventitious Na^+ and K^+ ions which were not evident with the other qDNA suggesting a higher affinity of this sequence for these ions. The adducts (ions to higher *m*/*z*) are evident in the spectrum shown in Fig. 2(E). Ion mobility mass spectra of Q1 under gentle solution and instrument conditions using a travelling wave ion mobility cell (Waters Synapt™ mass spectrometer) showed a change to longer drift time for Q1 ions when analysed under conditions where it was expected to be unfolded, supporting that Q1 was folded under the more gentle conditions of these experiments.[14]

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