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

Karina Gornall  
*University of Wollongong, kcg83@uow.edu.au*

Siritron Samosorn  
*University of Wollongong, siritron@uow.edu.au*

Bongkot Tanwirat  
*Srinakharinwirot University, Thailand*

Apichart Suksamrarn  
*Ramkhamhaeng University, Thailand*

John B. Bremner  
*University of Wollongong, jbremner@uow.edu.au*

*See next page for additional authors*

Follow this and additional works at: [https://ro.uow.edu.au/scipapers](https://ro.uow.edu.au/scipapers)

Part of the Life Sciences Commons, Physical Sciences and Mathematics Commons, and the Social and Behavioral Sciences Commons

**Recommended Citation**

Gornall, Karina; Samosorn, Siritron; Tanwirat, Bongkot; Suksamrarn, Apichart; Bremner, John B.; Kelso, Michael J.; and Beck, Jennifer L.: A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives 2010, 6602-6604.  

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives

Abstract
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. © 2010 The Royal Society of Chemistry.

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

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

Publication Details

Authors
Karina Gornall, Siritron Samosorn, Bongkol Tanwirat, Apichart Suksamrarn, John B. Bremner, Michael J. Kelso, and Jennifer L. Beck

This journal article is available at Research Online: https://ro.uow.edu.au/scipapers/419
A mass spectrometric investigation of novel quadruplex DNA-selective berberine derivatives†

Karina C. Gornall,ab Siriton Samosorn,ab Bongkol Tanwirat,b Apichart Suksamrarn,c John B. Bremner,a Michael J. Kelsoa and Jennifer L. Beck*ab

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. 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. 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. It has been suggested that the stabilisation of qDNA structures inhibits telomere extension. 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.

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. The alkaloid berberine (Berb, Fig. 1) and its 13-piperidino derivative show moderate and approximately equal abilities to bind three different qDNA (Q4, Q1, Q2) and duplex DNA (D1, D2, F10) sequences.

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

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. 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 antiparallel qDNA consisting of one and two strands, respectively. This is in agreement with other work. Maintenance of the folded qDNA structures in vacuo was supported by...
derivatives did not bind appreciably to duplex DNA. The
over duplex DNA. In contrast, masses of all DNA (free + bound) in the ESI
complexes were summed and expressed as a percentage of the
for all mixtures. The intensities of all ions from DNA–ligand
(Fig. 2(E)). Fig. 3 provides a summary of the binding data
with Q1 as has been observed in other work, but ions
exceeding of Q1 with adventitious Na +
molecules to Q2. Ligand
also bound appreciably to Q1
abundant under the conditions. Berb (I) bound extensively
to Q4 as previously observed, and to every sequence tested
with the exception of Q2. Fig. 2(C) and (D) show the spectra
of Q2 with I and 6, respectively. Ligand 6 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 I 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
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 (I) 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. 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 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 monovalent cations that sit between the layers of G-quartets.1,2 In the ammonium acetate solutions necessary for ESI-MS, NH\textsubscript{4}\textsuperscript{+} is retained in the structure.3,16,17 Q4 has five G-tetrads and the predominant ions in the ESI mass spectra were from Q4 + 4NH\textsubscript{4}\textsuperscript{+} with Q4 + 3NH\textsubscript{4}\textsuperscript{+} at lower abundance.2 In ESI mass spectra of Q2 alone, Q2 + 2NH\textsubscript{4}\textsuperscript{+} represented ~60% of the DNA with Q2 + 3NH\textsubscript{4}\textsuperscript{+} present at ~30%. It is not clear whether the presence of various species with different numbers of bound NH\textsubscript{4}\textsuperscript{+} reflects 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\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} without dissociation to single strands. The binding of the qDNA-selective ligands, but not berberine (1), stabilised qDNA against dissociation of NH\textsubscript{4}\textsuperscript{+}. For example, the ratio of the abundances of ions Q4 + 4NH\textsubscript{4}\textsuperscript{+}/Q4 + 3NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} over Q4 + 3NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} + n (7) were observed consistent with stabilisation of Q4 + 4NH\textsubscript{4}\textsuperscript{+} 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\textsuperscript{+} 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 others19 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 Q220 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\textsubscript{2}G\textsubscript{5}T\textsubscript{2})\textsubscript{4}; Q1, G\textsubscript{3}(T\textsubscript{2}AG\textsubscript{3})\textsubscript{3} is used as a model for the human telomeric sequence; Q2, (G\textsubscript{2}T\textsubscript{2}G\textsubscript{2})\textsubscript{2}. The structures of these or similar qDNA sequences have been determined.15 The duplex DNA (one strand only shown) was: D1, CTCCTCCTGGACCTTCC, and D2, GCTGCGAAATCACCT. Forced DNA, F10, contained a 16-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: TGTCGCGACGAAAAA.

§ Q1 was associated with adventitious Na\textsuperscript{+} and K\textsuperscript{+} 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\textsuperscript{TM} 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

2 S. Neidle, FEBS J., 2009, 277, 1118 (and references therein).