THE STRUCTURE OF GUANOSINE QUADRUPLEXES AND THEIR POTENTIAL AS TARGETS IN CANCER THERAPY

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INTRODUCTION

In the presence of monovalent cations such as Na^+ and K^+, guanosine and its derivatives can assemble into tetrameric units that are held together in a planar fashion by Hoogsteen interactions where each unit participates in four hydrogen bonds as both a donor and acceptor (Fig. 1). These tetrad units can self aggregate via pi-stacking interactions to form temperature-dependent, stable multiplexes that are called guanosine quadruplexes.[1] Guanosine-rich polynucleotides can form inter and intra molecular quadruplex structures that stack onto one another in a helical fashion. Depending on such conditions as coordinating cation or ligand, concentration, and pH, quadruplexes have been reported to adopt a variety of topologies that can be composed of one, two, or four nucleotide strands.[2]

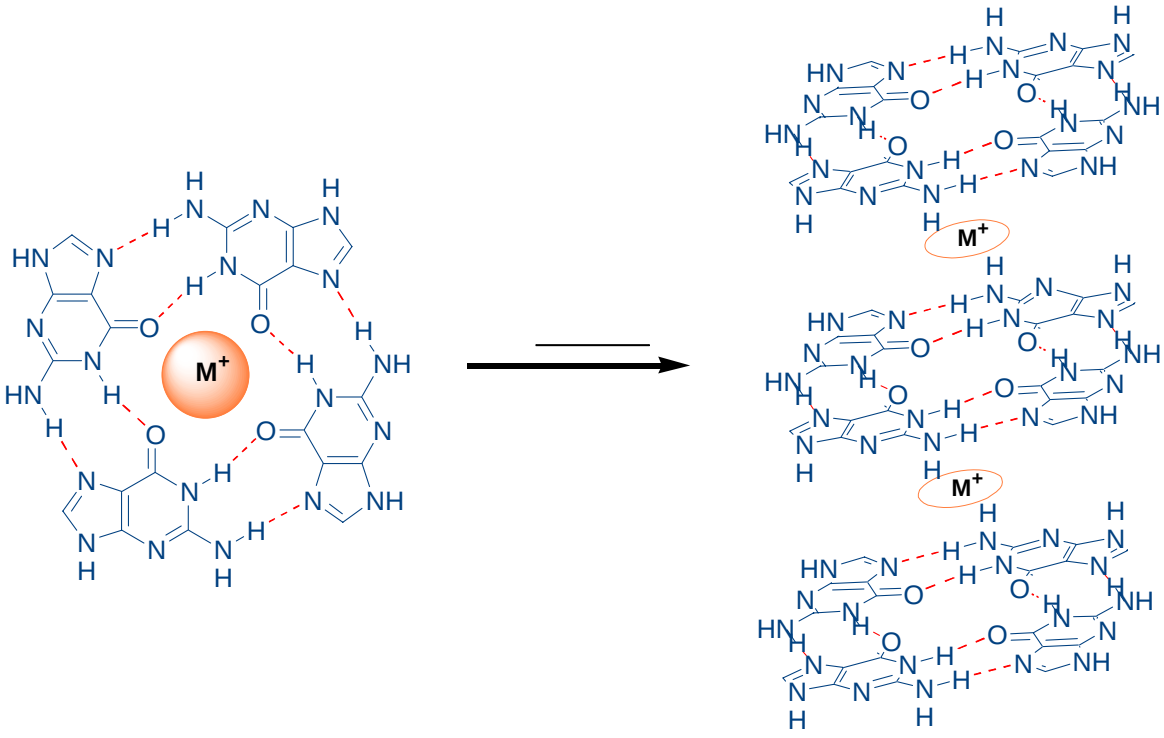

Figure 1. Hoogsteen interactions that stabilize the guanosine tetrad which can stack on itself to form the quadruplex. A cation can be positioned to stabilize quadruplex stacks.

Guanosine quadruplexes have attracted interest in the areas of supramolecular chemistry and nanotechnology, because of their potential applications for developing ion channels, wires, and

molecular switches. Because quadruplex structures form under physiological conditions, they are also considered attractive species for biological studies and intriguing pharmaceutical targets that might enable novel approaches to therapy in cancer. For example, polynucleotide processing enzymes can be prevented from binding their substrate if the primer sequences that they recognize are inaccessible due to their involvement in quadruplex secondary structure.[3] As a result, processes such as DNA replication and transcription are blocked. This form of indirect enzyme inhibition has potential in cancer therapy where cells proliferate indefinitely at extremely rapid rates and consequently are highly sensitive to the blockade of DNA replication.

BIOLOGICAL USES OF GUANOSINE QUADRUPLEXES

The lifespan of a somatic cell is dependent upon the length of its telomere. Telomeres generally consist of guanosine-rich tandem repeats that range from 5 to 50 kilobasepairs in length, with a 3' single stranded overhang 100 to 200 bases long. Between 50 to 200 basepairs are lost during each round of cell division, because DNA polymerase cannot fully replicate both strands at the ends of each chromosome. Therefore, after a certain number of cell divisions, the telomeres become depleted, and cellular DNA can no longer be replicated efficiently, resulting in programmed cell death. Tumor cells, however, evade this fate by extending their telomeres through the activity of telomerase.[4]

Telomerase is a ribonuclease enzyme that is present in stem cells and is normally turned off after birth; however, roughly 85% of all tumor cells exhibit telomerase activity in PCR assays.[5] Telomerase recognizes the 5'-TTAGGG-3' repeats in human telomeres, binds the single stranded 3' overhang, and extends the telomere in a recursive fashion. When the 3' overhang is folded into a quadruplex structure, however, telomerase is unable to bind to and act on its substrate, and in most cases dissociates from its recognition sequence.[6]

The development of drugs to induce the formation of and stabilize the quadruplex structure has been the fundamental approach for using guanosine quadruplexes in cancer therapy. Many of these molecules have exhibited encouraging activities, including telomerase inhibition, telomeric disruption, and induction of apoptosis.[7] The success of this approach of using guanosine quadruplexes as a target for cancer therapy depends, of course, on the ability of small molecules to induce and stabilize specific quadruplex structures from among varied possibilities.

VARIED STRUCTURES OF THE QUADRUPLEX

Under neutral or moderately basic conditions, oligonucleotides can form quadruplex structures that display a remarkable degree of polymorphism. The type of structure that forms depends on the number and orientation of DNA strands involved, the concentration and type of counterion present, and the nature of the intervening sequences between the guanosine columns. In order for the cyclic arrangement to be electronically favorable, a cation such as Na^+ or K^+, positioned as shown in Figure 1, must be present for coordination.

Under moderate sodium concentrations (~50 mM), the 5' end of the immunoglobulin switch region, which consists of 20–50 base pairs of GC tandem repeats, forms a four-stranded quadruplex in which all of the DNA strands are oriented symmetrically to one another (Fig. 2a). This structure is thought to participate in the pairing of homologous chromatids during meiosis.[8] The human telomere repeat sequence d(TAGGGTTAGGGT) can fold back to form hairpin structures that can then dimerize to form quadruplexes in which the dimer subunits are aligned parallel or antiparallel to one another (Fig 2b).[9]

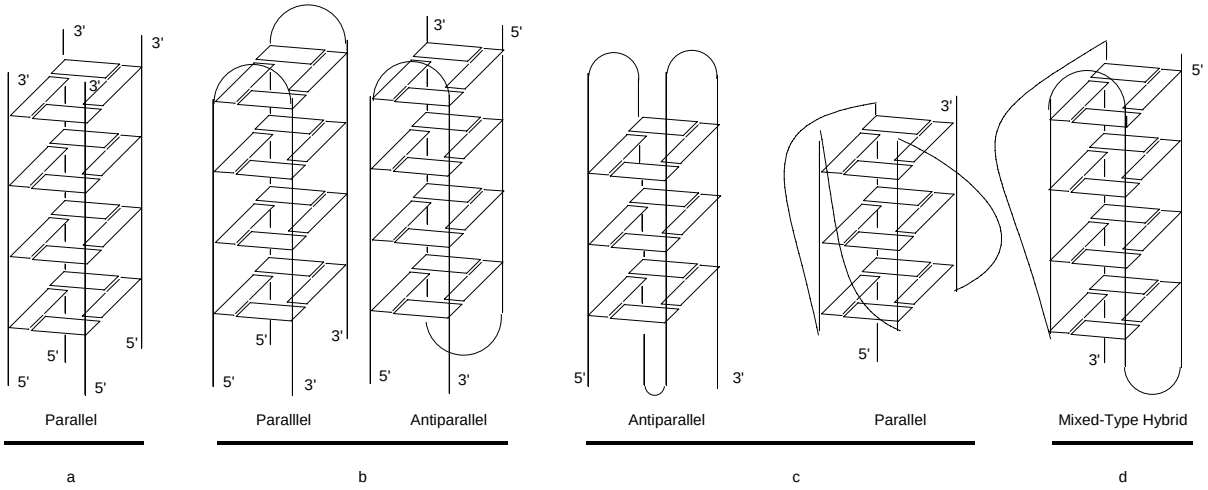

Figure 2. Structures found for telomeric DNA quadruplexes: a) four-stranded b) hairpin dimers c) intramolecularly single stranded human telomeres d) intramolecular single stranded Tetrahymena telomeres.

NMR studies reveal that both structures coexist and likely interconvert in solution. At temperatures below 50 °C single stranded telomeres fold more rapidly to form the predominating antiparallel structure, whereas at higher temperatures, the parallel form predominates.[10]

A single strand of human telomeric DNA can form a quadruplex containing three stacked tetrads, in which the intervening nucleotide sequences (composed of adenines and thymines) between

the guanines form loops. The orientation of the strands and loops varies with the counterion.[11] At K^+ concentrations similar to intracellular ionic environments (100 mM), the strands are oriented parallel to one another, with the loops protruding around the side of the structure like a propeller. In Na^+ environments, the strands are oriented antiparallel to one another and are connected by a diagonal loop (Fig. 2c). The lengths and nature of these loops can play a strong role in stabilizing or destabilizing the folded structures due to intraloop interactions such as hydrogen bonding and pi stacking.[12] Through recent studies using ^{125}I-radioprobing, the existence of a third type of intramolecular quadruplex, the mixed type hybrid, has been discovered (Fig. 2d). All three types of structures are thought to coexist in solution, with their relative proportion being determined by the nature and concentration of counterions.[13]

The alignment of the nucleotide strands and the orientation of the guanosine base with respect to the ribose sugar are interdependent. When nucleotide strands are aligned parallel to one another, then the conformation of the guanines must be the same, either *syn/syn* (Fig. 3A) or *anti/anti*. Consequently, when adjacent strands are antiparallel, their glycosidic conformations must be opposite (Fig. 3B). The structural requirements for the geometry of the glycosidic bonds play a role in the stability of the guanosine quadruplexes, as can be viewed in Fig. 3D where the 5' to 3' strand polarity is represented as going into (●) or out (+) of the plane of the paper.[14]

Quadruplexes are also diverse in their cation-binding properties. Ions and ligands may bind in a 1:1, 1:2, or 2:1 stoichiometry, resulting in different geometries that all have different characteristics. X-ray crystallography has determined that K^+ resides between the different layers of tetrads.[15] Other geometries are still being elucidated.

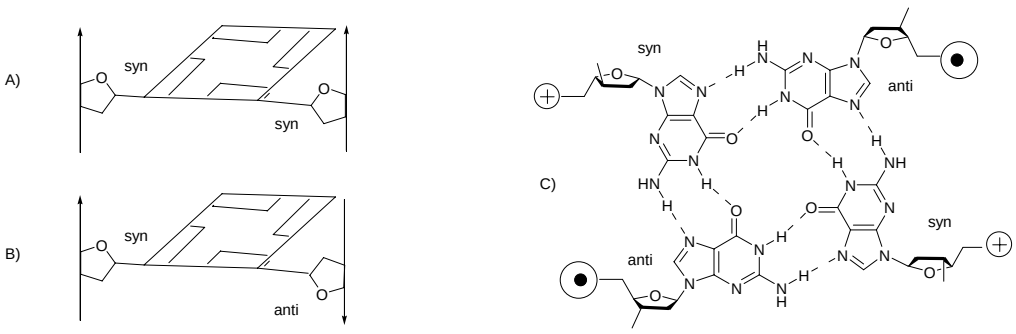

Figure 3. Diagrams of the *syn/syn* (A) and *syn/anti* (B) structural geometries. C) A structural diagram illustration of the strand alignment and glycosidic bond orientation dependence on one another.

The polymorphism of quadruplexes complicates characterization of their structure, and it presents a special challenge in using them as targets for cancer therapy. The most common approach

for using quadruplexes in cancer therapy has been to design drugs that induce and stabilize these structures. Many of these drugs interact differently with quadruplexes, depending on their specific structure.

GUANOSINE QUADRUPLEX INTERACTIVE AGENTS WITH POTENTIAL FOR THERAPEUTIC APPLICATIONS

Small molecules that stabilize guanosine quadruplexes in a manner analogous to alkali cations can inhibit telomerase by trapping the telomeres so that they are unable to function as primers. For these ligands to be effective, they must stabilize only the quadruplexes formed from 3'overhang in telomeres, and not duplex DNA, so that only telomere elongation in cancer cells is affected, not the replication of somatic cells. The Telomeric Repeat Amplification Protocol (TRAP) assay technique can be used to monitor telomerase activity with high sensitivity. TRAP uses PCR (polymerase chain reaction) to amplify telomerase-synthesized telomere repeats that can then be visualized by electrophoresis. It is also necessary to assess the activity of *Taq polymerase* through PCR to ensure that the replication of normal DNA by DNA polymerase is not inhibited. The selectivity of drugs in their binding to quadruplexes versus duplex DNA can also be analyzed by a technique called Surface Plasmon Resonance (SPR), which can detect changes in the index of refraction that occurs when a ligand binds to DNA. The signal derived from the change in refractive index is proportional to the concentration of bound ligand, which can be used to calculate the equilibrium binding constant.

Several different classes of quadruplex-stabilizing molecules have been proposed, most possessing large aromatic cores that can interact with the tetraplexes through pi stacking, and positively charged side chains that can interact with the negatively charged grooves of the quadruplex. Some examples of the more potent and promising classes are summarized below.

Perylenes (Fig. 4A) pi stack on the large surface area of the terminal tetrad, and their side chains are thought to interact with the quadruplex by intercalating into the side grooves. A number of perylene derivatives, where R= pyridine, piperidine, pyrrolidine, or morpholine with either ethyl or propyl groups connecting them to the aromatic core have been tested. Using Poly-Acrylamide Gel Electrophoresis (PAGE) assays, researchers determined that compounds that efficiently facilitated the formation of the quadruplex also inhibited the activity of telomerase. Compounds that were inefficient at facilitating the quadruplex displayed no telomerase inhibition activity, thus establishing a direct correlation between quadruplex formation and telomerase inhibition.[16]

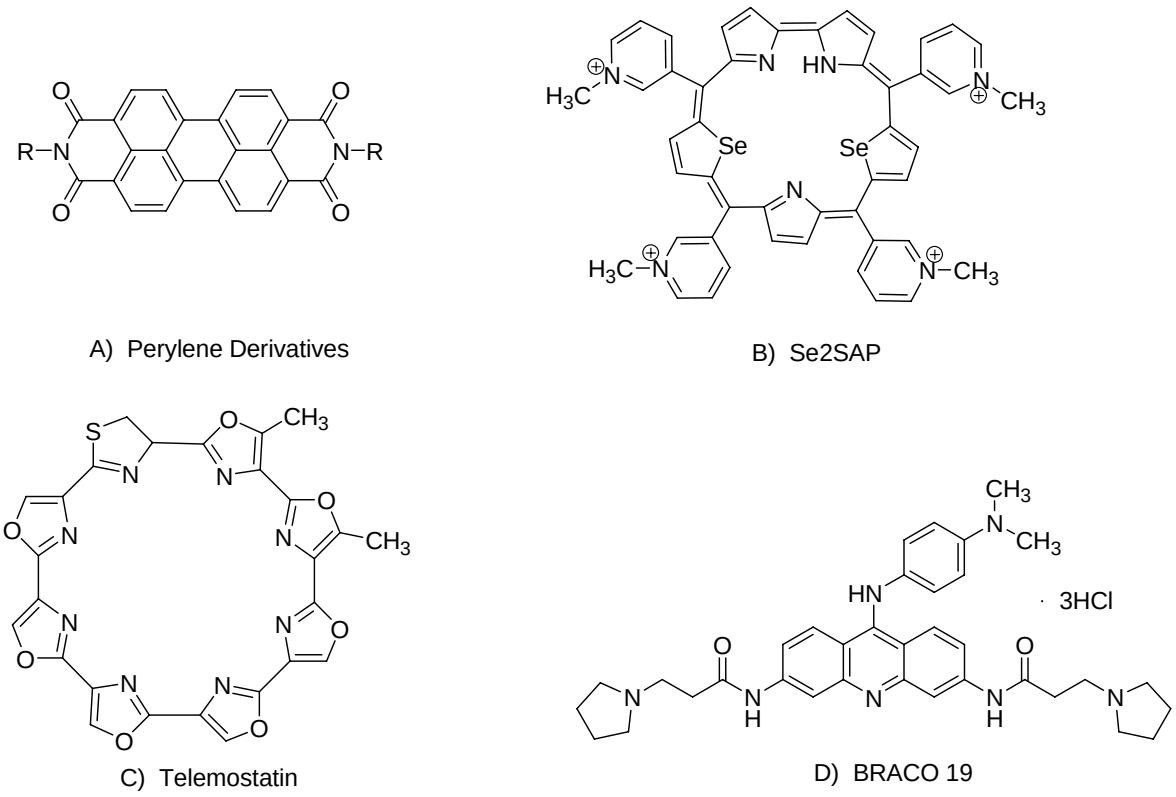

Figure 3. The chemical structures of A) perylene B) Se2SAP C) Telemostatin D) BRACO 19.

Cationic porphyrins can stabilize intramolecular and intermolecular tetraplexes through end stacking or intercalative interactions.[17] However, porphyrins can also act as photosensitizers, resulting in the cleavage of the DNA. A synthetically expanded and selenium-substituted porphyrin, deseleno Sapphyrin (Se2SAP, Figure 3B), is less toxic than the conventional porphyrin, and it is able to convert with high selectivity the preformed antiparallel intramolecular quadruplex into the mixed type hybrid in the presence of K^+, producing a CD spectra that is a mix of the distinctive parallel and antiparallel intramolecular quadruplexes. TRAP assays revealed an IC_{50} for this compound of less than 50 nM in 100 mM KCl. In 100 mM NaCl, the conditions at which the antiparallel quadruplex predominates, it is active only at the much higher concentration of 8 μM. SPR also confirmed that Se2SAP binds selectively to the quadruplex form under K^+ conditions, with a four fold higher binding constant ($K = 1.8 \times 10^7\ M^{-1}$).[18]

Telemostatin, a natural product, can drive the formation of the antiparallel intramolecular single stranded guanosine quadruplex from a random coil of human telomeric DNA. This quadruplex formation is uniquely observed even in the absence of any alkali cations, and at concentrations as low as 5 nM.[19]

BRACO 19 (Figure 3D) exhibits telomerase inhibition with an IC_{50} ~0.12 μM in cell-free assays, where concentrations greater than 10 μM are required to induce acute cytoxicity in tumor cell lines. Administration of non-toxic doses (2 mg/kg) of BRACO 19 to mice bearing advanced-stage A431 human vulval carcinoma subcutaneous xenografts and previously treated with paclitaxel showed a significant increase in antitumor effect beyond that observed with paclitaxel treatment alone.[20]

Numerous different classes of compounds that interact selectively with specific quadruplexes have been reported. Generally, their IC_{50} values are in the sub-micromolar range, this being about one hundred times less than the concentration requirement for cytoxicity. Their affinity for quadruplex DNA is about one order of magnitude greater than for duplex DNA.

CONCLUSION

Guanosine nucleotides can fold themselves into interesting secondary structures that are potentially very useful in cancer therapy. Different classes of compounds that interact selectively with specific quadruplex structures have been reported, many of which have shown encouraging results in various *in vitro* and *in vivo* bioassays. Nevertheless, to advance this field further, it will be necessary to elucidate the topology of quadruplexes greater detail, to understand how the various quadruplex forms are affected by nucleotide sequence and are stabilized by cations, and especially to determine the molecular details by which organic ligands interact with and stabilize the various quadruplex forms. A greater understanding of the structural and energetic aspects of quadruplex-stabilizing ligand interactions is needed for them to be optimized as *in vivo* biological agents and as pharmaceuticals for the treatment of cancer.

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