Meeting report: Guanosines and quadruplexes (London, September 15–17, 2010)

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Guanine

1. Introduction

The meeting entitled “Guanosines and quadruplexes”, organized by Shozeb Haider and Mateus Webba da Silva, was held at The London School of Pharmacy from 15 to 17 September 2010. It corresponded to the annual meeting of The European Cooperation in Science and Technology (COST) ACTION MP0802.

Guanosine (G) molecules show a remarkable ability to self-assemble into highly complex patterns. The most common basic motif is the G-quartet, a hydrogen-bonded array of four guanosine molecules, that is formed also in a variety of guanosine derivatives (Fig. 1). Stacking of G-quartets leads to the formation of G-quadruplexes; complex and highly ordered helical structures. The extent of stacking, and consequently the length of these supramolecular structures, can be controlled by temperature, pH value, solution concentration and by cations added to the solution. Other self-assembling motifs, like G-ribbons, were also identified in lipophilic guanosine derivatives. Switching between G-ribbon and G-quadruplex structures has been observed as a function of the solvent and cations in the solution [1].

Formation of quartets is not limited to isolated bases, nucleosides or nucleotides: DNA and RNA guanine-rich sequences may form quadruplex structures stabilized by G-quartets [2] (see Fig. 2 for an illustration of an intramolecular parallel quadruplex). G-quadruplexes can be very stable under physiological conditions and evidence for quadruplex formation in vivo is accumulating: i) quadruplex-specific antibodies provide strong evidence for quadruplex formation at ciliate telomeres [3]. ii) Radioactively labeled quadruplex ligand tends to accumulate at the termini of chromosomes [4]. However, this staining is not exclusive and these ligands may also actively induce G-quadruplex formation. iii) Genome-wide analyses have implicated G4 DNA in regulation of gene transcription and recombination [5]. G-quadruplex DNA motifs are conserved cis-regulatory elements in human and related species. iv) Altered gene expression in the Werner and Bloom syndromes or in G4 ligand treated cells correlates with the G4 forming potential of genes [6]. v) Intracellular formation of G-rich DNA induces the formation of “G-loops”, structures that contain G-quadruplexes, as evidenced by electron microscopy [7]. vi) Quadruplex ligands induce an uncapping of the telomeres. vii) A number of cellular and viral proteins bind to, induce, cleave, or unfold quadruplexes. viii) Sequences that are prone to form quadruplex are sites of genomic instability. Several helicases have been implicated in the maintenance of G-rich regions in vitro and/or in vivo. A. Nicolas (Institut Curie) showed that the Pif1 helicase is necessary for the stability of G-prone minisatellite repeats in yeast [8]. ix) G-rich oligonucleotides form quadruplexes, interact with nucleolin, and have interesting cellular and anticancer effects (a quadruplex-forming oligonucleotide AS1411 is currently in Phase II clinical trials) [9]. x) H.S. Seifert and colleagues have genetically defined a cis-acting G4-prone DNA sequence located near the antigenically variable pilin expression locus of the strict human pathogen Neisseria gonorrhoeae; this region is required for the gene conversion reactions leading to pilus antigenic and phase variation. Mutation of any one of 12 guanines blocks pilin antigenic variation supporting the hypothesis that G-quadruplex formation is functionally important [10].

This growing interest for G-quadruplexes is shown by an exponential increase in the number of publications in the field and a number of recent dedicated issues [11,12]. It was therefore an excellent idea to organize this meeting in 2010, as an appetizer for the third international conference on G-quadruplexes, to be hosted by Antonio Randazzo in Naples, Italy, in June 2011. The two previous international conferences on quadruplexes were organized by Jonathan B. Caires in 2007 [13] and 2009 [14] in Louisville, KY, USA.

The London event, a little bit more European-centered, nevertheless attracted a number of prestigious participants from all over the world, combining researchers interested in nucleoside/nucleotide self-assembly and nucleic acids structures, working in fields ranging from chemistry, biology, physics, theory to material sciences and nanotechnology. One of the strengths of this COST Action is to bring together scientists from different horizons, and this mix was also found in the meeting. The size and format of
the event allowed for interesting discussions during the 2.5 days and a memorable cruise dinner on the Thames River. Over 30 lectures and 22 posters were presented. For space consideration, we limited this report to the oral presentations and arbitrarily reorganized the talks. We apologize for failing to cover most posters: we will simply mention that three of those were awarded a price (two “Quadruplex Nucleic Acids” books [2] sponsored by the Royal Chemical Society of UK, and one “Quadruplex DNA” book sponsored by Springer). The winners were Pierre Murat (Grenoble, France) Gavin Collie (London, UK) and Tani Agarwal (Dehli, India).

2. Self-assembly

Assemblies of lipophilic guanosines and other purine derivatives were the topic of several communications.

Gian Piero Spada (Bologna University, Italy) presented data concerning the interconversion between motifs of lipophilic guanosines. Depending on metal ion content ($K^+$) one may obtain an octamer or a polymer formed by stacking of hydrogen-bonded G-quartets. In the absence of $K^+$, a G-ribbon (an infinite H-bonded motif) may occur. Supramolecular organization of modified guanosine nucleobases also depends on the nature of the solvent and may be controlled by photoisomerization [15].

Artur Ciesielki (ISIS, Strasbourg, France) presented data concerning supramolecular chemistry at the solid-liquid interface. H-bonding is of special interest for controlled assembly as it is reversible and specific. Light or other stimuli (cation chelants, pH) were used to control the conformation of these molecules, which may be visualized by scanning tunneling microscopy (STM) [16,17].

Lajos Kovacs (University of Szeged, Hungary) presented theoretical and experimental data on higher order structures based on purines (uric acid derivatives and protonated/methylated xanthines). Homo- and hetero-tetrads may be formed with these bases, as demonstrated by MS and NMR [18].

Andrew Marsh (University of Warwick, UK) showed some chimeric nucleosides that form cyclic supramolecular assemblies (“rosettes”) based on the pterin subunit. The interconversion between different forms (monomer, assembled or rosettes), induced by organic solvents or by temperature, was followed by fluorescence, solution DOSY and solid state DQ-MAS NMR.

Miha Devetak (Jozef Stefan Institute, Slovenia) analyzed Langmuir–Blodgett films of lipophilic G- and A-derivatives. A simple device was designed to successively limit surface or increase surface tension. dG or dA nucleosides with 1–3 lipophilic chains (C10) were compared. Interestingly, guanosine and adenosine behaved differently (in terms of reversibility) and profound differences were also found between derivatives with a different number of alkanoyl groups.

Alexander Kotlyar (Tel Aviv, Israel) demonstrated that Thiazole Orange interacts with long (hundreds of tetrads) monomolecular G-quadruplexes lacking core cations [19]. The intercalated highly fluorescent complex between the dye and the DNA is very stable and does not dissociate during electrophoresis or gel permeation chromatography. He also presented results on preparation and properties of discrete G4–DNA–nanoparticle conjugates that may possess interesting electrical conductive and photonic properties.

3. Modified bases and backbones

Nathan Luedtke (University of Zurich, Switzerland) demonstrated that a fluorescent analog of guanine, bearing a pyridine...
group at the 8-position, has increased fluorescent quantum yield and can serve as an efficient energy transfer acceptor when incorporated in intramolecular quadruplexes. In some cases, little or no change to the structure or stability of the G-quadruplex was observed. Using this probe together with PEG-mediated quadruplex folding, cation-dependent energy transfer reactions in three different G-quadruplex structures were discovered.

A similar position-dependent effect was found by Ramon Eritja (IRB, Barcelona, Spain) when considering bi-cycloderivatives having a North or South preferred configuration. He investigated the thrombin binding aptamer (“TBA” d-GGTGGTGTGGTTGCG) in which the sugar of one guanine is replaced by a bicyclo-hexane analog; difference in Tm could reach 26 °C for the most destabilizing case [20].

Daniela Montesarchio (University of Naples, Italy) presented results on modified d(TGGGAT) oligomers targeting HIV gp-120 [21]. The addition of large aromatic groups at the 5′-end results into an increase in stability of the tetramolecular G-quadruplex complex due to hydrophobic stabilisation and π–π stacking. Such 5′ modifications, which may be combined with the addition of monosaccharides at the 3′ extremity to improve exonuclease resistance, lead to species having an excellent activity against HIV-1 in a cell-based assay.

4. Quadruplex assays

Janez Plavec (National Institute of Chemistry, Slovenian NMR Center, Ljubljana, Slovenia) presented a detailed analysis of cation exchange within quadruplexes [22]. Even in structures in which imino protons are protected for weeks, cations may still diffuse and exchange between sites, or between the quadruplex and the solvent. Depending on the accessibility of the terminal quartet and on the position of the cation, the exchange rate may vary between 0.1 and 45 s⁻¹.

Jean-Louis Mergny (IECB, Pessac, France) discussed sequence effects for DNA but also RNA quadruplexes [23,24]. Many biologically relevant RNA sequences form extremely stable quadruplexes under near-physiological conditions [25].

Souvik Maiti (Institute of Genomics and Integrative Biology, Delhi, India) analyzed the difference in stability between duplexes and quadruplexes [26]. He found that the duplex is the thermodynamically stable species and that the quadruplex is a kinetically trapped species that happens under specialized conditions [27]. Increase in loop length favors the duplex and out competes quadruplex; on the contrary, an increase of the number of quadruplex repetitions in a putative quadruplex sequence favors the quadruplex. This duplex–quadruplex competition was also studied in the presence of silver ions for poly dG-poly dC by Alexander Kotlyar (Tel Aviv University, Israel).

5. In silico studies

A number of quadruplex-prone sequences are found in the human genome. Over 370,000 have been reported. Alan Todd (School of Pharmacy, London, UK) presented the method he developed to categorize and map the sequence space of quadruplex-prone sequences. Full results will soon be available on a dedicated web server.

Nancy Maizels (University of Washington, Seattle, USA) analyzed the local enrichment for quadruplex-prone regions around the transcription start site (TSS). While the peak upstream of the TSS could be attributed to the presence of CpG dinucleotides and transcription factor binding motifs (Sp1 for example), the G-richness downstream of the TSS is not masked by such factors and is conserved in a variety of species. In many human genes, this downstream enrichment is located at the 5′-end of the first intron, on the non-template DNA strand. A correlation was found between sites of RNA polymerase II pausing and G-richness, as shown by a study performed on 17,000 genes. Interestingly, a component of TFIH (one of several general transcription factors that make up the RNA pol II preinitiation complex) called XPB was found to bind to quadruplex DNA with nanomolar affinity. A single point mutation was sufficient to abolish binding. A microarray analysis in XPB deficient cells revealed that paused genes correspond to genes undergoing misregulation (either up or down) in the absence of XPB. This observation opens fascinating possibilities for a possible regulation of transcription pausing by quadruplexes modulated by protein recognition.

6. Quadruplex ligands

A number of researchers are interested in the development of small molecules capable of selective binding to quadruplexes. Initially developed as telomerase inhibitors [28], these molecules may exhibit a number of interesting properties, either as drugs or as probes for quadruplex formation.

Marie Paule Teulade-Fichou (Institut Curie, Orsay, France) reminded us that many duplex ligands are also quadruplex ligands. This is the case for example for thiazole orange, which was developed to target affinities of molecules in a displacement assay (FID). Fortunately, not all quadruplex ligands are duplex ligands. Since 1997, an impressive number of quadruplex ligands have been developed [29]. The most recent compounds exhibit enhanced selectivity for quadruplexes (as compared to duplexes) that opens perspectives for using small molecules as quadruplex probes in a biological context. In particular Bisquinoilinium derivatives have been shown to evidence the involvement of quadruplex structures in the genetic instability of minisatellites sequences inserted in a yeast genome [30]. However, the “Holy Grail” of selectivity (the recognition of a unique quadruplex topology) has not been reached yet, even if progresses have recently been made. Two fluorescent-based assays were presented by Jean-Louis Mergny (IECB, Pessac, France) to analyze binding to a variety of quadruplexes.

If small molecules have problems to specifically target one G-rich sequence, larger conjugates could do the trick. Sylvain Ladame (Imperial college, London, UK; formerly in ISIS, Strasbourg, France) illustrated two clever uses of PNA to recognize G-quadruplexes. Both approaches took advantage of sequences, adjacent to a G-quadruplex core motif: short complementary PNA may hybridize on both sides, and this may be used to reveal the presence of a target quadruplex [31] or specifically target it. G-quadruplex recognition is followed by fluorescence exaltation either induced by the specifically labeled nearby PNA sequences or by direct interaction of a ligand with the structure. Appropriate controls demonstrate the gain in selectivity provided by the PNA “side chains”.

Ilse G.J. Manet (ISOF, Bologna, Italy) demonstrated that a porphyrazine derivative binds to human telomeric quadruplexes with a dissociation constant in the μM range and induces a parallel G4 conformation in the complex displaying a melting temperature above 85 °C. This molecule has potentials both as G4 stabilizer and as singlet oxygen photosensitizer.

Ramon Vilar (Imperial College, London, UK) presented monomodal and di-metallic metal complexes to target the c-myc promoter. The terpyridine motif provides a very flexible scaffold to generate families of ligands. The geometry of the complex may be tuned by the nature of metal: Pt (square planar), Cu (square based pyramidal) and Zn (distorted trigonal pyramidal) complexes were investigated. Some cyclometallated platinum complexes display fluorescent properties and could be interesting DNA optical probes.
It was shown that the presence of a second metal increased considerably the affinity of the system towards quadruplex DNA.

Nathan Luedtke (University of Zurich, Switzerland) presented results obtained on a series of phthalocyanines. Impressive Kds in the 1–100 nM range were found [32]. An excellent specificity for quadruplexes was demonstrated, as shown by much weaker binding to tRNA and calf thymus DNA. Such compounds can readily enter cells, specifically accumulate in the nucleus, and can cause changes in c-Myc and RAS expression [32,33]. Promising in vitro activities were observed in a mouse model for metastatic melanoma.

Mark Searle (University of Nottingham, UK) presented results obtained with ligands targeting a quadruplex sequence found in the 5’UTR of the human estrogen receptor [34]. The transcribed G-rich RNA motif was found to form a more stable quadruplex than the corresponding DNA. Novel telomestatin analogs were also presented. These acyclic compounds are not completely an intrinsic CD spectrum. Interestingly, one may observe cation-mediated binding, as revealed by electrospray mass spectrometry.

Two small molecule ligands were also presented which display selectivity towards parallel and antiparallel G-quadruplex structures [35].

Stephen Neidle (School of Pharmacy, London, UK) provided an overview of the quadruplex ligands for which in vivo results are currently available. He also reported on the use of structure-based design in the development of a naphthalene diimide derivative which gave a 50% reduction in tumour volume (MiaPaCa-2 xenografts) at 3 mg/kg every other day. Another study used the Bracco-19 (a reference quadruplex ligand) co-crystal structure with a human telomeric quadruplex as the basis for the rational design of non-polycyclic molecular mimetics.

7. Structural studies

Peter Varnai (University of Sussex, Brighton, UK) presented in silico thermodynamic and kinetic analysis of mechanical unfolding of a G-quadruplex sequence. The insulin linked polymorphic region (ILPR) was chosen, using two repeats of the ACAGGGGTGTGGGGG motif.

Gary Parkinson (School of Pharmacy, London, UK) presented an overview of the crystal structures for quadruplex ligands. Strikingly, all five structures currently available (with four different molecules) have been obtained with a parallel quadruplex. While the core structure is unmodified, the loops may be rearranged in the presence of quadruplex ligands. The unpublished structure of the complex between a G4 ligand (FC4ND-01) and an intramolecular DNA quadruplex (d-TAGGGTTAGGGTTAGGG) was also presented. This structure shows a few end-stackings and a loop stacking, highlights the potential role of the connecting loops in ligand binding to G-quadruplex structures.

Dinshaw Patel (MSKCC, NY, USA) masterfully demonstrated that quadruplex structures are still full of surprises. He presented three new quadruplex motifs, one corresponding to an RNA aptamer–peptide complex, the others to the DNA motif found in the 5’ intron of the Chl1:FancJ helicase gene and the ckit2 promoter. In several cases, guanines involved in tetrad formation may originate from isolated guanines in the primary sequence. This observation reminds us that the sequence repertoire of quadruplex-prone sequences does not only include sequential segments of guanines. He also presented the effect of the cation concentration on the determination of the G-quadruplex structure type.

8. Methods

Different new methods have been presented to investigate G-quadruplex formation. Viktor Viglasky (Safarik University, Kosice, Slovakia) demonstrated that Temperature Gradient Gel Electrophoresis (TGGE) may reveal the existence of several distinct quadruplex conformations melting at different temperatures. The method can be applied generally for variously folded G-quadruplexes [36]. Laura Tugulea (University of Bucharest, Romania) presented results obtained on poly dG and d-G10 with attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT-IR).

Gian Piero Spada (Bologna University, Italy) demonstrated that strand orientation (parallel or antiparallel) is not the most appropriate parameter to describe circular dichroism (CD) quadruplex signatures. What matters is the relative disposition of the quartets. Although often correlated (in most parallel quadruplexes all guanines are anti) the two parameters (strand orientation and quartet stacking) may not coincide, as one may design a parallel quadruplex incorporating modified bases with a CD spectra with an “antiparallel” character. Such shape with a positive maximum around 295 nm reflects quartets stacked in a head-to-head orientation [37].

Dipankar Sen (Simon Fraser University, Canada) presented new catalysts and electronic switches made from G-quadruplexes. Interestingly, one can find “natural” quadruplex ligands within a cell, such as Heme. The strong peroxidase activity found in many quadruplex–heme complexes has found a number of analytical applications. But this G4–heme complex may also catalyze other oxidative reactions. These are being actively investigated.

9. Towards nanotechnological applications

Concerning the surface assembly of G-rich DNA oligonucleotides into long G-wires, Thomas Marsh (University of Saint Thomas, Saint Paul, MN, USA) presented results obtained on more than 30 different sequences obeying the general formula XGnTmGnY (with X & Y: non G; n = 3–5 and m = 0–4). General rules start to emerge concerning the G-wire potential of these motifs that can be strongly influenced by cations.

Danny Porath (Jerusalem, Israel) presented recent advances on the measurements of the electrical properties of single DNA molecules. While charge can be transported along short or single DNA polymers, no charge transport can be supported in long DNA wires due to structural defects. Electronic properties of long G-wires were also investigated.

Jussi Toppari (University of Jyväskylä, Finland) trapped DNA origamis between nanoscale electrodes in order to measure the electrical conductivity of DNA structures by AC impedance spectroscopy [38,39] (Fig. 3). Under dry conditions they behaved as total insulators. However, this behavior greatly depends on the amount of water molecules attached to the DNA origami structure.

Wolfgang Fritzsche (Institute of Photonic Technology, Jena, Germany) described functionalized silver and gold nanoparticles. Their optical properties may be altered by functionalization, analyte capture and forming networks with other nanoparticles. DNA-nanoparticles were specifically targeted to specific sites on metaphase chromosomes [40].

Silvia Armini (IMEC, Leuven, Belgium) proposed self-assembled monolayers as one of the most relevant and reliable functionalization approaches to achieve selectivity in the bio- and chemosensing applications.

10. Final word

Overall, this congress offered an excellent opportunity to discover the most recent developments in the field. Many presentations involved exciting unpublished data and we are looking forward to new discoveries: a century after the report by I. Bang
in 1910 that guanine form gels under certain conditions [41], we are still fascinated by the seemingly endless possibilities offered by this base. No doubt the 3rd International Quadruplex Meeting in Naples in 2011 will reveal new surprises!

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