Anticancer Agents

Deutsche Ausgabe: DOI: 10.1002/ange.201511999
Internationale Ausgabe: DOI: 10.1002/anie.201511999

Determinants for Tight and Selective Binding of a Medicinal Dicarbene Gold(I) Complex to a Telomeric DNA G-Quadruplex: a Joint ESI MS and XRD Investigation

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Abstract: *The dicarbene gold(I) complex [Au(9-methylcaffein-8-ylidene)$_2$]BF_4 is an exceptional organometallic compound of profound interest as a prospective anticancer agent. This gold(I) complex was previously reported to be highly cytotoxic toward various cancer cell lines in vitro and behaves as a selective G-quadruplex stabilizer. Interactions of the gold complex with various telomeric DNA models have been analyzed by a combined ESI MS and X-ray diffraction (XRD) approach. ESI MS measurements confirmed formation of stable adducts between the intact gold(I) complex and Tel 23 DNA sequence. The crystal structure of the adduct formed between [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ and Tel 23 DNA G-quadruplex was solved. Tel 23 maintains a characteristic propeller conformation while binding three gold(I) dicarbene moieties at two distinct sites. Stacking interactions appear to drive noncovalent binding of the gold(I) complex. The structural basis for tight gold(I) complex/G-quadruplex recognition and its selectivity are described.*

Gold compounds are a new class of inorganic medicinal agents of great interest for cancer treatment because they present innovative modes of action with respect to the classical platinum chemotherapeutic compounds.[1–3] In this regard, the established antiarthritic gold drug auranofin was recently repurposed as an anticancer agent candidate and is currently undergoing evaluation in three distinct US clinical trials.[4] Other gold(I) agents, and specifically a number of monocarbene and dicarbene gold(I) complexes, seem promising as they manifest relevant cytotoxic properties while being acceptably stable under physiological conditions.[5–8] This behavior might result in a higher selectivity for cancer cells and a lower systemic toxicity compared to other cytotoxic gold drugs, which typically manifest more pronounced and general reactivity with biomolecules.

The modes of action of cytotoxic gold(I) compounds are still a matter of intense debate. However, there is now quite a wide consensus on the concept that their behavior diverges profoundly from that of cisplatin and analogues, mainly grounded on a documented poor reactivity with double-helix DNA. On the other hand, there is good evidence that they often produce severe mitochondrial damage.[1] As an example, several cytotoxic gold(I) *N*-heterocyclic carbene (NHC) complexes are potent inhibitors of the mitochondrial selenoenzyme thioredoxin reductase, which is involved in the maintenance of the intracellular redox balance.[7,9,10] Nonetheless, alternative modes of action beyond mitochondrial insult have been proposed to explain their cytotoxicity within a probable multi-target scenario (these include proteasome inhibition and antitelomerase activity).[2] The latter hypothesis (that is, a pronounced antitelomerase activity) emerges distinctly from previous studies on auranofin.[11] Small molecules that are able to bind and block telomerase generally manifest remarkable anticancer properties. Moreover, G-quadruplex binding properties correlate well with antitelomerase activity.[12]

G-quadruplexes (also known as G4) are nucleic acid sequences, rich in guanines, capable of forming a characteristic four-stranded fold.[13] Four guanine bases may associate through Hoogsteen hydrogen bonding to produce a square planar motif, the so-called "guanine tetrad"; two or more guanine tetrads can stack on top of each other to form a G-quadruplex. The quadruplex structure is further stabilized by the presence of monovalent cations, which lie in the central channel between each pair of tetrads.[14]

Human telomeric DNA consists of tandem repeats of the hexameric sequence d(TTAGGG), located at the 3′-ends of chromosomes; the quadruplexes that arise from these repeats are described in detail thanks to NMR and X-ray diffraction structure determinations.[15–18] Interestingly, formation of quadruplexes causes a net decrease in the activity of the enzyme telomerase, which is responsible for maintaining the length of telomeres.[19] Therefore, molecules that template the formation or stabilize the structure of G-quadruplex DNA might lead to development of new effective anticancer drugs based on selective telomerase inhibition.[20–23]

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Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201511999.

While the majority of quadruplex DNA binders reported to date contain planar organic heteroaromatic systems, a variety of metal complexes (mostly bearing extended planar motifs) were also shown to exhibit large affinities for DNA G-quadruplexes.[12,24,25] An extensive overview of this topic is provided in two recent review articles, where relevant examples are illustrated.[26,27] However, structural information on adducts formed between metal complexes and G4-DNA is limited and would be of great help to develop selective G4 stabilizers. To date, crystal structures have only been reported for adducts of copper(II) and nickel(II) salphen complexes with human telomeric DNA.[28] Additionally, information on the interactions of gold compounds with G4-DNA is extremely scarce.[29,30] The above arguments prompted us to explore, at a molecular level, the interactions taking place between an organometallic gold(I) NHC compound and a representative DNA G-quadruplex (Tel 23 sequence (5′-TAGGG(TTAGGG)$_3$-3′)) by a combined ESI MS and X-ray diffraction investigative strategy. Based on recent work,[29] we selected the bis-carbene cationic complex ([Au(9-methylcaffein-8-ylidene)$_2$]$^+$; Figure 1) for this study, which was earlier reported to be a selective cytotoxic agent in cancer cells. This compound is able to bind quite strongly to a few representative G4-DNAs (more selectively than DNA duplexes), as well as 3- and 4-way DNA junctions.[30] Interactions were mainly characterized by spectrofluorimetric methods but thus far no structural data has been obtained for these systems.

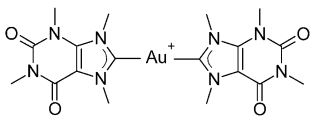

Figure 1. The [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ cation investigated.

Notably, the chemical features of the dicarbene gold(I) complex fulfill the basic requirements of an ideal G4 ligand: 1) it is planar; 2) it is positively charged and thus prone to electrostatic interactions with negatively charged DNA; and 3) it has two aromatic caffeine ligands (namely, a guanine analogue) that can associate to guanine moieties in G4-DNA through π-stacking interactions.

Currently, mass spectrometry methods offer a very powerful and straightforward tool for molecular level characterization of metal binding to G-quadruplexes; the suitability of ESI MS for these systems was recently highlighted.[31] The ESI MS spectrum of a solution containing both [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ and the Tel 23 sequence shows three main peaks, which are assigned to Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adducts of various stoichiometries (1:1, 2:1, and 3:1; Figure 2). It is inferred that [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ binds Tel 23 as an intact cation, at various molar ratios (up to a maximum of 3:1). The ammonium ion, which promotes G-quadruplex assembly (see Supporting Information), is also accommodated inside the structure (ESI MS spectrum; Supporting Information, Table S1), confirming maintenance of the quadruplex structure.

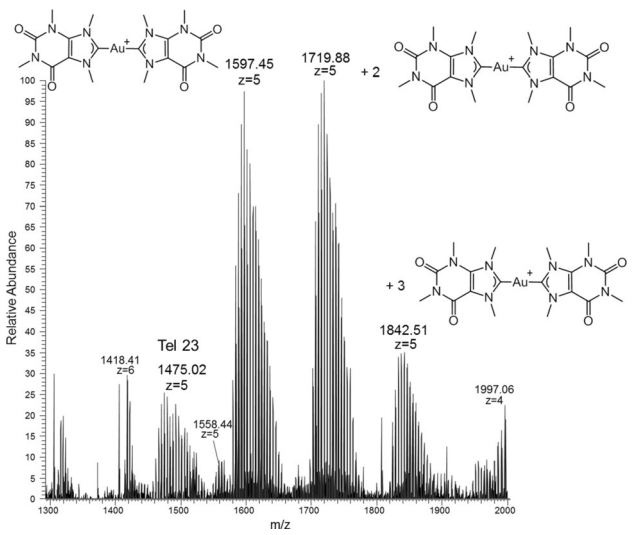

Figure 2. Section of the ESI MS spectrum of Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]BF_4 (25 μM in water in the presence of 60% EtOH, 3:1 metal complex/G4 molar ratio).

Subsequently, crystals of the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct were grown and analyzed by X-ray diffraction (orthorhombic system, space group $I2_12_12_1$).[32] The overall organization of the adduct in the crystal (Figure 3) reveals that the quadruplex adopts a parallel-stranded arrangement. TTA loops are better described using type-9 topology (Supporting Information, Tables S3 and S4), on the basis of the classification made by Collie and co-workers.[18] Parallel topology results in accessible external 5′ and 3′ planar G-tetrad surfaces for ligand stacking. The quadruplex units, which are symmetry related by two-fold rotation axes, repeat themselves in the crystal, and in so doing determine column growth in the [010] direction. Along these columns, [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ions are found at each interface between the adjacent two-fold symmetry related quadruplexes, and are stacked on their external tetrads about 3.4 Å apart. Notably, the present structure indicates that [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ binding is possible at the 3′-3′ (3′end-3′end) site, as well as at the 5′-5′ (5′end-5′end) site. Interestingly, these sites differ in the relative arrangement of the two tetrads. They are almost perfectly coplanar in the 5′-5′ site, and tilted with respect to each other in the 3′-3′ site, where G17 and G23 form a dihedral angle of about 38° (Figure 3c). Moreover, the two binding sites also differ in the overall number of hosted metal complexes: either one or two [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ions are located in the 3′-3′ and 5′-5′ sites, respectively (Figure 3a,c). It is worth mentioning that the number of complexes directly stacked on each single quadruplex unit is the same as that found in solution by ESI mass spectrometry measurements, thereby reinforcing the hypothesis that a maximal 3:1 gold/quadruplex binding stoichiometry is attained. At each binding site the stacked [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ions adopt a planar arrangement, with the linear geometry that is commonly expected from a two-coordinate Au^I complex. Overall, the structure found for each metal complex is quite similar to that previously obtained in the [Au(9-methylcaffein-8-ylidene)$_2$]-

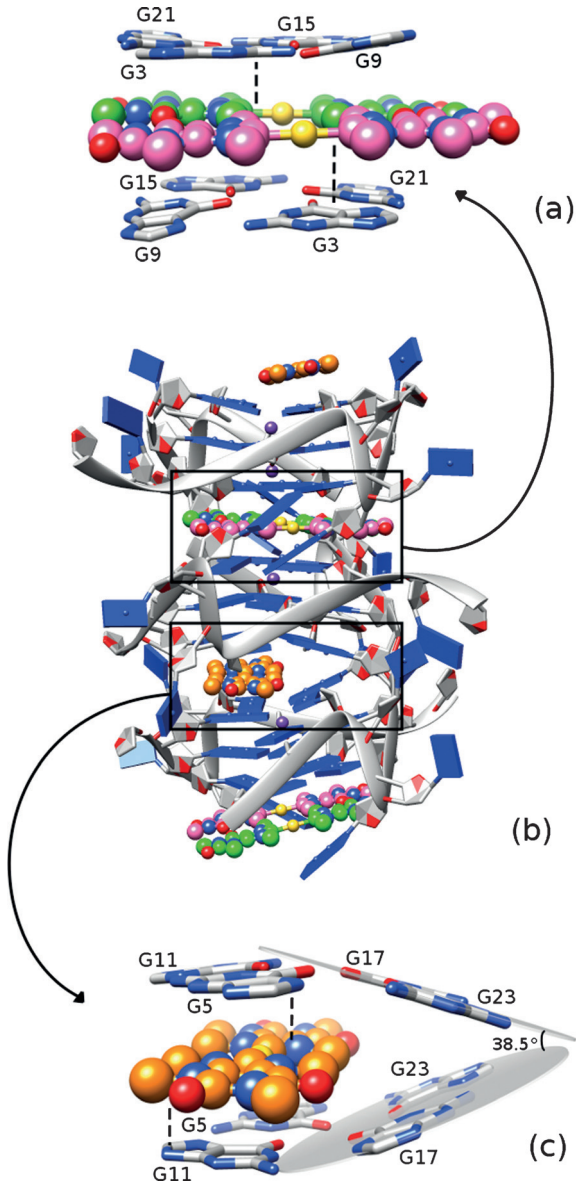

Figure 3. a) 5′-5′ End binding site of the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adducts; b) columnar disposition of the propeller Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adducts featuring the overall crystal packing; c) 3′-3′ end binding site of the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adducts. Dihedral angle formed by symmetry related G17 and G23 residues indicated. Interplanar distances (dashed lines) are ca. 3.4 Å. Different colors indicate symmetry independent complex molecules.

[BF_4] crystal structure.[29] Interestingly, caffeine ligands bound to Au^I ions are less prone to ligand exchange reactions than other ligands (chlorido and *N*-donor ligands for example), therefore preventing the Au^I center from establishing a direct coordination bond with G4 nucleobases.

The two-fold symmetry featured in the binding sites also applies to [Au(9-methylcaffein-8-ylidene)$_2$]$^+$. In the 3′-3′ end site the metal ion lies perfectly on the crystallographic axis while the caffeine units are disordered and shared between two-fold symmetry related positions (Supporting Information, Figure S2). In the 5′-5′ site the metal centers of both stacked [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ions are placed

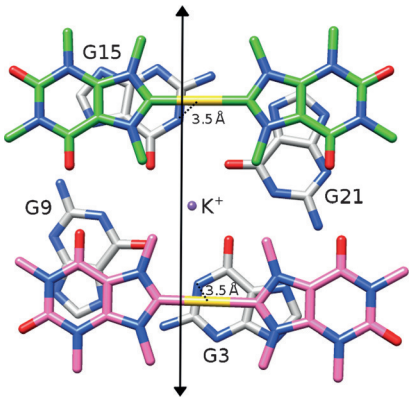

Figure 4. Gold(I) complex structural features in the 5′-5′ binding site.

near, but not above, the two-fold axis (Figure 4). This kind of two-fold pseudo-symmetric disorder, which manifests irrespective of the nature of the interacting ligand, is rather common and has been found in several crystal structures of human telomeric quadruplex/ligand adducts reported to date.[33–36] This observation supports the view that binding processes are mainly driven by non-directional forces, such as electrostatic, van der Waals and π–π stacking interactions, which require broad contact surfaces.

Nevertheless, as shown in Figure 4 and Figure S2 (Supporting Information), only one caffeine unit of two that are potentially available to each metal complex interacts with the guanine residues. The remaining unit protrudes and gives little or almost no overlap with the tetrad. The [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ion is longer than the side of the tetrad, but can provide good dimensional matching with the tetrad diagonal (about 16 Å for both the metal complex and the tetrad diagonal, versus 11 Å for the tetrad side). In the 3′-3′ end site, the 38.5° dihedral angle formed by symmetry related G17 and G23 residues (Figure 3c) prevents diagonal positioning of the [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ ion (Figure 3c). However, in the 5′-5′ end site the tetrad is completely available. Presumably partial coordination of two metal complexes provides greater stabilization than the stacking of two caffeine molecules belonging to only one metal compound (that possibly positioned diagonally with respect to the guanine tetrad). In this respect, it should be noted that all the gold(I) centers in the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct are located far from the central channel defined by the guanine carbonyl oxygen atoms. Instead, they are consistently sandwiched between two pyrimidine rings, one from each symmetric guanine tetrad, and about 3.5 Å from a nitrogen (Figure 4) or carbon atom (Supporting Information, Figure S2). Thus, cation–π contacts are deemed to be involved in and contribute to the overall stability of the adduct, though additional studies are needed to elucidate the real nature of these gold–guanine interactions. This feature highlights a significant departure from the salphen metal complexes previously reported by Campbell et al.,[28] where metal centers are placed almost in line with central ion channels, and lack specific contact with the quadruplex unit.

Notably, the symmetric binding sites found in the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct were not formed

by adjacent quadruplex units along the same telomeric polymer. Nevertheless, the actual structure for human telomeric DNA in vivo is still unknown, and different kinds of higher-order arrangements (afforded by non-consecutive G-quadruplexes belonging to long telomeric sequences) should be taken into account, as they may still produce the observed adducts.

In conclusion, we have described the adduct formed between a human telomeric DNA G-quadruplex and a cationic gold(I) dicarbene complex using a joint ESI MS and XRD investigation. In agreement with ESI MS results, XRD data indicate that three gold(I) complexes bind the G-quadruplex at two distinct sites. Details of the contacts between the metal complex and DNA G-quadruplex were carefully analyzed to dissect the molecular basis of such a tight interaction. The greater selectivity of [Au(9-methylcaffein-8-ylidene)$_2$]$^+$ for DNA G-quadruplex structures over double-helix DNA is tentatively explained as follows. The double-helix base pair, which is dimensionally similar to a G4 tetrad side, is shorter than the elongated size of our gold(I) complex. In ds-DNA sugar-phosphate backbones limit the space available to a parallel intercalating ligand. By comparison, in the Tel 23/[Au(9-methylcaffein-8-ylidene)$_2$]$^+$ adduct only one caffeine unit per ligand molecule interacts with the guanine residues. The second caffeine unit protrudes outside the tetrad surface, which is not hindered by lateral or diagonal loops in the all-parallel folding. The reported results shed further light on the possible mechanisms of anticancer activity of [Au(9-methylcaffein-8-ylidene)$_2$]$^+$, for which G4-DNA seems to be a relevant pharmacological target and inhibition of the enzyme telomerase a realistic mode of action.

Acknowledgements

We gratefully acknowledge ECRF-Ente Cassa di Risparmio di Firenze for a grant to F.P. Beneficentia Stiftung (Vaduz, Liechtenstein), COST Action CM1105 and AIRC16049 are also acknowledged for generous financial support.

Keywords: cancer · ESI mass spectrometry · gold · G-quadruplexes · X-ray diffraction

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 4256–4259
Angew. Chem. **2016**, *128*, 4328–4331

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Received: December 29, 2015
Published online: March 1, 2016

