G-Quadruplex Binding of an enantiomeric Osmium Polypyridyl Probe Revealed by Time-Resolved Infrared and NMR Solution Studies

Contact susan.quinn@ucd.ie

M. Stitch, S. J. Quinn
School of Chemistry, University College Dublin, Dublin 4, Ireland

M. Towrie
Central Laser Facility STFC, RAL, Harwell Campus, Didcot, OX11 0FA, Oxon, UK

K. Peterková, P. Podbevšek, J. Plavec
Slovenian NMR Center, National Institute of Chemistry, Hajdrihova 19, 1000, Ljubljana, Slovenia

R. Z. Boota, P. A. Scattergood, P. I. P. Elliott
Department of Chemical Sciences, University of Huddersfield, Huddersfield, HD1 3DH UK

Introduction

G-quadruplexes (**G4**) have emerged as prominent targets in cancer research[1,2] Therefore, understanding how new molecular probes bind to **G4**'s in solution is critical to the development of diagnostic and therapeutic agents.[1,2] This binding may be impacted by the particular type the **G4** structure, which may adopt different folded topology based on the arrangement of the loops around the stacked Hoogsteen bound guanine tetrads, see Figure 1. This study probes the binding of the Λ enantiomer of a NIR emitting $[Os(TAP)_2(dppz)]^{2+}$ complex (**1**) to two **G4** conformations formed by the cMYC and human telomer (hTel) sequences in solution. The combination of time-resolved infrared (TRIR) and NMR solution reveals the binding interactions within the physiologically relevant **G4** structures.

Results and Discussion

The G4 systems considered for this study were the parallel-type formed by a sequence derived from the **cMYC** promoter, forming a parallel **G4** conformation containing propeller loops and hybrid-1 type **hTel**, containing G-tetrads connected by one propeller and two edgewise loops, with these different loop arrangements resulting in the possibility of different binding interactions (Figure 1). It is expected that these complexes would have similar binding modes to the **G4** structures, with the extended dppz forming favourable stacking interactions with the G-tetrad core of the quadruplexes.

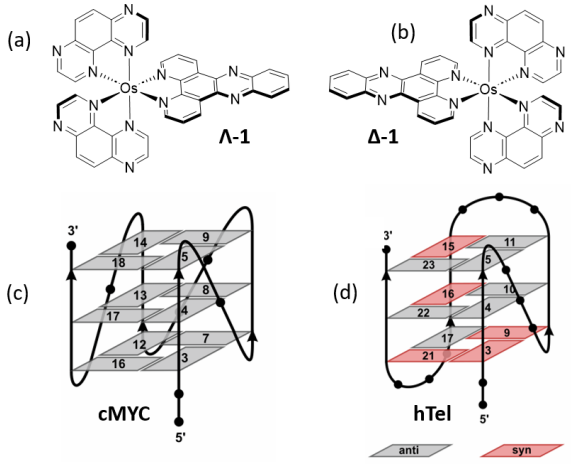

Figure 1: Molecular structures of probe **1** and schematic representation of (a) **cMYC** and (b) **hTel G4** structures.

Comparison of TRIR characterisation with hTel systems

Time resolved Infrared (TRIR) provides a detailed picture of the excited state dynamics of the probe on an ultrafast timescale,[3,4] whilst also reporting on the DNA bases (region 1600-1800 cm^{-1}) impacted by close proximity of the probe in its excited state, which has been termed the "site-effect". This effect has the advantage of exclusively monitoring the nucleobases in the binding site.

The TRIR spectra of the complex *rac*-**1** in D_2O (1300-1800 cm^{-1}) was recorded after 400 nm laser excitation (Figure 2a). There is a strong transient at 1465 cm^{-1} accompanied by bleaches associated with the ground state vibrations of the polypyridyl ligands at 1496 cm^{-1}, 1529 cm^{-1}, 1581 cm^{-1} and 1620 cm^{-1}. These bleach bands correspond to the depletion of the ground-state of the complex, notably, there are no vibrations in the "DNA region" (above 1622 cm^{-1}). This spectrum closely resembles that of the isostructural and extensively studied $[Ru(TAP)_2(dppz)]^{2+}$ complex.[5]

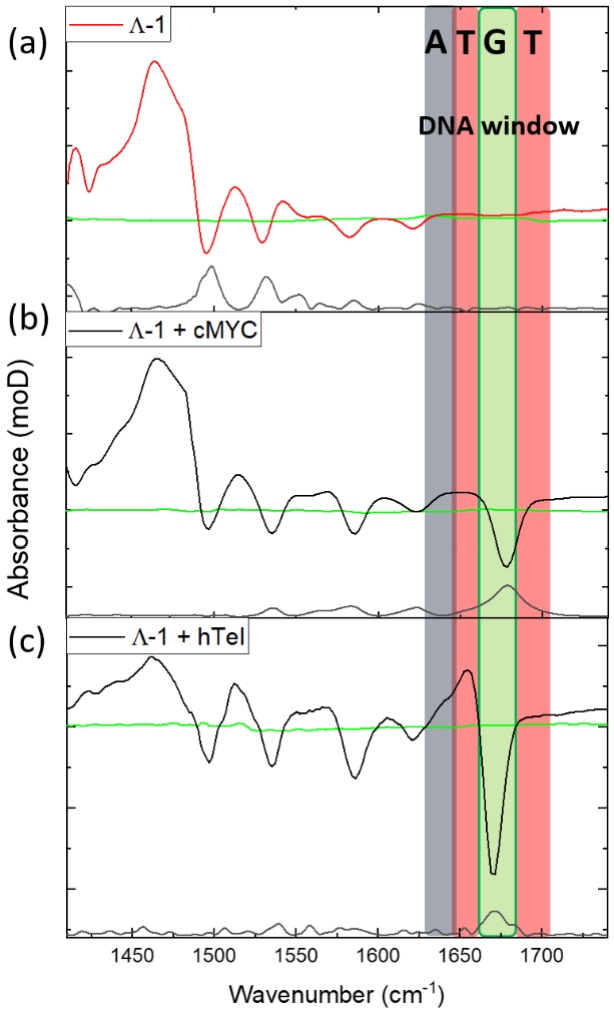

Figure 2: TRIR difference spectra recorded at 50 ps of (a) 0.4 mM of **1** and (b) in the presence of 0.4 mM of **cMYC,** (c) in the presence of 0.4 mM of **hTel** in 25 mM K-phosphate and 70 mM KCl, pH 7, 50 ps after ex l_{ex}= 400 nm, 2 kHz,150 fs).

When the TRIR measurement was taken for Λ-**1** in the presence of one equivalent of **cMYC G4** (Figure 2b) a new bleach band at 1675 cm^{-1} was observed. This strong bleach is associated with the guanine (G) carbonyl vibrations in the binding site and reflects a close interaction with Λ-**1** with one of the G-tetrads. Interestingly, there are no accompanying bleaches associated with the thymine or adenine nucleobases. This result indicated that binding was exclusively through guanine interactions.

Λ-**1** was further studied in the presence of the hybrid-1 **hTel** quadruplex forming sequence. The TRIR spectra recorded for Λ-**1** in the presence of **hTel** also reveals a strong bleach band associated with the G carbonyl, which is slightly shifted to 1668 cm^{-1} and a new transient band at 1650 cm^{-1}, which overlaps with the G vibrational bleach band (Figure 2c). The new transient is tentatively assigned to a perturbation of the thymine carbonyl ν(C4=O4) and G vibrations, which results in a shift in the ground state absorption. The TRIR spectra demonstrates a fully reversible process, with no permanent vibrational bands present after the initial decay processes or changes in the UV-vis and ground state FTIR after excitation, suggesting that no additional photodamage pathways are occurring in these measurements.

To further investigate this a complementary solution NMR titration study was undertaken. The NMR titration of Λ-**1** with 0.5 molar equivalents of **cMYC**, resulted in line broadening and upfield shifting of the methyl resonance located on the thymine nucleobase (T1) and Hoogsteen hydrogen bonded imino protons located on the G-tetrads (Figure 3a). Specifically, the imino resonances of G3, G7, G12 and to a lesser extent G8 broadened and shifted, as the [**G4**]:[Complex] ratio increased to 1:2. There were only minor chemical shift changes and line-broadening for the other residues. The results suggest that the main binding interaction site of Λ-**1** is with the 5'- guanine tetrad end of the **cMYC** structure (highlighted in red in Figure 3b). This is in excellent agreement with the TRIR results and can be used to rationalise the NIR emission light switch behaviour for Λ-**1** in the presence of increasing concentration of **cMYC** (Figure 3c).

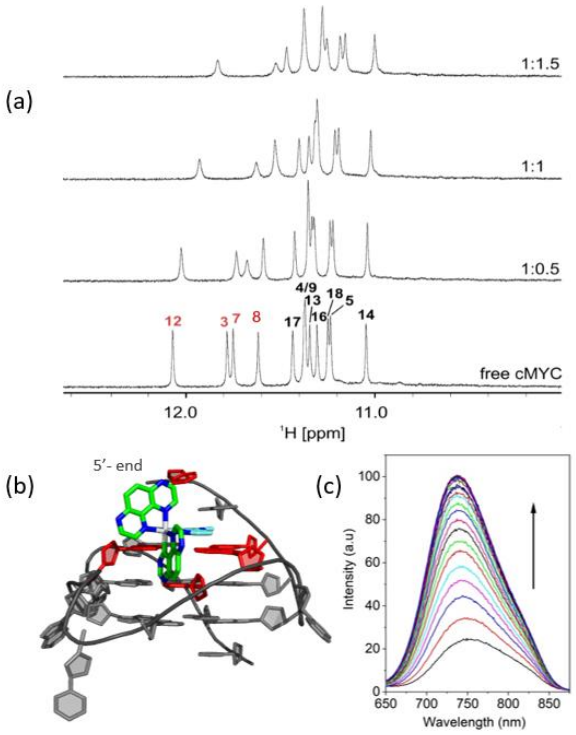

Figure 3: (a) ^{1}H NMR spectra of the **cMYC**, with increasing [Λ-**1**] molar equivalents with 70 mM KCl, 25 mM K-phosphate buffer, pH 7, 298 K, in 90% H_2O and 10% D_2O. (v) Modelling of Λ-**1** with the **cMYC** structure (PDB ID: 2LBY), nucleotides interaction highlighted in red. (c) Change in the emission of Λ-**1** in the presence of increasing [**cMYC**].

The ^{1}H NMR spectrum at a [**hTel**]:[Λ-**1**] ratio of 1:0.5, resulted in all G-tetrad imino resonances of the **hTel** quadruplex, except G10 and G11, broadening substantially.[6] This broadening of the majority of the G-tetrad imino resonances suggests Λ-**1** has a nonspecific binding interaction to the **hTel** sequence with multiple potential binding sites.[6] The reverse titration with Λ-**1** also induced selective broadening and chemical shifting of the dppz proton resonances, confirming that Λ-**1** interacts via dppz stacking interactions with the G-tetrad core of the **hTel G4**.

Summary

This study highlights the need to use a variety of spectroscopic techniques to fully understand the binding interaction of a complex to **G4** structures.[6] NMR and TRIR studies were used to gain insight into G4 binding of an enantiopure osmium NIR emitting probe in solution. Future work will consider the ability to exploit the structure of the osmium polypyridyl complexes to selectively target G4 systems over double-stranded DNA.[7]

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