A redox-activated G-quadruplex DNA binder based on a platinum(IV)-salphen complex

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Abstract

There has been increasing interest in the development of small molecules that can selectively bind to G-quadruplex DNA structures. The latter have been associated to a number of key biological processes and therefore are proposed to be potential targets for drug development. In this paper we report the first example of a reduction-activated G-quadruplex DNA binder. We show that a new octahedral platinum(IV)-salphen complex does not interact with DNA in aqueous media at pH 7.4; however, upon addition of bio-reductants such as ascorbic acid or glutathione, the compound readily reduces to the corresponding square planar platinum(II) complex. In contrast to the parent platinum(IV) complex, the *in situ* generated platinum(II) complex binds to HTelo and *c-Myc* G-quadruplex DNA with affinity constants up to 10^6 M^{-1} .

Graphical Abstract

Guanine-quadruplexes (G4) are tetra-stranded helical assemblies that can form from guanine-rich DNA sequences. These non-canonical DNA structures have been the subject of intense study since they have been associated with a number of biological processes such as telomere maintenance, gene regulation and replication.^[1,2] A number of recent studies have provided substantial evidence for their formation *in vivo*. More specifically, the development of G-quadruplex antibodies has allowed to study these structures in fixed cells^[3,4] while small-molecule optical probes have provided evidence for their formation in live cells.^[5,6] Furthermore, bioinformatic^[7-9] and sequencing^[10] studies have highlighted the widespread presence of quadruplex-forming sequences in the human genome. In particular, it has been shown that G-quadruplex forming regions are prevalent in oncogenes' promoters as well as in telomeres and these regions have been identified as attractive targets for anticancer drugs.^[2,11-13]

This has prompted the development of small molecules that can bind and stabilise G-quadruplexes selectively.^[13-15] This could provide an alternative to the development of anticancer agents and indeed several molecules have been extensively studied in this context. While a large number of small organic and metal-organic molecules have been reported to bind G-quadruplexes with different levels of selectivity, there are very few reports of compounds that are activated by an external stimulus and only then can bind to G4 DNA. Amongst the very few examples previously reported, there is an azobenzene derivative whose G-quadruplex binding can be switched on/off upon irradiation with UV/visible light^[16] and a ligand that regulates its affinity for G-quadruplexes by coordination to copper.^[17]

Previously it has been shown that square-planar metal-salphen complexes (such as **1**) are excellent G-quadruplex DNA binders.^[18-20] Some of these compounds have also been shown

to inhibit telomerase activity (mediated by the stabilisation of G-quadruplexes in the telomere)^[19] as well as to downregulate *c-Myc* expression (an oncogene with a guanine-rich promoter that forms G-quadruplexes).^[21] However, the use of this type of compounds as anticancer agents has the potential disadvantage of being unspecific for cancer cells.

As has been extensively investigated for cisplatin pro-drugs, since cancer cells are hypoxic (i.e. they are under a reducing environment), an attractive avenue to reduce the non-specific cytotoxicity of chemotherapies is to activate them by reduction.^[22] Several examples have been reported of inert platinum(IV) precursors of cisplatin, which under hypoxic conditions get reduced to the cisplatin which then displays its chemotherapeutic properties against cancer cells.^[23] With this in mind and inspired by the extensive literature of cisplatin prodrugs based on platinum(IV) compounds, we hypothesised that an octahedral platinum(IV) complex with a planar delocalised ligand (such as salphen – see complex **2**) would be inert and would not interact with G-quadruplexes, while its reduced, square-planar platinum(II) analogue would yield a good G4 binder (Figure 1).

Figure 1. Schematic representation of the redox-activated G-quadruplex DNA binder

While several Pt^{II}-salphen complexes are known,^[20,21] to the best of our knowledge no examples of Pt^{IV}-salphen complexes have been previously reported. To prepare this compound, we first synthesised the platinum(II) complex **1** (see Scheme 1) by adapting a previously reported synthetic procedure.^[20] The resulting complex was fully characterised by ¹H NMR and ¹⁹⁵Pt NMR spectroscopy as well as by mass spectrometry and elemental analyses. In the ¹H NMR spectrum, all the characteristic resonances for the salphen ligand (including the singlet at 9.30 ppm associated to the imine) were present and integrated correctly with respect to the resonances associated to the aliphatic region of the spectrum (see Figure S1). The ¹⁹⁵Pt NMR spectrum showed only one singlet at -1759 ppm which is characteristic of platinum(II) complexes (see Figure S3).

Scheme 1. Synthetic scheme of the ligand, compound **1**, **2** and **3**. Experimental conditions: (i) MeOH, 60 °C, 4 h, under N₂; (ii) DMSO, NaOAc, K₂PtCl₄, 50 °C, 15 h, under N₂; (iii) DMF, 1 eq. PhICl₂, 40 °C, 48 h, under N₂; (iv) DMF, 5 eq. PhICl₂, 40 °C, 48 h, under N₂

Having successfully synthesised the platinum(II) complex **1** we then investigated different conditions to oxidise it to the platinum(IV) analogue. We first carried out the oxidation of the compound using H₂O₂ with the aim of synthesising the platinum(IV)-salphen complex with axial OH groups (which could subsequently be functionalised). However, after several attempts using different experimental conditions, it was not possible to isolate the platinum(IV) complex resulting from the oxidation using H₂O₂. We therefore explored other oxidizing agents and were able to synthesise the platinum(IV) complex **2** in good yields using PhICl₂ as an oxidant (see Scheme 1). This compound was characterised by ¹H NMR and ¹⁹⁵Pt NMR spectroscopies and the formulation confirmed by mass spectrometry and elemental analyses. The ¹H NMR spectrum clearly showed a change in the chemical shifts of the aromatic resonances as compared to the platinum(II) analogue (see Figure 2). Interestingly, coupling between the imine proton and the platinum(IV) centre could also be observed (which was not seen in complex **1**); this was also confirmed by a 2D ¹H-¹⁹⁵Pt NMR study (see Figure S8 in the supplementary information). The ¹⁹⁵Pt NMR spectrum clearly showed that the platinum centre in **1** (δ = -1759 ppm) had been oxidised to yield **2** (δ = +507 ppm). In a subsequent attempt to increase the yield of **2**, a higher amount of oxidant PhICl₂ was used. Interestingly, this resulted in the formation of compound **3** in which not only the platinum(II) centre was oxidised to platinum(IV) but also an aromatic substitution with chlorides took place (see Scheme 1). The resulting compound was isolated, purified and fully characterised by ¹H and ¹⁹⁵Pt NMR spectroscopy, NOESY as well as mass spectrometry and elemental analyses. The stability of **2** at higher temperatures was also investigated with view to carrying out DNA melting studies (see below). Variable temperature ¹H NMR spectroscopy (in *d*₆-DMSO) showed that the compound is stable up to 100 °C with only some slight decomposition above 110 °C (see Figure S16).

The reduction potentials of complexes **2** and **3** were then determined using cyclic voltammetry. This showed an irreversible reduction at -234 and -140 mV vs. sat. Ag/AgCl ($v = 0.1$ V/s) for **2** and **3** respectively (Figure S17). These measurements were carried out in DMF (scan rate: 0.1 Vs^{-1} , 0.1 M TBAPF₆ supporting electrolyte). The reduction potential for **2** is similar to that of *cis*-[PtCl₄(NH₃)₂] ($E = -260$ mV), a compound that has been previously shown to reduce readily to cisplatin in the presence of bioreductants.^[24] We therefore focused on complex **2** for all subsequent studies.

The next step was to investigate whether complex **2** would be reduced with glutathione and ascorbic acid (both of them common bio-reductants) to yield the G4 DNA binder complex **1**. As can be seen in the ¹H NMR spectra shown in Figures 2 and S18, upon addition of ascorbic acid and incubation for 24 hours, all the resonances in the aromatic region for **2** shifted to the values associated to the reduced complex **1**. An analogous study was performed with glutathione which showed that after few minutes incubation complex **2** had been fully reduced to complex **1** (see Figure S19). Similarly, ¹⁹⁵Pt NMR spectroscopy showed the reduction of **2** to **1** upon addition of glutathione or ascorbic acid.

Figure 2. Aromatic region of the ^1H NMR spectrum for platinum(II) complex **1** (top), platinum(IV) complex **2** (medium) and product resulting from the reduction of complex **2** with ascorbic acid (bottom).

The reduction was also confirmed by recording the emission spectrum of **2** before and after addition of the reducing agent: as expected while no significant emission was recorded for **2**, addition of the reducing agents led to restoration of the emission in DMSO associated to the platinum(II) complex **1** (see Figure 3).

Figure 3. Emission spectra of platinum(II) complex **1** (4 μM), platinum(IV) complex **2** (4 μM) and **2** (4 μM) + glutathione (20 μM) (all spectra were recorded in DMSO- d_6).

The next step was to investigate the affinity of complexes **1** and **2** towards the three following G4 DNA structures: *c-myc* (parallel topology), HTelo in Na^+ (antiparallel topology) and HTelo in K^+ (hybrid topology); in addition, the affinity was also determined with duplex DNA (ds26). Spectrofluorimetric titrations with DNA were first carried out since it has been previously reported that platinum(II)-salphen complexes (such as **1**) are weakly emissive in aqueous media but their emission is switched-on upon interaction with DNA.[20,21] The binding isotherms in Figure 4 and affinity constants in Table 1, show that complex **1** has good affinity for G4 DNA. This is particularly the case for HTelo G4 DNA (in Na^+) over *c-Myc* G4 DNA, suggesting that complex **1** has a preference for antiparallel G4 topologies. No significant changes in the emission spectra of complex **2** (which, as described above, is poorly emissive) were observed upon addition of increasing amounts of DNA (therefore no affinity constants were determined). This indicates that **2** is not reduced to the platinum(II) complex **1** in aqueous solution (pH 7.4) in the presence of excess DNA.

Figure 4. Binding isotherms for complexes **1** and **2** (2 μM) upon addition of DNA in 10 mM lithium cacodylate (pH=7.3) containing 100 mM KCl (for HTelo-K, *c-Myc* and ds26) or 100 mM NaCl (for HTelo-Na). The isotherms were obtained by monitoring changes in the emission spectrum ($\lambda_{\text{ex}} = 364 \text{ nm}$; $\lambda_{\text{em}} = 581 \text{ nm}$) of the platinum complexes upon addition of increasing amounts of DNA.

Table 1. Binding constants between complex **1** and different DNA structures determined by fluorescence titrations upon addition of DNA in 10 mM lithium cacodylate (pH=7.3) containing 100 mM KCl (for HTelo-K, *c-Myc* and ds26) or 100 mM NaCl (for HTelo-Na). Binding constants were determined according to the independent-site model by nonlinear fitting of the data.^[25,26]

DNA structure	K (M^{-1})	DNA binding sites
HTelo in K^+	$6.9 \pm 0.3 \times 10^5$	2
HTelo in Na^+	$8.2 \pm 0.3 \times 10^5$	0.5
<i>c-Myc</i>	$6.8 \pm 0.4 \times 10^4$	1
ds26	$5.9 \pm 0.3 \times 10^4$	3

To compare the DNA affinities of complex **1** and **2** towards G4 DNA, we carried out CD melting studies using *c-Myc* G4-DNA, which has a single parallel conformation – unlike the hybrid topology of HTelo – and hence its thermal stability can be readily studied by CD spectroscopy. As can be seen in Figure 5, while addition of 1 μ M (5 equivalents) of complex **1** to *c-Myc* DNA led to a stabilisation of *ca.* 20 °C, the same concentration of octahedral complex **2** gave a very modest stabilisation (*ca.* 5 °C). Interestingly, upon addition of glutathione to the solution of **2** + *c-Myc* DNA, a significant increase in the thermal stability of *c-Myc* DNA was observed – the ΔT_m was determined to be 22 °C which is very similar to the value displayed by compound **1** (see Figures S21 and S23).

The thermal stabilisations of duplex DNA (ds26) with complexes **1** and **2** were also determined by CD melting assays (see Figures S25-S27). These studies showed that neither of the two complexes stabilised duplex DNA – in fact, a slight destabilisation was observed in both cases which might be associated to the higher affinity of these complexes for single stranded DNA than duplex DNA.

Figure 5. Thermal stability plots determined by CD spectroscopy of *c-Myc* G4 DNA (5 μ M) in the presence of complexes **1** and **2** (25 μ M) in 10 mM lithium cacodylate buffer containing 1 mM KCl and 99 mM LiCl (pH = 7.3).

Taken together, these studies show that the octahedral complex **2** has poor affinity towards G-quadruplex DNA (and does not bind to duplex DNA) but upon addition of a bio-reductant such as glutathione or ascorbic acid, it is converted to the square planar complex **1** which binds to G-quadruplex DNA with high affinity – particularly to *c-Myc* DNA. To the best of our knowledge this is the first example of a redox-triggered G-quadruplex binder which, in the future, could find applications towards targeting only hypoxic cells such as those found in tumours.

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