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Multi-nuclear platinum complexes encapsulated in cucurbit[*n*]uril as an approach to reduce toxicity in cancer treatment

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The dinuclear platinum complex *trans*-[{Pt(NH$_3$)$_2$Cl}$_2$μ-dpzm]$^{2+}$ (di-Pt) binds inside cucurbit[7]uril with slow exchange kinetics which does not significantly affect the cytotoxicity of the dinuclear complex but reactivity at the platinum centre is reduced.

The use of cisplatin in the treatment of human cancers is limited by a number of factors, including high toxicity.[1] One technique for lowering toxicity is the replacement of the chloro leaving groups with the slow ring opening oxalate ligand or by replacing one ammine ligand with a sterically bulky ligand.[2] Carboplatin and ZD 0473 are examples of complexes developed using these approaches. The lower toxicity of these drugs is attributed to their slower reaction rates, both with water and thiol containing proteins.[3,4]

Platinum drug resistance in human cancers has been shown to be overcome by the use of multi-nuclear platinum complexes,[5] however, these complexes are limited by high toxicity. Molecular encapsulation of these drugs provides a potential for reducing their toxicity through two mechanisms: 1) by decreasing the reactivity of the multi-nuclear platinum complex through hindered access and/or 2) to act as a slow release mechanism thereby limiting undesirable bio-reactions.

In this communication we report the preparation of two multi-nuclear platinum complexes encapsulated within cucurbit[7]uril Q[7] (Fig. 1), a relatively new molecular host.[6] The subsequent effect on reaction rates, DNA binding and cytotoxicity are discussed.

The encapsulated complexes (see Fig. 1) were prepared by dissolving equimolar amounts of the particular platinum complex[7–9] and Q[7] in hot 20 mM NaCl. Slow evaporation in all cases resulted in crystals of the encapsulated complexes. Encapsulation was confirmed in the ^{1}H NMR spectra where large up-field shifts of the non-exchangeable 4,4′-dipyrazolylmethane (dpzm) protons (H5, H3 and – CH_2–) were observed (*e.g.* Fig. 2). Similar chemical shift changes for a variety of organic compounds in Q[6] and Q[7] have been previously observed, where encapsulation has been confirmed by X-ray crystallography.[10,11] From the chemical shift changes of the dpzm resonances, and simple molecular modelling, it was concluded that the linking dpzm ligand sits within the cavity, while the cationic platinum centres sit near the Q[7] portals. Upon addition of further platinum complex to the 1 : 1 di-Pt–Q[7] mixture, separate resonances for the free and bound di-Pt were observed, indicating that the platinum complex is encapsulated by Q[7] with slow exchange (on the NMR timescale) kinetics.

As the rate of reaction of di-Pt (and cisplatin) with guanosine has been previously studied,[12] we examined the effect of encapsulation by following the rate of the reaction of the di-Pt–Q[7] complex with guanosine in a ratio of 1 : 2, at 60 °C, by ^{1}H NMR over 48 h (Fig. 3). Analysis of the data indicates at least a 3-fold reduction in the rate of reaction compared to the reaction of di-Pt with guanosine under the same conditions, but in the absence of Q[7].[12] From tentative assignments in the ^{1}H spectra it appears that the di-Pt complex binds at N7 of guanosine while still encapsulated in Q[7] (peaks 1, 4 and 6), although there appears to be evidence of guanosine bound by di-Pt not encapsulated by Q[7]. For the

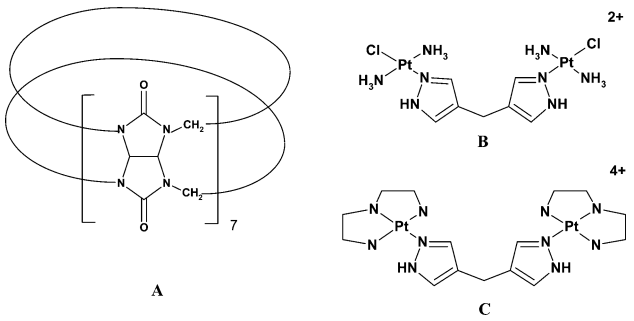

Fig. 1 (A) Cucurbit[7]uril (Q[7]), **(B)** *trans*-[{Pt(NH$_3$)$_2$Cl}$_2$μ-dpzm]$^{2+}$ (di-Pt) and **(C)** [{Pt(dien)}$_2$μ-dpzm]$^{4+}$.

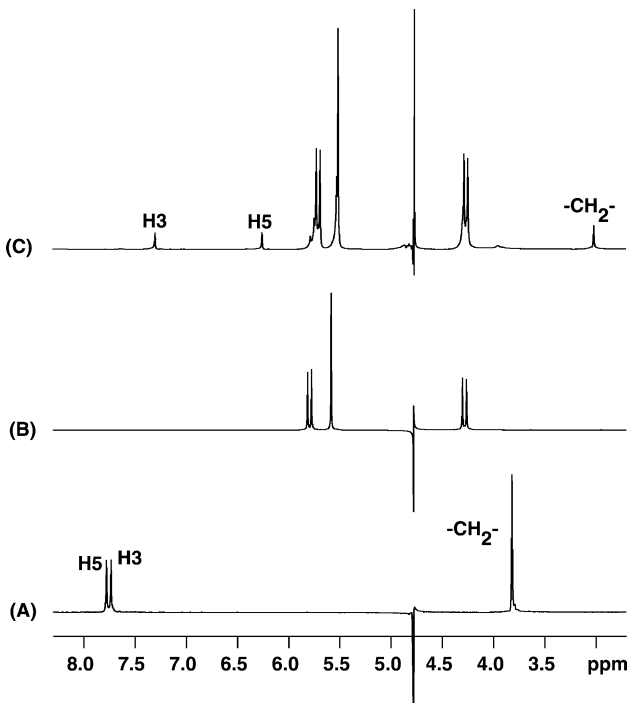

Fig. 2 ^{1}H NMR (D$_2$O) spectra showing **(A)** di-Pt, **(B)** Q[7] and **(C)** di-Pt encapsulated in Q[7]. The large up-field shifts of di-Pt indicate encapsulation of the linking ligand within the Q[7] cavity.

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reaction of di-Pt and guanosine at 60 °C, ~90% of the guanosine had reacted after just 5 h,[12] but for the di-Pt–Q[7] complex only ~40% of the guanosine had reacted in the same time. The di-Pt complex shows complete coordinate covalent binding by 24 h,[12] whereas the di-Pt–Q[7] complex had not fully reacted after 48 h (Fig. 3).

Having established that encapsulation of the platinum complex slows the reaction rate, it was then important to confirm that the encapsulated cationic platinum can be released from the Q[7] so that it could bind DNA in the same manner as a free drug. In order to determine this $[\{Pt(dien)\}_2\mu\text{-dpzm}]^{4+}$, an inert analogue of di-Pt, was encapsulated by Q[7] and added to a solution of the dodecanucleotide $d(CGCGAATTCGCG)_2$ in D_2O, and the results compared to an earlier study of the binding of the free platinum complex with the same dodecanucleotide.[8]

After 2 h at 37 °C an equilibrium was established between $[\{Pt(dien)\}_2\mu\text{-dpzm}]^{4+}$ encapsulated by Q[7] and $[\{Pt(dien)\}_2\mu\text{-dpzm}]^{4+}$ bound in the minor groove of the central A/T region of the dodecanucleotide. In the ^{1}H NMR spectra the H5 and H3 protons of the Q[7] encapsulated complex were observed as very sharp singlets at 6.33 and 7.19 ppm, whereas the non-encapsulated DNA bound metal complex peaks were observed at 8.04 and 7.74 ppm as slightly broad singlets, in agreement with our previous study.[8] At equilibrium, approximately 15% of the metal complex was not encapsulated but DNA bound and 85% encapsulated in Q[7]. No interaction of either the Q[7] or the Q[7] encapsulated metal complex with the dodecanucleotide was observed.

To determine the effect of encapsulation on the cytotoxicity, the di-Pt–Q[7] complex was tested against the murine leukaemia cancer cell line L1210 and the corresponding cisplatin resistant line L1210/DDP (Table 1). For the L1210 cell line no change in activity was observed, however a 2-fold decrease in activity is seen for

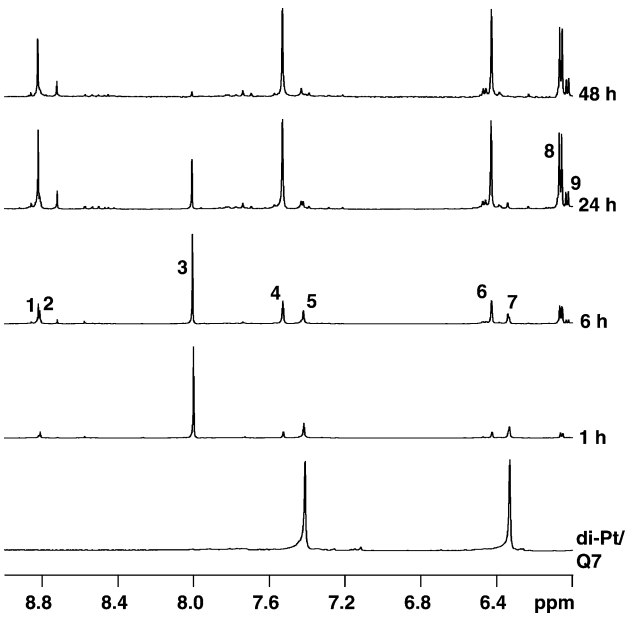

Fig. 3 ^{1}H NMR spectra (60 °C) showing the tentative assignments of the reaction of di-Pt–Q[7] with guanosine over 48 h. Based on the reference,[12] peaks **1** and **2** are bound guanosine, **3** is free guanosine, **4** and **6** are guanosine bound di-Pt–Q[7], **5** and **7** unbound di-Pt–Q[7], and **8** and **9** H1′ of adducts of guanosine bound di-Pt–Q[7].

Table 1 Cytotoxicity (IC_{50}) in the L1210 murine leukaemia cancer cell line and its cisplatin resistant line L1210/DDP, of free di-Pt and di-Pt–Q[7]. IC_{50} is defined as the concentration of complex (μM) required to inhibit cell growth by 50%. The resistance factor (Rf) is defined as IC_{50} resistant/ IC_{50} sensitive. IC_{50} values were determined from at least two independent experiments

Complex	L1210	L1210/DDP	Rf
Cisplatin	0.5	6.9	14
di-Pt	3.8	8.8	2.3
di-Pt–Q[7]	2.6	16.5	6.3

L1210/DDP sub-line. As the adducts formed by di-Pt–Q[7] are unlikely to be different to those formed by di-Pt, the difference in activity in the L1210/DDP line is probably due to reduced cellular uptake as a result of encapsulation.

The results of this study indicate that Q[7] has potential as a delivery system for DNA targeting multi-nuclear platinum complexes. The Q[7] encapsulates the complexes around the bridging ligand, slowing the rate of reaction by at least 3-fold, while having only a small effect on cytotoxicity. This approach may be particularly useful for the promising new drugs BBR3464 and BBR3571, where clinical trials showed that they are limited by their high *in vivo* toxicity and degradation by plasma proteins within the body.[13]

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