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Folding of dinuclear platinum anticancer complexes within the cavity of *para*-sulphonatocalix[4]arene

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ABSTRACT

The binding of three dinuclear platinum complexes, where the bridging ligand of the complexes is *N,N′*-(alkane-1,*n*-diyl)diisonicotinamide (*n* = 4, 6 or 8 for butane, hexane and octane, respectively) to the macrocycle *para*-sulphonatocalix[4]arene (sCX[4]) has been studied by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and molecular modelling. The NMR spectra show two important features, large upfield shifts of the methylene proton resonances of up to 1.8 ppm, which clearly places them within the shielding environment of the macrocycle's cavity, and a loss of chemical symmetry of the metal complexes with extra resonances observed upon sCX[4] binding. Molecular models of the platinum–sCX[4] host–guest complexes show significant folding of the metal complexes' aliphatic chain and a non-symmetrical interaction with the macrocycle. One side of the metal complexes forms three hydrogen bonds to sCX[4], whereas the opposite side of the metal complexes forms just one hydrogen bond, giving rise to the loss of chemical symmetry in the ^{1}H NMR spectra. As the dinuclear platinum complexes are model anticancer drugs, the effect of sCX[4] binding was investigated in vitro in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant sub-line A2780cp70. Whilst the free metal complexes are a magnitude of order more active than cisplatin in the A2780 cell line, they are all highly cross-resistant with cisplatin in the A2780cp70 line. Binding by sCX[4] has little affect on the metal complexes' cytotoxicity in the sensitive cell line, but has a large effect in the resistant cell line. The two shortest metal complexes become less active when bound by sCX[4], whereas the longest metal complex becomes more cytotoxic.

1. Introduction

Platinum-based anticancer drugs remain an integral component of many chemotherapy regimens despite their toxic side-effects and the ability of cancers to develop drug resistance against them [1]. Multinuclear platinum drugs are a class of chemotherapy agent which have attracted interest in their ability to overcome resistance developed by tumours to many other anticancer agents. The lead drug in this class is BBR3464 [2], a trinuclear complex, which was developed as far as Phase II in clinical trials. Recently, two dinuclear derivatives have been investigated as potential drug candidates [3]. Whilst these drugs are significantly more cytotoxic than cisplatin, they are rapidly degraded in vivo resulting in very little of the drug reaching tumours. Our group is interested in the use of macrocycles as protective delivery vehicles for multinuclear platinum drugs with a focus on the cucurbituril [4,5], cyclodextrin [6] and calixarene [6,7] families of cavitands. One of the key benefits of macrocycle binding to platinum drugs is that their encapsulation confers steric protection, which slows or prevents the drug's degradation by biological thiols like glutathione [8–11], or sequestration by proteins like human serum albumin [12–14].

Calixarenes are a family of macrocycles made from a hydroxyalkylation reaction between an aldehyde and phenol [15]. The resultant molecules, that generally contain 4, 6, 7, 8 or 9 subunits, form bowl shaped structures with a hydrophobic pocket and an extensively hydrogen bonded network of hydroxyl groups at its base [15]. The application of calixarenes to drug delivery stems from their ability to form host–guest complexes with a range of small molecule drugs and biological molecules [16,17]. Such complexes are stabilised by hydrophobic effects and/or ion–dipole interactions or hydrogen bonds.

Native calixarenes are poorly soluble in aqueous solutions but functionalisation with sulphonate groups yields derivatives that are highly soluble. *para*-Sulphonated calixarenes (Fig. 1), particularly the macrocycle made of four subunits (sCX[4]), have demonstrated many applications in drug delivery including: improved

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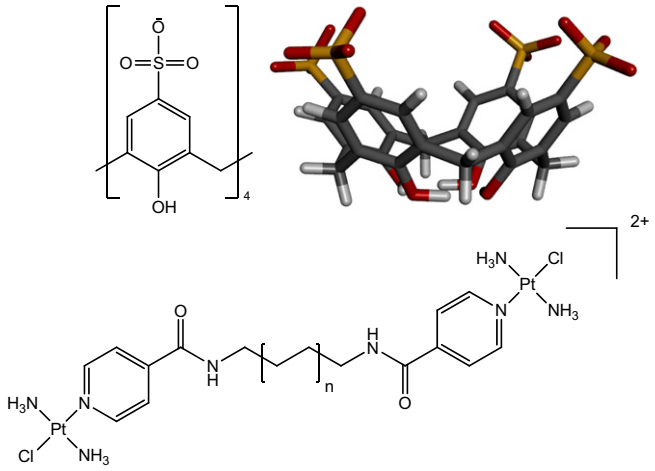

Fig. 1. The chemical structure of *para*-sulphonatocalix[4]arene (sCX[4]), a molecular model showing sCX[4]'s bowl-shaped structure, and the chemical structure of the dinuclear platinum complexes used in this study; n = 1, 2 or 3. Counter ions for both molecules have been omitted.

drug solubility, chemical stability, bioavailability, biodistribution and transport, and/or elimination of drug polymorphism in the solid state [16,18,19]. As an excipient for drugs, sCX[4] also displays relatively little cytotoxicity or toxicity in vivo [20].

Previously we have shown that sCX[4] is capable of forming host–guest complexes with different types of platinum anticancer complexes, including dinuclear agents [6,7]. In our recent study involving a dinuclear platinum complex with a rigid bridging linker, however, we found that the sCX[4] bound the platinum complex in a side-on manner and provided no barrier to drug degradation [7]. Despite this, the strong association constant, and the ease with which sCX[4] can be functionalised with cancer targeting groups, made us hypothesise that the macrocycle may still be useful as a platinum drug delivery vehicle. The sulphonate groups of sCX[4] can be readily functionalised with groups which can be used to actively target tumours. A recent example includes folate, a vitamin essential to cell growth [21], and which was conjugated to sCX[4] [22]. As such, we are interested in developing actively targeted sCX[4] derivatives which can be used to deliver multinuclear platinum drugs to cancers.

As a first step in our development of targeted calixarene-based delivery vehicles, in this paper we have investigated the binding of sCX[4] to a new group of dinuclear platinum complexes which have been shown to be up to 10-fold more active than cisplatin [23]. Their binding was examined by ^{1}H and DOSY NMR from which molecular models were developed. The effect of sCX[4] on the cytotoxicity of the dinuclear platinum complexes was evaluated using in vitro growth inhibition assays with the human ovarian carcinoma cell line A2780 and its cisplatin-resistance sub-line A2780cp70.

2. Experimental

2.1. Materials

para-Sulphonatocalix[4]arene, diethyl ether, triethylamine, transplatin, D_2O (99.9%), isonicotinic acid and 1,6-diaminohexane were purchased from Sigma Aldrich. T3P® was purchased from Alfa Aesar. The metal complexes (**1**–**3**) were made following a published method [23]. The characterisation data for **2** has not previously been reported and is given below.

2.2. Characterisation data for the ligand of complex (*2*)

^{1}H NMR (d_6-DMSO, ppm): 8.72 (t), 8.70 (d, J = 4.4 Hz), 7.72 (d, J = 4.6 Hz), 1.53 (m), 1.34 (m). ^{13}C NMR (d_6-DMSO): 165.1, 151.8, 142.1, 121.7, 29.45, 26.74. ESI-MS $[M+H]^+$ expected: 327.18 m/z, found: 327.60 m/z. Elemental *Anal.* for $C_{18}H_{22}N_4O_2$. Expected: C, 66.24; H, 6.79; N, 17.17. Found: C, 65.85; H, 6.71; N, 16.84%.

2.3. Characterisation data for complex (*2*)

^{1}H NMR (D_2O, ppm): 8.97 (d, J = 7.0 Hz), 8.96 (t), 7.78 (d J = 6.6 Hz), 3.38 (m), 1.61 (m), 1.38 (m). ^{195}Pt NMR (D_2O, ppm): −2301. ESI-MS $[M+H]^+$ expected: 855.16 m/z, found: 855.20; $[M]^{2+}$ expected: 427.08 m/z, found: 428.47 m/z. Elemental *Anal.* for $C_{18}H_{34}Cl_4N_8O_2Pt_2{\cdot}3H_2O$. Expected: C, 22.05; H, 4.11; N, 11.43. Found: C, 21.93; H, 4.09; N, 11.21%.

2.4. ^{1}H NMR

One dimensional NMR spectra were obtained in D_2O on a JEOL JNM-LA400 referenced internally to the solvent peak at 4.78 ppm. Diffusion ordered spectroscopy experiments in D_2O were obtained on a Bruker Avance 400 using 600 μL of sample in a Wilmad NMR tubes rated for 400–500 MHz use. The pulse sequence used was the standard DOSY sequence provided by Bruker and analysed with the Bruker relaxation software using a non-linear least-squares fit of the data to the equation: $I = e(\gamma^2 g^2 \delta^2 D(\Delta - \delta/3))$. Each diffusion coefficient was determined using a Δ of 80 ms, a δ of 5 ms, and recycle time of 5 s and at a fixed temperature of 25 °C.

2.5. Molecular modelling

Calculations were performed on a dual-Xeon processor, workstation using the DMol^3 (Delley, Delley) program in Accelrys' Materials Studio (Accelrys). Individual geometry optimisations for the three platinum complexes were undertaken at the LDA PWC level (Perdew) using a DND basis set (Double Numerical plus d-functions on all non-hydrogen atoms) in order to locate structurally stable conformers. Bond distances were determined using the measurement tool of the modelling software program: Accerlys Discovery Studio 3.1 visualizer.

2.6. In vitro growth assays

The cytotoxicity of the metal complexes and the platinum–sCX[4] host–guest complexes were conducted using MTT-based in vitro growth inhibition assays using the A2780 and A2780/cp70 human ovarian carcinoma cell lines as previously described [24]. The A2780 and A2780cp70 ovarian cancer lines were grown in RPMI media containing 10% foetal calf serum, penicillin streptomycin and L-glutamate in a 5% CO_2 atmosphere. The cells were trypsinised, counted and adjusted to 500–1000 cells per well in 96 well plates. Metal complex/sCX[4] stock solutions were diluted with RPMI to prepare a dilution series based on the total platinum concentration (0.1–100 μM). Ten microliters of aliquots were taken from these solutions and added in triplicate to each well along with RPMI only. The plate was then cultured for 24 h, after which MTT (50 μL of a 5 mg mL^{-1} solution) was added to the 200 μL of medium in each well and the plates were incubated at 37 °C for 4 h in the dark. Medium and MTT were then removed and the MTT-formazan crystals dissolved in 200 μL DMSO. Glycine buffer (25 μL per well, 0.1 M, pH 10.5) was added and the absorbance measured at 570 nm in a multiwell plate reader.