Two antitumour ruthenium(III) complexes showing selectivity in their binding towards poly(dG)·poly(dC) and poly(dA)·poly(dT)

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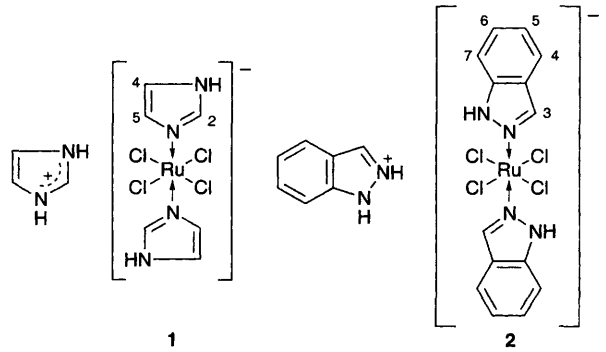
The antitumour-active complexes *trans*-Him[$Ru^{III}Cl_4(im)_2$] (Him = imidazole) and *trans*-Hind[$Ru^{III}Cl_4(ind)_2$] (Hind = indazole) bind at a higher binding rate to poly(dG)·poly(dC), compared to poly(dA)·poly(dT); the covalent binding to the nucleobases requires a preceding aquation of the compounds, similar to cisplatin.

The new tumour-inhibiting ruthenium(III) complexes *trans*-Him[$Ru^{III}Cl_4(im)_2$] **1** and *trans*-Hind[$Ru^{III}Cl_4(ind)_2$] **2** (Fig. 1) show a remarkable antitumour activity in different tumour models, especially against colon carcinomas.[1] To obtain an insight into the mode of action of these compounds, the aquation chemistry of the complexes has been investigated as well as their interaction with plasma proteins. For **1**, the *trans* geometry was confirmed by X-ray crystallography.[2] In aqueous solution, the compound forms mono- and di-aqua species by chloride exchange, analogously to cisplatin, [$Pt^{II}Cl_2(NH_3)_2$].[3] In physiological buffer at pH 7.4, both complexes show a remarkable difference in stability as well as in their binding rates to apotransferrin.[4] X-Ray crystallography of the homologous apolactoferrin adducts of **1** and **2** gave more precise information on the binding to transferrin, showing that both compounds bind to histidines at iron(III) binding sites, but **1** can also reach less specific binding sites.[5] For **1** it has been reported that the complex binds, after aquation, to DNA and blocks its template-primer properties for DNA polymerase-catalysed DNA synthesis.[6]

Here we report that the compounds bind covalently to calf-thymus DNA as well as to the synthetic double-stranded homopolymers poly(dG·dC) and poly(dA·dT). Both complexes show a binding preference for poly(dG·dC), compared to poly(dA·dT). In 10 mmol dm^{-3} NaCl, the imidazole complex binds to the three polynucleotides at higher rates than its indazole analogue. We used ICP-AES† and UV–VIS spectroscopy for the investigation of DNA binding. The application of ICP-AES has been reported for the DNA binding of metallocenes[7] and is a useful technique in measuring the non-labile DNA binding of metal complexes at concentrations used in therapeutical applications. To start our investigations, we chose double-stranded poly(dG·dC) and poly(dA·dT), because both homopolymers differ not only completely in their composition, but also slightly in their conformation[8] therefore favouring initial investigations into the selectivity of drug binding to polynucleotides.

Fig. 2 shows the binding rates for **1** (*a*) and **2** (*b*) to calf-thymus DNA, poly(dG·dC) and poly(dA·dT). 10 mmol dm^{-3} NaCl reaction solutions, containing DNA or polynucleotides (200 μmol dm^{-3}) and metal complexes (20 μmol dm^{-3}), were incubated at 37 °C for several hours. DNA purification and precipitation of reaction aliquots followed literature methods.[7]‡ Concentrations of the polynucleotides were determined at 260 nm.[9]

After 8 h of incubation, almost three-quarters of **1** were bound to poly(dG·dC) and also to calf-thymus DNA whereas the amount of **1** bound to poly(dA·dT) was < 50%. It can be clearly seen that the reaction is dependent on the formation of an active complex by hydrolysis. After 1 h of incubation, the generation of aqua species leads to an accelerated binding to polynucleo-



1 **2**

Fig. 1 Structures of *trans*-Him[$Ru^{III}Cl_4(im)_2$] **1** and *trans*-Hind[$Ru^{III}Cl_4(ind)_2$] **2**. The pH was adjusted to 5.5 with 10 mmol dm^{-3} NaOH in aqueous solutions (100 μmol dm^{-3}) of **1** and **2**, buffered by the imidazolium and indazolium counter ion, respectively. The complexes were dissolved and aquated (30 min at 37 °C) and then frozen in liquid nitrogen until use. The still frozen aliquots (1 cm^3) of **1** and **2** were then thermostatted for 30 min in a water bath at 37 °C, the total aquation time amounting to 1 h.

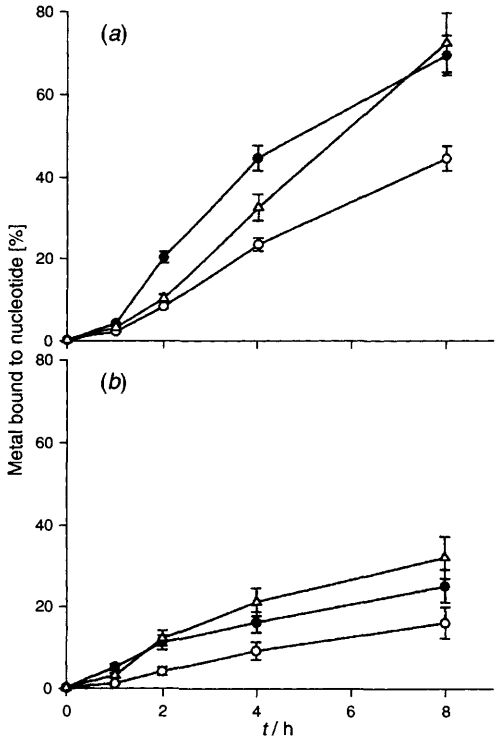


Fig. 2 Binding rates for the reaction of **1** (*a*) and **2** (*b*) with the sodium salts of calf-thymus DNA (●), poly(dG·dC) (Δ) and poly(dA·dT) (○) at a N : M (nucleotide/metal) ratio of 10 : 1 at 37 °C, pH 6. The obtained values were averaged, using three identically treated aliquots of independent reaction series.

tides. From previous studies[3,10] it is evident that the aquation reaction of **1** follows first-order kinetics, with a rate constant of 5.6×10^{-5} s^{-1} at 37 °C (0 mmol dm^{-3} NaCl, pH 5.6).[3] The corresponding half-life (3.4 h) changes only slightly when the chloride concentration is increased (4.6 h at 37 °C, 150 mmol dm^{-3} NaCl, pH 5.8).[3] The aquation of **2** occurs more slowly (11.2 h at 37 °C, 0 mmol dm^{-3} NaCl, pH 5.4), but the complex reaction pattern does not follow simple-order kinetics.[11] For the reaction at pH 6, 37 °C and at a low chloride concentration (10 mmol dm^{-3}) it can therefore be assumed that the observed binding to DNA and polynucleotides depends on previous aquation. After hydrolysis, the nature of the incoming ligand determines the rate of substitution. After 8 h, the preference of **2** for poly(dG·dC) as against poly(dA·dT) (32 *vs.* 16%) is evident. Calf-thymus DNA shows a medium binding rate (25%).

Under identical experimental conditions, UV–VIS spectroscopic measurements§ showed that the absorbance maxima at 340 nm for **1** and 357 nm for **2**, probably related to a LMCT transition,[4,5] disappeared after 2–3 h. Also, at 598 nm (**1**) and 588 nm (**2**) respectively, a new band appears. This band shows a remarkable intensity with quite high absorption coefficients ranging from 1900 to 4100 dm^3 mol^{-1} cm^{-1}. The absorption coefficients for this CT or d–d transition, calculated for **1** and **2** with the concentrations of bound metal measured by ICP-AES, correspond with ε values from the literature (600–6200 dm^3 mol^{-1} cm^{-1}), which have been reported for low-symmetry octahedral ruthenium(III) complexes and which have been assigned as d–d transitions.[12] The binding of **2** to histidine 253 of apolactoferrin also exhibited a d–d transition at 585 nm.[5] Therefore we assume that the observed d–d band gives evidence for a covalent binding of **1** and **2** to the nucleotides, preferably to a nucleophilic site of the nucleobases like N^7 of guanine. This transition cannot be observed during the ageing of aqueous solutions of **1** and **2**. In contrast to the apotransferrin binding studies,[4] where substitution of bicarbonate by the complexes was observed, no further buffer ions were present in our studies so reactions other than aquation can be excluded. Therefore, we conclude that the observed binding preference for poly(dG·dC) is related to the composition of the polynucleotide; the most nucleophilic site in DNA, N^7 of guanine, which is also the specific DNA binding site for cisplatin,[13] can be regarded as a possible target. Complementary HPLC experiments¶ with 9-methylguanine, using a diode-array detector for monitoring of UV peak spectra (200–360 nm), confirmed this assumption. For **1**, which does not absorb significantly between 220 and 320 nm, two neutrally charged, isomeric species in addition to hydrolysis products have been detected, containing a new LMCT transition as well as the strong absorption band ($\pi \rightarrow \pi^*$) of the nucleobase at 275 nm. Also an additional positively charged species, corresponding to a bis-guanine adduct, was present to a minor extent. It can be concluded that the observed binding to DNA and polynucleotides may also involve bifunctional crosslinking at a later stage as in the case of cisplatin.

For **1**, which does not show a significant UV absorption around 260 nm, the binding to poly(dG·dC) produces a hypochromic effect and leads to a 5 nm red shift and a decrease of band intensity of around 20%. With calf-thymus DNA and poly(dA·dT), no decrease of intensity but only a diminished 3 nm red shift for DNA binding can be observed. Although it has been known for a long time that poly(dG·dC) reacts sensitively to small changes in experimental parameters and can adopt a variety of molecular forms,[14] this feature requires further investigation including CD and LD studies, to understand if and how complex binding disturbs the solution conformation of G–C-containing polynucleotides. Therefore we are currently working on further studies into binding of ruthenium complexes towards polynucleotides, including investigations with sequence isomeric polymers and examination of possible conformational effects. Also studies with pure homopolymers are planned, to qualify the preferred binding site of each complex.

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Footnotes

† ICP-AES (induced coupled plasma-atomic emission spectroscopy) measurements were carried out on a Perkin Elmer plasma 400 emission spectrometer using a Babington nebulizer. Ruthenium was detected by the 240.3 nm line. Concentrations were determined in triplicate for each aliquot.

‡ Calf-thymus DNA was purified by ethanol precipitation before use. After incubation from 1 to 8 h, 0.5 ml reaction aliquots were added to 1.5 ml of ethanol saturated by sodium acetate. After 30 min cooling at −18 °C, the DNA and polynucleotides were pelleted by ultracentrifugation for 30 min. The hydrolytic cleavage of the pellets occurred overnight in 2 ml of 18% HCl. A 0.5 ml aliquot of the initial solution in 1.5 ml of 18% HCl served to determine the whole metal concentration in solution (100%). Precipitation experiments with polymers alone and the data treatment eliminate variation from DNA loss due to incomplete precipitation.

§ UV–VIS absorption spectra were recorded on a Perkin Elmer Lambda 12 spectrometer with a temperature-constant measuring cell (37 °C).

¶ HPLC studies were performed using a Merck-Hitachi L3000 diode array detector and a Nucleosil (100 Å–5 μm) diol column (250 × 8 mm) at ambient temperature. The used mobile phase (flow rate 0.5 cm^3 min^{-1}) consisted of 70% MeCN–30% 5 mmol dm^{-3} KNO_3 in doubly distilled water, pH 7. The complexes (500 μmol dm^{-3}), aquated as described before, were incubated at 37 °C for 20 h with 2 equiv. of 9-methylguanine (10 mmol dm^{-3} NaCl).

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