Supermacrochelate Complexes Containing an Artificial Nucleic Acid Backbone and Derived from Excellent Ligands Formed by Treating Platinum Anticancer Agents with Nucleotide Triphosphates

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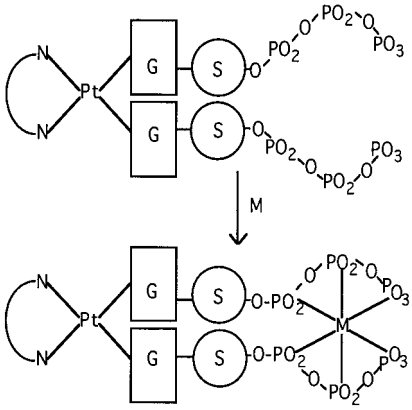
We have discovered that a diverse class of potent metal-ion chelators, *cis*-$A_2Pt(NTP)_2$, form novel *cis*-$A_2Pt(NTP)_2M$ complexes with unusual properties (A_2 = two amines or a diamine, NTP = nucleotide triphosphate). Formed by two metals and two NTPs, *cis*-$A_2Pt(NTP)_2M$ are named "supermacrochelate complexes" by analogy to typical MNTP macrochelate complexes formed by one metal and one nucleotide (Scheme 1). These "supermacrochelate ligands" are readily prepared in aqueous solution by exploiting the well-developed chemistry of *cis*-type Pt anticancer compounds, *cis*-A_2PtX_2.[1−12]

We illustrate the properties of supermacrochelate ligands with **en**Pt(5′-GTP)$_2$ (5′-GTP = guanosine 5′-triphosphate; **en** = ethylenediamine);[13] at pH 7 and below, this ligand competes for La^{3+} with the very strong ligand EDTA (log K = 15 for La-(EDTA)).[14] The linking of two nucleotides in this way by La^{3+} creates an unusual artificial metal-linked sugar−phosphate backbone; the "backbone" link in **en**Pt(5′-GTP)$_2$La persists intact even at pH 1.5. The supermacrochelate complex can be viewed as a model of the cross-link formed by *cis*-type Pt anticancer drugs and N7 of adjacent **G** residues in DNA (**G** = guanine nucleotide).[15] The simplest *cis*-PtA_2G_2 cross-link models do not have the **G**'s linked except by Pt; such models exist as just one C_2-symmetrical head-to-tail (HT) form with Δ chirality in crystalline solids.[5,16−19] In solution, the presence of only one H8 ^{1}H NMR signal has long been presumed to indicate that the models exist as an ∼1:1 mixture of Δ and Λ HT atropisomers that rapidly equilibrate by rotation about the Pt−N7(**G**) bonds.[4,5,20] The ^{1}H NMR spectrum of the supermacrochelate ligand, **en**Pt(5′-GTP)$_2$, has one major H8 singlet at 8.48 ppm (Figure 1) shifted ∼0.4 ppm downfield from 8.09 ppm for 5′-GTP. Thus, **en**Pt(5′-GTP)$_2$ is representative of the previously studied simple models that presumably exist as a rapidly interconverting mixture of Δ and Λ HT atropisomers.

Scheme 1



When La^{3+} was titrated into an **en**Pt(5′-GTP)$_2$ solution, new signals assignable to H1′ and to one H5′/5″ proton of **en**Pt(5′-GTP)$_2$La emerged at 5.94 and 4.00 ppm. At an ∼1:1 **en**Pt(5′-GTP)$_2$:La^{3+} ratio, the new H1′ signal dominated (Figure 1), and above this ratio the spectral changes ceased, demonstrating a 1:1 stoichiometry. This observation of separate resonances indicates slow La^{3+} exchange on the NMR time scale. Compelling ^{17}O, ^{31}P, and ^{1}H NMR spectroscopic evidence on La(5′-ATP)$_2$ demonstrated that La^{3+} was bound to oxygens of all three phosphate groups of both 5′-ATP's.[21] Since La(5′-ATP)$_2$ is in fast exchange, we believe our observation of slow exchange for the supermacrochelate **en**Pt(5′-GTP)$_2$La complex is strong evidence for the binding of phosphate groups from both 5′-GTP's.

Models with all six phosphate groups bound to La place the γ phosphate groups close to the respective C5′H$_2$ group. Such proximity could account for the strong shift of only one sugar ^{1}H NMR signal, which is an H5′/5″ signal. Furthermore, when the pH was decreased from 7.0 to ∼2, the only 5′-GTP ^{1}H resonance to shift appreciably was this one upfield-shifted H5′/5" signal; thus, even at low pH, **en**Pt(5′-GTP)$_2$La is very stable and probably protonated at the γ phosphate(s). A more dramatic indication of the stability of **en**Pt(5′-GTP)$_2$La derives from competition studies with 0.5 equiv of EDTA at pH 7.0 (Figure 2). The ^{1}H NMR signals of free and bound EDTA are well known,[22] and both types were observed, demonstrating that only 80% of the EDTA was bound to La; a similar percentage of the H1′ resonance for **en**Pt(5′-GTP)$_2$ was observed. Both La(EDTA) and **en**Pt(5′-GTP)$_2$La are in slow exchange on the NMR time scale at room temperature and pH 7. In a similar experiment

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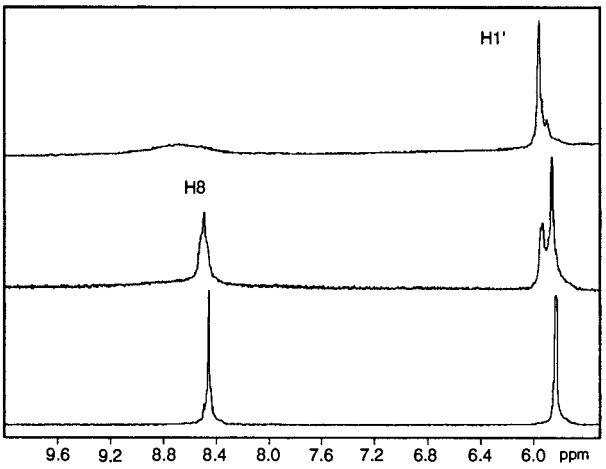

Figure 1. H8 and H1′ ^{1}H NMR signals of **en**Pt(5′-GTP)$_2$ (4.48 mM) at pH 7: (bottom) no La^{3+}; (middle) 0.5 equiv of La^{3+}; (top) 1 equiv of La^{3+}. The chemical shifts (ppm) not given in text follow in the order **en**Pt(5′-GTP)$_2$La/**en**Pt(5′-GTP)$_2$/free 5′-GTP: H1′, 5.93/5.87/5.89; H2′, under H_2O/4.67/4.74; H3′, 4.48/4.55/4.55; H4′, 4.28/4.20/4.32; H5′/5″, 4.14,4.00/4.20/4.20.

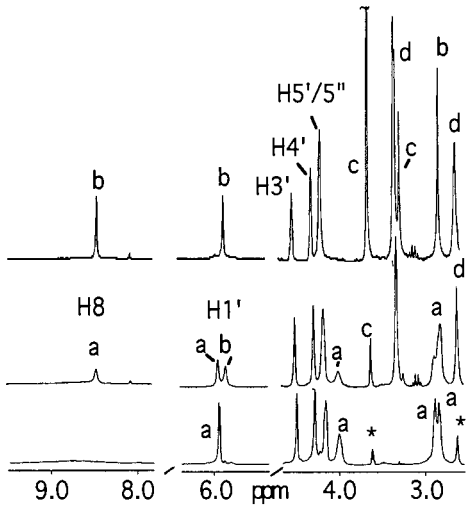

Figure 2. ^{1}H NMR signals of: **en**Pt(5′-GTP)$_2$La (bottom), and with 0.5 equiv (middle), and 1.5 equiv (top) of EDTA. Resonance assignments are as follows: (a) = **en**Pt(5′-GTP)$_2$La; (b) = **en**Pt(5′-GTP)$_2$; (c) = free EDTA; and (d) = La(EDTA). Minor inert impurities from 5′-GTP or **en**$PtCl_2$ are marked with *.

conducted at pH 5 (not shown), only 34% of the EDTA was bound, indicating a greater stability for **en**Pt(5′-GTP)$_2$La than for La(EDTA) at this pH.

At 20 °C the H8 resonance of **en**Pt(5′-GTP)$_2$La (4.30 mM) was so broad as to be almost undetectable (Figure 1). When the temperature was incrementally increased, the H8 resonance could be clearly observed at ∼8.7 ppm at 45 °C. This signal became sharper when the temperature was raised to 75 °C. No significant shift change was observed in this signal or in any ribose resonances as the temperature was increased. We attribute the broad H8 signal at room temperature to an intermediate rate for atropisomerization of the Δ and Λ HT forms, which, as mentioned above, are presumed to exist in dynamic *cis*-PtA_2**G**$_2$ complexes. The H8 signal sharpening results from the increasing rate of atropisomerization with temperature. The broadening is clearly not due to La^{3+} exchange.

To examine solutions below 0 °C, a 5:2 D_2O/acetone-d_6 mixture was employed (Figure 3). In this solvent mixture, two very broad

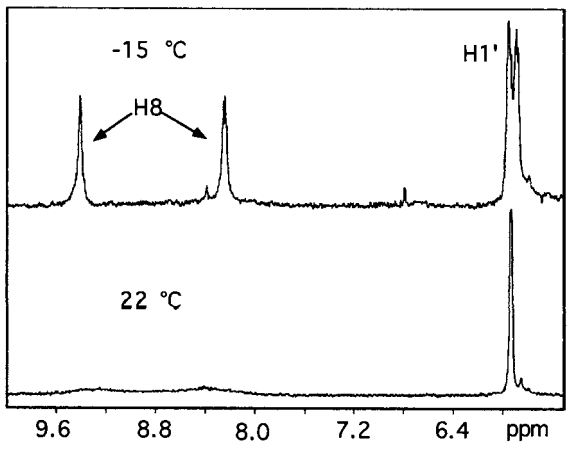

Figure 3. H8 and H1′ ^{1}H NMR signals of **en**Pt(5′-GTP)$_2$La (2.14 mM) in 5:2 D_2O/acetone-d_6 (pH 7.0 before addition of acetone).

H8 resonances observed for **en**Pt(5′-GTP)$_2$La at 22 °C sharpened and shifted <0.05 ppm to 9.43 and 8.25 ppm at −15 °C. The signals were similar but not of identical intensity, indicating that they arise from HT species. At −15 °C, **en**Pt(5′-GTP)$_2$ has a somewhat broad H8 signal with a shift of 8.73 ppm, a value similar to the average H8 shift (8.84 ppm) of **en**Pt(5′-GTP)$_2$La. These results indicate that **en**Pt(5′-GTP)$_2$ exists as a mixture of HT forms (as long suspected for simple *cis*-PtA_2**G**$_2$ cross-link models) with an atropisomerization rate starting to slow at −15 °C and that the La^{3+} serves the role of trapping the two HT forms. The rather upfield shift of one HT form of **en**Pt(5′-GTP)$_2$La at 8.25 ppm suggests that the **G** bases are tilted into a pseudo stacked form.[23] The other HT form has a quite unusually downfield H8 signal attributable to the absence of tilt and/or a deshielding effect of the phosphate groups.

In conclusion, we have identified a new type of complex with a chelate ligand incorporating Pt as a constituent. The ligand, named a supermacrochelate, has a high affinity for La^{3+}. Complexation creates a rather unique symmetrical backbone containing phosphate and natural sugar groups, as well as La.[24] A symmetrical backbone allows unambiguous evaluation of dynamic motion and atropisomer distribution, and the novel complex studied here provided such evidence. In contrast, the natural backbone of *cis*-PtA_2(dGpG) is unsymmetrical, with a 3′ and a 5′ nucleotide. The appearance of sharp signals for *cis*-PtA_2-(dGpG) cross-link models can be due either to fast exchange of multiple conformers or to one nonexchanging conformer. Therefore, the nature of the dynamic motion of these natural cross-links is unclear, unlike for the artificial cross-linked species studied here. Finally, the novel complex has an advantage over simpler models. The complex provides a new strategy for sampling the rotamer distribution of *cis*-PtA_2**G**$_2$ complexes, which atropisomerize too rapidly in the solution phase and inexplicably adopt the ΔHT form in the solid phase. Our study has led to the first compelling evidence that, for nonbulky A_2 ligands, both HT forms of *cis*-PtA_2**G**$_2$ exist and are present in an approximately 1:1 mixture.

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