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Platination of Nucleobases To Enhance Noncovalent Recognition in Protein–DNA/RNA Complexes

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Fluorescence quenching experiments show that the stacking interaction between nucleic acid bases and l-tryptophan is enhanced significantly upon base coordination to a metal center such as Pt(II), and the biological implications of such enhancement are discussed.

Stacking $\pi-\pi$ interactions play a fundamental role in DNA/RNA−protein selective recognition.[1] These interactions generally involve the planar protein residues tryptophan-(phenylalanine) and guanine(cytosine) on the nucleic acid. Methylation of purine and pyrimidine nucleic acid bases enhances $\pi-\pi$ stacking interactions to l-tryptophan (Trp) in both solid state and solution, attributable to a lowering in energy for the π-acceptor LUMO in the methylated nucleobase, thus improving the acceptor properties toward the π-donor HOMO of the amino acid.[2] In this contribution, we report that the analogy between the electrophiles Me^+ and Pt^{2+} may be extended so that platination of simple nucleic acid bases exerts a similar enhancement in stacking interactions. The results suggest a novel structural motif for design of metallodrugs capable of selective ternary DNA(RNA)−protein interactions and capable of serving as novel probes for the tryptophan environment in protein systems.

The systems examined were $[Pt(dien)(L)]^{2+}$ (where L = 9-ethylguanine, (9-EtGH, **1**)), 5′-guanosine monophosphate (5′-GMP, **2**), 1-methylcytosine (1-MeCyt, **3**), and 5′-cytidine monophosphate (5′-CMP, **4**). In the absence of a reactive leaving group, only noncovalent hydrogen-bonding and stacking interactions need to be taken into account (Figure 1). All complexes were synthesized using $[Pt(dien)(NO_3)]NO_3$ as a starting material. ^{195}Pt NMR spectroscopy of complex **3** gave two peaks at −2895 and −2912 ppm ($\Delta\delta$ = 17 ppm) at 20 °C coalescing to one broad peak at 80 °C, indicative of the presence of rotamers in solution due to hindered rotation about the Pt−N3 axis, Figure 2.[3] The comparative values for **4** are −2901 and −2916 ppm ($\Delta\delta$ = 15 ppm). The low field ^{1}H NMR spectrum for **3** (9.5−5.5 ppm) in d_7-DMF shows that the unique secondary dien-NH amine proton in the dien moiety, and *trans* to the 1-MeCyt ligand, exhibits a broad doublet where the signals are of unequal intensity, with coalescence of the signal occurring at 40 °C (Supporting Information). A further

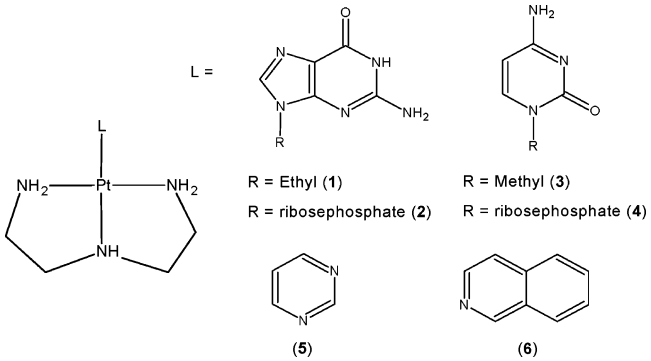

Figure 1. Schematic structures of complexes used in this study.

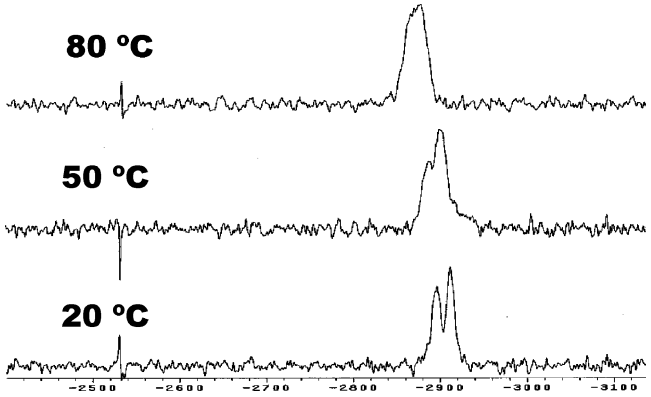

Figure 2. Coalescence of ^{195}Pt NMR spectra for $[Pt(dien)(1\text{-MeCyt})]^{2+}$.

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(1) (a) Hélène, C.; Lancelot, G. *Prog. Biophys. Mol. Biol.* **1982**, *39*, 1. (b) Hamilton, A. D.; Phillips, S. E. V.; Moras, D. *Curr. Opin. Struct. Biol.* **1993**, *3*, 11. (c) Nowakowski, J.; Shim, P. J.; Prasad, G. S.; Stout, C. D.; Joyce, G. F. *Nat. Struct. Biol.* **1999**, *6*, 151. (d) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210.

(2) (a) Ishida, T.; Ueda, H.; Segawa, K.; Doi, M.; Inoue, M. *Arch. Biochem. Biophys.* **1990**, *278*, 217. (b) Ishida, T.; Tarui, M.; In, Y.; Ogiyama, M.; Doi, M.; Inoue, M. *FEBS Lett.* **1993**, *333*, 214. (c) Kawai, H.; Tarui, M.; Doi, M.; Ishida, T. *FEBS Lett.* **1995**, *370*, 193.

(3) (a) Hitchcock, A. P.; Lock, C. J. L.; Pratt, W. M. C.; Lippert, B. In *Platinum, gold and other chemotherapeutic agents: chemistry and biochemistry*; Lippard, S. J., Ed.; American Chemical Society: Washington, DC, 1983; p 209. (b) Brüning, W.; Ascaso, I.; Freisinger, E.; Sabat, M.; Lippert, B. *Inorg. Chim. Acta* **2002**, *339*, 400.

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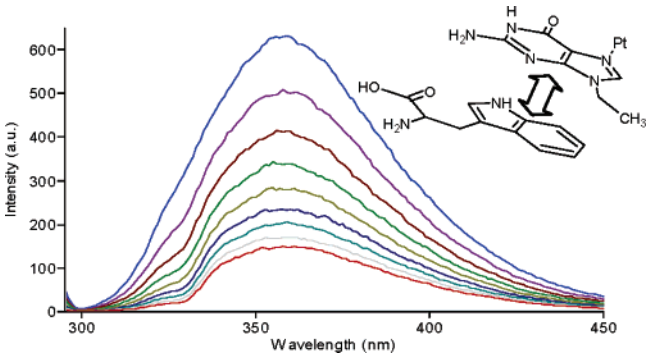

Figure 3. Quenching observed in Trp fluorescence spectrum upon increasing concentration of [Pt(dien)(9-EtGH)].

feature found in the ^{1}H NMR spectrum of **3** is the already reported chemical inequivalence among the C(4)−NH_2 protons in 1-MeCyt, 8.85 and 8.42 ppm, due to hindered rotation.[4]

These protons also show a marked acidity in comparison to the free 1-MeCyt with a large shift ($\Delta\delta \approx 2$ ppm) exhibited upon platination. Similar shifts are observed for the 5′-CMP analogue. The coupling constant ^3J (H5, H6) does not change significantly upon coordination; both **3** and **4** present values of 7−8 Hz. An explanation for the presence of rotamers is given from the X-ray crystal structure for the analogous complex [Pd(dien)Cytosine]$^{2+}$, where the cytosine plane is found almost perpendicular to the chelating dien ligand.[3b] The presence of rotamers is also in agreement with results reported for [Pt(dien)adenosine]$^{2+}$. In the present case, the energy barrier required for coalescence of the dien-NH signal appears to be greater (25 vs 40 °C for the adenosine and 1-methylcytosine respectively), probably because the amino group in the adenosine is farther from the dien-NH_2 protons than in the case of Cyt.[5]

Fluorescence spectroscopy can be used with high sensitivity to monitor small changes occurring on the π-cloud of the l-tryptophan indole ring due to noncovalent interactions, and the degree of quenching in the Trp spectrum is an estimate of the strength of the $\pi-\pi$ stacking interactions occurring (Figure 3).[6] Association constants (K_a's) were obtained for the alkylated nucleobases (9-EtGH and 1-MeCyt) and the corresponding nucleotides (5′-GMP and 5′-CMP) with solutions of Trp. On average, the values obtained from Eadie−Hofstee plots were approximately 3.0×10^3 M^{-1} for all nucleobases, except for 1-MeCyt which exhibited a significantly higher value of 6.0×10^3 M^{-1}. The association constants for complexes **1**−**4** revealed significantly higher values for the platinated nucleobases in all cases, Table 1. In general, the results obtained were very similar for complexes **1**, **2**, and **4** (the average increase upon platination was $\Delta K_a = 3.7 \times 10^3$ M^{-1}) showing that the presence of the ribose monophosphate moiety does not affect considerably the stacking interaction, at least in this simple system. These results are somewhat different from those reported for [Pt(dien)Cl] and 9-EtGH and 5′-GMP, but it is important to note that only in the 9-EtGH case was there a well-defined complex studied.[7] Complex **3** exhibited the overall highest association constant, albeit with a somewhat lower increase ($\Delta K_a = 2.7 \times 10^3$ M^{-1}) compared to the free base. ^{1}H NMR studies of **3**/*N*-acetyltryptophan (*N*-AcTrp) systems allowed assignments of chemical shift changes induced by the $\pi-\pi$ stacking interactions (Figure 4). Upfield chemical shifts have been observed for *trans*-[Cp*Rh(η^1(N3)-1-methylcytosine)-(μ-OH)]$_2$(OTf)$_2$ involving both 1-MeCyt and Trp in a noncovalent interaction.[8]

Table 1. Association Constants for Nucleobases and Nucleotides Used in This Study with Tryptophan[a]

system studied[a]	$K_a \times 10^3$ M^{-1}	SD
9-EtGH + Trp (1 + Trp)	3.3 (6.8)	0.1 (0.3)
5′-GMP + Trp (2 + Trp)	3.1 (6.9)	0.3 (0.2)
1-MeCyt + Trp (3 + Trp)	6.0 (8.8)	0.1 (0.2)
5′-CMP + Trp (4 + Trp)	3.1 (7.0)	0.1 (0.5)
3 + HSA (pH 5.2)	8.6	0.6

[a] Values in parentheses are for the corresponding platinated base.

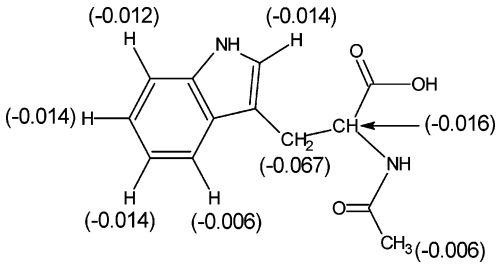

Figure 4. Upfield chemical shifts observed in *N*-acetyltryptophan protons after titration with complex **3** ($\Delta\delta = \pm 0.001$ ppm).

The importance of the "natural" nucleic acid bases in the stacking interaction was further assessed by examination of two model compounds [Pt(dien)(pyrimidine)$^{2+}$] (**5**) (lacking the exocyclic substituents of cytosine) and [Pt(dien)(isoquinoline)]$^{2+}$ (**6**) (containing a planar heterocycle). In neither case was any significant enhancement seen over the free ligands (data not shown), even at comparatively high concentrations. Contribution to $\pi-\pi$ stacking interactions from the cytosine functional groups C2-carbonyl and C4-amine could further decrease π−acceptor LUMO energy or play an important role by themselves in steric and H-bonding interactions.

The enhancement of $\pi-\pi$ stacking interactions, and especially the high K_a associated with the 1-MeCyt system, may have interesting applications. Recently, the recognition between the viral nucleocapsid protein (NCp7) of HIV-1 to single strand RNA and DNA has been shown to involve tryptohan(phenylalanine)/guanine(cytosine) stacking interactions.[9] The understanding of the protein/RNA(DNA) recog-

(4) Sartori, D. A.; Miller, B.; Bierbach, U.; Farrell, N. *J. Biol. Inorg. Chem.* **2000**, *5*, 575.
(5) Arpalathi, J.; Klika, K. D.; Sillampää, R.; Kivekäs, R. *J. Chem. Soc., Dalton Trans.* **1998**, *8*, 1397.
(6) (a) Pokalsky, C.; Wick, P.; Harms, E.; Lytle, F. E.; Van Etten, R. L. *J. Biol. Chem.* **1995**, *270*, 3809. (b) Hotopp, J. C. D.; Auchtung, T. A.; Hogan, D. A.; Hausinger, R. P. *J. Inorg. Biochem.* **2003**, *93*, 66.
(7) Kawai, H.; Tarui, M.; Doi, M.; Ishida, T. *FEBS Lett.* **1995**, *370*, 193.
(8) Elduque, A.; Carmona, D.; Oro, L. A.; Eisenstein, M.; Fish, R. H. *J. Organomet. Chem.* **2003**, *668*, 123.
(9) (a) De Guzman, R. N.; Wu, Z. R.; Stalling, C. C.; Pappalardo, L.; Borer, P. N.; Summers, M. F. *Science* **1998**, *279*, 384. (b) Morellet, N.; Déméné, H.; Teilleux, V.; Huynh-Dinh, T.; De Rocquigny, H.; Fournié-Zaluski, M. C.; Roques, B. P. *J. Mol. Biol.* **1998**, *283*, 419.

nition is important in this case for a better understanding of how to rationally design selective Zn-finger inhibitors (usually acting through active-site Zn ejection), a target of increasing interest in antiviral therapy.[10] In parallel, the potential for simple platinum complexes to eject zinc from zinc-finger environments has been demonstrated.[11] Complexes of general formula [*SP-4-2*]-[PtCl(nucleobase)(NH_3)-(L)]$^+$, where L = a planar heterocyclic ligand isoquinoline, quinoline, etc., have some anti-HIV activity and high specificity toward S-donor ligands, and Zn is displaced from the NCp7 C-terminal finger.[4b] The complex [*SP-4-2*]-[PtCl-(NH_3)(9-EtGH)(quinoline)] gave a K_a of 12.9 $\times$ 10^3 M^{-1} (at 20 °C), and in this case, the Pt(9-EtGH)/Trp interaction may be considered to serve as a template for specific recognition: sequence-selective targeting of zinc-fingers would be feasible when the platinated nucleobase is incorporated into consensus oligonucleotide strands.[12]

The applicability of complex **3** as a probe for the protein environment of l-tryptophan was studied. The 66 kDa protein human serum albumin (HSA) has one Trp residue on position 214 which has been used extensively to study conformational changes in the protein upon binding events to a host of ligands including metal complexes and organic drugs.[13] The fluorescence quenching of HSA by **3** leads to a blue shift from 350 to 344 nm with a calculated association constant of 8.6(6) $\times$ 10^3 M^{-1}, very close to that for the Trp-only system. The binding constant reported for *cis*-DDP and HSA is an order of magnitude smaller, 0.85 $\times$ 10^3 M^{-1}.[14] The K_a value and the instant quenching upon interaction of complex **3** with HSA suggest a direct access to the Trp residue within the hydrophobic pocket in the protein's IIA subdomain, one of the principal regions for ligand binding sites in HSA. It is possible that small, diffusible molecules such as **3** can find further use as probes for reporter tryptophan in proteins and protein−DNA interactions.

In a broad sense, bioinorganic chemistry is the application and use of intrinsic properties of metal complexes (redox properties, substitution kinetics, ligand donor preferences, and steric effects) to effect biological responses. The above results suggest further systematic manipulation of metal−ligand properties may serve as a motif for molecular recognition in protein−nucleic acid complexes.

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Supporting Information Available: Experimental section, synthesis, and characterization of complexes **1**−**6**, spectroscopic evidence of rotamers in complexes **3** and **4**, and fluorescence quenching experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) (a) Rein, A.; Henderson, L. E.; Levin, J. G. *Trends Biochem. Sci.* **1998**, *23*, 297. (b) Stephen, A. G.; Rein, A.; Fisher, R. J.; Shoemaker, R. H. *Current Drug Discov.* Aug 2003, p 33.

(11) (a) Darlix, J. L.; Gabus, C.; Nugeyre, M. T.; Clavel, F.; Barre-Sinoussi, F. *J. Mol. Biol.* **1990**, *216*, 689. (b) Volckova, E.; Evanics, F.; Yang, W. W.; Bose, R. N. *Chem Commun.* **2003**, *10*, 1128.

(12) Louie, A. Y.; Meade, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6663.

(13) Espósito, B. P.; Najjar, R. *Coord. Chem. Rev.* **2002**, *232*, 137.

(14) Neault, J. F.; Tajmir-Riahi, H. A. *Biochim. Biophys. Acta* **1998**, *1384*, 153.