Guanidyl Groups: New Metal-Binding Ligands in Biomolecules. Reactions of Chloro(2,2′:6′,2″-terpyridine)platinum(II) with Arginine in Two Cytochromes *c* and with Other Guanidyl Ligands

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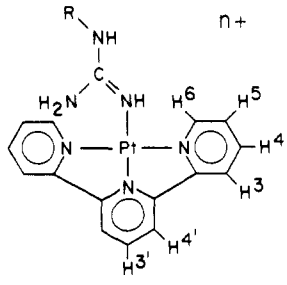
The recognized functions of arginine (Arg) residues in proteins—binding of cofactors and anions—involve electrostatic attraction to the guanidinium cation.[1,2] Although transition metals are common in metalloproteins and in metal-activated enzymes, their covalent binding to Arg side chain has not been proposed. Indeed, metal–guanidyl complexes are barely known.[3] The precedent reported herein shows that Arg can bind metals, and that inorganic complexes hold promise as heavy-atom and spectroscopic tags for Arg.[4,5]

Compound $[Pt(trpy)Cl]^+$ reacts readily and noninvasively with His residues at pH 5.0. The yield of $[Pt(trpy)His]^{2+}$ depends on the accessibility and the pK value of the imidazole.[6,7] The tag is stable, and its strong UV–vis bands are characteristic of His and of its environment.[6,7] Cytochromes *c* from horse and tuna were incubated with $[Pt(trpy)Cl]^+$ at pH 7.0 as they were at pH 5.0, and the products were separated efficiently on CM52 cellulose and identified, as before.[6] The derivatives tagged at His 26 and His 33 in the horse protein and at His 26 in the tuna protein (which lacks His 33) formed at pH 7.0 as they did at pH 5.0.[6] The following additional derivatives formed at pH 7.0: a singly-tagged, a doubly-tagged, and a triply-tagged of the horse protein and a singly-tagged and a doubly-tagged of the tuna protein. The covalently bonded cationic tags remained after the cation-exchange chromatography, and the elution order was consistent with overall charges and charge distributions.[6,7] The increase in the incubation pH from 5.0 to 7.0 evidently created (by deprotonation) a new binding site. Difference UV–vis spectra, recorded as before,[6,7] showed it to be the same in all the new derivatives.[8]

The binding site was identified indirectly, but conclusively, by control experiments and structural considerations. Mixtures of $[Pt(trpy)Cl]^+$ with every amino acid containing heteroatoms in the side chain were monitored at pH 7.0 by UV–vis or ^{1}H NMR spectroscopy. Amino acids Lys, Trp, Asp, Asn, Glu, Gln, Pro, Thr, Ser, Tyr, and Met and their short peptides did not react even after long incubation; Cys and His reacted readily, but the horse and tuna cytochromes *c* lack free Cys; and Arg reacted upon standing or heating. The new ligand must be accessible in both horse and tuna cytochromes *c*, and there must be few such ligands (because only one reacts). Only three residues—Tyr 74, Tyr 97, and Arg 91—satisfy both requirements.[9,10] Further tests, with heating, confirmed the unreactivity of Tyr (as well as of Gly and Lys) and reactivity of Arg. Guanidine (Gua), methylguanidine (MeGua), and *N*-acetylarginine (AcArg) yielded homologous

Chart I



R	*n*	L	pK^a
H	2	Gua	13.5
CH_3	2	MeGua	13.5
$(CH_2)_3CH(NH_3)(COO)$	2	Arg	12.5
$(CH_2)_3CH[CH_3C(O)NH](COO)^-$	1	AcArg	12.5
$O(CH_2)_2CH(NH_3)(COO)$	2	Can	7.0

[a] For guanidine in the free ligands.

complexes (Chart I),[11] whose UV–vis spectra confirmed guanidyl coordination in the new protein derivatives.[8,12] The aforementioned new derivatives contain $Pt(trpy)^{2+}$ tags at the following residues: at 91, at 33 and 91, and at 26, 33, and 91 in the horse protein; at 91 and at 26 and 91 in the tuna protein.

Why Arg 91 reacts at pH 7.0 despite the guanidine basicity is evident from the cytochrome *c* structure.[9,10] Barely exposed on the surface (hence its low labeling yield of 10%), Arg 91 abuts the N-terminus of the α-helical segment 92–102. Both the helical macrodipole and the hydrophobic environment lower its pK value.[13–17] Indeed, canavanine (Can), whose pK is 7.0,[18] formed $[Pt(trpy)(Can)]^{2+}$ readily under the conditions of protein labeling. This model complex was characterized by UV–vis spectrophotometric titration and by ^{1}H NMR and IR spectroscopy. The conveniently separate aromatic and aliphatic ^{1}H signals showed the trpy and Can moieties in the 1:1 ratio; the former were shifted downfield by 0.04–0.10 ppm, whereas the latter were unperturbed, a proof of the guanidyl coordination.[19]

This report of Pt(II) binding to Arg in proteins and a previous report of the bioactive metals Cu(II), Co(II), Zn(II), and Cr(III) binding to tetramethylguanidine[20] together demonstrate that Arg side chain is a potential ligand. Most likely to bind metals is a guanidyl group whose basicity is diminished owing to its environment; for the binding to occur on the protein surface, this group should also be accessible. Since guanidine probably is a π acceptor, its coordination may be facilitated if other ligands bonded to the

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(8) For example, the horse cytochrome *c* in 85 mM phosphate buffer at pH 7.0 (λ_{max} in nm, ϵ_{max} in mM^{-1} cm^{-1}). Singly-tagged minus native: 345, 10; 327, 11; 277, 25; and 249, 30. Triply-tagged minus doubly-tagged (at His 33 and His 26): 346, 12; 330, 12; 275, 29; and 244, 29.
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(11) The reactions, understandably, are slow without heating (30–80 °C), or basicity (pH 8.5–10.5), or both. The yellow $[Pt(trpy)L]^{2+}$ and the red $[Pt(trpy)]_2(\mu_2\text{-}L)^{4+}$ are separated on the CM52 cellulose. The presence of guanidyl ligands L in them was proved by UV–vis spectrophotometry and by thin-layer chromatography; acid-cleaved complexes and the free compounds L gave identical ninhydrin reactions and R_f values.
(12) For example, $[Pt(trpy)(MeGua)]Cl_2$ in 0.10 M phosphate buffer at pH 8.0 (λ_{max} in nm, ϵ_{max} in mM^{-1} cm^{-1}): 345, 10; 329, 12; 277, 27; and 247, 34.
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(19) The ^{1}H NMR spectrum (ppm downfield from DSS) of $[Pt(trpy)(Can)]Cl_2$ in 0.10 M phosphate buffer in D_2O at the (uncorrected) pH of 8.0: 2.26, q, β-CH_2; 3.88, t, α-CH; 4.09, m, γ-CH_2; 7.25, t, H^5; 7.60, d, H^6; 7.80, d, $H^{3,3'}$; 7.97, dd, H^4; and 8.00, t, $H^{4'}$. The IR spectrum of $[Pt(trpy)(Can)](BPh_4)_2$ contains the amino, carboxyl, and guanidyl bands, which are absent from the spectrum of $[Pt(trpy)Cl]BPh_4$. For the Can complex in Nujol (cm^{-1}): 3450 (s) and 3350 (s), NH; 3088 (s), aromatic CH; 1640 (sh), COO^-; 1607 (s) and 1580 (s), C=N and C=C; 1261 (w) and 1250 (w), C–N; 773 (s), 746 (s), and 716 (s), CH in Ph; 627 (m), 612 (m), and 602 (m), trpy; 514 (vw) and 464 (w), Pt–N; and 489 (w), Pt–py.
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same metal are π donors. Therefore Arg is particularly likely to bind metals in conjunction with Cys or Tyr. Our research on guanidyl complexes and on new heavy-atom tags for Arg residues continues.

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Supplementary Material Available: Attempts at direct identification of the Arg binding site by peptide mapping and by blocking, lability of the $[Pt(trpy)L]^{n+}$ complexes in acid, and ^{13}C NMR evidence for the Arg coordination via its guanidyl group (4 pages). Ordering information is given on any current masthead page.

Calculations of the Geometric and Electronic Structure of Trichloromethyltitanium: Is There an Agostic Hydrogen Interaction?

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A complete geometry optimization on Cl_3TiCH_3 shows no flattening of the methyl hydrogens toward the metal and no titanium–hydrogen interaction. From the optimized geometry of calculations at several different wave functions, we predict a Ti–C–H bond angle of 107 ± 1° which is significantly larger than the electron diffraction (ED) result of 101 ± 2°. The anomalously low methyl rock frequency of the titanium complex in comparison to the germanium complex is correctly predicted by the full second derivatives of the energy and is shown to be due to titanium's empty d-orbitals, which allow rehybridization of the carbon–metal bond during the rocking motion. The naturally weak scattering by hydrogen atoms, which hinders accurate determination of hydrogen positions, could account for the observed difference between the ED and theoretical Ti–C–H angle.

A recent preliminary communication on the ED[1] of Cl_3TiCH_3 suggests that the methyl hydrogens are symmetrically "flattened" away from a normal tetrahedral geometry by 8.5° (2.2°), which was interpreted as evidence of an agostic hydrogen interaction between the methyl hydrogens and titanium. Spectroscopic data show a very low-energy methyl rocking vibration for Cl_3TiCH_3, in comparison to Cl_3GeCH_3, which Berry et al.[1] interpreted as the result of hydrogen flattening. Eisenstein and Jean[2] using extended Huckel calculations found that rocking the methyl in both staggered and eclipsed conformations of H_3TiCH_3 is weakly destabilizing. Here, we report the results of Hartree–Fock–Roothaan (HFR) calculations which were done to determine if there is a large degree of flattening of the three hydrogens and if not, why there is a large difference in methyl rocking vibrational frequency between Cl_3TiCH_3 and Cl_3GeCH_3.

We optimized the geometry of Cl_3TiCH_3 by using four different basis sets. Basis set I is a "double ζ" modified Huzinaga basis set used in previous geometry optimizations.[3] Basis sets II–IV have the same Ti basis, but different basis for Cl, C, and H. The Ti basis is a (5333-53-5)[4] modified to a (533211-5211-3111) by splitting off the most diffuse s, p, and d functions and adding additional s, p, and d functions with exponent values one third the value of the most diffuse functions. Basis set II is Cl-(5321-521), C(331-31), and H(31)[4], basis set III is Cl(531111-4211), C(721-41), and H(31),[5] and basis set IV is basis set II with polarization functions[4] added. The geometry optimizations were done in C_{3v} symmetry in a staggered conformation. Basis set I was used for all calculations on H_3TiCH_3 and H_3GeCH_3. The generalized valence bond (GVB) calculations involved perfect-pairing for all seven σ bonds.[6]

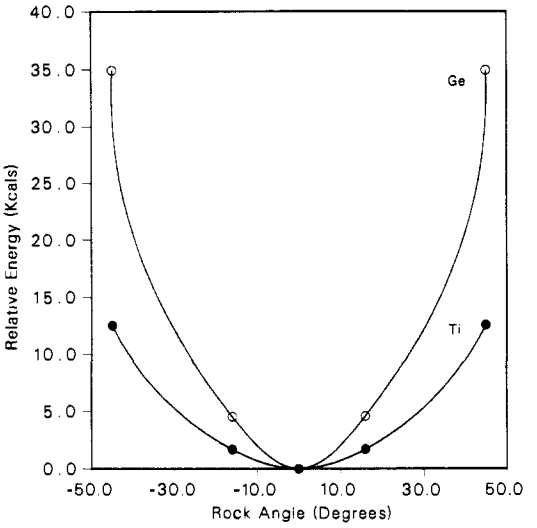

Figure 1. Relative energy of H_3TiCH_3 and H_3GeCH_3 for the methyl rock.

The results of complete geometry optimizations on Cl_3TiCH_3 using basis sets I–IV gave calculated bond lengths and bond angles which varied slightly (see Table I). The addition of polarization functions to basis set II (basis set IV) shortened the Ti–Cl distance and increased the Cl–Ti–C angle closer to the ED values; however, the Ti–C–H angle did not change. The calculated bond lengths from the GVB optimizations are larger than the SCF bond lengths, which is expected for this level of electron correlation. As a result of the long Ti–C distance, the $TiCl_3$ and CH_3 moieties favor a more radical-like flattened geometry as seen by the decrease of the Ti–C–H and C–Ti–Cl angles. However, when the Ti–C, Ti–Cl, and C–Ti–Cl parameters are fixed at the ED values, the Ti–C–H angle increased by 2.7°. Further calculations show that the major parameter influencing the Ti–C–H angle is the Ti–C bond distance. If any hydrogen flattening were due to direct titanium–hydrogen interactions, one would expect the Ti–C–H angle to increase as the Ti–C bond lengthened; however, the GVB results showed the opposite; thus, we conclude there to be no direct interaction between Ti and H.

The frequency of the methyl rock for Cl_3TiCH_3 in comparison to the analogous frequency in Cl_3GeCH_3 is anomalously low. Berry et al.[1] presumed this anomalously low frequency to result from flattening of the hydrogens. After optimizing the geometries of the model complexes H_3TiCH_3 and H_3GeCH_3, we calculated the vibrational frequencies of each complex (see Table II) by taking finite differences of energy gradients. Although these absolute frequencies show the error expected of results at the HFR level, comparison of the change in frequency when changing the metal from Ge to Ti are in good agreement with the experimental values. The calculated difference of the methyl rocking modes between the germanium and titanium hydride complexes of 401 cm^{-1} is much larger than the differences of other modes which was the observation made by Berry et al. for the germanium and

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