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Communication

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Synthesis and Characterization of a Chromium(V) *cis*-1,2-Cyclohexanediolato Complex: A Model of Reactive Intermediates in Chromium-Induced Cancers

Ruben Bartholomäus,[†,‡] Klaus Harms,[†] Aviva Levina,[‡] and Peter A. Lay*[,‡]

[†]Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein Strasse, D-35032 Marburg, Germany

[‡]School of Chemistry, The University of Sydney, Sydney NSW 2006, Australia

Supporting Information

ABSTRACT: Stabilization of chromium(V) by biological 1,2-diolato ligands, e.g., carbohydrates and glycoproteins, is postulated to be crucial for both the chromium(VI)-induced carcinogenicity and chromium(III) antidiabetic activities. Close structural mimics of biologically relevant chromium(V) 1,2-diolato complexes, $M[Cr^{V}O(chd)_2]$ [chd = *cis*-1,2-cyclohexanediolato(2−)], were synthesized and characterized by X-ray crystallography (M = Na) or by spectroscopic techniques, including X-ray absorption spectroscopy (M = K). This is the first structurally characterized chromium(V) complex with a cyclic diolato ligand.

The toxicity and carcinogenicity of chromium(VI) [Cr(VI)][1] and the controversial role of chromium(III) [Cr(III)] as an antidiabetic agent[2] were traditionally regarded as independent biological activities. However, recent evidence suggests that they both arise from varying amounts of Cr(VI) and reactive intermediates, e.g., chromium(V) [Cr(V)] species, which can be formed in biological systems either by the reduction of Cr(VI) or by the oxidation of Cr(III).[3] These reactive Cr(V) intermediates are stabilized by a variety of biological ligands, predominantly the highly abundant 1,2-diols (carbohydrates, sialic acids, and glycoproteins).[3−5] In particular, steady-state Cr(V) diolato species have been detected in single cells and in multicellular organisms (including live mice and rats) treated with Cr(VI)[6,7] and in cultured adipocytes (fat cells) treated with an antidiabetic Cr(III) complex.[8] Previous electron paramagnetic resonance (EPR) studies of Cr(V) complexes with carbohydrates and model 1,2-diols have centered around their generation in solutions [typically, by the reactions of Cr(VI) with thiol reductants in the presence of large excesses of diols].[4,5,7] Their isolation in a pure form was not previously considered feasible since they are reactive intermediates.[4,5,7] In the current work, the photocatalyzed reduction of Cr(VI) by *cis*-1,2-cyclohexanediol [$chdH_2$; a close structural mimic of Cr(V)-binding carbohydrates][4] in nonaqueous media was used for the first high-yield isolation and structural characterization of a Cr(V) complex with a cyclic diolato ligand. Because of the high reactivity of most Cr(V) complexes, their crystal structures are scarce.[9] The only known structure of a Cr(V) complex with 1,2-diol has been obtained with the unreactive hexafluoropinacol.[10]

The Cr(V) complexes $Na[Cr^{V}O(chd)_2]\cdot DMF$ (**1a**, single crystals) and $K[Cr^{V}O(chd)_2]$ (**1b**, powder) were isolated from the reactions of $M_2Cr_2O_7$ (M = Na and K) with slight molar excesses of $chdH_2$ in *N*,*N*-dimethylformamide (DMF) at 295 K under ambient light [see the Supporting Information (SI) for details of their syntheses and characterizations]. The reactions leading to the isolation of **1a** and **1b** had many features in common with the previously described Cr(VI) reactions with biologically relevant reductants, including thiols,[5] amino acids,[11] peptides,[12] and hydroxamic acids.[13] First, of all of the common solvents, the best yields of Cr(V) intermediates were obtained in DMF.[5,13] Second, the reactions of Cr(VI) with $chdH_2$ in DMF solutions led to a color change from yellow to orange and then to dark-green[5,13] due to the formation of $[Cr^{VI}(O)_3(chdH)]^-$ and $[Cr^{V}O(chd)_2]^-$ [identified by electrospray mass spectrometry (ESMS), m/z −214.1 and −296.2, respectively, in negative-ion mode]. Formation of the green Cr(V) species was relatively slow (hour time scale)[13] and was markedly catalyzed by fluorescent light.[11,12] Electronic absorption and EPR spectroscopies showed $[Cr^{V}O(chd)_2]^-$ was stable in solutions for several days under ambient conditions.

The crystal structure of **1a** (Figure 1; see the CIF file in the SI for details) showed a Cr(V) geometry that is intermediate between square-pyramidal and trigonal-bipyramidal (axial O1 and O8 donors),[9,10,14] with one oxido and two diolato ligands. The bond lengths, 1.55 Å for Cr^{V}−oxido and 1.85−1.89 Å for Cr^{V}−diolato (Figure 1), were typical for Cr(V) complexes[9,10,14] with a bond order of 3 (σ and two π bonds) and greater than 1 (σ and shared π bonds), respectively.[15,16] The Na^+ ions were coordinated to the deprotonated diolato ligands (Cr^{V}−Na^+ distance, 3.35 Å), as well as to the carbonyl donors of the DMF molecules, leading to a chain arrangement of the $[Cr^{V}O(chd)_2]^-$ moieties (Figure 1).

Complex **1b**, isolated as a green solid from the reaction of $K_2Cr_2O_7$ with $chdH_2$ in DMF, was characterized by the following techniques (see the SI for details): (i) elemental analysis (corresponding to the formula $K[Cr^{V}O(chd)_2]$); (ii) the magnetic moment (μ_{eff} = 2.03 μ_B) that is typical for monomeric Cr(V) complexes;[13,14] (ii) ESMS (dominated by the $[Cr^{V}O(chd)_2]^-$ signal; Figure 2a and the S1); (iii) solid-state EPR spectroscopy at 77 K, showing a single sharp signal at $g \sim 1.980$, which is characteristic for monomeric Cr(V) complexes (Figure

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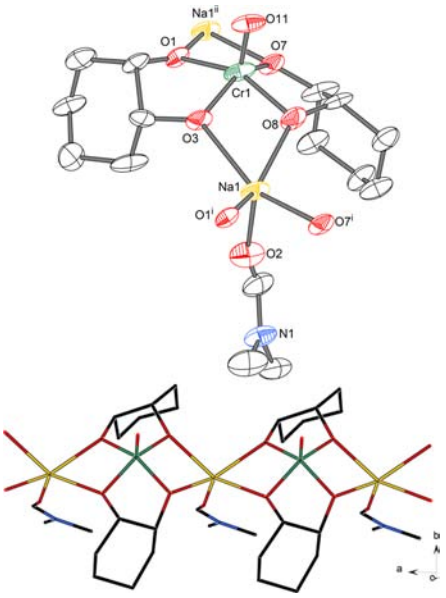

Figure 1. ORTEP depiction (showing 50% probability ellipsoids) of the crystal structure of **1a** and chain arrangement of cations and anions (see the CIF file in the SI for details).

2b);[7,12,13] (iv) electronic absorption spectroscopy, showing a characteristic d−d transition band of Cr(V) (Figure 2c; λ_{max} = 640 nm, $\varepsilon_{max} = 2.6 \times 10^2$ M^{-1} cm^{-1});[5,11−14] (v) X-ray absorption near-edge structure (XANES) spectroscopy, showing typical features of chromium(V) oxido complexes (such as a crystallographically characterized $Na[Cr^VO(ehba)_2]$, where ehba = 2-ethyl-2-hydroxybutanoato),[14,16] including a moderately high preedge absorbance peak (Figure 2d). Solution EPR spectra of **1b** in H_2O or DMF solutions ([Cr] ∼ 5 mM, 295 K, no added ligand; Figure S1 in the SI) were identical with those reported previously[4,17] for the Cr(V) complexes generated by the reduction of Cr(VI) by thiols in aqueous solutions in the presence of 100−1000-fold molar excesses of $chdH_2$. The EPR signals of **1b** were stable for hours in DMF solutions but disappeared within ∼10 min (295 K) in H_2O solutions (pH ∼ 6). These spectra have superhyperfine coupling to the ring protons and several overlapping signals from geometric isomers (Figure S1 in the SI).[4,7,17] Somewhat different g_{iso} values are observed in DMF and H_2O due to specific solvent interactions as discussed for $[Cr^VO(ehba)_2]^-$ previously.[18]

Multiple-scattering structural characterization of **1b** from extended X-ray absorption fine structure (EXAFS) (Figure 3 and Tables S1 and S2 in the SI),[19] used the crystal structure of **1a** (Figures 1 and S2 in the SI). The atomic coordinates of **1a** were left unchanged during the fitting (except for those of the K^+ ions; Figure 3a), which led to a good fit to the experimental data (solid red lines in Figure 3b,c; R = 19.2%; fits with R < 20% are considered acceptable).[19] The fitted Cr−K distances in **1b** [3.43(2) Å] were slightly longer than the Cr−Na distances in the crystal structure of **1a** [3.35(1) Å]. The addition of DMF molecules coordinated to K^+ ions to the EXAFS model (by analogy with **1a**, Figure 1) did not improve the fit because the atoms of the solvent molecule were more than 4.5 Å apart from the Cr center and their contributions to the fit were negligible. Furthermore, coordination of the solvent molecules in **1b** is not supported by elemental analyses (see the SI). Removal of K^+ from the EXAFS model (Figure 3a) led to a significant deterioration of the fit (R = 22.2%; dashed red lines in Figure

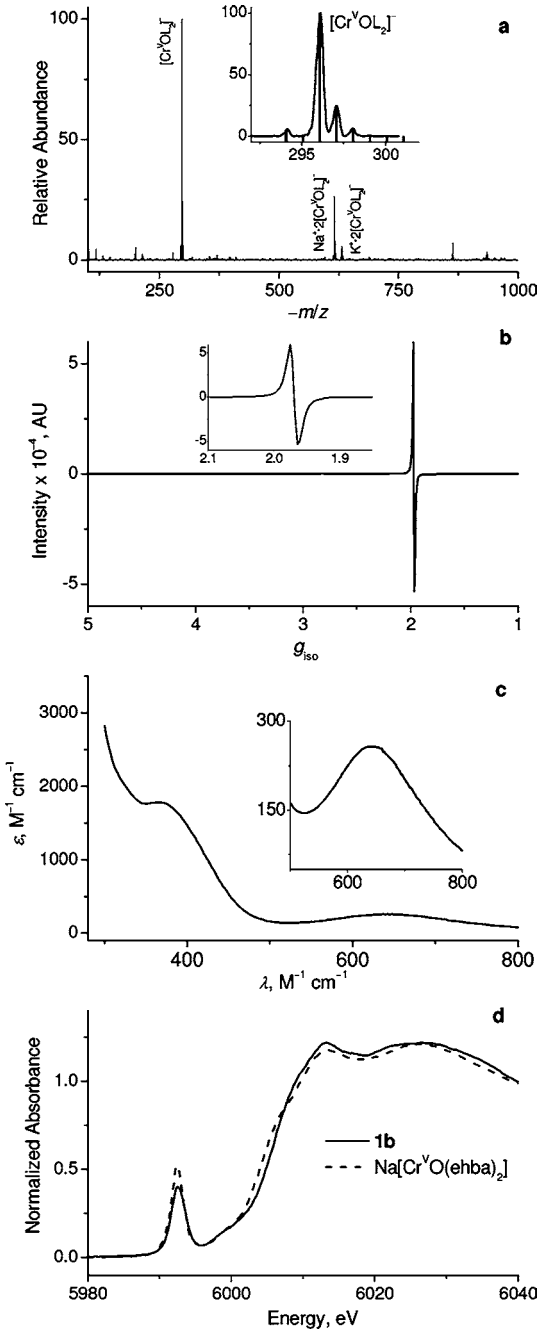

Figure 2. Spectroscopic characterization of complex **1b** (see also Figure S1 in the SI): (a) negative-ion ESMS data, solution in DMF, [Cr] = 1.0 mM, 295 K; (b) solid-state EPR spectrum (neat solid, 77 K); (c) electronic absorption spectrum of **1b** in a DMF solution, [Cr] = 1.0 mM, 295 K; (d) solid-state XANES spectra of **1b** and $Na[Cr^VO(ehba)_2]$ (mixtures with boron nitride, 10 K).[16]

3b,c). These data suggest that coordination of K^+ to the diolato ligands in **1b** is likely, although it cannot be established with certainty. In summary, the results of EXAFS analyses (Figure 3 and Tables S1 and S2 in the SI), together with the data of other spectroscopic techniques (Figure 2) and elemental analyses, show that the isolated bulk solid (**1b**) is essentially pure (≥95%) $K[Cr^VO(chd)_2]$, with the coordination environment of Cr(V) close to that described by the crystal structure of its Na^+ analogue (**1a**; Figure 1).

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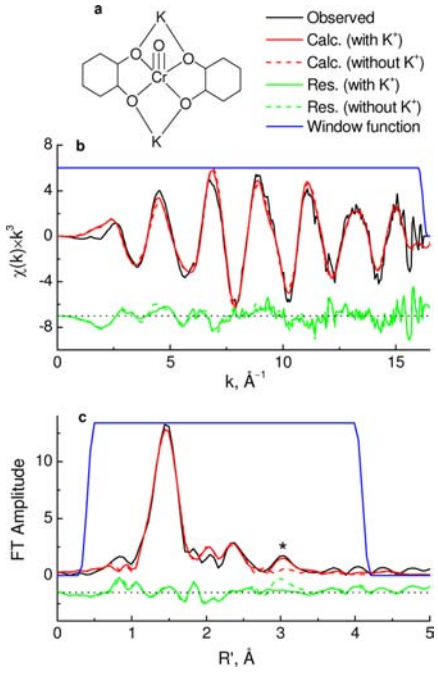

Figure 3. Results of multiple-scattering analysis of the XAFS of **1b** (solid mixture with boron nitride, 10 K): (a) proposed structure of **1b**, based on the crystal structure of **1a** (Figure 1); (b) observed and calculated XAFS spectrum of **1b**; (c) observed and calculated Fourier-transformed XAFS spectrum of **1b**. The dashed lines in parts b and c show the fits from which the K^+ ions were excluded (the main difference in the fits is indicated with an asterisk in part c). Details of the XAFS fitting are given in Tables S1 and S2 and Figure S2 in the SI.

The first definitive structure of a Cr(V) complex with a cyclic 1,2-diolato ligand adds confidence to the proposed structures of chromium(V) carbohydrato intermediates that are formed in biological systems exposed to carcinogenic Cr(VI), based on EPR spectroscopic studies.[3,4,7,17] Similar synthetic methods are being explored for the isolation and studies on the reactivities and DNA cleaving abilities of Cr(V) complexes with sugars.[20]

■ ASSOCIATED CONTENT

Supporting Information

Details of the experimental procedures and the equipment used, positive-ion ESMS and solution EPR spectroscopic data for **1b**, details of the multiple-scattering XAFS fitting for **1b**, and a CIF file for the crystal structure of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: peter.lay@sydney.edu.au. Tel: 61-2-9351 4269. Fax: 61-2-9351 4269.

Notes

The authors declare no competing financial interest.

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