Suppression by Vanadium(IV) of Chromium(V)-Mediated DNA Cleavage and Chromium(VI/V)-Induced Mutagenesis. Synthesis and Crystal Structure of the Vanadium(IV) Complex $(NH_4)[V(O)\{HOC(Et)_2COO\}\{OC(Et)_2COO\}]$‖

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Cr(V) is generated by reactions of Cr(VI) with intracellular reductants,[1-4] and Cr(V), but not Cr(III) or Cr(VI), rapidly causes DNA strand breaks in vitro.[1] Together with other results,[1] this points to Cr(V) being an intermediate in Cr(VI)-induced mutagenesis and carcinogenesis. In this context, V(IV) is of interest because it rapidly reduces Cr(V) to Cr(III),[5] and because it has similar ligand-exchange chemistry,[6] V(IV) is likely to compete with Cr(V) complexes for binding to DNA. Moreover, V(IV) and V(V) are strong inhibitors of both nuclease activity[7,8] and the formation of cancers in mammalian systems.[9,10] Reported here are the effects of V(IV) on Cr(V)-induced DNA cleavage and on the frequency of Cr-induced reversion mutations in bacteria. The crystal structure of ammonium (2-ethyl-2-hydroxybutanoato(1–))(2-ethyl-2-hydroxybutanoato(2–))oxovanadate(IV), $NH_4[V(O)(ehbaH)(ehba)]$, used in these studies is also discussed.

$Na_2[V(O)(ehba)_2]\cdot 0.5[Na(ehbaH)]\cdot 3.5H_2O$ (I), obtained from $VOSO_4\cdot 5H_2O$ and Na(ehbaH) in basic aqueous acetone, was recrystallized from saturated aqueous NH_4Cl as $(NH_4)[V(O)(ehbaH)(ehba)]\cdot 0.5NH_4Cl$ (II).[11] Slow evaporation of an aqueous solution of II under Ar yielded crystals of $(NH_4)[V(O)(ehbaH)(ehba)]$ (III). III exhibits strong IR bands at 1563, 980, and 952 cm^{-1} due to ν_{CO}(carboxylate, ν_{as}(V=O), and ν_{CO}(alkanolate),[12-14] respectively. The anion of III[15] (Figure 1) has protonated (ehbaH) and deprotonated (ehba) ligands, with

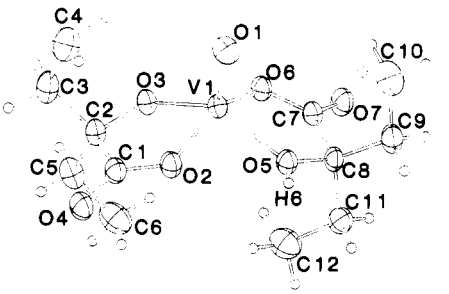

Figure 1. ORTEP diagram of the anion of $(NH_4)[V(O)(ehbaH)(ehba)]$, showing the atom-labeling. Selected bond lengths (Å) and angles (deg) are as follows: O(1)–V(1) = 1.594 (3), O(2)–V(1) = 1.953 (2), O(3)–V(1) = 1.882 (2), O(5)–V(1) = 1.987 (2), O(6)–V(1) = 1.977 (2), C(1)–O(2) = 1.296 (4), C(2)–O(3) = 1.419 (4), C(1)–O(4) = 1.218 (4), C(2)–C(1) = 1.538 (5); O(2)–V(1)–O(1) = 104.1 (1), O(3)–V(1)–O(1) = 113.0 (1), O(3)–V(1)–O(2) = 82.2 (1), O(5)–V(1)–O(1) = 110.7 (1), O(5)–V(1)–O(2) = 87.8 (1), O(5)–V(1)–O(3) = 136.3 (1), O(6)–V(1)–O(1) = 107.9 (1), O(6)–V(1)–O(2) = 147.8 (1), O(6)–V(1)–O(3) = 88.5 (1).

longer V–O bonds than analogous bonds in isoelectronic $[Cr(O)(ehba)_2]^-$ [16] but comparable with other V(IV) complexes.[17,18] The trans isomer is expected due to steric demands,[16,18] and the distorted square pyramid geometry is common with V(IV).[17,18] The protonated alcohol group forms a much weaker V–O bond (1.987 (2) Å) than does the deprotonated alcohol

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‖ The views expressed in this article are those of the authors and do not necessarily reflect those of the National Occupational Health and Safety Commission.

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(15) $(NH_4)[V(O)(ehbaH)(ehba)]$, $C_{12}H_{29}NO_7V$, MW = 350.3, monoclinic, space group $P2_1/c$, a = 11.320 (1) Å, b = 14.032 (5) Å, c = 12.910 (5) Å, β = 109.51 (2)°, U = 1932.99 Å^3, D_c (Z = 4) = 1.204 g cm^{-3}, $F(000)$ = 912, μ_{Mo} = 9.54 cm^{-1}. Range of hkl –13 to 11, 0 to 15, and 0 to 15, R = 0.035, R_w = 0.039, residual extrema 0.27 and –0.20. Cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections. Data were measured on an Enraf-Nonius CAD4-F four-circle diffractometer employing Mo Kα radiation (0.7017 Å) and a graphite monochromator and operating in the ω–θ scan mode. Data were reduced and Lorentz, polarization and decomposition and absorption corrections were carried out using the Enraf-Nonius Structure Determination Package. Of the 3005 collected independent reflections not systematically absent, 2292 with $I > 2.5\sigma(I)$ were considered observed and used in solution of the structure. The structure was solved by direct methods and refined by a full-matrix least-squares analysis using SHELX 76 (Sheldrick, G. M. SHELX-76, A Program for X-Ray Crystal Structure Determination. University of Cambridge, England, 1976). Hydrogen atoms were included at calculated sites (C–H = 0.97 Å) and refined with isotropic thermal parameters, and all other atoms were refined anisotropically. Scattering factors and anomalous dispersion corrections for vanadium were taken from: Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, England, 1974; Vol. IV. For all others, the values supplied in SHELX 76 were used.
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(1.882 (2) Å), whereas the V–O(carboxylate) bond lengths (1.953 (2) and 1.977 (2) Å) are intermediate between these two extremes. Oxo/ehba and ehba/ehbaH contacts are close to van der Waals distances (C–O > 3.558, C–C > 3.830, and H–H > 2.51 Å). This is believed to contribute to the unusual stability of the V(IV), and related Cr(V),[16] Ru(V), and Os(V) five-coordinate complexes.[19,20]

The magnetic susceptibility (aqueous solution, Evans method,[21] 1.62 μ_B) of III is consistent with mononuclear V(IV). The UV/Vis spectrum of I (0.02 M) in 10-fold excess ligand buffer (0.2 M ehbaH/ehbaH$_2$, pH 3.5) has four d–d bands and one charge-transfer band (λ (nm)/ϵ (M^{-1} cm^{-1}): 240 (1226), 410 (30), 524 (26), 600 (24), 816 (17)). This solution oxidizes over a period of 7 d to form $(NH_4)_2[V(ehba)(O)_2]_2$, which is a structural model for the active sites of V proteins.[22] In 100-fold excess of ligand, the spectrum is similar, but the solution is more stable. A spectrophotometric titration (412 nm) yielded pK_a values of 3.2 and 4.1 (25 °C, I = 0.02 M, ligand buffer), due to consecutive deprotonations of $[V(O)(ehbaH)_2]$. This is consistent with observations with other bis(2-hydroxyacid) complexes[23,24] and inconsistent with deprotonations of aqua ligands, which occur at much higher pH values for V(IV) complexes.[25] The four-band spectrum is indicative of the predominance of the trans isomer in solution.[18,23] The characteristic eight-line EPR spectrum of I (room temperature, H_2O, X-band (9.431 GHz), g_{iso} = 1.973, A_{iso} = 86.83 G, based on second-order analysis) is typical of analogous bis(2-hydroxyacid)oxovanadate(IV) complexes.[23,24] The UV/vis spectra, the EPR results, the two pK_a values, the insensitivity of the spectral properties to excess ligand (at constant pH), and the fact that the same complex is formed when the two acac ligands of $[V(O)(acac)_2]$ are replaced by two ehba ligands (EPR, electrochemistry),[26] all show that the complex exists in solution as $[V(O)(ehba)_2]^{2-}$. This is consistent with the many detailed studies on the titrations of 2-hydroxyacid ligands with V(IV).[23,24] Therefore, the assumption that the ehba–V(IV) complex is a monochelate in solution[5,27] is incorrect and part of the mechanism of reduction of Cr(V) by V(IV)[5,27] needs to be reinterpreted. The nature of the solution structure is also important in interpreting the DNA cleavage experiments reported below.

$[Cr(O)(ehba)_2]^-$ (60 μM) was pretreated for various times with $VOSO_4 \cdot 5H_2O$ or $[V(O)(ehba)(ehbaH)]^-$ (0–60 μM) and then assayed for its ability to cleave DNA using techniques described previously[1] (Figure 2, [V] = [Cr] = 40 μM after dilution with DNA). While $[Cr(O)(ehba)_2]^-$ promotes cleavage of covalently-closed, supercoiled plasmid pUC9 DNA in vitro[1] (lanes 3–5), $VOSO_4 \cdot 5H_2O$ and $[V(O)(ehba)(ehbaH)]^-$ do not (lanes 6–9). At [V(IV)] = 40 μM and time 0, the extent of Cr(V)-induced DNA cleavage increased compared to Cr alone, but at longer preincubation times, the presence of V(IV) inhibited Cr-induced cleavage (lanes 10–17). $VOSO_4 \cdot 5H_2O$ was much more powerful at potentiating the DNA cleavage than $[V(O)(ehba)(ehbaH)]^-$ (lanes 10 and 14), but they have similar abilities

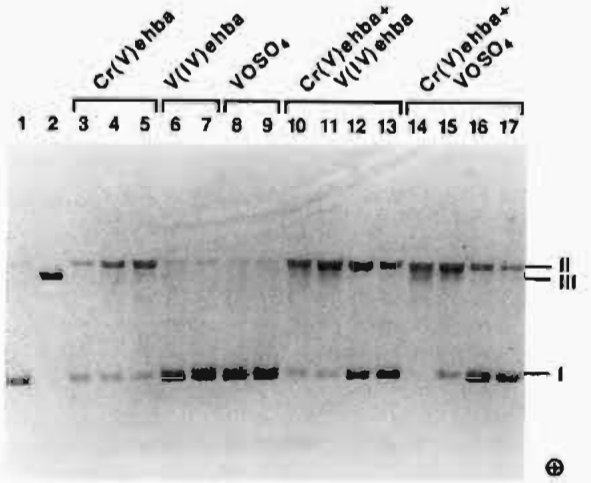

Figure 2. In vitro reactions of Cr(V) and V(IV) with covalently-closed circular plasmid pUC9 DNA at 37 °C, analyzed by agarose gel electrophoresis. Reactions were carried out in a 50 mM acetate buffer, pH 3.7.[1] They contained, in addition to DNA (lane 1, control), 40 μM $[Cr(O)(ehba)_2]^-$ (lanes 3–5), 40 μM $[V(O)(ehba)(ehbaH)]^-$ (lanes 6 and 7), or 40 μM $VOSO_4$ (lanes 8 and 9). Reactions were allowed to proceed for 5 min (lane 3), 40 min (lanes 4, 6, and 8), or 120 min (lanes 5, 7, and 9). For samples in the rightmost eight lanes, 60 μM $[Cr(O)(ehba)_2]^-$ was first reacted with 60 μM $[V(O)(ehba)(ehbaH)]^-$ (lanes 10–13) or 60 μM $VOSO_4$ (lanes 14–17), for 0, 5, 30, or 60 min, respectively. DNA was then added (reducing the concentrations of Cr and V to 40 μM), and the reactions were allowed to proceed for a further 30 min. Lane 2 contained pUC9 that had been linearized with restriction endonuclease *Hin*dIII. The distances of migration of DNA from the origin (top of figure) are indicated: I, covalently-closed DNA; II, relaxed (nicked) DNA; III, linear, unit length, double-stranded DNA. Electrophoresis in the presence of ethidium bromide and photography was as described,[1] except that the data are presented as the photographic negative.

to inhibit cleavage at longer preincubation times. Since V(IV) and the products of the redox reaction (Cr(III) and V(V)) are all inactive at cleaving DNA under the reaction conditions, the potentiation that occurs at low [V(IV)] and early preincubation times must involve intermediates in the reaction. The intermediate that is produced in the reaction of $[Cr(O)(ehba)_2]^-$ with the V(IV)/ehba complex is a $(ehba)_2Cr^{IV}$ species,[5] which has been characterized further using other chemical reductants[28] and pulse radiolysis.[29] This Cr(IV) intermediate is a much stronger oxidant than Cr(V)[28] and hence is likely to bring about more rapid and extensive oxidative cleavage of DNA. At longer preincubation times, Cr(IV) disproportionates to Cr(III) and Cr(V),[28] and hence the potentiation is lost. When V(IV) is in excess (molar ratio V:Cr > 2:1), the Cr(IV) intermediate is rapidly consumed by excess V(IV) to produce the inactive Cr(III) and V(V) products, and hence only inhibition is observed under these conditions. $VOSO_4 \cdot H_2O$ also has these effects, but additionally, it sequesters released ehbaH. This increases the concentration of the monodissociated form of the Cr–ehba complex required to bind to DNA[1] (Scheme I). That ligand exchange between Cr(V) and V(IV) is contributing to these effects is supported by the observation[1] that excess ehbaH_2 rapidly quenches DNA cleavage by $[Cr(O)(ehba)_2]^-$.

The influence of V(IV) on cellular DNA damage caused by Cr(V) (0.5 μmol/plate) and Cr(VI) (0.2 μmol/plate)[1] was determined by monitoring the reversion frequencies of the *Salmonella typhimurium his* auxotrophic strain TA100 (Ames mutagenicity test). Standard procedures[1,30] were used to assay reversions by scoring *his*$^+$ colonies on minimal agar plates. When a TA100 culture was treated simultaneously with micromolar

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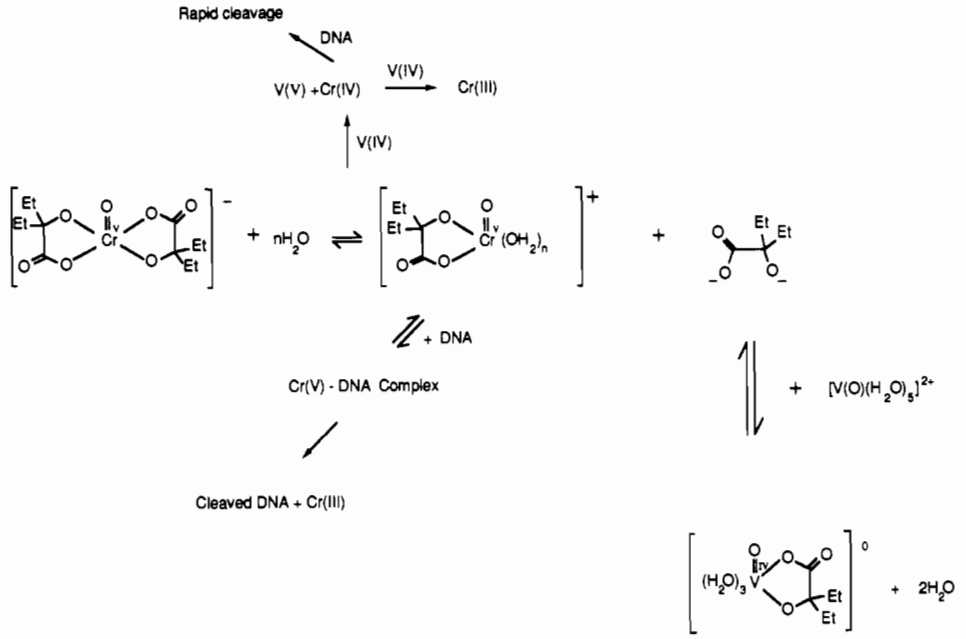
Scheme I. Potentiation and Inhibition of the in Vitro Cleavage of DNA by $[Cr(O)(ehba)_2]^-$ in the Presence of Vanadyl Sulfate[a]



[a] V(IV) exists mainly as the mono-ehba-complex under these conditions, i.e. less than a 2:1 stoichiometric ratio of ehba:V. When the reaction is performed with $[V(O)(ehba)(ehbaH)]^-$, the vanadium is already complexed and does not act to strip the ligand from the Cr complex. Under the conditions used in the Ames test, V is completely complexed by glucose and other intercellular molecules. Therefore, the chemistry (to produce Cr(IV)) is identical, irrespective of whether vanadyl sulfate or $[V(O)(ehba)(ehbaH)]^-$ is used as the source of vanadium.

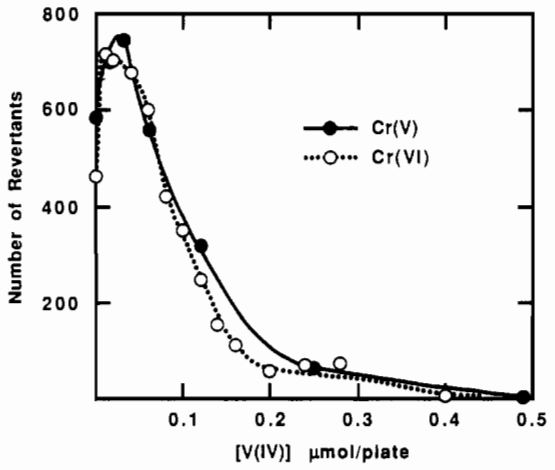


Figure 3. Effect of $VOSO_4 \cdot 5H_2O$ on the mutagenicity of $Na[Cr^V(O)(ehba)_2] \cdot H_2O$ and $Na_2Cr^{VI}_2O_7 \cdot 2H_2O$ in *S. typhimurium* TA100 ([Cr(V)] = 0.05 μmol/plate, [Cr(VI)] = 0.2 μmol/plate, background spontaneous reversion level = 133 ± 15). All analyses were performed in triplicate and colonies scored after incubation at 37 °C for 6 days.[30]

concentrations of Cr(V) and V(IV) in a ~2:1 molar ratio (0.2 μmol/plate of V(IV)), the number of revertants observed was lowered to background levels (Figure 3). At <1:1 molar ratios ($0.25 \leq [V(IV)] < 0.05$ μmol/plate), even spontaneous revertants failed to be produced in significant numbers. Separate assays showed this is not due to either cytostasis or enhanced toxicity of V(IV) toward the *his*$^+$ revertants.[31] At very low concentrations of V(IV), with Cr(V) in excess, there is significant potentiation of Cr(V) mutagenesis, which parallels the transient effects seen in the DNA-cleavage experiments. This is consistent with the formation of the more reactive Cr(IV) intermediates at low concentrations of V(IV) (low V(IV):Cr(V) mole ratios). Unlike the DNA-cleavage experiments, identical results were obtained with either source for V(IV), which is consistent with the known rapid ligand-exchange chemistry[6] that occurs with ligands in the cell medium to produce identical V(IV) complexes. Similar behavior was observed with V(IV) inhibition of Cr(VI)-induced mutations (Figure 3), which supports the hypothesis[1] that common Cr(V) intermediates are responsible for Cr-induced mutations. The lower V(IV):Cr ratios required to prevent mutations than required to prevent in vitro DNA cleavage result from the known ability of both the medium and cells to reduce some of the Cr-(V).[31]

V(IV) also prevents background reversions and those induced by other potent mutagens.[30] With NaN_3 (50 nmol/plate, 426 ± 28 revertants), the number of revertants decreased to background levels (166 ± 24) at 0.2 μmol/plate of V(IV) (4 molar excess) and then to zero at $0.5 < [V(IV)] < 0.75$ μmol/plate. With 4-nitroquinoline *N*-oxide (1.5 nmol/plate, 264 ± 19 revertants), 0.4 μmol/plate of V(IV) (270 molar excess) was required to reduce reversion to background levels (166 ± 24), and at $0.5 < [V(IV)] < 0.75$ μmol/plate, no revertants were observed. Potentiation at low [V(IV)] is specific to Cr-induced mutations (since it is not observed with other mutagens). V(IV) is also more potent in preventing Cr-induced mutagenesis (at higher [V]) than those produced by other chemical mutagens. This supports a scheme similar to Scheme I as operating in cells, except that all of the ehba is replaced by intercellular ligands by ligand-exchange reactions.[6,31] However, the results with other mutagens show that additional V(IV) suppression mechanisms operate, apart from simple reduction of Cr(V) by V(IV).

The chemistry and results of DNA cleavage and reversion assays reported here are all consistent with the mechanistic scheme proposed previously[1] for induction of mutagenesis by Cr and further implicate Cr(V) as a key intermediate in the potentiation of carcinogenesis. In agreement with known Cr chemistry,[5,28,29,32] transient Cr(IV) complexes (generated in situ) are implicated as being even more damaging to DNA than Cr(V).

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Supplementary Material Available: Tables listing positional parameters, thermal parameters, and interatomic distances and angles (5 pages). Ordering information is given on any current masthead page.

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