Synthesis, X-ray structure, and hydrolytic chemistry of the highly potent antiviral polyniobotungstate A-α-[$Si_2Nb_6W_{18}O_{77}$]$^{8-}$

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Received (in Bloomington, IN, USA) 14th May 1999, Accepted 5th July 1999

Potently antiviral polyniobotungstates have been structurally characterized; the dimer A-α-[$Si_2Nb_6W_{18}O_{77}$]$^{8-}$ cleaves cleanly to the monomer A-α-[$SiNb_3W_9O_{40}$]$^{7-}$ within 1 min in aqueous solution buffered at physiological (neutral) pH establishing that the monomer and not the dimer is pharmacologically relevant.

In recent years, several classes of early transition-metal oxygen-anion clusters or polyoxometalates (POMs) have been documented to exhibit antiviral properties.[1] The size, shape and functional group complementarity of these nanometer-sized inorganic compounds and key enzymatic targets, including the active sites of HIV-1 reverse transcriptase[2] and HIV-1 protease,[3] is substantial. This fact coupled with the growing ability to systematically vary the physical and electronic structures and other properties of POMs,[4] has increased interest in POMs as potential antiviral agents. The double-Keggin POMs of formula A-α- or A-β-[$Si_2Nb_6W_{18}O)_{77}$]$^{8-}$ (**1**) are as promising as any of the 200+ POMs investigated to date as antiviral agents. The A-β isomer was first prepared by Finke and Droege in 1984,[5] and subsequently shown by our group and others to strongly inhibit a number of viruses including HIV-1, HIV-2, respiratory syncytial virus (RSV) and several strains of influenza and herpes while being essentially non-toxic in mammals.[6] The A-α isomer, A-α-[$Si_2Nb_6W_{18}O_{77}$]$^{8-}$ (A-α-**1**) has comparable pharmacological profiles (therapeutic indices) to A-β-**1**.[1] Despite the interest in A-α- and A-β-**1**, the structure or hydrolytic form of these dimers in aqueous solution under physiological conditions has never been characterizaed. We report here, that the dimers (A-α- or A-β-**1**) are not present under physiological conditions, because cleavage to the corresponding monomers is thermodynamically and kinetically favorable at serum pH values. We focus here on the A-α system for which X-ray structures of both A-α-**1** and its corresponding monomer A-α-[$SiNb_3W_9O_{40}$]$^{7-}$ (A-α-**2**) have been obtained. The A-β system exhibits effectively identical aqueous speciation chemistry.

The organic-solvent-soluble tetrabutylammonium (TBA) salt of **1** (A-α-TBA**1**) can be prepared by the peroxide-bisulfite method that Finke and Droege used to make the analogous TBA salt of the A-β isomer.[7] Efforts to use this method[7] to obtain water-soluble forms of either isomer of **1** were hampered by co-precipitation of sulfate-salt byproducts. This problem was overcome in two ways: by selective precipitation using K^+ and Cs^+ salts under carefully controlled conditions and by use of a new sulfate-free synthesis. Selective precipitation was accomplished by adding saturated methanolic solutions of either CsCl (8 equiv.) or of CF_3CO_2K (10 equiv. of neat CF_3CO_2H followed by 24 equiv. of methanolic CF_3CO_2K) to 10 mM acetonitrile solutions of A-α-TBA**1**. Powders of the respective salts obtained were washed with methanol, followed by acetonitrile, to remove excess salts. The Cs^+ salt, (A-α-Cs**1**),‡ was obtained in 90% yield based on A-α-TBA**1**. The K^+ salt (34% yield from A-α-TBA**1**) was partially hydrolyzed by reversible cleavage of one of the three Nb–O–Nb μ-O linkages:‡ subsequent dissolution in 1.0 M HCl and passage of the solution through a Dowex-50 proton exchange resin gave the free-acid form of re-condensed **1**, A-α-H_8[$Si_2Nb_6W_{18}O_{77}$] (A-α-H**1**)‡ in effectively quantitative yield.

The Cs^+ salt of A-α-**1** was also prepared by direct condensation of the monomeric triperoxoniobium precursor A-α-[$Si(NbO_2)_3W_9O_{37}$]$^{7-}$: a yellow solution of A-α-Cs_7[$Si(NbO_2)_3W_9O_{37}$] [12 mM in 2.0 M HCl(aq)] was refluxed until it was colorless and CsCl (22 equiv.) was added to give A-α-Cs**1** in 86% yield. Reflux of a yellow acetonitrile solution of A-α-$(TBA)_4H_3$[$Si(NbO_2)_3W_9O_{37}$] in the presence of HCl followed by diffusion of diethyl ether into the reaction mixture gave X-ray quality crystals of A-α-TBA**1**§ in 74% yield. The solid state (KBr pellet) IR spectra of all the A-α-**1** salts prepared using these methods exhibit strong Nb–O–Nb bands in the 680–700 cm^{-1} region; the spectra of the monomer, A-α-**2**,‡ does not.

X-Ray crystal structures¶ of A-α-TBA**1** and the A-α-Cs**2** confirm the A-α-isomeric assignments. The structure of A-α-**1** with principal bond distances and angles is given in Fig. 1. Bond valence sum calculations[8] indicate that all the niobium atoms in both A-α-**1** and A-α-**2** are in the +5 oxidation state, a result consistent with the NMR spectra (both POMs are diamagnetic). The 'double Keggin' structure in **1** is known in three other structurally characterized POMs: A-α-[$H_9Si_2Cr^{III}{}_6W_{18}O_{77}$]$^{11-}$,[9] a tri-μ-hydroxo compound, and A-β-[$Si_2Ti_6W_{18}O_{77}$]$^{14-}$,[10] and A-α-[$Ge_2Ti_6W_{18}O_{77}$]$^{14-}$,[11] both tri-μ-oxo compounds.

With both the dimer, A-α-**1**, and monomer, A-α-**2**, structurally characterized in both the solid state and in solution, the pH-dependent aqueous speciation chemistry of these POMs and the form present at physiological (neutral) pH was readily established. It is well documented that various bases cleave the Nb–O–Nb unit in both metal oxide materials[12] and POMs.[5,13] A combined pH–conductometric titration of A-α-H**1** confirmed that 14 equiv. of hydroxide were required to arrive at the inflection point. This is consistent with eqns. (1) and (2) and the

$$H_8Si_2Nb_6W_{18}O_{77} + 8\ OH^- \rightarrow Si_2Nb_6W_{18}O_{77}{}^{8-} + 8\ H_2O \quad (1)$$

$$Si_2Nb_6W_{18}O_{77}{}^{8-} + 6\ OH^- \rightarrow 2\ SiNb_3W_9O_{40}{}^{7-} + 3\ H_2O \quad (2)$$

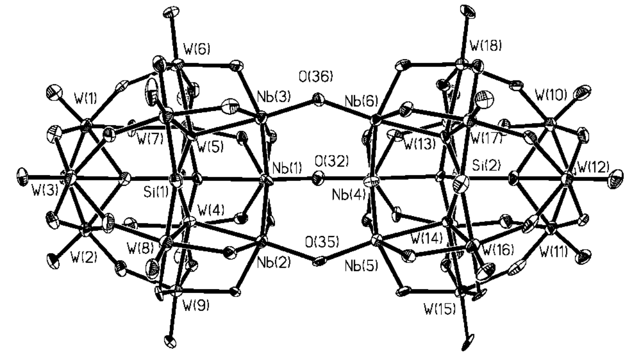

Fig. 1 The ORTEP drawing of A-α-[$Si_2Nb_6W_{18}O_{77}$]$^{8-}$. Selected bond lengths (Å) and angles (°): Nb(1)–O(32) 1.904(12), Nb(2)–O(35) 1.915(12), Nb(3)–O(36) 1.893(11), Nb(4)–O(32) 1.922(12), Nb(5)–O(35) 1.907(12), Nb(6)–O(36) 1.919(12), Nb(1)–O(32)–Nb(4) 136.6(7), Nb(5)–O(35)–Nb(2) 137.3(6), Nb(3)–O(36)–Nb(6) 137.1(7).

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fact that **1** is a tri-μ-oxo dimer in acidic aqueous solution. Examination of A-α-**1** and A-α-**2** by both ^{183}W NMR and FTIR in D_2O buffered at pD 7.0 using 3 different systems, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), 3-[*N*-morpholino]propanesulfonic acid (MOPS), or phosphate, indicated that only monomer, A-α-**2**, was present in all cases. While these measurements indicated the thermodynamic instability of the dimer relative to the monomer at physiological pH, they did not provide the rate of dimer cleavage, the issue of most relevance to the use of A-α-**1** as an antiviral agent. Unfortunately, overlapping absorbances or instrument-limited acquisition times rendered all the obvious spectroscopic techniques, including FTIR on aqueous buffer solutions, inadequate to assess the rate. However, it was determined that the Nb–O–Nb stretching region of the mid-IR could be used to follow this hydrolytic cleavage process provided D_2O was used as the solvent. Dimer cleavage was assessed by adding 0.156 g (0.0303 mmol) of A-α-**1** to 3.00 mL of 0.609 M MOPS buffer in D_2O to give a clear, colorless solution with a pD of 7.0. An aliquot of this solution was added to an AgBr IR solution cell and the spectrum, obtained in < 1 min showed that no dimer Nb–O–Nb band remained. The same experiments using 0.609 M HEPES or phosphate buffer in place of MOPS yielded the same results. ^{183}W NMR and FTIR established that when the pH of the hydrolyzed neutral solution was decreased to 0, A-α-**1** was re-formed in very high yield (Fig. 2). The corresponding experiments with the A-β system gave analogous results and, in neither system was Baker–Figgis (α–β) isomerisation[14] observed.

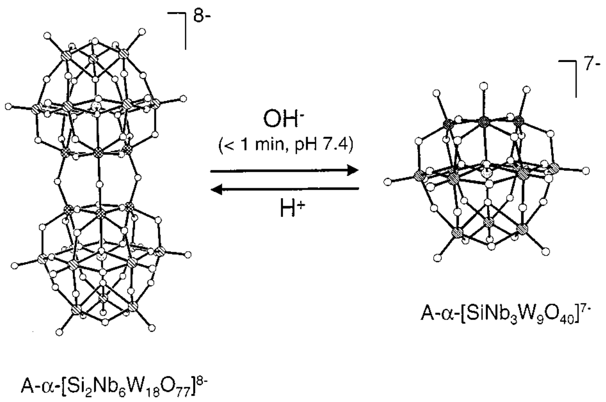

Fig. 2 Summary of dimer–monomer interconversions [eqn, (2) is the balanced reaction].

In summary, the tri-μ-oxo linkages in the double Keggin complexes, A-α-**1** or A-β-**1**, are cleaved quickly and with effectively quantitative selectivity to the corresponding monomers at physiological pH. In consequence, it is highly unlikely that the double Keggin POM structure accounts for any of the extensive biological data reported for these complexes.

This work was supported by the National Institutes of Health (R01 AI32903-04A1), and the Molecular Design Institute (Office of Naval Research, grant N00014-95-1-1116). We thank Dr Don VanDerveer (Georgia Inst. Tech.) for collecting the X-ray data.

Notes and references

‡ A-α-Cs**1**: anal. Calc. for $Cs_8[Si_2Nb_6W_{18}O_{77}]\cdot18H_2O$: H, 0.55; Cs, 16.3; Nb, 8.52; W, 50.6. Found: H, 0.50; Cs, 16.3; Nb, 8.31; W, 50.6%. FTIR (KBr): 687s, *ν*(Nb–O–Nb). FT Raman (solid): 984vs, 910w, 860(sh).

α-K dimer: unlike **1**, which contains three μ-oxo Nb–O–Nb linkages between the Keggin $SiNb_3W_9$ units, the K^+ dimer contains two μ-oxo linkages, which results in *syn* and *anti* orientations between the two $SiNb_3W_9$ units. Anal. Calc. for $K_{10}[Si_2Nb_6W_{18}O_{78}]\cdot25H_2O$: H, 0.84; K, 6.50; Nb, 9.27; W, 55.0. Found: H, 0.83; K, 6.68; Nb, 8.98; W, 54.6%. ^{183}W NMR (lithiated 0.07 M in D_2O, pD = 0.4 with DCl; ref. 2.0 M Na_2WO_4 in D_2O): *syn*-di-μ-dimer, δ −99.0 (4W), −119.3 (4W), −128.7 (4W), −130.0 (2W), −146.4 (4W) (80 mol%), *anti*-di-μ-dimer, δ −101.2 (4W), −111.3 (2W), −125.4 (4W), −130.8 (4W), −143.6 (4W) (20 mol%). FTIR (KBr): 683s, *ν*(Nb–O–Nb). FT Raman (solid): 983vs, 904w.

A-α-H**1**: Anal. Calc. for $H_8[Si_2Nb_6W_{18}O_{77}]\cdot20H_2O$: H, 0.87; Nb, 10.0; W, 59.9. Found: H, 0.78; Nb, 9.83; W, 59.6%. FTIR (KBr): 683s, *ν*(Nb–O–Nb). FT Raman (solid): 989vs, 903w. ^{183}W NMR (0.08 M in D_2O, $[D^+]$ = 1.2 M with DCl; ref. 2.0 M Na_2WO_4 in D_2O): δ −124.1 (6W), −141.2 (12W).

A-α-Cs**2**: anal. Calc. for $Cs_7SiNb_3W_9O_{40}\cdot10H_2O$: Cs, 25.2; Nb, 7.54; W, 44.8. Found: Cs, 24.7; Nb, 7.40; W, 45.0%. FTIR (KBr): 1003w, 963m, 905s, 778vs, 538m. ^{183}W NMR (0.08 M in D_2O, pD = 6.0 with LiOH, ref. 2.0 M Na_2WO_4 in D_2O): δ −106.8 (6W), −148.7 (3W).

§ A-α-TBA**1**·Et_2O: anal. Calc. for $C_{100}H_{228}N_6Si_2Nb_6W_{18}O_{77}$: C, 17.96; H, 3.44; N, 1.26; Si, 0.84; Nb, 8.34; W, 49.5. Found: C, 17.92; H, 3.37; N, 1.36; Si, 0.67; Nb, 8.43; W, 49.7%. FTIR (KBr): 688s, *ν*(Nb–O–Nb). FT Raman (solid): 988vs, 973m, 921 (sh), 909w, 885(sh). FAB-MS: *m*/*z* (intensity), [assignment]: 5410 (28), $[M + Q + 6H]^-$ 4709 (100), $[M + 7H - 2WO_3]^-$; 4496 (64), $[M + 7H - W_3O_8]^-$; 4275 (47), $[M + 7H - W_4O_{10}]^-$; 4070 (35), $[M + 7H - W_5O_{12}]^-$; 3836 (24), $[M + 7H - W_6O_{15}]^-$. ^{183}W NMR (0.2 M in 1 : 1 CD_3CN–DMF; ref. 2.0 M Na_2WO_4 in D_2O): δ −110.30 (6W), −130.45 (12W).

¶ *Crystal data*: A-α-TBA**1**·Et_2O: $C_{100}H_{228}Nb_6O_{78}Si_2W_{18}$, M = 6685.81, orthorhombic, space group $Pca2_1$, a = 29.4854(3), b = 20.3867(3), c = 28.4247(10) Å, V = 17086.4(3) Å^3, D_c = 2.57 g cm^{-3}, T = 293 K, Z = 4, F(000) = 12232, μ(Mo-Kα) = 12.540 mm^{-1}, Siemens SMART CCD, 87737 reflections measured, 28106 unique (R_{int} = 0.0895) which were used in all calculations. The final R_1 = 0.0506 and wR_2 = 0.1107.

A-α-Cs**2**·$4H_2O$: $Cs_6H_9Nb_3O_{44}SiW_9$, M = 3471.98, tetragonal, space group $P4_2/ncm$, a = 21.0827(4), c = 10.4262(3) Å, V = 4634.2(2) Å^3, D_c = 4.99 g cm^{-3}, T = 293 K, Z = 4, μ(MoKα) = 27.726 mm^{-1}, Siemens SMART CCD, 38780 reflections measured, 2944 unique (R_{int} = 0.0828) which were used in all calculations. The final R_1 = 0.0363 and wR_2 = 0.0991. The 3 Nb and 9 W atoms are statistically distributed among the 12 positions in the Keggin unit due to a crystallographically imposed $2/m$ symmetry passing through Si atom. CCDC 182/1313. See http://www.rsc.org/suppdata/cc/1999/1651/ for crystallographic files in .cif format.

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Communication 9/03983J