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Tuning the interactions of decavanadate with thaumatin, lysozyme, proteinase K and human serum proteins by its coordination to a pentaquacobalt(II) complex cation†

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The decavanadate anion, $H_xV_{10}O_{28}^{(6-x)-}$ (V_{10}), is one of the most studied vanadium polyoxometalate species. In recent decades several works have pointed to its biological relevance coming mainly from its ability to bind to proteins (such as actin, myosin or ion pumps). On the other hand, non-functional binding was observed in several protein crystal structures, where V_{10} was incorporated “accidentally” resulting from the presence of Na_2VO_4 as a phosphatase inhibitor. In this work we broaden the potential biological applications of V_{10} by presenting the synthesis and characterization of two decavanadate species where the anion acts as a ligand: $(2\text{-hepH})(NH_4)[\{Cu(H_2O)_2(2\text{-hep})\}_2V_{10}O_{28}] \cdot 4H_2O$ ($V_{10}Cu$) and $(2\text{-hepH})_2[\{Co(H_2O)_5\}_2V_{10}O_{28}] \cdot 4H_2O$ ($V_{10}Co$) (2-hep = 2-hydroxyethylpyridine). Unlike free decavanadate, the complex anions stay intact in model buffer solutions (0.1 M 2-(*N*-morpholino)ethanesulfonic acid, 0.5 M NaCl, pH = 5.8 and 8.0). It has been shown that $V_{10}Co$ is stable also in the presence of proteins and for the first time it was possible to study the interaction of decavanadate with proteins without the interference of lower vanadate oligomers. This allowed comparison of interactions of V_{10} and $V_{10}Co$ with the model proteins thaumatin, lysozyme, proteinase K, human serum albumin and transferrin under conditions close to biological ones (0.1 M 2-(*N*-morpholino)ethanesulfonic acid, 0.5 M NaCl, pH = 5.8). The linewidths of the signals at half-height in ^{51}V NMR spectra reflect the strength of interaction of a vanadium species with a protein, and thus it was shown that V_{10} and $V_{10}Co$ both bind strongly to thaumatin, V_{10} binds to lysozyme and $V_{10}Co$ binds to proteinase K. V_{10} interacts with both human serum albumin and transferrin, but surprisingly $V_{10}Co$ exhibits high affinity to transferrin but does not interact with albumin.

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1 Introduction

Polyoxometalates (POMs) are an important group of metal oxide clusters¹ exhibiting diverse archetypal structures of, particularly, vanadates, molybdates and tungstates.^{2–4} The tremendous variability of POMs has given rise to their application in distinct areas of materials science,^{5,6} catalysis,^{7–10} electrochemistry and redox processes,^{11,12} photochemistry^{13–15} and magnetism.^{16–18} In recent years, the roles of POMs in biological systems have been intensively investigated. Such studies may be divided

into two groups: functional binding and interaction of POMs in biological systems widening the borders of medicinal chemistry,^{19–24} and non-functional interaction with biomolecules, such as proteins, enhancing the current possibilities in macromolecular crystallography by promoting the crystallization or being useful in obtaining the initial phases while solving the structures of proteins.^{25–28}

Decavanadate, $H_xV_{10}O_{28}^{(6-x)-}$ (V_{10}), is the predominant species formed in vanadate solutions at vanadium(v) concentrations above 1 mM in the pH range of $\approx 2\text{--}6$.^{29,30} The structure of V_{10} consists of ten face-sharing octahedra (Scheme 1). The symmetrically non-equivalent vanadium atoms V_A , V_B and V_C give rise to three different signals in ^{51}V NMR spectra depending on the conditions: while the low-field signal of V_A atoms stays in a narrow region around -425 ± 3 ppm, the other two peaks representing V_B and V_C atoms are more sensitive to changes in acidity and exhibit signals at approximately -505 ± 10 ppm and -525 ± 10 ppm, respectively. The oxygen atoms O_B and O_C are potential sites for protonation, and the atoms O_C , O_F , O_G and O_D are the most potential sites for ligation to transition metal ions.^{31,32}

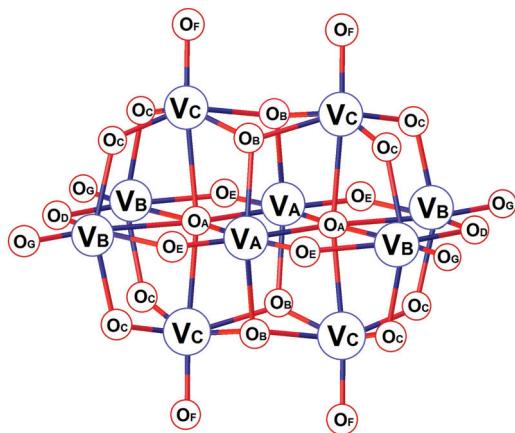
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Scheme 1 The structure of $[V_{10}O_{28}]^{6-}$ showing different types of vanadium and oxygen atoms.

As such, changes in ^{51}V NMR parameters reflect the versatility of structural modifications of V_{10} . While protonation and coordination of V_{10} manifest mostly in peaks' movement, the interaction and binding to proteins result in significant peak broadening defined by linewidths at half-height of the signals, $W_{1/2}$. This is caused by the ligand bulkiness and decreased symmetry of the V_{10} species upon interaction.³²

Vanadium is naturally omnipresent in biological matrices^{33,34} in a few enzymes such as vanadium dependent haloperoxidases and nitrogenases³⁵ or acts as a crucial component in the energetic metabolism of *Ascidians*.³⁶ Some artificial vanadium compounds, on the other hand, exhibit insulin mimetic properties, antitumor activity, antibacterial activity or anti-HIV activity.^{35–39}

Specifically decavanadate itself has also been studied with respect to many biological aspects,^{40–43} and it was shown that V_{10} binds to several proteins such as actin,⁴⁴ myosin,⁴⁵ ion pump Ca^{2+} -ATPase,⁴⁶ bovine serum albumin and gelatine,⁴⁷ and microtubule-associated proteins.⁴⁸ Protein crystallography revealed the presence of V_{10} in the crystal structures of acid phosphatase A (*F. tularensis*),⁴⁹ human activated receptor tyrosine kinase,⁵⁰ NTPDase1 (*L. pneumophila*),⁵¹ NTPDase1 (*R. norvegicus*),⁵² and human TRPM4 channel.⁵³ In all cases, V_{10} was formed from the initially employed Na_3VO_4 (used as a phosphatase inhibitor) and its role may be explained as stabilization and rigidification of the protein structure.²⁵

In this work we compare the interaction of both free and ligated decavanadate with commercially available model proteins thaumatin, lysozyme, proteinase K, as well as human serum albumin and transferrin. We utilize ^{51}V NMR spectroscopy as a powerful tool to investigate the stability of two decavanadates coordinated to metal centres $\text{Cu}(\text{II})$ and $\text{Co}(\text{II})$ under conditions usually used for protein crystallization.

2 Experimental

2.1 Materials and methods

All chemicals were of analytical grade and used as received without further purification. All proteins were supplied as lyophilized powders (supplier, reference code): thaumatin from

Thaumatococcus daniellii (Sigma, T7638; a mixture of thaumatin I and thaumatin II with traces of other sweet proteins), proteinase K from *Tritirachium album* (Sigma, P6556), lysozyme from chicken eggwhite (Carl Roth GmbH & Co. KG, 8259.3), albumin from human serum (Sigma, A1653) and apo-transferrin from human serum (Sigma, T1147). The determination of C/H/N was carried out by using an 'EA 1108 CHNS-O' elemental analyzer by Carlo Erba Instruments at the Mikroanalytisches Laboratorium, University of Vienna. Metal elements' analyses were performed in aqueous solutions containing 2% HNO_3 using inductively coupled plasma mass spectrometry (PerkinElmer Elan 6000 ICP MS) for Mo and V, and atomic absorption spectroscopy (PerkinElmer 1100 Flame AAS) for Cu and Co. Standards were prepared from single-element standard solutions of concentration 1000 mg L^{-1} (Merck, Ultra Scientific and Analytika Prague). FT-IR spectroscopy was performed on a Bruker Vertex 70 IR Spectrometer equipped with a single reflection diamond-ATR (attenuated total reflectance) unit in the range of $4000\text{--}100 \text{ cm}^{-1}$.

2.2 ^{51}V NMR spectroscopy

^{51}V nuclear magnetic resonance spectroscopy measurements of aqueous solutions were performed on a Bruker Avance II 500 MHz instrument operating at 131.60 MHz for ^{51}V nucleus (2000 scans, accumulation time 0.05 s, relaxation delay 0.01 s). Chemical shift values are given with reference to VOCl_3 ($\delta = 0 \text{ ppm}$) as a standard. The solutions were in general prepared by dissolving 0.01 mmol of the given decavanadate in 700 μL of the buffer solution (0.11 M MES, 0.55 M NaCl, pH = 5.8; MES = 2-(*N*-morpholino)ethanesulfonic acid) and addition of 100 μL of D_2O used for locking. Next, the solution was either made up to 1000s μL with the buffer (Section 3.3.1) or a solution of a protein in the same buffer was slowly added. The protein solutions were prepared by dissolution of the given amount of solid protein in 200 μL of the buffer solution. The solution of proteinase K was prepared as 3.5 M solution in 0.1 M TRIS (tris(hydroxymethyl)aminomethane) buffer (pH = 7.0), and subsequently 1 μL of this solution was diluted in 200 μL of the MES buffer solution. The spectra were collected at RT one hour after preparation of the solutions.

2.3 X-ray diffraction on single crystals

The X-ray diffraction data were collected on Bruker X8 APEXII ($V_{10}\text{Cu}$, Mo $K\alpha$) and Bruker D8 Venture ($V_{10}\text{Co}$, Cu $K\alpha$) instruments equipped with multilayer monochromators, Incoatec Microfocus sealed tubes, and Kryoflex and Oxford cooling devices. The structures were solved by direct methods and refined by full-matrix least-squares. Nonhydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with riding coordinates except for solvent molecules and H atoms in NH_4^+ in $V_{10}\text{Cu}$. This was not done because of disorder and partial occupancies of O and N atoms in question. The following software was used for the structure solving procedure: Bruker SAINT software package⁵⁴ (frame integration, cell refinement), SADABS⁵⁵ (absorption correction), SHELXS-2013⁵⁶ (structure solution), SHELXL-2013⁵⁷ (structure refinement), OLEX2⁵⁸ (user interface and publication



material) and Diamond⁵⁹ (graphics). Experimental data and CCDC-codes can be found in Table 2.

2.4 Syntheses

(NH₄)₆[V₁₀O₂₈]·6H₂O (**V₁₀**): the compound was prepared as per the previously described procedure⁶⁰ and the composition was confirmed by FT-IR spectroscopy and elemental analysis. Analytical data for V₁₀O₃₄N₆H₃₆ in % (calc.): V 46.3 (43.4). IR: [V₁₀O₂₈]⁶⁻: 958vs, 816m, 727s, 588m, 516s, 452s, 392vs; NH₄⁺: 1405s, 3151sh, 3009sh, 2008sh.

(2-*hepH*)(NH₄)₂[(Cu(H₂O)₂(2-*hep*))₂V₁₀O₂₈]·4H₂O (**V₁₀Cu**): NH₄VO₃ (0.468 g, 4 mmol) was dissolved in 30 mL of distilled water by heating. After cooling to RT, the pH of the solution was adjusted to 4.6 with 5 M HCl. Then, 2-*hep* (2-*hep* = 2-hydroxyethylpyridine) was added (0.45 mL, 4 mmol) and the pH was adjusted to 4.6 with 5 M HCl again. Finally, Cu(NO₃)₂·6H₂O was added (0.484 g, 2 mmol). After dissolution, the pH dropped to 4.0. The clear orange-green solution was left to crystallize at 18 °C. After three days, green crystals of **V₁₀Cu** were isolated. Yield 250 mg (39% based on V). Analytical data for V₁₀Cu₂O₃₉C₂₁H₄₈N₄ in % (calc.): V 30.7 (31.5), Cu 7.76 (7.86), C 15.36 (15.60), H 3.02 (3.00), N 3.41 (3.46). IR: [V₁₀O₂₈]⁶⁻: 983s, 959vs, 937s, 850s, 793s, 721s, 512s, 429s, 386s; 2-*hep*: 1609m, 1488m, 1313m, 1248w, 1109w, 1073w, 1051w, 1025w; NH₄⁺: 1439s, 3210sh.

(2-*hepH*)₂[Co(H₂O)₅]₂V₁₀O₂₈]·4H₂O (**V₁₀Co**): a procedure similar to that in the previous synthesis was employed, except that upon addition of Co(NO₃)₂·6H₂O (0.596 g, 2 mmol) it was necessary to adjust the pH of the solution to 4.0 with 5 M HCl. The clear red solution was left to crystallize at RT. After three days, red crystals of **V₁₀Co** were isolated. Yield 400 mg (63% based on V). Analytical data for V₁₀Co₂O₄₄C₁₄H₄₈N₂ in % (calc.): V 31.7 (32.3), Co 7.17 (7.48), C 10.55 (10.67), H 3.11 (3.07), N 1.82 (1.78). IR: [V₁₀O₂₈]⁶⁻: 989s, 967vs, 921s, 811s, 724s, 596s, 554s, 505s, 446m, 398s; 2-*hep*: 1608m, 1772m, 1248w, 1024w. Crystals suitable for X-ray diffraction were grown by prolonged crystallization at 18 °C in a flask closed with perforated Parafilm.

3 Results and discussion

3.1 Syntheses

To the best of our knowledge, the work of Schwendt *et al.*⁶¹ presenting the synthesis and characterization of (2-*hepH*)₂[(Cu(H₂O)₂(2-*hep*))₂V₁₀O₂₈]·6H₂O is the only one reporting a coordination compound of decavanadate that stays intact in aqueous solution. Inspired by this work, the synthetic protocol was modified in order to prepare new stable complexes of decavanadate. A reversed addition of the individual reaction components and stepwise acidification of the solution prevented formation of heavy precipitates that were described in the original procedure⁶¹ and such an approach resulted in successful isolation of **V₁₀Cu** containing the [(Cu(H₂O)₂(2-*hep*))₂V₁₀O₂₈]²⁻ complex anion and a new compound **V₁₀Co** composed of the purely inorganic complex anion [(Co(H₂O)₅]₂V₁₀O₂₈]²⁻. In this case, 2-*hep* did not coordinate to the cobalt(II) center and acted only as a cation. Syntheses employing Ni^{II}, Zn^{II}, Mn^{II}, La^{III} and Ce^{III} nitrates were not successful. Despite the systematic study it was

Table 1 Coordination compounds of H_xV₁₀O₂₈^{(6-x)-}

Compound	Type of coordinated O atom ^a	Ref.
[Cu(2,2'- <i>bipy</i>) ₂][H ₂ V ₁₀ O ₂₈]·(2,2'- <i>bipy</i>)·H ₂ O	O _C	63
{[(CuL) _{0.5} (H ₂ L) _{1.5}][H ₂ V ₁₀ O ₂₈]·6H ₂ O} _n	O _F	64
(NH ₄) ₂ [Cu ₂ (NH ₃ CH ₂ CH ₂ COO) ₄ (V ₁₀ O ₂₈)]·10H ₂ O	O _G	65
(H ₂ pz) ₂ [(Cu(pz) ₄] ₂ V ₁₀ O ₂₈]·2H ₂ O	O _G , O _F	66
{Cu(pz) ₄ }[Cu(pz) ₃] ₂ V ₁₀ O ₂₈ }	O _G , O _C	66
(2- <i>hepH</i>) ₂ [(Cu(H ₂ O) ₂ (O,N-2- <i>hep</i>)) ₂ (V ₁₀ O ₂₈)]·6H ₂ O	O _C	61
(2- <i>hepH</i>)(NH ₄) ₂ [(Cu(H ₂ O) ₂ (2- <i>hep</i>)) ₂ V ₁₀ O ₂₈]·4H ₂ O	O _C	This work
[Cu(2- <i>amp</i>) ₂ (H ₂ O)] ₂ H ₂ V ₁₀ O ₂₈ ·4H ₂ O	O _C	61
(H ₃ O) ₂ [(Cu(en) ₂ (H ₂ O)) ₂ V ₁₀ O ₂₈]·3H ₂ O	O _C , O _D	67
{[Cu(en) ₂] ₃ (V ₁₀ O ₂₈)]·6H ₂ O	O _C , O _D	67
[Zn ₂ (H ₂ O) ₁₄ (V ₁₀ O ₂₈)]·H ₂ ppz	O _D	68
[Zn(H ₂ O) ₆][Zn ₂ V ₁₀ O ₂₈ (H ₂ O) ₁₀]·6H ₂ O	O _F	69
[Zn(en) ₂] ₃ [V ₁₀ O ₂₈]·5H ₂ O	O _C	70
[Zn(im) ₂ (DMF) ₂] ₂ [H ₂ V ₁₀ O ₂₈]·im·DMF	O _C	71
{[Zn ₃ (trz) ₃ (H ₂ O) ₄ (DMF)] ₂ [V ₁₀ O ₂₈]·4H ₂ O} _n	O _C	71
(2- <i>hepH</i>) ₂ [(Mn(H ₂ O) ₅] ₂ V ₁₀ O ₂₈]·4H ₂ O	O _F	72
{[Mn(<i>mim</i>) ₄] ₂ (H ₂ V ₁₀ O ₂₈)]	O _C , O _F	73
(NMe ₄) ₂ [(Mn(H ₂ O) ₅] ₂ V ₁₀ O ₂₈]·5H ₂ O	O _F	76
[NH ₃ C(CH ₂ OH) ₃] ₂ [(Mn(H ₂ O) ₅] ₂ V ₁₀ O ₂₈]·2H ₂ O	O _F	76
[Ag(<i>btz</i>)] ₄ H ₂ V ₁₀ O ₂₈ ·2H ₂ O	O _C	74
{[Na ₃ (H ₂ O) ₈ (μ ₂ -H ₂ O) ₆ Ag ₂]HV ₁₀ O ₂₈]·6H ₂ O	O _C , O _G	75
(2- <i>hepH</i>) ₂ [Co(H ₂ O) ₅] ₂ V ₁₀ O ₂₈]·4H ₂ O	O _F	This work

^a As in Scheme 1. Abbreviations: 2,2'-*bipy* – 2,2'-bipyridine, *btz* – 1,4-*bis*(triazol-1-ylmethyl)benzene, *ppz* – piperazine, *pz* – pyrazole, *im* – imidazole, *mim* – 1-methylimidazole, *trz* – 1,2,4-triazole, *en* – ethylenediamine, *amp* – 2-(aminomethyl)pyridine, 2-*hep* = 2-hydroxyethylpyridine, *L* – 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane.

possible to isolate only two complexes of decavanadate, and even they differed in the coordination mode of decavanadate and in the structure of the coordinated transition metal. Table 1 summarizes 22 compounds where decavanadate coordinates to transition metal cations. There are ten Cu^{II}, four Zn^{II}, four Mn^{II} and two Ag^I complexes. Compound **V₁₀Co** is the first complex of decavanadate involving Co^{II}. There are about 100 salts of decavanadate and some of them contain a transition metal complex in the cationic part, including the [Co(H₂O)₆]²⁺ complex cation.^{62a-c} Therefore, in general it is still not clear what conditions are required for decavanadate to act as a ligand.

3.2 Crystal structures

The structure solution and refinement details for compounds **V₁₀Cu** and **V₁₀Co** are summarized in Table 2. The crystal structure refinement of **V₁₀Cu** revealed the presence of the complex anion [(Cu(H₂O)₂(2-*hep*))₂V₁₀O₂₈]²⁻ that has been already reported (Fig. 1).⁶¹ The decavanadate anion [V₁₀O₂₈]⁶⁻ is a bridging ligand between two [(Cu(H₂O)₂(2-*hep*))₂]²⁺ fragments and binds to the Cu1 atom through oxygen atom O12 (O_C in Scheme 1) at 1.9612(8) Å. The organic ligand is chelating the Cu1 atom by the N atom of the pyridine ring [N1–Cu1 1.9802(11) Å] and the O atom of the hydroxyl group [O13–Cu1 1.9857(9) Å]. The tetragonal pseudo-plane around the atom Cu1 is completed by a water molecule [O15–Cu1 1.9824(10) Å]; the water molecule in the axial position completes the pyramidal geometry [O14–Cu1 2.2860(10) Å].

The closest contact in the *trans* position towards this water molecule is oxygen atom O8 of [V₁₀O₂₈]⁶⁻ (O_G in Scheme 1).



Table 2 Crystallographic and refinement data for compounds $V_{10}Cu$ and $V_{10}Co$

	$V_{10}Cu$	$V_{10}Co$
CCDC No.	1909251	1909250
Empirical formula	$V_{10}Cu_2O_{39}C_{21}H_{48}N_4$	$V_{10}Co_2O_{44}C_{14}H_{48}N_2$
M_r	1605.02	1575.80
Crystal system, space group	Monoclinic, $C2/c$	Triclinic, $P\bar{1}$
Temperature (K)	100	100
a (Å)	27.0345(15)	9.4219(5)
b (Å)	9.9170(5)	11.2451(6)
c (Å)	20.5851(12)	11.9820(7)
α (°)		78.5909(17)
β (°)	119.341(5)	75.1180(17)
γ (°)		66.3817(17)
V (Å ³)	4810.9(5)	1117.66 (11)
Z	4	1
Radiation type	Mo K α	Cu K α
μ (mm ⁻¹)	2.82	23.51
Absorption correction	Multi-scan	Multi-scan
T_{min} , T_{max}	0.505, 0.747	0.534, 0.754
$F(000)$	3160	782
Theta range	2.2–35.7°	5.7–72.6°
Crystal size in mm	0.45 × 0.25 × 0.22	0.11 × 0.11 × 0.04
Index ranges	$h = -44 \rightarrow 44$ $k = -16 \rightarrow 16$ $l = -33 \rightarrow 33$	$h = -11 \rightarrow 11$ $k = -13 \rightarrow 13$ $l = -12 \rightarrow 14$
No. of measured, independent and observed [$I > 2s(I)$] reflections	343 398, 11 216, 9986	16 530, 4079, 3882
R_{int}	0.062	0.127
No. of reflections, parameters, restraints	11216, 421, 9	4079, 337, 20
Goodness-of-fit on F^2	1.10	1.16
$R[F^2 > 2s(F^2)]$	0.024	0.098
$wR(F^2)$	0.071	0.222
Largest diff. peak and hole	0.79 e Å ⁻³ , -0.65 e Å ⁻³	1.79 e Å ⁻³ , -1 e Å ⁻³

The short distance O8–Cu1 2.889 Å indicates some weak attraction. The crystal structure of $V_{10}Cu$ differs from the previously described one⁶¹ in the presence of the NH_4^+ cation that was confirmed by elemental analysis and IR spectroscopy.

The asymmetric unit of $V_{10}Co$ contains one half of the centrosymmetric anion $[\{Co(H_2O)_5\}_2V_{10}O_{28}]^{2-}$ (Fig. 1), one molecule of $(2\text{-hepH})^+$ balancing its charge and two water molecules of crystallization. The decavanadate anion $[V_{10}O_{28}]^{6-}$ is acting as a bridging ligand for two $\{Co(H_2O)_5\}^{2+}$ fragments. The slightly irregular octahedral coordination sphere of the Co1 atom is completed by one oxygen atom O1 coming from the terminal V=O group of the decavanadate (atom O_F in Scheme 1). Such a coordination fashion is not unknown and was already reported for manganese(II)^{72,76} and zinc(II) derivatives.^{68,69} The bond length O1–Co1 2.089(3) Å indicates a relatively strong coordination bond of decavanadate to the metal centre. The bond lengths of the Co1 atom and oxygen atoms of the coordinated water molecules are in the range of 2.105 – 2.115 Å for the equatorial ligands and 2.057(4) Å for the Co1–O15 bond of the water molecule in the *trans* position towards the decavanadate ligand.

The supramolecular structure of $V_{10}Co$ is stabilized by a rich network constructed from hydrogen bonds in which all the individual components are involved. Due to the presence of $\{Co(H_2O)_5\}^{2+}$ fragments the most prominent hydrogen bonds are formed between the adjacent $[\{Co(H_2O)_5\}_2V_{10}O_{28}]^{2-}$ anions. Three oxygen atoms of the decavanadate O5, O6 and O11 are connected with hydrogen bonds to three water molecules of $\{Co(H_2O)_5\}^{2+}$ at contact distances O5··O17 2.636 Å, O6··O17 2.636 Å and O11··O15 2.744 Å.

3.3 Solution studies

3.3.1 Stability in a buffer solution. For the solutions studies 1 mM decavanadate solutions of V_{10} , $V_{10}Cu$ and $V_{10}Co$ were firstly prepared in a medium containing 0.1 M MES buffer and 0.5 M NaCl at pH = 5.8. These conditions were used to simulate the environment of protein crystallization where usually higher buffer concentrations and high ionic strengths are necessary. The acidic regime is preferred to ensure that the protein is positively charged and may therefore more profoundly interact with negatively charged POMs. The ⁵¹V NMR spectra of the given solutions are shown in Fig. 2. In the spectrum of V_{10} the expected distribution of vanadium into several species can be observed. Due to hydrolysis, the equilibrated species include not only the originally employed decavanadate (–422.5, –498.2 and –513.8 ppm), but also monovanadate $H_2VO_4^-$ (V_1 , –558.5 ppm), divanadate $H_2V_2O_7^{2-}$ (V_2 , –571.2 ppm), tetravanadate $V_4O_{12}^{4-}$ (V_4 , –575.0 ppm) and pentavanadate $V_5O_{15}^{5-}$ (V_5 , –583.0 ppm). However, due to the high ionic strength decavanadate is still the dominant species and consumes about 80% of V^V present in the solution.

The spectra of $V_{10}Cu$ and $V_{10}Co$ exhibit only peaks that can be assigned to vanadium atoms arising from decavanadate: –422.3, –496.9 and –511.9 ppm for $V_{10}Cu$ and –422.3 and –495.9 ppm (broad peak) for $V_{10}Co$. This is the first indication that the complex anions stay intact and do not dissociate off the Cu(II) and Co(II) centres. As a matter of fact, the presence of free decavanadate would evoke vanadate self-condensation reactions and the overall picture of the present species should be similar



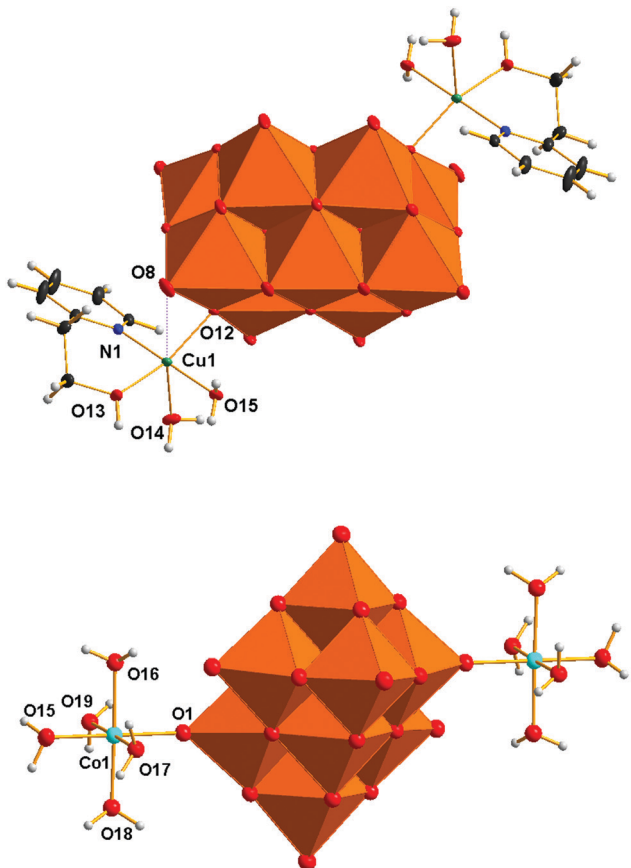


Fig. 1 Molecular structures of the anions $[\{Cu(H_2O)_2(2\text{-hep})\}_2V_{10}O_{28}]^{2-}$ ($V_{10}Cu$) and $[\{Co(H_2O)_5\}_2V_{10}O_{28}]^{2-}$ ($V_{10}Co$) as revealed by X-ray crystallography showing atom labelling. Colour code: orange octahedra $\{VO_6\}$, green Cu, light blue Co, blue N, red O, black C, gray H. Ellipsoids are displayed at a 30% probability level.

to that of V_{10} . Next, similar to Schwendt *et al.*,⁶¹ we observed significant movement of the high-field signal of the V_C atom in $V_{10}Cu$ to -511.9 ppm compared to -513.8 ppm in V_{10} , and the signal of the V_B atom is shifted by 1.3 ppm. For $V_{10}Co$, the shift of the peak is much more obvious and instead of two independent signals for V_B and V_C atoms we observe only a broad peak with the maximum at -495.9 ppm having a high-field shoulder. Based on peak integration, the integral intensities of the two present signals are in the ratio 2 : 8. In addition, significant peak broadening of the individual signals was observed (Table 3). This can be explained by at least two factors. Firstly, vanadates usually provide narrower lines in comparison to common vanadium(v) complexes because of higher symmetry (*i.e.* D_{2h} for ideal $[V_{10}O_{28}]^{6-}$). Thus, peak broadening originates in the decrease of symmetry of the coordinated decavanadates. It is also important to note that for $V_{10}Co$ the low field signal is broadened by only 10% in comparison to free decavanadate, while the second signal is broader by more than 220% compared to the sum of individual signals of V_B and V_C in V_{10} . This difference is naturally caused by the fact that the V_C atoms are the closest ones to the $Co(II)$ atom and the inner V_A atoms are less affected by the coordination. The compound $V_{10}Cu$ formed a cloudy precipitate in the

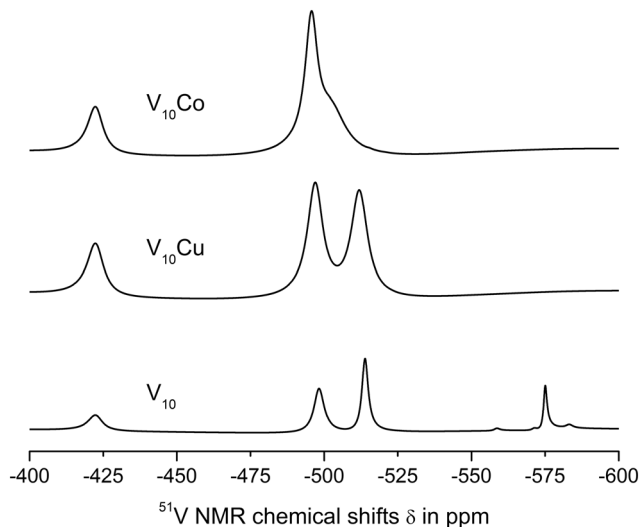


Fig. 2 ^{51}V NMR spectra of 1 mM aqueous solutions of V_{10} , $V_{10}Cu$ and $V_{10}Co$ in 0.1 M MES buffer, 0.5 M NaCl at pH = 5.8.

buffer solution which might have also influenced the peaks' width, but despite this, similar peak broadening as for $V_{10}Co$ was observed – 14% and 200%, respectively. This leads to the conclusion that the peak coalescence is a consequence of its coordination to the $Cu(II)$ center and not the presence of a precipitate. However, we excluded $V_{10}Cu$ from further examination to prevent misinterpretation of protein interaction experiments.

On the other hand, peak broadening may also originate in the presence of paramagnetic centres $Cu(II)$ (d^9) and $Co(II)$ (d^7). The experience has shown, however, that if an extraneous paramagnetic species interferes in a ^{51}V NMR experiment, this manifests itself also in a lower signal-to-noise ratio and uneven lines (this was not the case).

At pH = 8.0 (0.1 M MES, 0.5 NaCl) profound decomposition of V_{10} into lower vanadates was observed, $V_{10}Cu$ dissociated off the $Cu(II)$ complex cation, while $V_{10}Co$ was still the only species present (see Fig. S1 and Table S1, ESI[†]).

3.3.2 Interaction of decavanadates with thaumatin, lysozyme and proteinase K. The interaction of V_{10} and $V_{10}Co$ (1 mM) with model proteins thaumatin (10 μM), lysozyme (10 μM) and proteinase K (3.5 μM) was inspected by ^{51}V NMR

Table 3 The ^{51}V NMR parameters of peaks in the spectra of solutions of V_{10} , $V_{10}Cu$ and $V_{10}Co$ in 0.1 M MES, 0.5 M NaCl at pH = 5.8. The chemical shifts are given in ppm (upper) and the linewidths at half-height in Hz (lower). The linewidths are not given for species with concentrations < 5%

	$H_xV_{10}O_{28}^{(6-x)-}$						
	V_A	V_B	V_C	$H_2VO_4^-$	$H_2V_2O_7^{2-}$	$V_4O_{12}^{4-}$	$V_5O_{15}^{5-}$
V_{10}	-422.5 636.22	-498.2 483.86	-513.8 309.16	-558.5	-571.2	-575.0 176.77	-583.0
$V_{10}Cu$	-422.3	-496.9	-511.9	—	—	—	—
	727.51	775.17	806.75				
$V_{10}Co$	-422.3	-495.9	—	—	—	—	—
	702.38	1794.05 ^a					

^a The linewidth was calculated from the half-height for the low-field and high-field components of the merged broad signal.



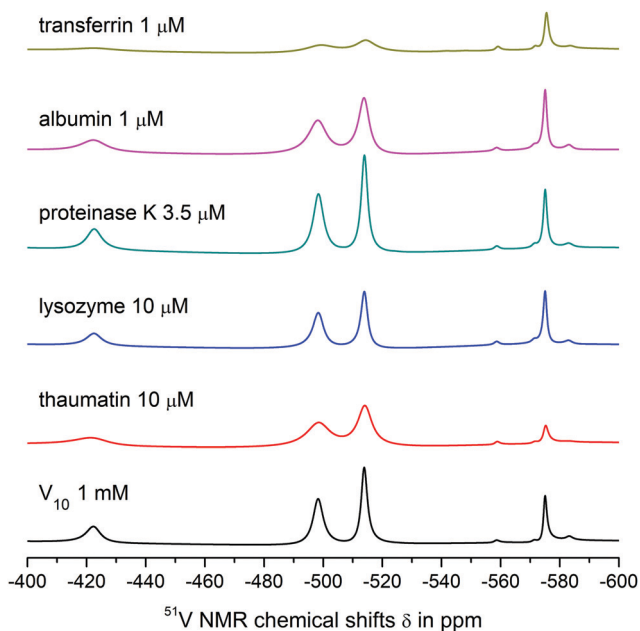


Fig. 3 ^{51}V NMR spectra showing interactions of 1 mM aqueous solutions of V_{10} with model proteins under given concentrations in 0.1 M MES buffer, 0.5 M NaCl at pH = 5.8. The black line represents the spectrum of the referent V_{10}Co and the coloured lines show the spectra of solutions of V_{10}Co in the presence of different proteins at given concentrations.

spectroscopy in 0.1 M MES buffer, 0.5 M NaCl at pH = 5.8 one hour after careful addition of the protein solution to the solution of POMs (Fig. 3 and 4). Table 4 summarizes the data on chemical shifts and linewidths at half-height for the observed peaks. During the experiments, a peak movement larger than ± 1 ppm was not observed indicating that no changes in protonation

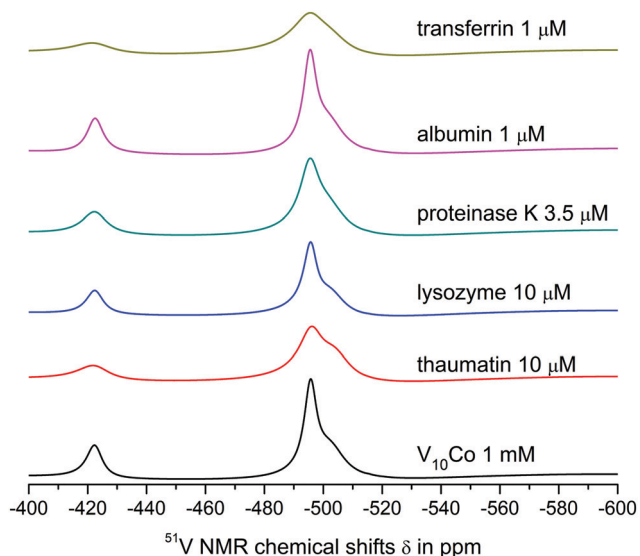


Fig. 4 ^{51}V NMR spectra showing interactions of 1 mM aqueous solutions of V_{10}Co with model proteins under given concentrations in 0.1 M MES buffer, 0.5 M NaCl at pH = 5.8. The black line represents the spectrum of the referent V_{10}Co and the coloured lines show the spectra of solutions of V_{10}Co in the presence of different proteins at given concentrations.

Table 4 The ^{51}V NMR parameters of peaks in the spectra of solutions of V_{10} and V_{10}Co in the presence of model proteins ($c_v = 10$ mM, 0.1 M MES, 0.5 M NaCl, pH = 5.8). The chemical shifts are given in ppm (upper) and the linewidths at half-height in Hz (lower)

Protein	Decavanadate	$\text{H}_x\text{V}_{10}\text{O}_{28}^{(6-x)-}$			
		V_A	V_B	V_C	$\text{V}_4\text{O}_{12}^{4-}$
None	V_{10}	-422.5 636.22	-498.2 483.86	-513.8 309.16	-575.0 176.77
	V_{10}Co	-422.3 702.38	-495.9 1794.05 ^a		—
Thaumatococcus 10 μM	V_{10}	-421.5 1144.08	-498.5 1198.98	-514.1 746.33	-575.2 274.76
	V_{10}Co	-421.8 1265.82	-495.9 2254.31 ^a		—
Lysozyme 10 μM	V_{10}	-422.5 580.67	-498.3 530.95	-4513.9 342.1	-575.0 199.5
	V_{10}Co	-422.4 732.6	-495.7 1810.91 ^a		—
Proteinase K 3.5 μM	V_{10}	-422.6 653.95	-498.4 510.83	-513.9 326.98	-575.1 194.65
	V_{10}Co	-422.3 977.23	-495.6 2041.13 ^a		—
Albumin 1 μM	V_{10}	-422.3 943.72	-498.2 851.65	-513.8 532.05	-575.0 192.97
	V_{10}Co	-422.5 709.7	-495.6 1629.61 ^a		—
Transferrin 1 μM	V_{10}	-422.6 967.87	-499.5 —	-513.8 983.55	-575.5 230.59
	V_{10}Co	-421.6 1268.63	-495.6 2404.42 ^a		—

^a The linewidth was calculated from the half-height for the low-field and high-field components of the merged broad signal.

occurred. Table 4 includes data only for decavanadates and $[\text{V}_4\text{O}_{12}]^{4-}$ as peaks corresponding to other vanadates had integral intensities < 5%. Importantly, no visible reduction of vanadium(v) was recognized after 1 week of standing solutions.

Both V_{10} and V_{10}Co bind strongly to thaumatin resulting in significant peak broadening. In the case of lysozyme, very weak binding to V_{10} may be deduced from slightly broadened higher-field peaks, but no obvious interaction was observed for V_{10}Co . Finally, proteinase K seemed not to interact with free decavanadate, but binds strongly to V_{10}Co . In this case we used about 3 times lower protein concentration; therefore, after extrapolation of the data it can be assumed that the binding of V_{10}Co to proteinase K is comparatively stronger than to thaumatin.

3.3.3 Interaction of decavanadates with human serum albumin and transferrin. Using the same experimental conditions, the interaction of V_{10} and V_{10}Co with the main proteins present in human plasma, namely, human serum albumin (1 μM) and transferrin (1 μM) (Fig. 3, 4 and Table 4), was also examined. Interestingly, free decavanadate V_{10} binds strongly to albumin, but V_{10}Co does not interact. It seems that the Co(II) centers coordinated to V=O groups of decavanadate occupy the binding sites for interaction of decavanadate with this protein and block the interaction. In fact, this was the only case where the interaction was observed only for the free decavanadate but not for the coordinated one. The interaction of $\text{V}_4\text{O}_{12}^{4-}$ with human serum albumin is much weaker than that of decavanadate based on only about 10% increase in the peak width.



The interaction of V_{10} with transferrin is even stronger, and a significant binding to $V_4O_{12}^{4-}$ was also observed. The shift of the peak corresponding to the V_B atom of the decavanadate by 1 ppm made it impossible to determine its half-height width. As expected, the presence of Co(II) causes extremely strong binding of $V_{10}Co$ to transferrin, resulting in peak broadening comparable to that observed in the case of thaumatin, but at $100\times$ lower protein concentration.

The stability of $V_{10}Co$, $V_{10}Cu$ and potentially other coordinated decavanadates in the examined medium at 10 mM total vanadium concentration, in line with the potential of functionalized decavanadates to interact with various proteins, open new possibilities for the investigation of decavanadate's effects in biological systems – for the first time without the side effects of the always present lower oligovanadates (decavanadate may be the only species present in multicomponent solvents).⁷⁷ On the other hand, at physiological concentrations ($c_V = 1 \mu M$ and less), vanadate exists only as a monomeric species (VO_2^+ , $H_2VO_4^-$, HVO_4^- depending on the pH).³² We therefore checked the stability of $V_{10}Co$ at $1 \mu M$ concentration (10 μM total vanadium) by ^{51}V NMR (Fig. S2, ESI†). The chemical shifts corresponding to the decavanadate species (–421.2, –497.9 and –513.1) represent an undisturbed anion indicating that the Co(II) centers are no longer involved in coordination.

4 Conclusions

In this work, we showed that the complex anions $[\{Cu(H_2O)_2(2\text{-hep})\}_2V_{10}O_{28}]^{2-}$ and $[\{Co(H_2O)_5\}_2V_{10}O_{28}]^{2-}$ involving coordinated decavanadate are stable in aqueous solution and do not decompose in the buffer solution compatible with proteins (0.1 M MES, 0.5 NaCl) at pH = 5.8 making them promising candidates for biological studies. For the first time, an interaction between modified decavanadate and biomolecules without the participation of lower oligovanadates was performed. The pilot interaction studies with several proteins used in model protein crystallization research showed that V_{10} and $V_{10}Co$ bind to thaumatin, V_{10} binds also to lysozyme and $V_{10}Co$ binds to proteinase K. As expected, V_{10} interacts with human serum albumin and transferrin, but surprisingly $V_{10}Co$ exhibits high affinity to transferrin but does not interact with albumin. The isolation and structural characterization of the crystalline products is the ultimate goal necessary for more precise understanding of the interaction between POMs and proteins. It is expected that, in addition to electrostatic interaction of V_{10} with proteins, the presence of heterometals may induce a complementary interaction – even a covalent bond – when the coordinated transition metal contains labile ligands such as water molecules (as in $V_{10}Co$ and $V_{10}Cu$) or a vacant accessible coordination position (as in $V_{10}Cu$). Furthermore, both $V_{10}Cu$ and $V_{10}Co$ contain biogenic transition metals that are known to interact with biomolecules to a great extent. In conclusion, the high potential of ligated decavanadate in medicinal chemistry and protein crystallography necessitates the challenging development of synthetic methods leading reliably to stable complexes of decavanadate (*i.e.* decavanadato complexes).

Conflicts of interest

There are no conflicts to declare.

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