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

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The decavanadate anion, \(H_2V_{10}O_{28}^{6-}\) (\(V_{10}\)), 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 \(Na_3VO_4\) 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: \(\{2\text{-(hep)}\}[\text{NH}_4]\{\text{Cu}(H_2O)_2(\text{2-hep})_2V_{10}O_{28}\cdot4H_2O\} (V_{10}\text{Cu})\) and \(\{2\text{-(hep)}\}_2\{\text{Co}(H_2O)_2\}V_{10}O_{28}\cdot4H_2O \ (V_{10}\text{Co})\) \(\{2\text{-(hep) = 2-hydroxyethylpyridine}\). Unlike free decavanadate, the complex anions stay intact in model buffer solutions \(0.1 \text{M} (N\text{-morpholino})\text{ethanesulfonic acid, 0.5 M NaCl, pH} = 5.8 \text{ and 8.0})\). It has been shown that \(V_{10}\text{Co}\) 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 \(V_{10}\text{Co}\) with the model proteins thaumatin, lysozyme, proteinase K, human serum albumin and transferrin under conditions close to biological ones (0.1 M \(2\text{-}N\text{-morpholino})\text{ethanesulfonic acid, 0.5 M NaCl, pH} = 5.8\)). The linewidths of the signals at half-height in \(^{51}\text{V}\) NMR spectra reflect the strength of interaction of a vanadium species with a protein, and thus it was shown that \(V_{10}\) and \(V_{10}\text{Co}\) both bind strongly to thaumatin, \(V_{10}\) binds to lysozyme and \(V_{10}\text{Co}\) binds to proteinase K. \(V_{10}\) interacts with both human serum albumin and transferrin, but surprisingly \(V_{10}\text{Co}\) exhibits high affinity to transferrin but does not interact with albumin.

1 Introduction

Polyoxometalates (POMs) are an important group of metal oxide clusters exhibiting diverse archetypal structures of, particularly, vanadates, molybdates and tungstates. The tremendous variability of POMs has given rise to their application in distinct areas of materials science, catalysis, electrochemistry and redox processes, photochemistry and magnetism. 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 and non-functional interaction with biomolecules, such as proteins, enhancing the current possibilities in macro-molecular crystallography by promoting the crystallization or being useful in obtaining the initial phases while solving the structures of proteins.

Decavanadate, \(H_4V_{10}O_{28}^{6-\cdot}\) \((V_{10})\), is the predominant species formed in vanadate solutions at vanadium(\(V\)) concentrations above 1 mM in the pH range of \(\approx 2-6\). The structure of \(V_{10}\) consists of ten face-sharing octahedra (Scheme 1). The symmetrically non-equivalent vanadium atoms \(V_a\), \(V_c\) and \(V_c\) give rise to three different signals in \(^{51}\text{V}\) NMR spectra depending on the conditions: while the low-field signal of \(V_a\) atoms stays in a narrow region around \(-425 \pm 3 \text{ ppm}\), the other two peaks representing \(V_b\) and \(V_c\) atoms are more sensitive to changes in acidity and exhibit signals at approximately \(-505 \pm 10 \text{ ppm}\) and \(-525 \pm 10 \text{ ppm}\), respectively. The oxygen atoms \(O_h\) and \(O_c\) are potential sites for protonation, and the atoms \(O_f\), \(O_r\), \(O_i\) and \(O_d\) are the most potential sites for ligation to transition metal ions.
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.  

Vanadium is naturally omnipresent in biological matrices in a few enzymes such as vanadium dependent haloperoxidases and nitrogenases or acts as a crucial component in the energetic metabolism of Ascidia. Some artificial vanadium compounds, on the other hand, exhibit insulin mimetic properties, antitumor activity, antibacterial activity or anti-HIV activity.

Specifically decavanadate itself has also been studied with respect to many biological aspects and it was shown that $V_{10}$ binds to several proteins such as actin, myosin, ion pump Ca$^{2+}$-ATPase, bovine serum albumin and gelatine, and microtubule-associated proteins. Protein crystallography revealed the presence of $V_{10}$ in the crystal structures of acid phosphatase A (F. tularensis), human activated receptor tyrosine kinase, NTPDase1 (L. pneumophila), NTPDase1 (R. norvegicus), and human TRPM4 channel. In all cases, $V_{10}$ was formed from the initially employed Na$_3$VO$_4$ (used as a phosphatase inhibitor) and its role may be explained as stabilization and rigidification of the protein structure.

In this work we compare the interaction of both free and ligated decavanadate with commercially available model proteins thauamatin, 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 Cu$^{2+}$ and Co$^{2+}$ 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 danielli (Sigma, T7638; a mixture of thaumatin I and thaumatin II with traces of other sweet proteins), proteinase K from Trichirachium 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 4000–100 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$_3$ ($\delta = 0$ ppm) as a standard. The solutions were in general prepared by dissolving 0.01 mmol of the given decavanadate in 700 L 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 L of D$_2$O used for locking. Next, the solution was either made up to 1000s L 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 L 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 L of this solution was diluted in 200 L 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 ($V_{10}$Cu, Mo Kα) and Bruker D8 Venture ($V_{10}$Co, Cu Kα) 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 $V_{10}$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 package (frame integration, cell refinement), SADABS (absorption correction), SHELXS-2013 (structure solution), SHELXL-2013 (structure refinement), OLEX2 (user interface and publication...
3. Results and discussion

3.1 Syntheses

To the best of our knowledge, the work of Schwendt et al.\(^{51}\) presenting the synthesis and characterization of \((2\text{-heph})\text{[Cu(H}_2\text{O)}_2\text{V}_{10}\text{O}_{28}]\), \([\text{Co(H}_2\text{O)}_5\text{V}_{10}\text{O}_{28}]\) and \([\text{Zn(H}_2\text{O)}_4\text{V}_{10}\text{O}_{28}]\) 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 work.\(^{60}\) 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 V\(_{10}\)Cu and V\(_{10}\)Co are summarized in Table 2. The crystal structure refinement of V\(_{10}\)Cu revealed the presence of the complex anion \([(\text{Cu(H}_2\text{O)})_2\text{V}_{10}\text{O}_{28}]^{2+}\) that has already been reported (Fig. 1).\(^{61}\) The decavanadate anion \([\text{V}_{10}\text{O}_{39}]^{2-}\) is a bridging ligand between two \([(\text{Cu(H}_2\text{O)})_2\text{V}_{10}\text{O}_{28}]^{2+}\) fragments and binds to the Cu(I) atom through oxygen atom O12 (C2 in Scheme 1) at 1.9612(8) Å. The organic ligand is chelating the Cu(I) 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-cube 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 \([\text{V}_{10}\text{O}_{38}]^{8-}\) (O2 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                       | 19909250                      |
| Empirical formula| V_{10}Cu_{2}O_{6}C_{2}H_{4}N_{4} | V_{10}Co_{2}O_{6}C_{4}H_{4}N_{2} | 1575.80
| M_r              | 1605.02                       |                               |
| Crystal system, space group | Monoclinic, C2/c | Triclinic, PI |
| Temperature (K)  | 100                           | 100                           |
| a (Å)            | 27.0345(15)                   | 29.4219(5)                   |
| b (Å)            | 9.9170(5)                     | 11.2451(6)                   |
| c (Å)            | 20.5851(12)                   | 11.9820(7)                   |
| z (Å)            | 119.341(5)                    | 75.1180(17)                  |
| V (Å³)           | 4810.9(5)                     | 1117.66 (11)                 |
| Z                | 4                             | 1                             |
| Radiation type   | Mo Kz                         | Cu Kz                         |
| μ (mm⁻¹)         | 2.82                          | 23.51                         |
| Absorption correction | Multi-scan                 | Multi-scan                   |
| T_{min}          | 360                           | 782                           |
| T_{max}          | 2.2–35.7³                    | 5.7–72.6⁰                    |
| Theta range      | 0.45 × 0.25 × 0.22            | 0.11 × 0.11 × 0.04           |
| Crystal size in mm | 343,398, 11,216, 9986        | 16,530, 40,79, 3882          |
| Index ranges     | 0.062                         | 0.127                         |
| No. of reflections, parameters, restraints | 112,16, 421, 9               | 4079, 337, 20                |
| R_{int}          | 1.10                          | 1.16                          |
| No. of measured, independent and observed [I > 2σ(I)] reflections | 0.024                      | 0.098                         |
| R (I)            | 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 NH₄⁺ cation that was confirmed by elemental analysis and IR spectroscopy.

The asymmetric unit of V_{10}Cu contains one half of the centro-symmetric anion [[Co(H₂O)₅]₂V₁₀O₈]⁻ (Fig. 1), one molecule of (2-hepH)⁺ balancing its charge and two water molecules of crystallization. The decavanadate anion [V₁₀O₈]⁶⁻ is acting as a bridging ligand for two [Co(H₂O)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 O₅ 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 lengths 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}Cu is stabilized by a rich network constructed from hydrogen bonds in which all the individual components are involved. Due to the presence of [Co(H₂O)₅]²⁺ fragments the most prominent hydrogen bonds are formed between the adjacent [[Co(H₂O)₅]₂V₁₀O₈]⁻ anions. Three oxygen atoms of the decavanadate O5, O6 and O11 are connected with hydrogen bonds to three water molecules of [Co(H₂O)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 V₁₀, V₁₀Cu and V₁₀Co 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 V₁₀ 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 H₂VO₄⁻ (V₁, −558.5 ppm), divanadate H₂V₂O₅⁻ (V₂, −571.2 ppm), tetravanadate V₄O₁₂⁴⁻ (V₄, −575.0 ppm) and pentavanadate V₅O₁₅⁵⁻ (V₅, −583.0 ppm). However, due to the high ionic strength decavanadate is still the dominant species and consumes about 80% of V¹ present in the solution.

The spectra of V₁₀Cu and V₁₀Co exhibit only peaks that can be assigned to vanadium atoms arising from decavanadate: −422.3, −496.9 and −511.9 ppm for V₁₀Cu and −422.3 and −495.9 ppm (broad peak) for V₁₀Co. 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.
to that of V10. Next, similar to Schwendt et al., we observed significant movement of the high-field signal of the VC atom in V10Cu to −511.9 ppm compared to −513.8 ppm in V10, and the signal of the VB atom is shifted by 1.3 ppm. For V10Co, the shift of the peak is much more obvious and instead of two independent signals for VB and VC 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. D2h for ideal [V10O28]6−). Thus, peak broadening originates in the decrease of symmetry of the coordinated decavanadates. It is also important to note that for V10Co 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 VB and VC in V10. This difference is naturally caused by the fact that the VC atoms are the closest ones to the Co(ii) atom and the inner VA atoms are less affected by the coordination. The compound V10Cu formed a cloudy precipitate in the buffer solution which might have also influenced the peaks’ width, but despite this, similar peak broadening as for V10Co 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 V10Cu 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) (d9) and Co(ii) (d7). The experience has shown, however, that if an extraneous paramagnetic species interferes in a 51V 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 V10 into lower vanadates was observed, V10Cu dissociated off the Cu(ii) complex cation, while V10Co 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 V10 and V10Co (1 mM) with model proteins thaumatin (10 mM), lysozyme (10 mM) and proteinase K (3.5 mM) was inspected by 51V NMR in 0.1 M MES buffer which might have also influenced the peaks’ width, but despite this, similar peak broadening as for V10Co 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 V10Cu from further examination to prevent misinterpretation of protein interaction experiments.

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### Table 3

The 51V NMR parameters of peaks in the spectra of solutions of V10, V10Cu and V10Co in 0.1 M MES, 0.5 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%

| V10 | V10Cu | V10Co |
| --- | --- | --- |
| H1V10O286− | H1V10O286− | H1V10O286− |
| VA | VB | VC | VA | VB | VC | VA | VB | VC |
| H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− | H2V10O2− |
| −422.5 | −498.2 | −513.8 | −558.5 | −571.2 | −575.0 | −583.0 | −422.3 | −496.9 | −511.9 |
| 636.22 | 483.86 | 309.16 | 176.77 | 176.77 | 176.77 | 176.77 | 727.51 | 775.17 | 806.75 |
| 702.38 | 1794.05 | 1794.05 | 1794.05 | 1794.05 | 1794.05 | 1794.05 | 702.38 | 1794.05 | 1794.05 |

| V10 | V10Cu | V10Co |
| --- | --- | --- |
| H3V10O42− | H3V10O42− | H3V10O42− | H3V10O42− | H3V10O42− | H3V10O42− |
| −575.0 | −583.0 | −558.5 | −571.2 | −575.0 | −583.0 |

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*The linewidth was calculated from the half-height for the low-field and high-field components of the merged broad signal.*
spectroscopy in 0.1 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 occurred. Table 4 includes data only for decavanadates and [V\(_{4}\)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 \(\mu\)M) and transferrin (1 \(\mu\)M) (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(\(n\)) centers coordinated to \(\text{V}=\text{O}\) 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 V\(_{4}\)O\(_{12}\)\(^{4-}\) with human serum albumin is much weaker than that of decavanadate based on only about 10% increase in the peak width.

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**Table 4** The \(^{51}\text{V}\) NMR parameters of peaks in the spectra of solutions of V\(_{10}\) and V\(_{10}Co\) in the presence of model proteins (c\(_p\) = 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 | V\(_{A}\) | V\(_{B}\) | V\(_{C}\) | V\(_{4}\)O\(_{12}\)\(^{4-}\) |
|-------------|--------------|----------|----------|----------|-----------------|
| None        | V\(_{10}\)   | -422.5   | -498.2   | -513.8   | -575.0          |
|             | V\(_{10}Co\) | 636.22   | 483.86   | 309.16   | 176.77          |
| Thaumatin   | V\(_{10}\)   | -421.5   | -498.5   | -514.1   | -575.2          |
|             | V\(_{10}Co\) | 1144.08  | 1198.98  | 746.33   | 274.76          |
| Lysozyme    | V\(_{10}\)   | -421.8   | -495.9   | —        | —               |
|             | V\(_{10}Co\) | 1265.82  | 2254.31  | —        | —               |
| Proteinase K| V\(_{10}\)   | -422.6   | -498.4   | -513.9   | -575.0          |
|             | V\(_{10}Co\) | 653.95   | 510.83   | 326.98   | 194.65          |
| Albumin     | V\(_{10}\)   | -422.3   | -495.6   | —        | —               |
|             | V\(_{10}Co\) | 977.23   | 2041.13  | —        | —               |
| Transferrin | V\(_{10}\)   | -422.6   | -499.5   | -513.8   | -575.0          |
|             | V\(_{10}Co\) | 943.72   | 851.65   | 532.05   | 192.97          |

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

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**Fig. 3** \(^{51}\text{V}\) 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.

**Fig. 4** \(^{51}\text{V}\) 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.
The interaction of V_{10} with transferrin is even stronger, and a significant binding to V_{10}O_{12} 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× 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 functionalyzed 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). On the other hand, at physiological concentrations (c_v = 1 µM and less), vanadate exists only as a monomeric species (VO_3^-), H_2VO_4^- and HVO_4^- depending on the pH. We therefore checked the stability of V_{10}Co at 1 µM 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+}(2-heap)]_2V_10O_{28}]^{2+} and [[Co(H_2O)_{3-3+}V_10O_{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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