Magnetic Properties of Tunicate Blood Cells. I. *Ascidia nigra*

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**ABSTRACT**

The magnetic properties of intact and freeze-dried blood cells of the tunicate *Ascidia nigra* and of model vanadium(III) and (IV) compounds as polycrystalline solids and in aqueous solution have been measured up to 50 kOe with a SQUID susceptometer. Corrections for the samples' diamagnetism were extracted from the temperature dependence of the data without any further assumptions. For vanadium(IV), measured values of the magnetic moment at different values of the applied magnetic field over the temperature range 2–100 K obey a Brillouin function with spin 1/2. For vanadium(III), the magnetic moment data did not obey a Brillouin function and were analyzed in terms of a spin Hamiltonian with \( S = 1 \). Measurements on both whole and freeze-dried blood samples give consistent results with vanadium(III) the predominant species. These results are discussed in terms of the mechanisms of vanadium accumulation and the use of vanadium oxidation states as criteria of ascidian taxonomy.

**ABBREVIATIONS**

B.M., Bohr magneton; cat, \([o-C_6H_4(O)_2]^{2-}\) (o-dihydroxybenzene dianion); ESR, electron spin resonance; XAS, x-ray absorption spectroscopy; SQUID, superconducting quantum interference device.

**INTRODUCTION**

Ever since Henze first discovered vanadium in the blood cells of the tunicate *Phallusia mammillata* and reported the element to be present as vanadium(III) [1–3], there has
been much discussion of the oxidation state of the accumulated vanadium [4]. Tunicates (sea squirts) are sessile marine invertebrates that sequester metal ions, especially iron and vanadium, storing them in reduced form in certain blood cells [5–8]. However, the +3 oxidation state of vanadium is strongly reducing and its presence in a living system, except as a transient intermediate, is unusual. Early studies such as Henze's were performed on cell lysates using chemical techniques, not on intact blood cells. Due to the susceptibility of the lysates to oxidize and undergo other chemical changes rapidly, artifacts may well have been introduced into these gross chemical analyses.

A better determination of intracellular oxidation state would be obtained if a noninvasive technique were utilized, thus keeping the blood cells intact during the measurement. Three such techniques that have been used are magnetic susceptibility, ESR, and XAS. Boeri and Ehrenberg [9] studied *P. mammillata* and *Ascidia obliqua* blood cells and lysates with magnetic susceptibility and concluded that endogenous vanadium present in these cells is in the +3 oxidation state. In an XAS study of *A. ceratodes* blood cells [10], however, the intracellular vanadium was found to be present in two oxidation states: about 90% vanadium(III) ion and 10% or less of vanadium(IV) ion. The XAS study confirmed a previous detection of vanadium(IV) in the blood cells of the same species by ESR [6]. The relative amount of vanadium(IV) measured was similar to that reported in a subsequent ESR study of *A. nigra* blood cells [7].

Though they represent improvements over the lysate studies, each of the previous noninvasive studies have drawbacks. Vanadium(III) is in a $d^2$ oxidation state and is therefore ESR-silent. XAS is sensitive to oxidation state; however, an interpretation of XAS spectra requires knowledge of the element's coordination number, binding site geometry, and nature of dentate atoms. These factors are usually evaluated on the basis of model compound studies, but a precise knowledge of the intracellular environment of the accumulated vanadium is not available, and the model compounds chosen for this purpose, i.e., sulfato and acetylacetonato complexes [10], may not be appropriate.

Compared with the other two methods, magnetic susceptibility has advantages. It can be used to detect any paramagnetic oxidation state, and is independent of model compound selection. However, care must be exercised in interpreting the magnetic susceptibility data. Most biological specimens consist mainly of diamagnetic substances. Since this material dominates the total susceptibility at room temperature, the paramagnetic contribution is obtained by subtracting the larger, diamagnetic term from the total susceptibility.

In correcting their data for diamagnetism, Boeri and Ehrenberg subtracted terms for sulfuric acid, sulfate salts, and protein. However, the intracellular material of the blood cells of several species appear not to contain sulfuric acid [11], and protein, though certainly present, may not be the main organic intracellular component [12]. Another equally serious problem with the earlier work is that the magnetic moments of the vanadium ions were inferred from data collected at a single temperature and a single applied magnetic field. Without varying these parameters, it is difficult to quantify the description of the spin oxidation state.

Because of the difficulty in interpreting much of this early work, we have undertaken a complete study of the magnetic properties of tunicate blood cells from *A. nigra*. Magnetic properties were also determined for $K_2[VO(cat)_2]$ and $K_3[V(cat)_3]$ in polycrystalline solid and solution forms. These compounds are models for vana-
dium(IV) and vanadium(III), respectively. Diamagnetic corrections were obtained from the susceptibility results without further assumptions, as discussed below. It will be shown that the temperature dependence of the paramagnetic moment in different applied magnetic fields is diagnostic for the oxidation state of vanadium and can be used to ascertain the predominant vanadium oxidation state in tunicate blood cells.

EXPERIMENTAL

The compounds K₂[VO(cat)₂], I, and K₃[V(cat)₃], II, model complexes for vanadium(IV) and vanadium(III), respectively, were prepared according to a previous procedure [13]. Magnetic measurements were made for samples in sealed Delrin holders. Since both model compounds and blood cells are air-sensitive, preparation of solutions and transfers of samples to the SQUID sample holder were carried out in a glove bag filled with an inert gas such as analytical grade N₂ or Ar. The amount of metal in the model compounds was obtained from elemental analysis, which also served to confirm the identities of these two catecholato complexes. Solutions of the model compound samples were made in distilled water (concentrations: V(III) compound 0.313 M; V(IV) compound 0.482 M; total volume of each 140 μL).

Blood was taken under anaerobic conditions from the hearts of A. nigra [12] (gathered off Key Biscayne, FL) and centrifuged. The plasma was discarded and the blood pellet collected. Concentrations of V, Fe, and Mn were obtained using a Spectrametrics Spectrospan IIIIB DC plasma emission spectrometer after cold digestion with concentrated nitric acid (blood sample: 86.85 mg wet weight; V, 8.0 ± 0.2; Fe, 0.202 ± 0.002; Mn, 0.0025 ± 0.0007 μg/mg). The freeze-dried blood sample was prepared by anaerobically lyophilizing the centrifuged blood pellet (freeze-dried blood cells: 31.34 mg dry weight: V, 38.0 ± 0.7; Fe 0.130 ± 0.004, Mn 0.005 ± 0.002 μg/mg). The whole blood sample and the freeze-dried blood cells were obtained from different groups of individual tunicate specimens.

Magnetic moment measurements at applied fields between 5 and 50 kOe were carried out with an S. H. E. VTS-905 SQUID magnetometer operating between 2 and 100 K.

DATA TREATMENT AND RESULTS

Model Compounds

The magnetic moment per molecule Mₚ in B.M. for compound I is plotted as a function of H/T in Figure 1, where H is the applied magnetic field intensity (kOe) and T is the absolute temperature (K). The experimental data were corrected for diamagnetism by noting that in the high temperature (T > 30 K) Curie region, the paramagnetic moment Mₚ varies as 1/T, whereas the diamagnetic moment M_D is temperature independent. Thus, the total moment M_T is given by

$$M_T = M_P + M_D = \frac{C}{T} + M_D$$  \hspace{1cm} (1)

where C is the proportionality factor relating M_P to 1/T. A plot of the product M_T(T) versus T for 30 ≤ T ≤ 100 K yields a straight line with slope M_D. If there were significant interactions between vanadium ions, M_P would vary as 1/(T − θ) and M_T(T) would not be linear in T. M_D is proportional to H and includes contributions
from the sample and the container. Diamagnetic corrections for all the other samples were determined similarly.

The paramagnetic moment in Bohr magnetons per vanadium ion can be calculated from the expression

$$M_p = g \langle S_H \rangle_T$$

(2)

where \( g = 2 \) is the electronic g-factor and \( \langle S_H \rangle_T \) is the thermal average of the spin \( S \) projected along the direction of the applied magnetic field. The molar susceptibility \( \chi_M \) is related to \( M_p \) by

$$\chi_M = N_0 \beta M_p / H$$

(3)

where \( N_0 \) is Avogadro's number and \( \beta \) is the Bohr magneton; \( N_0 \beta = 5585 \) in cgs units. The quantity known as the effective moment, \( \mu_{\text{eff}} \), is related to \( M_p \) by

$$\mu_{\text{eff}} = [(3kT/\beta H)M_p]^{1/2}$$

(4)

where \( k \) is Boltzmann's constant.

Knowledge of \( \langle S_H \rangle_T \) permits the calculation of all of the above quantities. If the ground state multiplet splits isotropically in an applied magnetic field, i.e., no zero-field splittings, the thermal average is given by the Brillouin function

$$\langle S_H \rangle_T = SB_1(x) = S \left[ \frac{(2S+1)}{2S} \coth \left[ 1/2(2S+1)(x) \right] - \frac{1}{2S} \coth \left[ 1/2(x) \right] \right]$$

(5)

where \( x = g\beta H/kT \) and \( 0 \leq B_1(x) \leq 1 \). In this case \( M_p \) is a unique function of the ratio \( H/T \) and a plot of \( M_p \) versus \( H/T \) for different choices of \( H \) and \( T \) should follow equation (5). At low values of \( H/T \), \( M_p \) is linear in \( H/T \) with the slope proportional to \( g^2 S(S + 1) \); at high values of \( H/T \), \( M_p \) asymptotically approaches \( gS \).
This behavior is illustrated by the data for the solid sample, compound I, in Figure 1. Vanadium(IV) is a $d^1$ system; hence, the ground state spin $S = 1/2$. The spin value $S = 1/2$ is always isotropic and $M_P$ at 25 and 50 kOe follows a Brillouin function of $S = 1/2$, saturating at 1 B.M. per vanadium ion.

For compound I in solution, $M_P$ is plotted as a function of $H/T$ in Figure 2. The data also follow a Brillouin function with $S = 1/2$, but the fit requires that 7% of the vanadium ions have $S = 0$, i.e., are diamagnetic. The necessity for incorporating the higher oxidation state into the fit is likely to have resulted from a partial oxidation of the sample to vanadium(V) ($d^6$) during handling. In practice, underestimation of the number of moles of vanadium(IV) in the sample is indistinguishable from partial oxidation. Nevertheless, the $S = 1/2$ Brillouin function’s dependence of the data identifies the paramagnetic vanadium ions in the sample as vanadium(IV).

For the solid sample, compound II, $M_P$ is plotted as a function of $H/T$ in Figure 3. Here the value of $M_P$ at a given value of $H/T$ depends on the magnitude $H$. This result indicates magnetic anisotropy of the ground state and is consistent with $S = 1$. Now $\langle S_H \rangle_T$ must be determined from the spin Hamiltonian

$$3\mathcal{C} = D[(S_x)^2 - (1/3)S(S+1)] + E[(S_y)^2 - (S_y)^2] + \beta(g_x H_x S_x + g_y H_y S_y + g_z H_z S_z)$$

(6)

with $S = 1$. For a given orientation of the applied field with respect to the crystalline xyz-axis system, the spin Hamiltonian is diagonalized and the projection of the spin along the applied field is calculated. A polycrystalline average is obtained by stepping the applied field direction in equal increments of solid angle over an octant of the unit sphere. In the present case, a $40 \times 40$ grid was used.

The solid lines in Figure 3 are a fit to the data using a simplex least-squares-fitting algorithm with variable parameters $D$, $E$, $g_x$, $g_y$, and $g_z$ (Table I). Given the large
FIGURE 3. Magnetic moment per ion versus \( H/T \) of a vanadium(III) solid, \( K_3[V(\text{cat})_3] \) powder. The solid lines are calculations in 50, 25, and 12.5 kOe using a spin Hamiltonian, as explained in the text.

The number of variables, single crystal data would be required to obtain a unique fit. Nevertheless, the data and fit illustrate the point that the \( S = 1 \) ground state of vanadium(III) has magnetic anisotropy that is easily distinguished from the isotropic \( S = 1/2 \) ground state of vanadium(IV). Note that in the present case the initial slope is proportional to \((g_{av})^2S(S + 1)\), where

\[
(g_{av})^2 = \frac{1}{3}[(g_x)^2 + (g_y)^2 + (g_z)^2] \tag{7}
\]

### TABLE 1. Spin Oxidation States and Fitted Parameters

<table>
<thead>
<tr>
<th>System</th>
<th>Oxidation state</th>
<th>Fitted Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_3[\text{VO(cat)}_3] ) (s)</td>
<td>100% V(IV), ( S = 1/2 )</td>
<td>( D = 4.6 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td>( 0.482 \text{ M (aq.)} )</td>
<td>93% V(IV), ( S = 1/2 )</td>
<td>( E = 1.1 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td>( K_3[V(\text{cat})_3] ) (s)</td>
<td>7% V(V), ( S = 0 )</td>
<td>( g_x = 1.97, g_y = 1.89, g_z = 1.46 )</td>
</tr>
<tr>
<td>[( V(\text{cat})_3 )] ( 2^- )</td>
<td>100% V(III), ( S = 1 )</td>
<td>( D = -7.1 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td>( 0.313 \text{ M (aq.)} )</td>
<td>11% V(IV), ( S = 1/2 )</td>
<td>( E = -2.2 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td>Freeze-dried ( A. \text{nigra} ) blood</td>
<td>80% V(III), ( S = 1 )</td>
<td>( g_x = 1.97, g_y = 1.83, g_z = 2.00 )</td>
</tr>
<tr>
<td>( 20% \text{ V(IV), } S = 1/2 )</td>
<td>( D = -25.0 \text{ cm}^{-1}, )</td>
<td></td>
</tr>
<tr>
<td>Whole ( A. \text{nigra} ) blood</td>
<td>90% V(III), ( S = 1 )</td>
<td>( E = -1.7 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td>( 10% \text{ V(IV), } S = 1/2 )</td>
<td>( g_x = 2.00, g_y = 2.00, g_z = 2.00 )</td>
<td>( D = -25.5 \text{ cm}^{-1}, )</td>
</tr>
<tr>
<td></td>
<td>( g_x = 1.67, g_y = 1.22, g_z = 2.66 )</td>
<td>( E = -1.8 \text{ cm}^{-1}, )</td>
</tr>
</tbody>
</table>
For compound II in solution, $M_p$ is plotted as a function of $H/T$ in Figure 4. As in the polycrystalline solid sample, the value of $M_p$ at a given value of $H/T$ depends on the magnitude of $H$, indicating a zero-field splitting of compound II in solution. The solid lines are a least-squares fit of Equation (6) to the data. However, unlike the solid sample, the solution data require that a portion of the vanadium ions have $S = 1/2$ or $S = 0$, again indicating partial oxidation of the sample to vanadium(IV) or vanadium(V). Fits could be obtained assuming either oxidation state. An ESR signal indicating vanadium(IV) was detected (but not integrated) in a sample subjected to similar handling as the magnetic measurement sample. Therefore, the fit with $S = 1/2$ oxidation product is shown in Figure 4 and given in Table I. The same caveats as those cited for the fit to the solid sample data apply here. Nevertheless, the data clearly indicate vanadium(III) as the predominant oxidation state in solution.

**Freeze-dried and Whole Blood**

As a rule, ascidians that accumulate vanadium primarily also accumulate lesser amounts of metals such as iron and manganese, and the samples we have studied are not exceptions. In the freeze-dried blood cell sample, the amount of manganese is an insignificant 0.013% of the amount of vanadium. Iron, which is 0.34% of the vanadium in the same sample, could contribute to the magnetic signal, depending on its combination of oxidation and spin states. If, for example, all the vanadium were +3, $S = 1$ and all the iron were +3, $S = 5/2$, then the iron could contribute up to 1.5% of the magnetic signal because the contribution goes as $S(S + 1)$. Our data fits did not require the inclusion of this contribution; moreover, there is evidence that iron accumulates in tunicate blood cells in reduced form as iron(II) [8], which is diamagnetic in the low-spin electronic configuration.

For the freeze-dried blood cell sample, $M_p$ is plotted as a function of $H/T$ in Figure
5. The data for the whole blood sample are shown in Figure 6. As in Figures 3 and 4, the value of $M_p$ at a given value of $H/T$ depends on the magnitude of $H$, indicating a zero-field splitting of the accumulated vanadium. Therefore, vanadium(III) is the predominant form of vanadium in both samples. The solid lines in the figures are least-squares fits of Equation (6) to the data, assuming a single species of vanadium(III) in each sample. The fit also requires some oxidized vanadium; assuming this species to

FIGURE 5. Magnetic moment per ion versus $H/T$ of $A.\ nigra$ freeze-dried blood cells. The solid lines are calculations in 50, 25, and 12.5 kOe using a spin Hamiltonian, as explained in the text.

FIGURE 6. Magnetic moment per ion versus $H/T$ of whole $A.\ nigra$ blood cells. The solid lines are calculations in 50, 25, and 12.5 kOe using a spin Hamiltonian, as explained in the text.
be vanadium(IV) \((S = 1/2)\) corresponds to 80\% vanadium(III) and 20\% vanadium(IV) in the freeze-dried sample, and 90\% vanadium(III) and 10\% vanadium(IV) in the whole blood sample.

**DISCUSSION**

The model compound studies provide an effective test of the theoretical and experimental approaches to using magnetic data for the determination of intracellular oxidation states of vanadium. The superposition of the data for different combinations of \(H/T\) onto a single curve in Figures 1 and 2 shows that it is possible to extract reliable values for the diamagnetic contribution and the spin state of a paramagnetic ion in dilute solution using the SQUID susceptometer. For this case the saturation value of the magnetization identifies the metal ion as vanadium(IV). The small amount of vanadium(V) needed to fit the data indicates how important it is to exclude atmospheric oxygen in preparing samples for analysis.

Different values of the magnetization at the same value of \(H/T\) are given by the three nonsuperposable curves of Figures 3 and 4. This result identifies the presence of a paramagnetic ion with an anisotropic ground state that is split at zero field and necessarily excludes \(S = 1/2\) as a possibility for the spin of the ground state. Unlike the case \(S = 1/2\), the saturation value alone of the magnetization at a single value of the applied field is not sufficient to determine \(S\). Rather, a set of curves is needed to determine the details of the zero field splitting. Regardless of these details, it is thus clear from Figures 5 and 6 that the +3 oxidation state predominates for vanadium in the blood cells of *A. nigra*.

It is interesting to consider whether the lesser amounts of vanadium(IV) needed to fit the data are endogeneous or result from inclusion of atmospheric oxygen during sample preparation. Our data are not conclusive in this regard; however, should a single cell contain vanadium in two oxidation states, the implication could be that the organism maintains a precise value of the oxidation-reduction potential in the cell rather than a strongly reducing potential intended to maintain the lower oxidation state exclusively. Another possibility is that accumulation of vanadium takes place in a stepwise fashion either within the same cell or within a different blood cell.

Recent analyses of the metal contents of sorted *A. nigra* blood cells [14] and the sorted cells of *A. ahodori* [15] indicate that vanadium is stored chiefly in signet ring cells, but some vanadium also occurs in morula cells. It is possible that the measured distribution of accumulated vanadium's oxidation states is the same in both cells. However, it is also possible that the lesser amount of vanadium residing in the morula cells is present in the higher oxidation state \((+4)\), and the greater amount of vanadium accumulated in the signet ring cells is present in the +3 oxidation state. The studies reported here are based on whole blood and do not distinguish vanadium in different cell types. SQUID studies of sorted cells have the potential to solve this problem, in which vanadium oxidation states occur in each type of blood cell where the element is accumulated.

The taxonomy of ascidians is traditionally based on morphological features [16]. Data on vanadium oxidation states, based on atomic absorption and ESR measurements, have been used to reexamine ascidian classification [17]. It was proposed that the suborder Phlebobranchia, of which *A. nigra* is a member, contains species that accumulate vanadium in the +3 oxidation state and that a second suborder, Aplousobranchia, contains vanadium in the +4 oxidation state. Since this discrimina-
tion was based on ESR data, and the concentration of vanadium(III) was only inferred, our future experiments with SQUID on freeze-dried blood cells, for a variety of ascidians, will provide a critical test of the validity of using this biochemical criterion of taxonomy.

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