Reactivity of Tunichromes: Reduction of Vanadium(V) and Vanadium(IV) to Vanadium(III) at Neutral pH

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Ascidians accumulate vanadium ions to extraordinarily high concentrations (up to 1 M)[1a] from sea water, where vanadium is present in the +5 oxidation state.[1] In ascidian blood cells, however, the vanadium was found to be in the +3 and/or +4 states[2]—in *Ascidia nigra*, at least 90% of the total vanadium is in the oxygen-sensitive +3 state.[2a–c] Organic ligands are thought to maintain the solubility of concentrated vanadium at biological pH values.[1a,3] These ligands may belong to a class of oxygen-sensitive pigments 1 and 2, called tunichromes,[4–6] whose polyphenolic moieties suggest a role in vanadium accumulation by complexation and/or reduction. It is known that tunichromes and catechols can reduce V^V to V^{IV} in vitro;[4,7,8] however, proof of any

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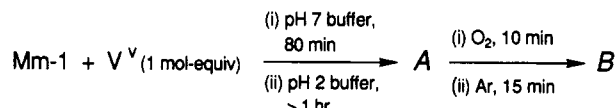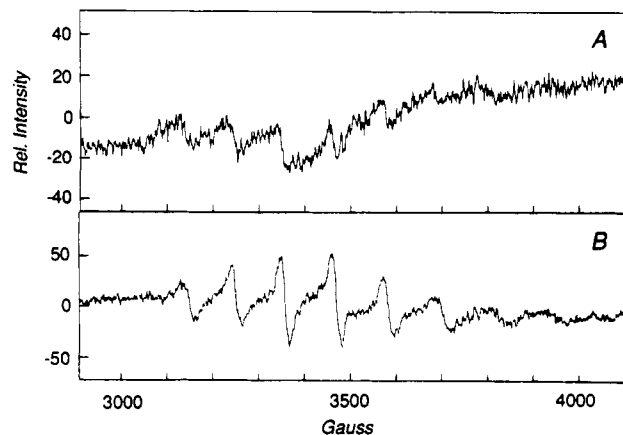

Figure 1. EPR spectra of Mm-1 treated with 1 mol equiv of V^V in pH 7 buffer (adjusted to pH 2 before EPR) before (A) and after (B) oxygenation.

Table I. Ratio of V^{IV} versus V^{III} Found by EPR Analysis of Mm-1 Treated with V^V or V^{IV} (1, 2, and 4 mol equiv) at pH 7[a]

entry	starting metal	V^{IV} found vs V^{III} found
1	1 mol equiv of V^{IV}	85:15
2	2 mol equiv of V^{IV}	90:10
3	4 mol equiv of V^{IV}	60:40
4	1 mol equiv of V^V	trace:<100
5	2 mol equiv of V^V	60:40
6	4 mol equiv of V^V	93:7

[a] Adjusted to pH 2 before EPR measurement at room temperature.

relationship between tunichrome and vanadium in vivo has been elusive.
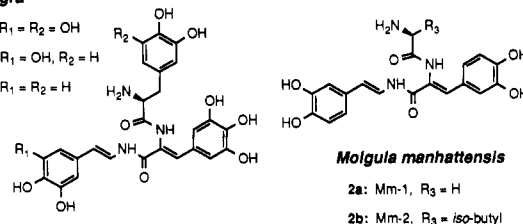
In studies of the general reactivity of tunichrome in vitro,[9] *we have obtained the first evidence (using EPR spectroscopy) that tunichrome can reduce V^V, and also V^{IV}, to the +3 oxidation state, V^{III}.* These reactions were conducted in neutral aqueous media. Similar redox reactivity was previously observed under anhydrous conditions: V^{IV} was reduced by pyrogallol in THF[10] and by 3,5-di-*tert*-butylcatechol in toluene or methanol.[11] The present results corroborate the hypotheses that tunichrome could generate V^{III} in vivo and that an oxidized form of tunichrome could sequester native V^{III}.

The reaction between the simplest tunichrome 2a (synthetic Mm-1[12] in methanol/phosphate buffer pH 7) and V^V (from V_2O_5) or V^{IV} (from $VOSO_4$) was studied by EPR spectroscopy to determine the oxidation state(s) of product vanadium. EPR measures the V^{IV} oxidation state selectively,[13] but the levels of V^{III}

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in a product can also be determined. Any V^{III} in a product was converted to V^{IV} by bubbling O_2 through the sample;[14] the subsequent increase in the V^{IV} signal indicates the amount of V^{III}. An important consideration was that the V^{IV} signal strength was dependent on pH with a total loss of signal at pH 7. This pH effect is due to the formation of EPR-silent, spin-coupled V^{IV} dimers and oligomers at pH 3–11 (predominant at neutral pH).[3b,15] The signal was strongest at pH < 3 where V^{IV} is monomeric; therefore, samples were buffered at pH 2 before measurement (after reaction at pH 7). Redox reactions between tunichrome and V^V or V^{IV} go to completion at pH 7 as confirmed by similar results when EDTA (pH 7), instead of pH 2 buffer, was added in excess to allow EPR measurements of V^{IV} at neutral pH.

The mole equivalents of V^V or V^{IV} were varied as follows: V:Mm-1 = 1:1, 2:1, and 4:1. After complete reaction at pH 7, the signal was measured at pH 2 before and after oxygenation of V^{III} (e.g., Figure 1). A parallel control, having V^{IV} but no Mm-1, was also measured to calibrate total vanadium. Using the measurements of V^{IV}, V^{III}, and total vanadium, the %V^{IV} and %V^{III} in a product mixture were estimated.[16] (Due to ca. 20% error in comparing a product mixture to its control, the percentages are not shown.) The percentage of V^V was estimated by subtracting the sum of %V^{IV} and %V^{III} from 100% total vanadium. The percentages showed that in each case essentially all (within 100 ± 20%) of the product vanadium was present as V^{IV} and V^{III}, except from 4 mol equiv of V^V in which remaining V^V was found (5–20%). The most reliable calculations are made using EPR measurements taken from an individual sample. Therefore, for each product mixture, the measurements of V^{IV} and V^{III} were compared to calculate a ratio (Table I). These ratios are qualitatively reproducible, but their quantitative aspect should not be overemphasized.

The EPR studies reveal that tunichrome can reduce V^V or V^{IV} to appreciable levels of V^{III}. Mm-1 can donate up to four electrons to a suitably strong oxidant, such as V^V (entry 6). In this example, all four available electrons from Mm-1 were necessary to reduce all but 5–20% of the V^V reactant (4 mol equiv) to V^{IV} and V^{III}.

Interestingly, the generation of V^{III} is more facile from V^V than from V^{IV}. Comparing 1 mol equiv of each (entries 1 and 4), one finds that V^{IV} reactant was mostly not reduced (85%), whereas V^V reactant was fully reduced to V^{III}. Hence, V^V must be reduced to a transient V^{IV} intermediate which is reduced further. It is perhaps surprising that this intermediate V^{IV} is reduced, in contrast to V^{IV} reactant (1 mol equiv). However, the intermediate V^{IV} could interact with its co-intermediate—a tunichrome semiquinone—which is a powerful reductant.[17] This situation could facilitate the formation of V^{III}. We know that the reduction and/or complexation of V^V is rapid,[4,8,9] which may prevent it from equilibrating with newly formed V^{III}. In contrast to a semiquinone, the Mm-1 starting material is presumably a much weaker reductant such that it does not readily reduce V^{IV} reactant.[17]

Although Mm-1 reduced V^V at all mole equivalents tested, the reduction of V^{IV} occurred according to a particular pattern. The first 2 mol equiv of V^{IV} with Mm-1 were mostly not reduced (entry 1, 2); however, 2 additional mol equiv were significantly reduced

to V^{III} (entry 3). The results suggest that both catecholic groups are preferentially complexed by V^{IV} prior to the activation of redox reactivity. The activation may be due to complete complexation of tunichrome and/or to formation of V^{IV} pairs. However, no spectroscopic evidence of tunichrome–vanadium complexes was found in these studies, including EPR analyses at pH 7 (no EDTA). The addition of pH 2 buffer to product mixtures—dark green solutions containing dark suspensions—gave clear, pale green solutions. At pH 2, the spectra of product mixtures and V^{IV} controls were superimposable, indicating that the V^{IV} species are predominantly free pentaaquovanadyl ions, $VO(OH_2)_5^{2+}$ (peak to peak line separation ~110 G), rather than catecholate–V^{IV} complexes which typically have smaller line separations.[18] Similarly, for the experiments with EDTA added at pH 7, the spectra of product mixtures and EDTA–V^{IV} controls were superimposable. It is possible that tunichrome–vanadium complexes exist at pH 7 but are EPR-silent due to spin-coupled interactions between metal centers.

A fortuitous finding is that V^V is almost completely reduced to V^{III} by using 1 mol equiv of Mm-1 (entry 4; Figure 1). In our ongoing studies, these conditions will be useful for generating tunichrome–V^{III} products for structural characterization and comparison to the native V^{III} complex.

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Protein Electrochemistry at High Pressure

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Various techniques coupled with high-pressure methods have proven to be useful for the characterization of proteins.[1–3] For the first time we have combined protein electrochemistry with high pressures. In this paper, we discuss the implications of the effects of compression on the reduction potential of horse heart cytochrome c. We derive the corresponding volumes of reaction and determine the difference in the compressibility between ferri- and ferrocytochrome c. By measuring the change in the standard potential of a reaction ($E°$) as a function of pressure, one can obtain the volume of reaction ($\Delta V°$): $(\partial E°/\partial P)_T = -(1/nF)\Delta V°$, where $\Delta V°$ equals the sum of the standard molar volumes of the products minus the sum of the standard molar volumes of the reactants, $\Delta V° = \sum v°_p - \sum v°_r$.

Thus, a process that involves a net decrease in volume ($\Delta V° < 0$) will be favored by compression. If we consider a reduction process, such an effect will cause a shift in the standard potential of the couple in the positive direction.

Previous investigations of electrode processes at high pressure[4,5] dealt mainly with pressures below 3 kbar using experimental details

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