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Communications

Acidity and Vanadium Coordination in Vanadocytes

Sir:

A recent paper in this journal by Carlson and coworkers concluded that the globules within the vanadocytes of *Ascidia ceratodes* are acidic with a pH of 1.8 ± 0.1 and that the intracellular vanadium exists primarily as $[V(SO_4)(H_2O)_{4-5}]^+$ with less than 5% present as the aquaoxovanadium(IV) ion.[1] The reported acidity is in conflict with results that have been published recently[2-4] and with results reported here for other species of the Ascidiacea. Further, the electron spin resonance spectra of a series of phlebobranch and aplousobranch ascidians measured in our laboratory are consistent with complexes of vanadium(IV).

Early work showed that on lysis of ascidian blood cells copious acid was produced.[5,6] However, more recent experiments with intact blood cells using infused pH indicators,[2] ^{31}P chemical shift of intracellular inorganic phosphate,[2] and the distribution of ^{14}C-labeled methylamine and 9-aminoacridine between extracellular buffers and the cytoplasm[3,4] have shown that intact cells are not acidic to any marked degree with pH values mostly near neutral. Results for morula cells from some species indicated pH values down to 4.6, but these lower values can be explained by a number of factors, including a greater tendency for some species' cells to lyse under microscopic investigation thus creating acid in the plasma, which in turn lowers the intracellular pH of the intact cells on the slide, and the reaction of the indicator with intracellular material causing the indicator to suggest an artifactually lower pH (see below).

Carlson and co-workers based their conclusions on the line width of the $(-^7/_2)_\parallel$ line in the ESR spectrum of intracellular vanadium(IV).[1] If indeed the method can be used as a noninvasive probe of intracellular pH, it would only be valid if the spectrum originated from the uncomplexed aquaoxovanadium(IV) species. The low-temperature, anisotropic spectrum published by Carlson and co-workers for the blood cells of *A. ceratodes* is not consistent with published spectra of aquaoxovanadium(IV).[7] However, if it is assumed that the $(+^7/_2)_\parallel$ line had been inadvertently omitted from the figure, the parameters that are calculated from the figure are indeed consistent with the aquaoxovanadium ion (*A. ceratodes*: $A_\parallel = 199.4$, $A_\perp = 80.3$ G; $g_\parallel = 1.935$, $g_\perp = 1.992$. $[VO(H_2O)_5]^{2+}$: $A_\parallel = 201.7$, $A_\perp = 76.4$ G; $g_\parallel = 1.933$, $g_\perp = 1.985$[8,9]).

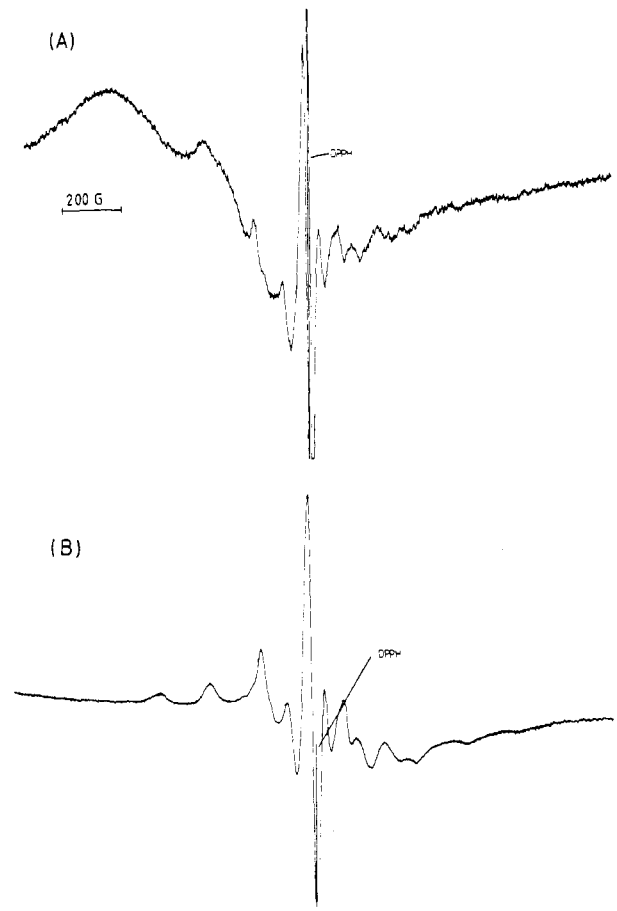

Figure 1. ESR spectra of *P. julinea*: (A) unoxidized whole animal, gain 6.3×10^5; (B) oxidized whole animal, gain, 4×10^4. Conditions: *T*, 110 K; power, 10 dB (19.8 mW); modulation, 4.0 G.

The spectrum for *A. ceratodes* could not be reproduced by us or other workers.[10] The ESR spectrum of this species' blood cells had a very weak vanadium(IV) spectrum at 77 K, and only upon exposure of the cells to oxygen was a spectrum obtained that was of intensity similar to that of Carlson and co-workers, but the ESR parameters differed from those of $[VO(H_2O)_5]^{2+}$ ($A_\parallel = 195.0$, $A_\perp = 65.0$ G; $g_\parallel = 1.941$, $g_\perp = 1.984$).[11] A set of 11 other

(1) Frank, P.; Carlson, R. M. K.; Hodgson, K. O. *Inorg. Chem.* **1986**, *25*, 470.
(2) Hawkins, C. J.; James, G. A.; Parry, D. L.; Swinehart, J. H.; Wood, A. L. *Comp. Biochem. Physiol. B: Comp. Biochem.* **1983**, *76B*, 559.
(3) Dingley, A. L.; Kustin, K.; Macara, I. G.; McLeod, G. C.; Roberts, M. F. *Biochem. Biophys. Acta* **1982**, *720*, 384.
(4) Agudelo, M. I.; Kustin, K.; McLeod, G. C. *Comp. Biochem. Physiol. A* **1983**, *75A*, 211.
(5) Henze, M. *Hoppe-Seyler's Z. Physiol. Chem.* **1911**, *72*, 215.
(6) See ref 2 for a review of early work.
(7) Borcherts, R. H.; Kikuchi, C. *J. Chem. Phys.* **1964**, *40*, 2270.
(8) At 1 mM concentration in 0.1 M H_2SO_4.
(9) Spectra were measured with a Bruker ER200D X-band spectrometer at 9.25 GHz at 77 or 110 K; DPPH was used as an internal reference, and the field for each line was measured with a gaussmeter. Parameters were calculated by spectrum simulation.
(10) Swinehart, J. H.; Biggs, W. R.; Halko, D. J.; Schroeder, N. C. *Biol. Bull. (Woods Hole, Mass.)*, **1974**, *146*, 302.

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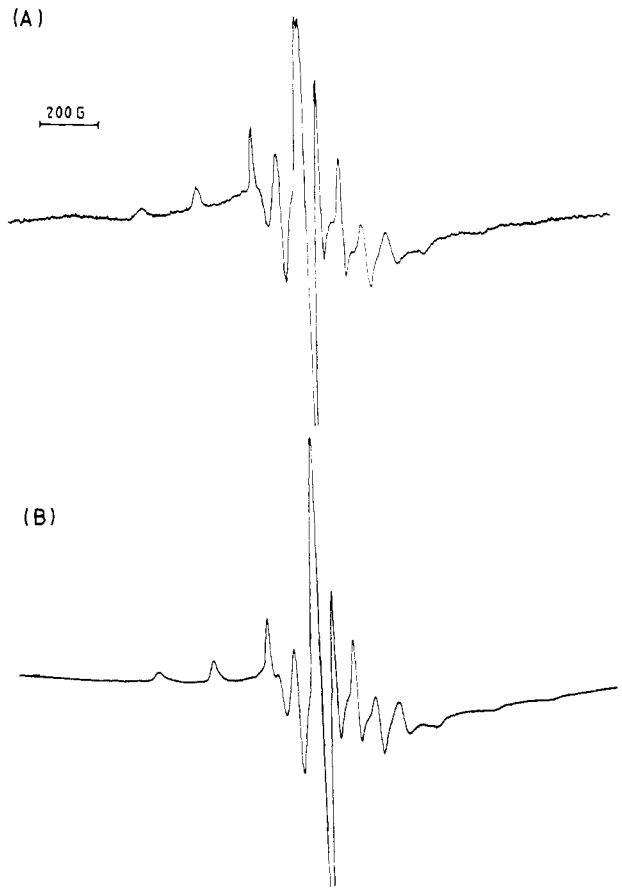

Figure 2. ESR spectra of *L. lissus*: (A) unoxidized section of animal, gain 2.5×10^5; (B) oxidized section of animal, gain 1.25×10^4. Conditions: T, 110 K; power, 10 dB (19.8 mW); modulation, 4.0 G.

phlebobranch species[12] have been found here to have identical "oxygenated" spectra[9] that again differ from that of $[VO(H_2O)_5]^{2+}$. The parameters obtained by simulation of the spectrum of *P. julinea* (Figure 1) are $A_x = 57.00$ G, $A_y = 56.00$ G, $A_z = 169.27$ G, $g_x = 1.992$, $g_y = 1.987$, and $g_z = 1.967$. Kustin and co-workers[13] have published spectra for the blood cells of *Ascidia nigra* at room temperature consistent with less than 5% of the vanadium in the IV oxidation state, but contrary to a statement by Carlson and co-workers[1] the parameters calculated from the spectra are not consistent with those for the uncomplexed vanadyl ion (*A. nigra*, $A_0 = 111.6$ G;[14] $[VO(H_2O)_5]^{2+}$, $A_0 = 115.7$ G[8]). It should be noted, however, that there is a second report in the literature of $[VO(H_2O)_5]^{2+}$ in phlebobranch blood cells.[15]

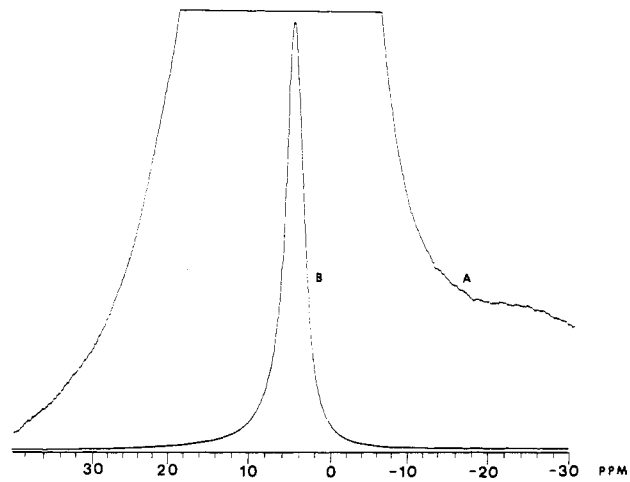

Figure 3. ^{1}H NMR spectra of *A. ceratodes* blood cells: (A) spectrum with y gain increased 100-fold; (B) normal spectrum.

In contrast to the Phlebobranchs, the ESR spectra of whole or sliced Aplousobranchs with significant vanadium concentrations have strong vanadium(IV) signals, even when kept under a nitrogen atmosphere.[16] The spectra of a set of eight species, which are practically superimposable on each other, differ from the spectrum of $[VO(H_2O)_5]^{2+}$.[17] The spectra of *L. lissus* are given in Figure 2 as an example. These give on simulation $A_x = 63.91$ G, $A_y = 61.21$ G, $A_z = 183.00$ G, $g_x = 1.980$, $g_y = 1.985$, and $g_z = 1.951$.

The spectra for the species of the two suborders require different rhombic sets of parameters for simulation, indicating different modes of coordination.

Carlson had previously used ^{1}H NMR spectroscopy to show that most of the vanadium in the vanadocytes of *A. ceratodes* is in the form of vanadium(III) with about five coordination sites occupied by water.[18] A weak signal approximately 21 ppm to lower shielding of the strong water signal was attributed to water bound to paramagnetic vanadium(III). However, when this experiment was repeated by one of the present authors a similar signal at about 22 ppm to higher shielding was obtained (Figure 3).[19] Swinehart[20] also has repeated the measurement, obtaining a spectrum similar to that of Carlson. Close inspection of all these spectra suggests that the weak signals arise from the failure to achieve perfect phasing based on the extremely intense water signal. The cells of *P. julinea* have been studied here, but again no real resonance for paramagnetically shifted water was observed.[21]

Carlson and his co-workers have gained support for his conclusion from an EXAFS experiment that showed vanadium surrounded by six oxygens at 1.99 Å with "no sign of an ordered chelate-imposed second atom shell".[1,22] The ESR-active vanadium(IV) complexes cannot be derived from the same compound because the above vanadium(IV) complexes formed by oxidation have ESR parameters inconsistent with simple aqua species.

When phlebobranch vanadocytes are lysed in water with oxygen *rigorously* excluded, no acid is produced in the lysate, no vanadium

(11) Specimens of *A. ceratodes* were collected at Bodega Bay, CA, and spectra recorded with gently packed cells (500 g for 1 min to give a 3-cm column) at the University of California, Davis, in conjunction with Professor J. H. Swinehart by using a Bruker ER200 X-band spectrometer at 9.25 GHz with DPPH as an internal reference. Parameters were calculated directly from the spectrum of oxygenated cells, assuming an axial spectrum.

(12) Specimens of *Ascidia liberata*, *Ascidia glabra* (red and yellow forms), *Ascidia capillata*, *Ascidia sydneiensis*, *Ascidia kreagra*, *Ascidia gemmata*, *Ascidia archaia*, *Phallusia julinea*, *Phallusia arabica*, *Ecteinascidia nexa*, and *Ecteinascidia rubricollis* were collected at Heron Island, Queensland, were snap frozen immediately after collection using liquid nitrogen or a dry-ice acetone slush, and were placed under a nitrogen atmosphere. Sections of animals, or whole bodies were transferred frozen to ESR tissue tubes in a VAC Dri Lab, and sealed. Oxygenation of the animals or sections was achieved by the snap freezing in liquid nitrogen which caused the condensation of oxygen in the tissues, or by cutting the animal prior to freezing in the presence of oxygen, or, after measurement in the deoxygenated state, samples were defrosted, exposed to oxygen for 5 h, refrozen and remeasured. Identical spectral parameters were obtained by these methods. The blood cells of *A. sydneiensis* are known to be more easily lysed than the others, and unless great care was taken with the animal it gave a spectrum compatible with aquaoxovanadium(IV).

(13) Dingley, A. L.; Kustin, K.; Macara, I. G.; McLeod, G. C. *Biochim. Biophys. Acta* **1981**, *649*, 493.

(14) Calculated here from a figure in ref 13.

(15) Bell, M. V.; Pirie, B. J. S.; McPhail, D. B.; Goodman, B. A.; Falk-Petersen, I.-B.; Sargent, J. R. *J. Mar. Biol. Assoc. U. K.* **1982**, *62*, 709.

(16) Hawkins, C. J.; Kott, P.; Parry, D. L.; Swinehart, J. H. *Comp. Biochem. Physiol. B: Comp. Biochem.* **1983**, *76B*, 555.

(17) Specimens of *Leptoclinides lissus*, *Leptoclinides reticulatus*, *Leptoclinides dubius*, *Eudistoma amplus*, *Eudistoma glaucus*, and two *Eudistoma* spp, were collected at Heron Island and treated as in ref 12.

(18) Carlson, R. M. K. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2217.

(19) Spectrum measured with gently packed cells (500 g for 1 min) in 5-mm tube (3-cm column) with Nicolet 200-MHz FT-NMR spectrometer (University of California, Davis).

(20) Swinehart, J. H., personal communication, 1981.

(21) Spectrum measured with gently packed cells (500 g for 1 min) in 5-mm tube (3-cm column) with JEOL FX100 FT-NMR spectrometer.

(22) Tullius, T. D.; Gillum, W. O.; Carlson, R. M. K.; Hodgson, K. O. *J. Am. Chem. Soc.* **1980**, *102*, 5670.

is detected, and no organic pigment susceptible to oxidation with oxygen is present in the lysate. In the presence of oxygen, cells from the same species on lysis yield acid, vanadium, and oxidizable pigment: the typical Henze solution[5] was obtained. It should be noted that for the deoxygenated lysate indicators gave a lower pH reading than a pH electrode. These experiments were performed with *P. julinea*. Blood, which had been removed by heart puncture using a sterile syringe and had been kept on ice, was gently centrifuged at 500 g for 1 min and the plasma removed under nitrogen. A small volume of deoxygenated distilled water was added to the cells for lysis, and the suspension was bubbled vigorously with nitrogen for 2 h. The pH of the lysate was monitored throughout but did not deviate from 6.5, the original pH of the water. The cell debris was removed by centrifugation, and the pale yellow supernatant was stored under nitrogen at −20 °C. Vanadium concentration was determined by graphite-furnace atomic absorption and the presence of oxidizable pigments was examined by UV–visible spectroscopy. These experiments further verify the absence of any significant concentrations of free acid and would suggest that the vanadium is predominantly present in an insoluble matrix as concluded previously from electron microscopy[23-25] and that the vanadium complex is made soluble by the oxidation process that produces acid.

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Registry No. Vanadium, 7440-62-2; aquaoxovanadium(IV), 15391-95-4.

(23) Pirie, B. J. S.; Bell, M. V. *J. Exp. Mar. Biol. Ecol.* **1984**, *74*, 187.
(24) Kalk, M. *Nature (London)* **1963**, *198*, 1010.
(25) Marshall, A. T., personal communication, 1985.

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Articles

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Vanadium(V) Oxyanions. Interactions of Vanadate with Oxalate, Lactate, and Glycerate

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The interactions between vanadate and oxalate, lactate, and glycerate in aqueous solution have been studied by ^{51}V nuclear magnetic resonance. Two octahedrally coordinated oxalate derivatives were formed, the mono- and bis(oxalato) compounds. Formation of the mono(oxalato) vanadate compound was accompanied by incorporation of a single proton with a second proton being required for the formation of the bis(oxalato) complex. Formation constants were obtained for the various equilibria. Vanadate, in the presence of lactate, forms a variety of derivatives including a tetrahedrally coordinated lactate ester and two trigonal bipyramidally coordinated products, both mononuclear in vanadium but containing either one or two lactate ligands. Also formed are two binuclear vanadium complexes, one with octahedral coordination about each vanadium nucleus and the other with one octahedral and one tetrahedral vanadium atom. Formation of the bipyramidal products does not require proton incorporation; however, formation of the octahedral derivatives does. Equilibrium constants for the formation of the various lactate derivatives were determined. Glycerate was found to behave similarly to lactate with both bipyramidal and octahedral products being formed. Since octahedral coordination is favored by protonation, lowering the pH serves to promote formation of the octahedral products at the expense of the other derivatives. A description of preferred coordination geometry in terms of electron availability is developed.

Introduction

The chemistry of aqueous solutions of vanadate oxyanions is very complex, with the formation of various oligomeric forms as a function both of pH and of concentration. When in combination with alcohols, vanadate spontaneously forms acyclic mono- and diesters,[1] while with vicinal diols, it forms both cyclic and acyclic compounds.[2] The geometry about vanadium is tetrahedral in the acyclic cases and has been proposed to be trigonal bipyramidal in the cyclic esters.[2] Vanadate in combination with oxalate forms a crystalline octahedral *cis*-bis(oxalate),[3] and it has been assumed that this structure is retained in solution.[4]

The facile formation of vanadate esters and the ease by which vanadate adopts different coordination geometries may lie at the source of the biological importance of vanadium. Vanadium is thought to be an essential element and has significant effects on the function of various enzymes.[5,6]

Vanadate can apparently play a role similar to that of phosphate by acting as a phosphate analogue. Vanadate spontaneously esterifies hydroxyl groups, which are then accepted as enzyme substrates in lieu of the normally phosphorylated substrate. This function has been demonstrated for the dehydrogenase activity of glucose 6-phosphate dehydrogenase, where glucose plus vanadate serves as a substrate to produce gluconic acid.[7] A va-

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