ESR Spectra of Vanadyl Ion Detected in Branchial Basket of an Ascidian, *Ascidia ahodori*

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Abstract

ESR spectra due to the vanadyl ion (VO^{2+}, +4 oxidation state) was detected in the branchial basket of *Ascidia ahodori*, which is reported to contain vanadium in high amounts. The branchial basket, washed with a medium containing 1 mM EDTA, and the supernatant showed different types of vanadyl ESR spectra. On further treatment with 100 mM EDTA the branchial basket gave a characteristic ESR spectrum, indicating that the vanadyl ion binds to a high molecular weight matrix, such as proteins, which makes up the branchial basket. Judging from the relationship of the ESR parameters, $g_{\parallel}$ *versus* $A_{\parallel}$, the vanadyl ion is assumed to ligate with moieties such as deprotonated hydroxyl, or nitrogenous or thiolato groups from oxy- or thiol-amino acid residues. The branchial basket was shown to have the ability to reduce added vanadate ion (+5 oxidation state) to the vanadyl form. On the basis of these observations, participation of the branchial basket in vanadium-accumulation by ascidians from seawater is suggested.

Introduction

The ability of ascidians to accumulate vanadium ion from seawater distinguishes them from other classes of marine animals [1]. Knowledge of the distribution of vanadium in ascidians is essential to understand the mechanism of accumulation of this ion, e.g., *Ascidia nigra* [1, 2]. Recently, Michibata *et al.* have thoroughly determined the vanadium contents in 15 species of solitary ascidians belonging to the suborders Phlebobranchia and Stolidobranchia as well as their organ distribution. They found that vanadium contents were higher in the Phlebobranchial species than in the Stolidobranchial species. The highest value, 21 μg/mg dry weight, was obtained from the blood corpuscles of *Ascidia ahodori* [3]. The second highest vanadium tissue content in this species was found in the branchial basket. This tendency was observed in most ascidians of Phlebobranchia, which means that the branchial basket may play an important role in concentrating vanadium ion from seawater before the ion is transferred to its final repository in specialized blood cells [4].

ESR spectra of blood cells of ascidians have been reported by several groups to characterize the vanadyl ion (VO^{2+} as the +4 oxidation state of vanadium) [5–12]. ESR spectrometry has proved to be a useful technique to estimate the valence state of vanadium ion as well as the coordination mode around the vanadyl ion in biological systems [13–15]. However, the ESR spectrum of the branchial basket of ascidians has not yet been studied, as far as we know. This paper reports the ESR spectra as well as the characterization of vanadyl ion detected in the branchial basket of *A. ahodori.*

Experimental

A. ahodori collected from the bottom of the marine tank at Ushimado Marine Biological Station (Okayama), Okayama University, Japan, was carried alive to the University of Tokushima to measure the ESR spectra. The branchial basket was removed from two or three animals and transferred to ESR quartz tubes after treatment at 10 °C, as described in the following section. Reagents used in the experiments were of special Reagent grade. The medium, pH 7.0, used contained the following components: 460 mM NaCl, 33 mM Na_2SO_4, 6 mM $NaHCO_3$, 1 or 100 mM EDTA and 5 mM HEPES (4-(2-hydroxyethyl)-1-piperizine-ethane-sulfonic acid). ESR spectra were recorded with a JES-FE1XG (X-band) spectrometer with 100 KHz field modulation at liquid nitrogen temperature (77 K), which was calibrated with a Takeda Riken frequency counter, TR 5212. As standards, Li-TCNQ and Mn(II) doped in MgO were used.

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Results and Discussion

As it can safely be assumed that blood cells flow through the branchial basket of ascidians, this part was washed several times with medium and incubated in the medium for 10 h at 10 °C. Nevertheless, the ESR signal due to VO^{2+} was observed in the supernatant of the incubation mixture (Fig. 1(A)), showing the occurrence of high amounts of vanadyl ion in the branchial basket. It is thus possible that the metal ion may be present in blood cells that remained after the washing, or that the metal ion is removed by the medium, which contains EDTA, from the

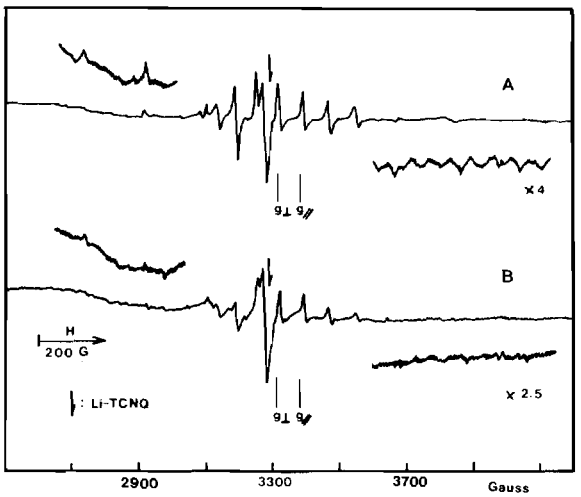

Fig. 1. ESR spectra of vanadyl species in supernatant of washed branchial basket of *A. ahodori* (A), and branchial basket after washing (B). Recording conditions: power, 5 mW; gain, 630 for (A) and 100 for (B); modulation, 6.3 G; frequency, 9.1699 GHz for (A) and 9.18724 GHz for (B); and temperature of 77 K.

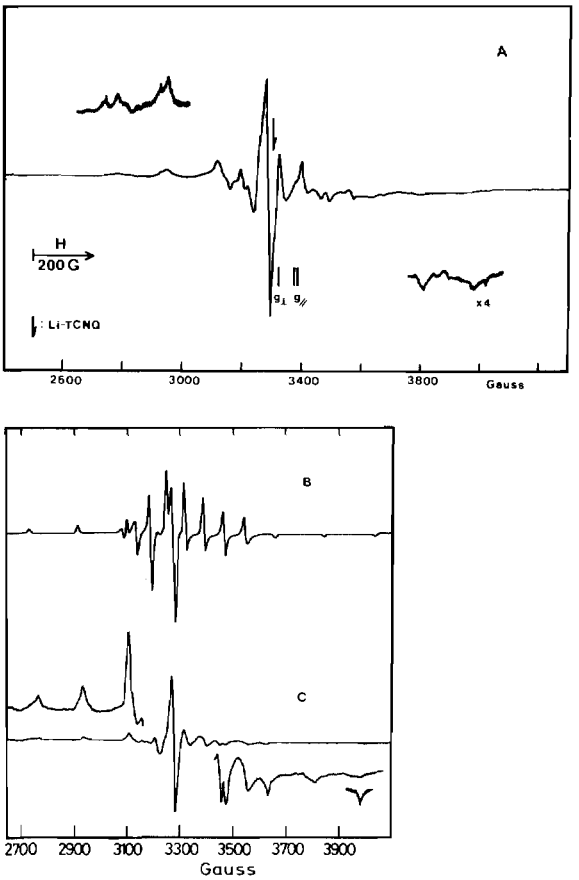

Fig. 2. ESR spectra of vanadyl species in branchial basket washed and treated with the medium containing 100 mM EDTA (A), $VOSO_4$ dissolved in the medium (B), and $VOSO_4$ dissolved in egg albumin solution at pH 7.8 (C). Recording conditions for (A): power, 5 mW; gain, 500; modulation, 6.3 G; frequency, 9.2080; and temperature of 77 K. Similar conditions were used for samples (B) and (C).

TABLE I. ESR Parameters[a] of Vanadyl Ions Detected in Branchial Basket of *A. ahodori*

System	g_0	$g_\parallel$	$g_\perp$	A_0 (Gauss/10^{-4} cm^{-1})	$A_\parallel$	$A_\perp$	No.[b]
Supernatant of washed branchial basket	1.976	1.949	1.989	107 (99)	190 (173)	66 (61)	31
Branchial basket (washed)	1.976	1.950	1.989	106 (98)	187 (170)	65 (60)	32
Branchial basket treated with 100 mM EDTA	1.979	1.951	1.993	101 (96)	182 (165)	61 (57)	33
	1.981	1.959	1.993	93 (89)	166 (151)	61 (57)	34
Branchial basket treated with 100 mM EDTA + $NaVO_3$ (after 5 h)	1.977	1.951	1.990	103 (99)	182 (168)	64 (59)	
	1.979	1.959	1.990	95 (91)	166 (153)	64 (59)	

[a]The g_0 and A_0 values were calculated by the equations $g_0 = (g_\parallel + 2g_\perp)/3$ and $A_0 = (A_\parallel + 2A_\perp)/3$, respectively, using sets of $g_\parallel$ and $g_\perp$ values and $A_\parallel$ and $A_\perp$ values, obtained from the ESR spectra in the frozen (77 K) state. [b]The numbers refer to Fig. 3.

branchial basket in which the vanadium is loosely bound.

On the other hand, an ESR signal due to VO^{2+} was also detected in the solid part of the branchial basket treated with medium (Fig. 1(B)), but this signal displays a pattern different from that found in the supernatant (A). As this spectrum (B) suggests the occurrence of vanadyl ion bound tightly to the branchial basket, this part was treated again overnight at 10 °C with medium containing 100 mM EDTA and washed with distilled water several times before the measurement of the ESR spectrum. The spectrum shown in Fig. 2(A) distinctly indicates the change of the signal pattern from the original branchial basket (Fig. 1(A) and (B)). The spectrum is characteristic and very similar to protein-bound vanadyl species such as the vanadyl–egg albumin complex formed at pH 7.8 (Fig. 2(B)), but not to aquo-vanadyl dissolved in the medium (Fig. 2(C)). These results suggest that the vanadyl ion is incorporated into the insoluble matrix of the branchial basket.

As seen in the ESR spectrum (Fig. 2(A)), the presence of two kinds of VO^{2+} signal were detected (Table I). The ESR parameters due to the vanadyl species, which are contained in relatively high amounts ($g_{\parallel} = 1.959$, $A_{\parallel} = 153 \times 10^{-4}$ cm^{-1}), were found to be similar to those detected in the blood cells of the same species ($g_{\parallel} = 1.957$, $A_{\parallel} = 151 \times 10^{-4}$ cm^{-1}) [16] and of Phlebobranchial species [12].

Correlations of ESR parameters such as g_0 *versus* A_0 and $g_{\parallel}$ *versus* $A_{\parallel}$ sets have successfully been used for estimating the coordination modes around the vanadyl ion in biological systems when reference compounds are used as model complexes having coordination sets such as $VO(O_4)$, $VO(O_2O_2^-)$, $VO(O_2N_2)$, $VO(N_4)$, $VO(O_2S_2)$, $VO(N_2S_2)$ and $VO(S_4)$ [13–18]. Thus the $g_{\parallel}$ *versus* $A_{\parallel}$ relationships of the measured ESR spectra were plotted for a number of model vanadyl complexes in aqueous frozen solution as well as for the parameters of vanadyl species detected in the branchial basket (Fig. 3, No. 31 and 32) and those treated with 100 mM EDTA-containing medium (Fig. 3, No. 33 and 34). These parameters (▼) fall in the region of $VO(O_2O_2^-)$, but not in the aquo-vanadyl region. The points No. 31–33 are also located in the $VO(O_2N_2)$ region, while point No. 34 lies quite near the VO^{2+}–thiolato complex ($VO(S_2O_2)$ or ($VO(S_2N_2)$) region. These results indicate that vanadyl ion ligates tightly with candidates such as deprotonated hydroxyl, nitrogenous or thiolato groups of oxy- or thiol-amino acid residues in high molecular weight components of the branchial basket.

Another interesting feature is the ability of the branchial basket to reduce the added vanadate ion

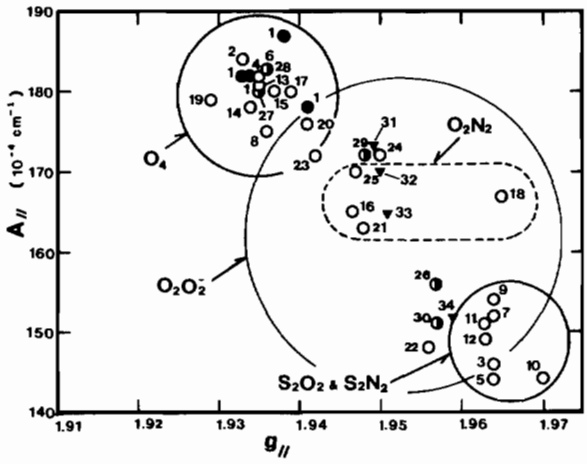

Fig. 3. Relationship between $g_{\parallel}$ and $A_{\parallel}$ for vanadyl species with various ligand fields, $VO(O_4)$, $VO(O_2O_2^-)$, $VO(O_2N_2)$, $VO(S_2O_2)$ and $VO(S_2N_2)$ as well as for vanadyl ion detected in branchial baskets of *A. ahodori*. (1) aquovanadyl (ref. 10, 12 and the present work); (2) VO^{2+}–cysteine at pH 3 and (3) at pH 10; (4) VO^{2+}–cysteine methyl ester at pH 3 and (5) at pH 10; (6) VO^{2+}–mercaptoethylamine at pH 3 and (7) at pH 10; (8) VO^{2+}–glutathione at pH 3 and (9) at pH 10; (10) VO^{2+}–mercaptoethanol at pH 10; (11) VO^{2+}–dithiothreitol at pH 10; (12) VO^{2+}–mercaptopropionic acid at pH 10; (13) VO^{2+}–ascorbic acid at pH 3.5; (14) VO^{2+}–catechol at pH 6.0; (15) VO^{2+}–serine at pH 3 and (16) at pH 10.5; (17) VO^{2+}–threonine at pH 4.0 and (18) at pH 10.5; (19) VO^{2+}(ATP) at pH 3.0, (20) at pH 7.7, (21) at pH 8.1 and (22) at pH 12.1 (ref. 20); (23) supernatant of rat liver (ref. 18); (24) mitochondria of rat liver (ref. 18); (25) radish leaves treated with sodium vanadate (ref. 21); (26) *P. julinea* (ref. 12); (27) *A. ceratodes* (ref. 10); (28) *A. sydneiensis samea* (ref. 11); (29) *A. ahodori* (Asamushi, Aomori, Japan) layer 4 (ref. 16); (30) *A. ahodori* (Ushimado, Okayama, Japan) layer 4 (ref. 16); (31–34) (these numbers refer to Table I).

(+5 oxidation state) to the vanadyl form, as monitored by ESR spectrometry. When sodium vanadate (1 mM, 0.3 ml) was added to the medium solution (2.7 ml) in which the branchial basket collected from two animals was immersed, the signal height due to the vanadyl ion increased approximately two-fold after incubation for 5 h at 10 °C (data are not shown), indicating clearly that the vanadate ion added was reduced to vanadyl ion by the branchial basket.

This finding is important not only as a possible function of the branchial basket of ascidians but also as a mechanism of vanadium accumulation from seawater through this part of animals. Macara *et al.* proposed a hypothesis that vanadate expressed as $H_2VO_4^-$ in seawater enters blood cells directly through anionic channels and that vanadate is reduced to the vanadic form (+3 oxidation state) inside the cells, where it accumulates [1, 19]. However, our present findings may suggest that some

of the vanadium ion is present already in the reduced vanadyl form in the branchial basket before the vanadate ions are transferred to blood cells. Therefore, we feel at present that the mechanism of vanadium accumulation by ascidians should be reinvestigated in the light of our present observations, including the participation of the branchial basket.

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