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Discovery of a New Vanadium Accumulator, the Fan Worm *Pseudopotamilla occelata*

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Many biologists, physiologists, and chemists have been interested in the significance of high vanadium concentrations in ascidians since the first report on its accumulation by Henze in 1911 [1]. In spite of many efforts to find other vanadium accumulators for a comparative study, it has been thought that only ascidians in the Animal Kingdom have the special ability to accumulate vanadium at a high level. During our screening work on elemental concentrations in marine organisms collected from the sea around Japan, we first noticed that a high level of vanadium is contained in the fan worm, *Pseudopotamilla occelata* (Fig. 1), classified into Annelida, Polychaeta. Here, we report the concentration, distribution, and oxidation state of vanadium in *P. occelata*.

Fig. 1. Lateral view of the head part of *P. occelata*. The branchial crown of this fan worm is composed of two opposing half circles of bipinnate radioles, forming a funnel when expanded outside the end of a tube

Specimens of *P. occelata* were collected from the Sanriku coast in the northeastern region of Japan. The whole body was dissected into the bipinnate radiole (Fig. 2A) and the trunk body. Vanadium concentrations in them were determined by ICP-AES (inductively coupled plasma atomic emission spectrometry; wavelength 310.2 nm).

In order to examine the vanadium distribution in the bipinnate radiole, a transverse fracture surface was made by the "katsudan" method using an anatomical knife and freeze-dried in a vacuum atmosphere after the whole body of *P. occelata* was quick-frozen in liquid nitrogen. Two-dimensional analysis of a fracture surface was carried out with a JEOL JXA-8600MX electron microprobe using an operating voltage of 15 kV and a probe current of 2×10^{-8} A. The characteristic X-ray of vanadium (wavelength V $K\alpha$ = 0.2505 nm) was measured with a PET analyzing crystal.

The EXAFS technique [2] was applied to investigate the oxidation state and coordination form of vanadium in *P. occelata*. X-ray absorption spectra of living *P. occelata* and six reference compounds of known structure [$V_2(SO_4)_3$, $V(acac)_3$, $VO(acac)_2$, $VOC_2O_4 \cdot nH_2O$, NH_4VO_3, and V_2O_5] were measured using the synchrotron light source at the Photon Factory in the National Laboratory for High Energy Physics, Tsukuba, Japan with a ring energy of 2.5 GeV. Data were collected in the fluorescence mode using a Si(111) two-crystal monochromator and a Lytle-type detector [3] on a bending magnet beam line.

The vanadium concentration in the bipinnate radiole determined by ICP-AES ranged from 3270 to 7150 μg/g on a dry weight basis. The average vanadium concentration of the bipinnate radiole of 15 individuals was 5100 ± 1400 μg/g s.d. and was 100 times higher than that (56 ± 30 μg/g s.d., n = 15) of the trunk body. Approximately 90% of the total body burden was concentrated in the bipinnate radiole, which represents only 6.8 ± 1.2% s.d. (n=24) of the whole body weight. The discovery of the new vanadium accumulator indicates that the specific accumulation of vanadium in the sea is not limited to only Protochordata, Ascidiacea. Furthermore, our discovery is useful in directing the attention of the toxicologist to possible health problems of some Japanese who eat this species as a marine food.

As can be seen from Fig. 2B, a great amount of vanadium was concentrated in the epidermis covering the dorsal half of the radiole. *P. occelata* is the first reported marine animal that can accumulate a markedly high level of vanadium in the epidermis. On the other hand, it was also found that very little vanadium was contained in connecting and supporting tissues, or in blood plasma and muscles. Furthermore, the vanadium content in the epidermis of the radiole was much higher than that of the pinnules. In contrast to *P. occelata*, it has been reported that vanadium existed in the blood cells, e.g., morula cells, signet ring cells, and granular amoebocytes of ascidians *Ascidia mentula* and *Ascidiella aspersa* [4]. In the latest paper, the vanadium-containing blood cell, the so-called vanadocyte, has been identified as the signet ring cell [5]. Further studies of the difference in vanadium distribution between *P. occelata* and ascidians will be helpful in clarifying the physiological roles of vanadium in marine organisms.

Based on morphological observation (Fig. 2A), when the total epidermis of the radiole was assumed to represent at most one-fifth of the whole bipinnate radiole, the vanadium concentration in

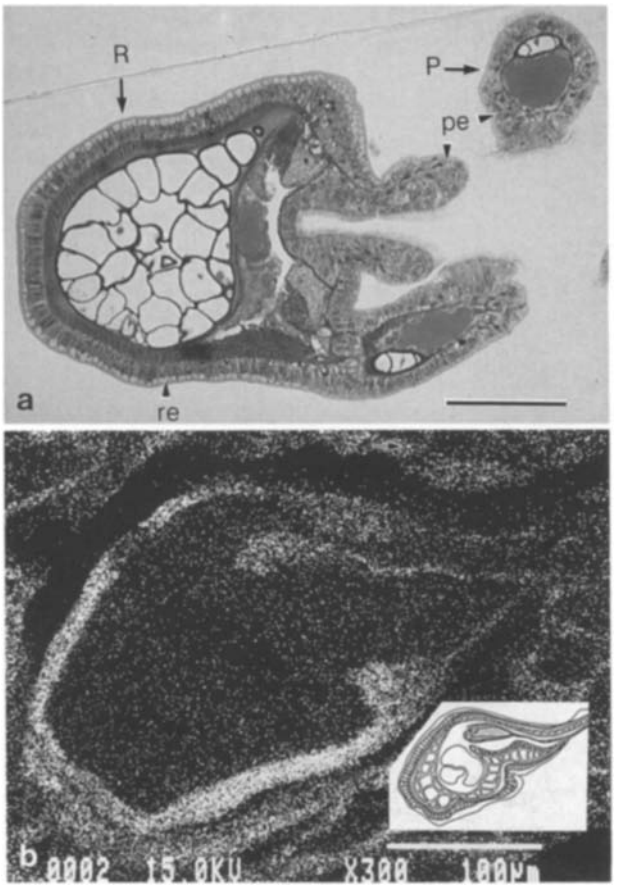

Fig. 2. Morphological observation and electron microprobe analysis of the bipinnate radiole of *P. occelata*. a) Light micrograph of a transverse section of the bipinnate radiole of *P. occelata*; *R* radiole, *P* pinnules, *re* radiole epidermis, *pe* pinnule epidermis, *scale bar* 100 μm. b) Area analysis of vanadium in a transverse fracture surface by an electron microprobe. The vanadium concentrated area is expressed by the white spots. It is apparent that most of the vanadium is contained in the epidermis of the bipinnate radiole when the analytical result is contrasted with Fig. 2a and Fig. 2b, which shows a schematic drawing of the section of the radiole

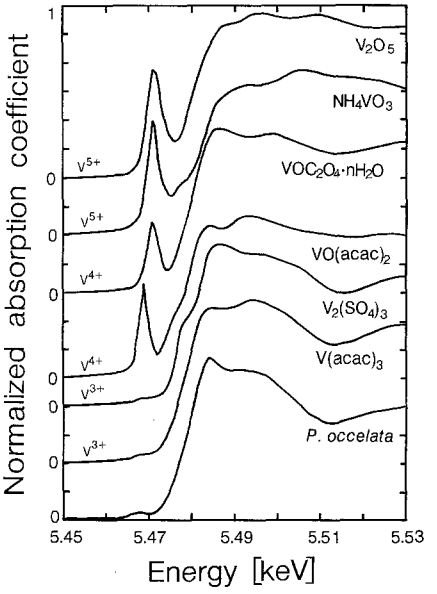

Fig. 3. Vanadium *K*-edge absorption spectra of the bipinnate radioles of living *P. occelata* and reference vanadium compounds with various oxidation states (III, IV, and V). Intense pre-edge peaks at around 5.47 keV were observed for compounds containing V(IV) or V(V) ions. The intensity of the pre-edge absorption in the spectrum of living *P. occelata* resembles those of $V_2(SO_4)_3$ and $V(acac)_3$. This indicated that vanadium in the samples was present as V(III) ions in a symmetrical octahedral coordination environment

the epidermal cells of the radiole was calculated to be 25.5 mg/g dry wt. This value was comparable to that in the blood cells of *Ascidia nigra* (26.8 mg/g dry wt) [6], *A. ahodori* (21.1 mg/g dry wt) [7], and *A. gemmata* (8.75 mg/g wet wt) [8], which show the highest concentrations of vanadium among ascidians.

As seen in Fig. 3, vanadium *K*-edge spectra of vanadium(IV) compounds exhibit a strong pre-edge peak at around 5.47 keV. This peak is ascribed to the 1s to 3d electron transition, which is formally forbidden by dipole selection rules if coordination about the vanadium displays octahedral (O_h) symmetry with a center of inversion [9]. If a vanadium compound contains a terminal oxo ligand (V=O), as in the vanadium(IV) compounds, the octahedral symmetry is lost and intense pre-edge absorption is exhibited. It has been reported that the intensity of the pre-edge absorption is roughly proportional to the number of oxo groups present in the molecule [10]. The intensity variation of the pre-edge peak across the series of the reference compounds from V(III) to V(V) was very distinct (Fig. 3).

The spectrum of *P. occelata* showed a pre-edge absorption that was negligibly small, resembling those of the symmetrically coordinated $V_2(SO_4)_3$ and $V^{III}(acac)_3$. This indicates that vanadium in the living *P. occelata* exists as the V(III) state in a highly symmetrical octahedral coordination environment with a lack of any significant quantity of the VO^{2+} component.

A preliminary result of our EXAFS analysis has indicated that the Fourier transforms of the EXAFS oscillation give a single main peak with the absence of any ordered second coordination shell of scattering atoms at longer distance. Since the *K*-edge spectrum of *P. occelata* has disclosed that the vanadium is in the symmetrical octahedral coordination, the present EXAFS analysis may indicate that vanadium in *P. occelata* exists in an ionic state in a solution. A similar coordination form of vanadium has been reported for living ascidian blood cells as determined by EXAFS [10] and NMR [11] analyses. Therefore, the possibility exists that vanadium might be present in a similar chemical form in both animals.

Although more than 80 years have passed since the first report on the high accumulation of vanadium in ascidians, the physiological roles of vanadium in these organisms remain unknown. Our discovery of the new vanadium accumulator, *P. occelata*, is expected to accelerate the clarification of the physiological roles and accumulation mechanisms of vanadium in marine organisms. We anticipate that many researchers in various fields will have a great interest in *P. occelata* and ascidians, and will join in the study on the significance of the high vanadium concentrations in these mysterious creatures.

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Functionalized Materials Based on Amino and Amide Polymers by an Easy One-Pot Preparation

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Functional polymers having many chemical, technological, and biomedical applications are an important class of material. Of them, the group of amino polymers and their derivatives has found increasing interest [1–3]. The condensation of low-molecular alkylamines with formaldehyde and phenol derivatives, though a long-known general organic-chemical reaction [4], has hardly been extended to macromolecular chemistry. Thus, α-aminomethyl phosphorous acids have been prepared by the condensation of amines, formaldehyde, and phosphorous acid [5] and, on the basis of this type of reaction, polymer-bound phosphonic acids were also synthesized in multistep reactions by the condensation of poly(ethyleneimine) with phosphorous acid [6]. Recently, such materials have found a technical application as ion-exchange materials for the recovery of uranium from seawater [7, 8]. From our interest in developing functional materials [1, 3, 9], we have found that amino or amide polymers can be easily modified in a general manner by condensation with two or more other components (Fig. 1).

We here describe the preparation of such functional and stable polymeric materials. The preparation is based on the principle of the Mannich reaction, in which formaldehyde (or another aldehyde) is condensed with ammonia and with a compound containing an active hydrogen [10].

The proposed preparation method is quick, simple, and very versatile with respect to the polymers that can be used as basis materials and to the functional compounds that can be introduced into the polymer material. Some examples are phosphorous acid, ureas, phenols, benzoic acids, aromatic sulfonic acids, surfactants, dyes, organic analytical reagents, and many others.

In order to obtain noncross-linked and defined products, it is necessary to remove remaining low-molecular components, especially aldehydes. Most advantageous and effective is the work-up in the homogeneous phase using membrane filtration. It is also the only way to obtain soluble polymer derivatives.

The versatility of this synthetic method for the preparation of polymeric materials is illustrated in Table 1.

Depending on the functional component introduced, the polymers can be applied as antimicrobial or metal-complexing agents, ion exchangers, biomaterials, polyelectrolytes, or other functional materials. For example, 8-hydroxyquinoline-5-sulfonic acid sodium salt reacted with poly(ethyleneimine) and formaldehyde in aqueous solution at 80 °C to form an oxine-functionalized

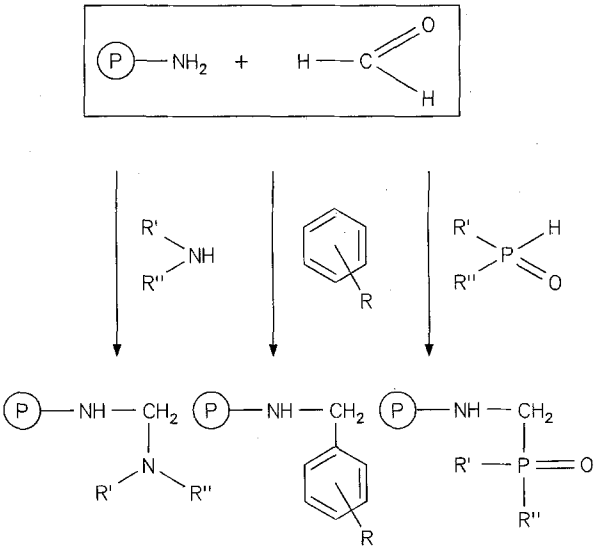

Fig. 1. Reaction scheme for the polymer-analogous functionalization of amino and amide polymers, (P) = polymer

