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Preparation and Localization of a Monoclonal Antibody against a Vanadium-Associated Protein Extracted from the Blood Cells of the Vanadium-Rich Ascidian, *Ascidia sydneiensis samea*

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ABSTRACT—Ascidians are known to accumulate high levels of vanadium in their blood cells. Recently, we found a vanadium-associated protein (VAP) in blood cells of a vanadium-rich ascidian, *Ascidia sydneiensis samea*. In this paper, we raised a monoclonal antibody against VAP, designated F8DH. Immunoblot analysis showed that F8DH recognized 2 related peptides of 15 kDa and 16 kDa of VAP. Using F8DH, VAP was shown to be in the cytoplasm of vanadocytes and compartment cells, both of which were reported to contain vanadium. F8DH also stained the vanadocytes distributed in the connective tissues around the alimentary canal, suggesting that vanadocytes in the connective tissue contained VAP. Furthermore, blood cells of 3 different species of ascidian having high levels of vanadium, *A. sydneiensis samea*, *A. ahodori*, and *Ciona intestinalis*, showed reactivity of F8DH but little reactivity was observed in 2 species having less vanadium, *Halocynthia roretzi* and *Pyura michaelseni*, suggesting that VAP recognized by F8DH is a common protein in vanadium-rich ascidians.

INTRODUCTION

An unusual phenomenon whereby some ascidians accumulate vanadium to levels more than ten million times, 10^7, higher than that in seawater has attracted researchers from various fields, from analytical chemists, chemists of natural products, physiologist, and biochemists (cf. Michibata, 1996; Michibata and Kanamori, 1997). However, the mechanism by which vanadium is accumulated has not yet been determined.

Recently, we reported that a vanadium-associated protein (VAP) was extracted from the blood cells of an ascidian, *Ascidia sydneiensis samea*. VAP was estimated to associate with vanadium at an approximate ratio of 1 mol : 16 mols. SDS-PAGE revealed that VAP is composed of at least 2 types of peptides estimated to be 12.5 kDa and 15 kDa with a minor peptide of 16 kDa (Kanda *et al.*, 1997).

In these experiments, we produced a monoclonal antibody against VAP. Using the monoclonal antibody obtained as a probe, we further examined the localization of the antigen recognized by the antibody among several types of blood cell in the ascidian, *A. sydneiensis samea* and surveyed the existence of VAP in the blood cells of some different species of ascidian.

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MATERIALS AND METHODS

Ascidians

Ascidia sydneiensis samea and *Halocynthia roretzi* were collected at Otsuchi Marine Research Center, Ocean Research Institute, the University of Tokyo, Otsuchi, Iwate Prefecture and at Asamushi Marine Biological Station, Tohoku University, Asamushi, Aomori Prefecture, Japan. *A. ahodori*, *Ciona intestinalis*, and *Pyura michaelseni* were collected at Mukaishima Marine Biological Laboratory, Hiroshima University, Hiroshima Prefecture, Japan. The ascidians were maintained in an aquarium that contained circulating natural seawater at 20°C.

Preparation of blood cells and isolation of the vanadium-associated protein (VAP)

Methods for the preparation of blood cells and isolation of a vanadium-associated protein from the blood cells have been described elsewhere in detail (Kanda *et al.*, 1997). In brief, blood cells of *A. sydneiensis samea* were suspended into 20 volumes of ice-cold buffer solution (buffer A) that contained 400 mM NaCl, and 50 mM Tris-HCl (tris-hydroxymethylaminomethane) at pH 8.5. After centrifugation, the pelleted cells including vanadocytes were suspended in another buffer solution (buffer B) that contained 1 mM DTT (dithiothreitol), 1 mM PMSF (phenylmethylsulfonylfluoride) and 20% (v/v) glycerol in buffer A. The suspended sample was rapidly frozen and then kept in a deep freezer at -85°C until use. The frozen blood cells were thawed on ice and centrifuged at 1,000 × g for 20 min and sequentially at 100,000 × g for 60 min at 4°C. The supernatant obtained was dialyzed against a 50 mM Tris-HCl buffer containing 1 mM PMSF, 20 μg/ml of pepstatin A, 10 μg/ml of leupeptin and 10 μg/ml of chymostatin at pH 7.4 for 24 hr at 10°C in order to remove the inorganic vanadium ions which

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were not associated with the proteins. The dialyzed sample was centrifuged at 100,000 × g for 60 min at 4°C and the supernatant was applied to a DEAE-Sephacel anion-exchange column (ø1.0 ×10 cm length) which had been equilibrated with a 50 mM Tris-HCl buffer at pH 7.4. Proteins were eluted in 3.4 ml-fractions from the column with an increasingly concentrated NaCl solution, step-wise from 0 to 400 mM in the buffer solution. The concentrations of the protein and vanadium in each fraction were determined by the method of Bradford (1976) using BSA (bovine serum albumin) as the standard and by an atomic absorption spectrometry (Michibata *et al.*, 1990), respectively. The first major peak that contained both vanadium and protein was obtained in fraction 3-9 (0 mM NaCl fractions) and designated peak 1 as described in Kanda *et al.* (1997).

SDS-PAGE

In addition to the blood cells including vanadocytes, giant cells, muscle, branchial basket, stomach, and connective tissues around the alimentary canal were dissolved, respectively, in a sample dissociation buffered solution containing 62.5 mM Tris-HCl at pH 6.8, 5% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, 2.3% (w/v) SDS and 0.05% bromophenol blue. Samples of protein were applied to 14% uniform SDS-polyacrylamide gels and subjected to electrophoresis in a 10 mA constant current. Coomassie Brilliant Blue R-250 was used to stain the proteins.

Preparing a monoclonal antibody

A 3.5 ml-aliquot of the peak 1 fractions that contained the vanadium-associated protein (ca. 100 mg protein/ml) after elution from a DEAE-Sephacel column was diluted with PBS in order to adjust the protein concentration to 40 μg/ml. A 0.5 ml-aliquot of antigen was mixed with 0.5 ml of Freund's complete/incomplete ajuvant and boosted 3 times into a BALB/c mouse. Spleen cells collected surgically were fused with P3U1 myeloma cells. Hybridomas obtained were cultured in ASF 103 medium (Ajinomoto Co. Ltd.). Hybridomas generating antibodies were screened in twice by immunoblot analysis.

Immunoblot analysis

Homogenates of blood cells, giant cells, peak 1 fractions, muscle, branchial basket, stomach, connective tissues around the alimentary canals, and homogenates of blood cells of several different species of ascidians were dissolved, respectively, in a sample dissociation buffered solution and subjected to SDS-PAGE. For immunoblot analysis, each sample subjected to SDS-PAGE was transferred to nitrocellulose paper. The paper was then soaked in TEN buffer for 3 hr, which consisted of 150 mM NaCl, 1 mM EDTA, 25 mM Tris-HCl buffer at pH 7.4 containing 0.5% BSA, and exposed to a monoclonal antibody (F8DH) contained in the supernatant of cultured medium for 1 hr, which was diluted at a ratio of 1 to 500 in TEN buffer containing 0.5% BSA. The nitrocellulose paper was washed twice with TEN buffer containing 0.01% Tween 20 for 15 min and incubated with the anti-mouse IgG (H+L)-HRP conjugate (Organon Teknika Corporation, Philadelphia, USA) for 30 min, which was diluted at a ratio of 1 to 2,500 with TEN buffer containing 0.01% Tween 20. After washing 10 times with the TEN buffer containing 0.01% Tween 20, the nitrocellulose paper was incubated with ECL Western blotting detection reagents (Amersham International, Plc., Buckinghamshire, England). Finally, the nitrocellulose blot was exposed to Hyperfilm-ECL (Amersham International, Plc.).

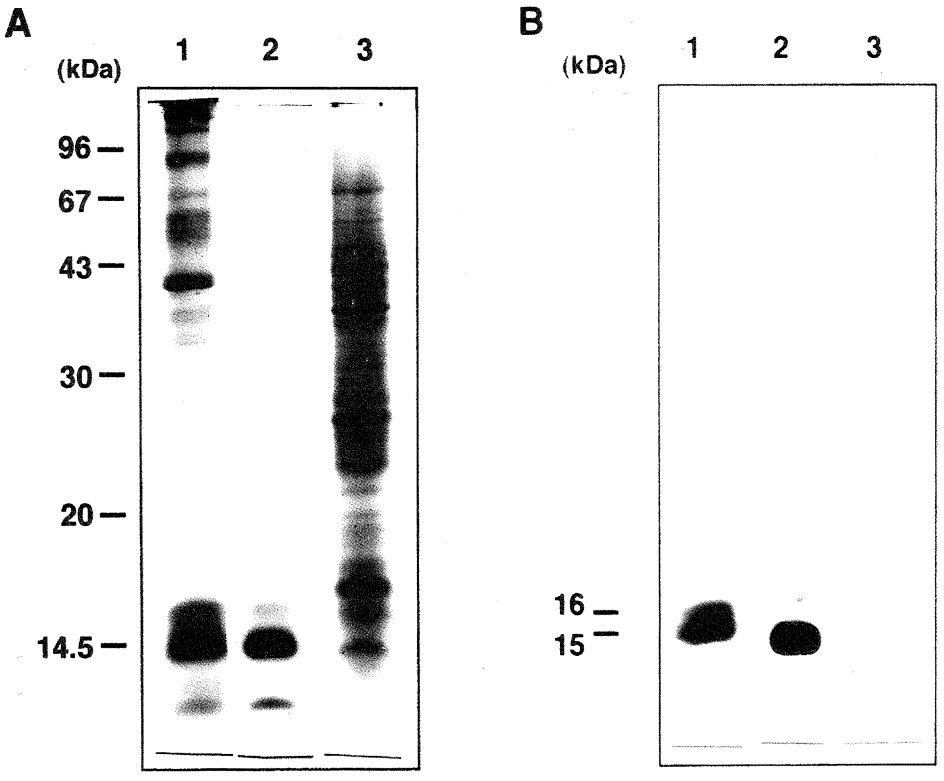

Fig. 1. SDS-PAGE (**A**) and immunoblot (**B**) analysis of the homogenates of blood cells and the VAP fraction. Each sample (30 μg protein) was subjected to SDS-PAGE on a 14% polyacrylamide separating gel. Lane 1, a homogenate of blood cells except giant cells; lane 2, VAP (the vanadium-associated protein eluted from a DEAE-Sephacel anion-exchange column, cf. Kanda *et al.*, 1997); lane 3, a homogenate of giant cells. Immunoblot analysis shows that a monoclonal antibody F8DH recognized 2 closely related peptides of 15 kDa and 16 kDa in the homogenate of blood cells except giant cells as it did not react with any protein in giant cells.

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Indirect immunofluorescence

Blood cells suspended in ASW and the connective tissue around the alimentary canal were smeared onto small coverslips and fixed in 75% acetate-ethanol for 5 min at 4°C, and subsequently in 70% ethanol for 5 min at 4°C. The samples prepared were washed with PBS. To reduce non-specific background, the samples were immersed in 0.05% Triton X-100 and 5% bovine serum albumin dissolved in PBS for 1 hr at room temperature and washed with PBS twice. The coverslips were then immersed in an appropriate dilute solution of either of the monoclonal antibodies, F8DH or S4D5. The coverslipes washed with PBS were immersed in a dilute solution of FITC/rhodamin-conjugated anti-mouse IgG antibody (Organon Teknika Corporation) for 30 min and washed again with PBS for 2 hr. The samples obtained were mounted on slideslips, sealed with 80% glycerol and observed by fluorescence microscopy with Nomarski differential-interference apparatus. The monoclonal antibody S4D5, specific for vanadocytes (Uyama *et al.*, 1991), was used to identify the vanadocytes.

RESULTS

SDS-PAGE and immunoblot analysis

A hybridoma cell line designated F8DH was newly prepared to secrete a monoclonal antibody that specifically recognized a vanadium-associated protein extracted from the homogenate of blood cells in vanadium-rich ascidian, *Ascidia sydneiensis samea*. To characterize the monoclonal antibody F8DH obtained, proteins extracted from blood cells of *A. sydneiensis samea* were immunoblotted. As shown in Fig. 1, F8DH reacted with peptides in the homogenate of blood cells including vanadocytes but did not with peptides in giant cells. This result was supported by the fact that giant cells, being very large and spherical shaped, 40 to 80 μm in diameter, and having a fluid-filled vacuole which occupied most of the cell, contained no vanadium (Michibata *et al.*, 1990; Wuchiyama and Michibata, 1995). The monoclonal antibody was further confirmed to reacted with 2 related peptides of 15 kDa and 16 kDa in the peak 1 fraction containing vanadium that was eluted from a DEAE-Sephacel column (Fig. 1). Although we have previously reported that VAP is composed of at least 2 types of peptides estimated to be 12.5 kDa and 15 kDa with a minor peptide of 16 kDa (Kanda *et al.*, 1997), these suggest that F8DH reacted with a common motif in the 15 kDa and 16 kDa peptides.

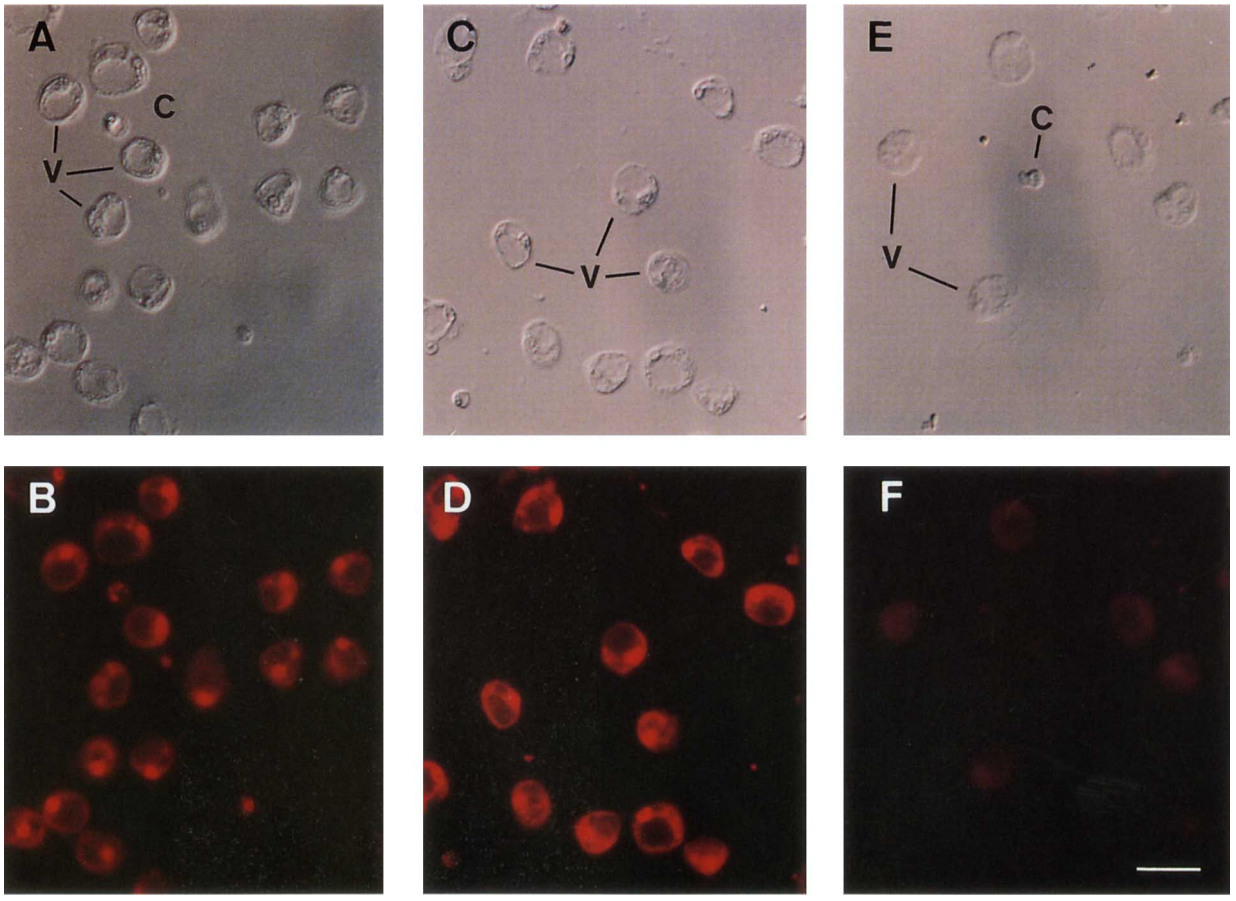

Fig. 2. Immunohistochemistry of blood cells reacted with the monoclonal antibody F8DH. Immunoreactivity was detected with rhodamin-labeled anti mouse IgG goat serum using fluorescence microscopy (**B**, **D**, and **F**). **A**, **C** and **E** are Nomarski differential-interference micrographs of the same field as **B**, **D** and **F**, respectively. **A-B**, using F8DH; **C-D**, using S4D5 so as to show the vanadocytes; **E-F**, normal mouse serum as a negative control. V, vanadocyte; C, compartment cell. Scale bar: 10 μm. Immunoreactivity of F8DH is shown in both the cytoplasm of vanadocytes and compartment cells, suggesting that VAP co-exists with the antigen recognized by S4D5 that is a 45 kDa peptide (Uyama *et al.*, 1991). Reactivity of F8DH appeared in the nucleus of some types of blood cell, it seems to be an artifact judging from changes in reactivity with an alteration in the concentration of the antibody.

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Localization of immunoreactivity among several types of blood cells

To determine the localization of VAP among several types of blood cell of *A. sydneiensis samea*, F8DH was subjected to immunohistochemistry. As a result, the reactivity of F8DH was observed in both types of blood cell; vanadocytes (signet ring cells) and compartment cells, while not in the other types of blood cell, as shown in Fig. 2, which results suggested that VAP is localized in both types of blood cells, vanadocytes and compartment cells, but not in other types of cell. Compartment cells, ovoid and 6 μm in diameter, account for 15 to 21% of the total population of blood cells in *A. sydneiensis samea* (Michibata *et al.*, 1990). Although the reactivity of F8DH appeared in the nucleus of some types of blood cell, as shown in Fig. 2, it seems to be an artifact judging from changes in reactivity with an alteration in the concentration of the antibody.

Localization of VAP in tissues

Since we have already shown that vanadocytes are densely distributed in the connective tissues around the alimentary canal where smaller signet ring cells, presumptive precursor cells of the vanadocytes that reacted with a monoclonal antibody S4D5 specific to the vanadocytes, were also found (Kaneko *et al.*, 1995), we examined whether some tissues; the connective tissues around the alimentary canal, intestines, branchial baskets, and muscles were recognized

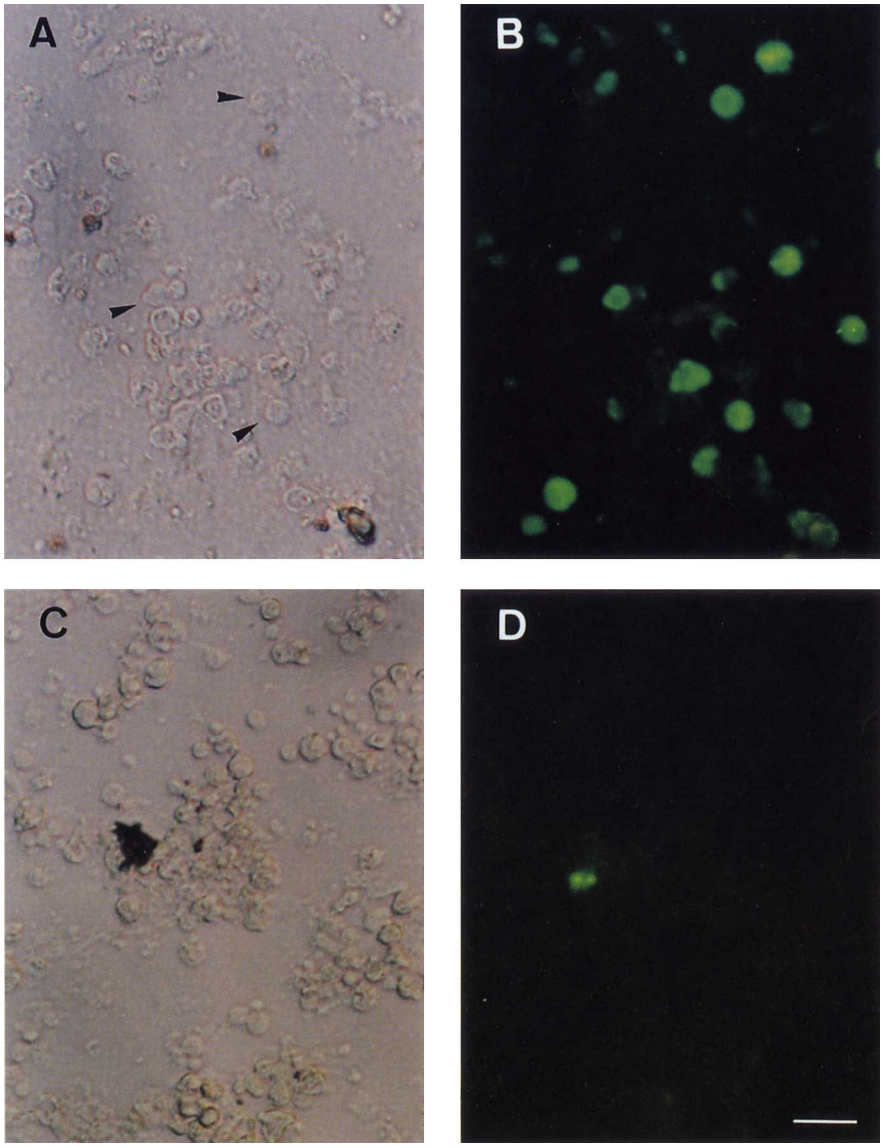

Fig. 3. Immunohistochemistry of the connective tissue around the alimentary canal with the monoclonal antibody F8DH. Immunoreactivity was detected with FITC-labeled anti mouse IgG goat serum using fluorescence microscopy (**B** and **D**). **A** and **C** are pictures taken by a Nomarski differential-interference micrographs of the same field as **B** and **D**, respectively. **A**-**B**, stained with F8DH; **C**-**D**, negative control. Arrowhead shown in **A** indicates the typical immunoreactive cells. Scale bar: 10 μm. Immunoreactivity was shown in small cells localized in the connective tissue, in which cells are also recognized by a monoclonal antibody S4D5 specific for vanadocytes (cf. Kaneko *et al.*, 1995). No other tissues were stained by F8DH.

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by the monoclonal antibody F8DH in these experiments. As a result, it was revealed that the vanadocytes distributed in the connective tissues around the alimentary canal were stained with both monoclonal antibodies, S4D5 and F8DH, suggesting that the vanadocytes contained the antigen, VAP, recognized by F8DH. No other tissues examined in these experiments were shown to react with the monoclonal antibody F8DH.

VAP in several ascidian species

Levels of vanadium vary depending on the ascidian species (Michibata *et al.*, 1986). Thus, in order to examine whether there might be a correlation between the levels of vanadium and the intensities of immunoreactivity in several ascidian species, *A. sydneiensis samea*, *A. ahodori*, *Ciona intestinalis*, *Halocynthia roretzi*, and *Pyura michaelseni* were submitted. The homogenates of blood cells from the 3 species, *A. sydneiensis samea*, *A. ahodori*, and *C. intestinalis*, were recognized by the monoclonal antibody F8DH but no bands from the latter 2 species, *H. roretzi* and *P. michaelseni* were stained with F8DH, which suggests that the intensities of the antigen (VAP) recognized by F8DH coincided with the levels of vanadium in the ascidian species.

DISCUSSION

A monoclonal antibody against a vanadium-associated protein (VAP) obtained in this experiment is an important molecular clue for resolving the mechanism of how ascidians selectively accumulate vanadium in their blood cells at a highest concentration of 350 mmol/dm^3 (mM) from seawater where vanadium is dissolved in very low concentration of 35 nmol/dm^3 (nM), namely, whose concentration is 10^7 times higher than that in seawater. Our previous report (Kanda *et al.*, 1997) indicated that VAP was isolated from the blood cells of the vanadium-rich ascidian, *Ascidia sydneiensis samea* using a DEAE-Sephacel anion-exchange column and the peak fraction containing VAP was composed of at least 2 different peptides of 12.5 kDa and 15 kDa with a minor peptide of 16 kDa. Immunoblot analysis using a polyclonal antibody prepared against VAP further revealed that VAP was not in the vacuoles of the vanadocytes but in the cytoplasm and might bind with cell membranes by ionic interaction.

A monoclonal antibody prepared against VAP in this experiment, designated F8DH, recognized 2 closely related peptides of 15 kDa and 16 kDa. Our preliminary data on the amino acid sequence of VAP show that 15 kDa and 16 kDa peptides have a common motif for the N-terminus (not shown). There is, therefore, a good possibility that the monoclonal antibody F8DH recognized a peptide including the common motif. On the other hand, a monoclonal antibody against the 12.5 kDa peptide with no significant motif matching those of the 15 kDa and 16 kDa peptides has been prepared yet. Such a monoclonal antibody is needed in order to not only determine the sequence of the amino acids but to also examine whether the peptide participates in the binding of vanadium ions.

As shown in Figs. 1 and 2, immunoblot analysis and immunohistochemistry using the monoclonal antibody F8DH revealed that VAP exists in the homogenate of blood cells including vanadocytes and F8DH reactivity was observed in both types of blood cells; vanadocytes (signet ring cells) and compartment cells, while it was not in the other types of blood cell. That is to say, the result pointed out a possibility that VAP localized in the vanadocytes and compartment cells but not in the other types of cells. Supporting this result, Scippa *et al.* (1988) pointed out that compartment cells and granular amoebocytes are cell types with vanadium besides vanadocytes, via X-ray microanalysis of cryo-fixed samples. However, there is little knowledge of the cell lineage of the vanadocytes from the stem cells. To determine when the accumulation of vanadium commences during the process of blood cell differentiation will clarify this problem.

The vanadocytes were densely distributed in the connective tissues around the alimentary canal where smaller signet ring cells, presumptive precursor cells of the vanadocytes, reacted with a monoclonal antibody (S4D5) specific for vanadocytes. There is, therefore, a strong likelihood that the vanadocytes are renewed in the connective tissues around the alimentary canal (Kaneko *et al.*, 1995). As shown in Figs. 3 and 4, the monoclonal antibody F8DH prepared in this experiments reacted with the same tissues as those recognized by S4D5. No other tissues examined in the present experiments were recognized by the monoclonal antibody F8DH. It is further conceivable that the vanadocytes, distributed in the connective tissues around the alimentary canal and recognized by S4D5, are presumptive precursor cells of the

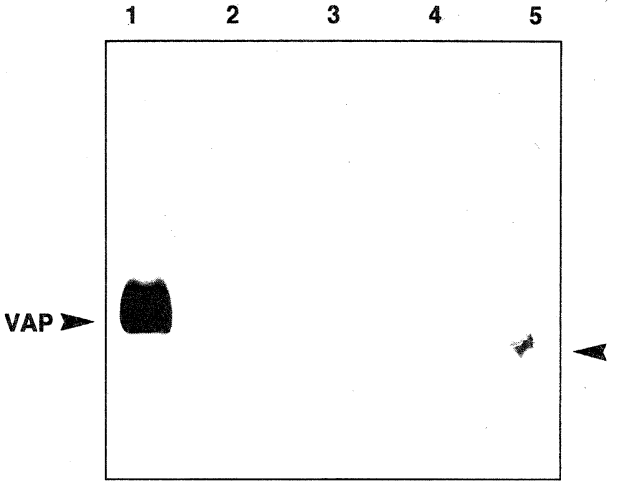

Fig. 4. Immunoblot analysis of several tissues of *A. sydneiensis samea*. Protein from the blood cells, muscle, branchial basket, stomach, and connective tissues around the alimentary canal (100 μg) was subjected to 14% polyacrylamide gel electrophoresis and then to immunoblot analysis with the monoclonal antibody F8DH. Lane 1, a homogenate of blood cell; lane 2, branchial basket; lane 3, muscle; lane 4, stomach; and lane 5, connective tissues around the alimentary canal. Immunoreactivity of F8DH was shown in the homogenate of blood cells and in that of the connective tissues around the alimentary canal. No reactivity of F8DH was observed in the homogenate of the other tissues examined.

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vanadocytes having the antigen, VAP, recognized by F8DH.

Levels of vanadium in the blood cells vary depending on ascidian species (Michibata *et al.*, 1986). The concentrations of vanadium in the blood cells of the ascidians examined are as follows; 12.8 mmol/dm^3, *A. sydneiensis samea*; 59.9 mmol/dm^3, *A. ahodori*; 0.7 mmol/dm^3, *Ciona intestinalis*; 0.007 mmol/dm^3, *Halocynthia roretzi* (Michibata *et al.*, 1986) and 0.006 mmol/dm^3, *Pyura michaelseni* (determined in the present experiment). As shown in Fig. 5, immunoblot analysis of the blood cells from 5 different species of ascidian, *A. sydneiensis samea*, *A. ahodori*, *C. intestinalis*, *H. roretzi*, and *P. michaelseni* clearly revealed that the former 3 species having higher levels of vanadium in their blood cells reacted with the monoclonal antibody F8DH, but little reactivity was observed in the latter 2 species, with the consequent, finding that the intensities of antigen (VAP) recognized by F8DH coincided with the levels of vanadium in the ascidian species.

At present, biochemical characterization of VAP and cloning of the gene encoded VAP are in progress, using the monoclonal antibody F8DH as a useful molecular indicator.

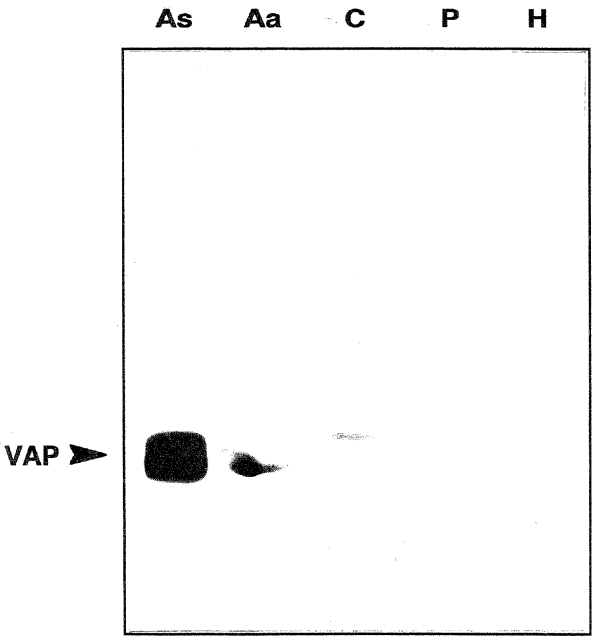

Fig. 5. Immunoblot analysis of the blood cells of several ascidians. For SDS-PAGE, 45 μg of protein from each homogenate of blood cells of *A. sydneiensis samea* and *A. ahodori* and 100 μg of each protein from those of *C. intestinalis*, *H. roretzi*, and *P. michaelseni* were applied, respectively. As, *A. sydneiensis samea*; Aa, *A. ahodori*; C, *C. intestinalis*; H, *H. roretzi*; and P, *P. michaelseni*. The former 3 species, *A. sydneiensis samea*, *A. ahodori*, and *C. intestinalis*, had higher levels of vanadium in their blood cells, VAP was recognized by the monoclonal antibody F8DH. While no immunoreactive signal of F8DH was detected in the blood cells of the other 2 ascidians, *H. roretzi* and *P. michaelseni*.

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REFERENCES

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein in utilizing the principal of proteindye binding. Anal Biochem 72: 248–254

Kanda T, Nose Y, Wuchiyama J, Uyama T, Moriyama Y, Michibata H (1997) Identification of a vanadium-associated protein from the vanadium-rich ascidian, *Ascidia sydneiensis samea*. Zool Sci 14: 37–42

Kaneko A, Uyama T, Moriyama Y, Michibata H (1995) Localization, with monoclonal antibodies and by detection of autonomous fluorescence, of blood cells in the tissues of the vanadium-rich ascidian, *Ascidia sydneiensis samea*. Zool Sci 12: 733–739

Michibata H (1996) The mechanism of accumulation of vanadium by ascidians: some progress towards understanding of this unusual phenomenon. Zool Sci 13: 489–502

Michibata H, Terada T, Anada N, Yamakawa K, Numakunai T (1986) The accumulation and distribution of vanadium, iron, and manganese in some solitary ascidians. Biol Bull 171: 672–681

Michibata H, Uyama T, Hirata J (1990) Vanadium containing cells (vanadocytes) show no fluorescence due to the tunichrome in the ascidian *Ascidia sydneiensis samea*. Zool Sci 7: 55–61

Michibata H, Kanamori K (1997) Selective accumulation of vanadium by ascidians from seawater. In "Adv Environm Sci and Technol" Ed by Nriagu JO, John Wiley & Sons Inc, New York, in press

Scippa S, Zierold K, de Vincentiis M (1988) X-ray microanalytical studies on cryofixed blood cells of the ascidian *Phallusia mammillata*. II. Element composition of the various blood cell types. J Submicrosc Cytol Pathol 20: 719–730

Uyama T, Nishikata T, Satoh N, Michibata H (1991) Monoclonal antibody specific to signet ring cells of *Ascidia sydneiensis samea*. J Exp Zool 259: 196–201

Wuchiyama J, Michibata H (1995) Classification, based on autonomous fluorescence, of the blood cells of several ascidians that contain high levels of vanadium. Acta Zool (Stockholm) 76: 51–55

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