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General Paper

Fluorescent Properties of Extract from Fired Purple Sea Urchin Shell

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Abstract

The fluorescence properties of an aqueous solution extracted from fired purple sea urchin shell (PSU) have been investigated. A pale blue-white fluorescence with a broad spectrum of 420 nm (peak) and 450 nm (shoulder) was observed by excitation at 370 nm for the extract from PSU fired at temperatures of 200, 250, 300, 350 and 400 °C. The highest fluorescence intensity was obtained for the extract from PSU fired at 300 °C. However, no fluorescence was observed from the extracts of the non-fired shells or from those fired at 600, 800 and 1000 °C, or from that fired at 300 °C which had been immersed in alkaline solution for 14 days. An oligopeptide with a molecular weight of ca. 910 and some trace metal ions of Cu and Zn were detected in the extracted aqueous solution by gel permeation chromatography (GPC), infrared (IR) spectroscopy and inductively coupled plasma-mass spectrometry (ICP-MS) analyses. These substances and calcium carbonate are considered to be the source of the fluorescence.

Key words: purple sea urchin shell, fluorescence, aqueous solution extract, IR, GPC, ICP-MS

Introduction

The catch amount of sea urchins in Japan was approximately 8.2 thousand tons in 2013 [1]. After removal of the edible gonads (ca. 5 wt %), the residual shells and spines of the sea urchins are discarded as food waste. Recently, seaweed beds have been lost by feeding damage due to the abnormal breeding patterns of sea urchins, so that urchins have been removed_from these areas for_extermination. However, there is a problem in that organic matter remaining in the discarded food waste decomposes and generates offensive odors; therefore, the cleaned urchin shells and spines are generally buried in landfills. Similar shell waste from molluscs such as scallops, turban snails, and blue mussels has been extensively studied for utilization of their fluorescent properties, and organic substances are known to be involved in fluorescence [2-7]. To achieve utilization of the shell waste from the purple sea urchin (PSU), *Anthocidaris crassispina* , methods for the production of calcium [8] has been proposed. In this study, the fluorescent properties of an aqueous solution extracted from fired PSU were investigated for the utilization of PSU.

Materials and Method

Materials and Extraction　PSU were captured off the

coast of Akune City Bay (Kagoshima, Japan) facing the East China Sea. After removal of the sea urchin gonads followed by boiling for 30 min and drying (Fig. 1), the PSU was fired at temperatures of 200, 250, 300, 350, 400, 600, 800 and 1000 °C in a muffle furnace. The fired PSU was then powdered using a SpectroMill Ball Pestle Impact Grinder (Model 1100, Chemplex Industry Inc., USA). The PSU powder (0.50 g) was extracted with 10.0 mL of pure water by ultrasonication for 10 min and allowed to stand for over 2 h. An aqueous extract was obtained after centrifugation of the supernatant and filtration through a 0.45 μm cellulose filter.

Fig. 1. Sample of the purple sea urchin shell (PSU) after boiling used in this study. The PSU was fired after separation of spines.

Characterization of PSU

Thermogravimetry-differential thermal analysis (TG-DTA; Thermo Plus TG8120, Rigaku Co., Osaka, Japan) was performed with a heating rate of 10 °C min^{-1} in an air atmosphere. The changes in the crystal structure of the powdered PSU by firing were observed using X-ray diffraction analysis (XRD; X'Pert PRO MRD, PN Aalytical B.V., Netherlands) with Cu Kα radiation.

Characterization of fluorescent material

Fluorescence spectra were recorded using a fluorescence spectrometer (FP8300, Jasco Co., Tokyo, Japan). Infrared (IR) spectra of the extracted solution were recorded on a Fourier transform IR spectrometer (6100SS, Jasco Co., Tokyo, Japan) using the attenuated total reflectance (ATR) method. The molecular weights of the fluorescent substances within the aqueous extract were determined using gel permeation chromatography (GPC) on a HPLC system (LC-20AD, Shimadzu, Kyoto, Japan with UV-vis (SPD-20AV) and fluorescence (RF-20A) detectors. The instrument conditions were as follows: eluent, phosphate buffered saline (PBS); gel column, Superdex 75 10/300GL and Superdex^{TM} Peptide 10/300GL (GE Healthcare Japan Co., Tokyo, Japan); temperature, 25 °C; flow rate, 0.5 mL min^{-1}; UV-detector (215 nm) or fluorescence-detector (λ_{ex}= 365 nm, λ_{em}= 440 nm). Standard protein (Bio-Rad Laboratories, Inc., USA), aprotinin from bovine lung, bradykinin and tryptophan (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as calibration standards. Trace metal elements were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; Triple Quad 8800, Agilent Technologies Inc., California, USA) according to a semi-quantitative method. Plasma conditions were as follows: RF power, 1.6 kW; plasma gas (Ar), 15.0 L min^{-1}; auxiliary gas (He), 0.91 L min^{-1}; carrier gas (Ar), 1.03 L min^{-1}.

The influence of organic substrates in the PSU on the fluorescence properties was examined according to ref. [4]. PSU powder (1.0 g) was added to 20.0 mL of sodium hydroxide solution (1.0, 3.0, 5.0, 10.0 w/v %) and immersed for 14 days. The PSU residues were then obtained by filtration and washed with pure water until the pH was less than 8.0. Biuret tests on the dried powders were conducted to confirm the removal of organic substrates such as protein and peptide; 4.0 mL of biuret reagent adjusted with copper sulfate, potassium sodium tartrate and sodium hydroxide was added to 200 mg of the treated PSU powder and left for 24 h. The absorbance at 540 nm was then measured with a UV-vis

spectrophotometer (UV2200, Shimadzu Co., Kyoto, Japan). Fluorescence spectra were also measured for the aqueous extract obtained from the powder fired at 300 °C that had been immersed in sodium hydroxide solution for 14 days.

Results and Discussion

Physical and chemical properties of PSU and fired PSU TG-DTA results for the PSU are shown in Fig. 2. Two mass-loss steps of ca. 5 and 45% were observed with endotherms at 600 °C and 700-820 °C, respectively. With respect to the thermal analysis of scallop shell [2,3] and taking the theoretical mass-loss percentage into account, the former step is considered to be due to decomposition of organic components and the latter step to the decomposition of calcium carbonate to calcium oxide.

Based on the thermal analysis data, only XRD patterns for the PSU fired at 600, 800 and 1000 °C are shown in Fig. 3. The PSU samples fired at 600 and 800 °C consisted of calcium carbonate with the calcite crystal system. In contrast, PSU fired at 1000 °C was identified as calcium oxide.

Characterization of fluorescent aqueous extract The extracts obtained from fired PSU at 200, 250, 300 300, 350, and 400 °C exhibited broad spectra with a peak at

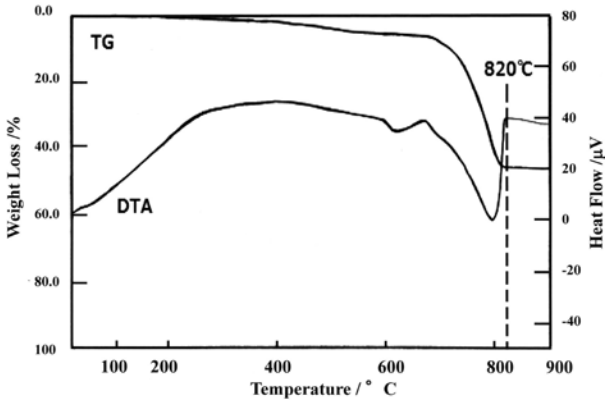

Fig. 2. TG and DTA profiles for PSU powder.

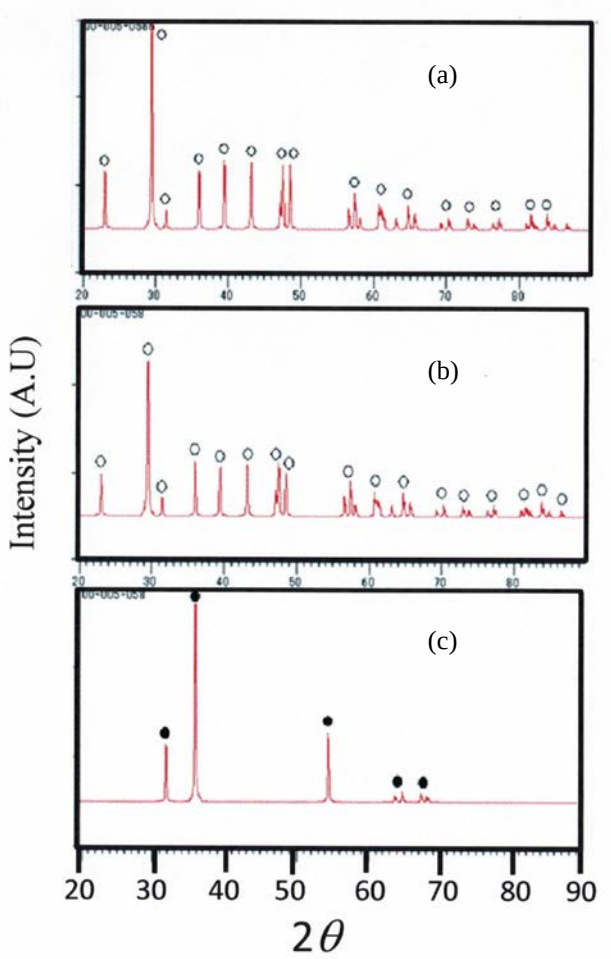

Fig. 3. XRD patterns for PSU-shell fired at (a) 600, (b) 800, and (c) 1000 °C. ○$CaCO_3$, ●CaO.

420 nm and a shoulder at 450 nm under excitation at 360 nm, as partially shown in Fig. 4. The highest intensity was obtained for PSU fired at 300 °C. However, no fluorescence was observed for the extracts from unfired PSU or from those fired at 600, 800 and 1000 °C. Figure 5 shows a photograph of the extracted solution fluorescing under UV lamp irradiation (365 nm). The spectrum is similar to that for aqueous extracts from scallop, turban snail and blue mussel shells around 435 nm (peak) and 485 nm (shoulder) [5].

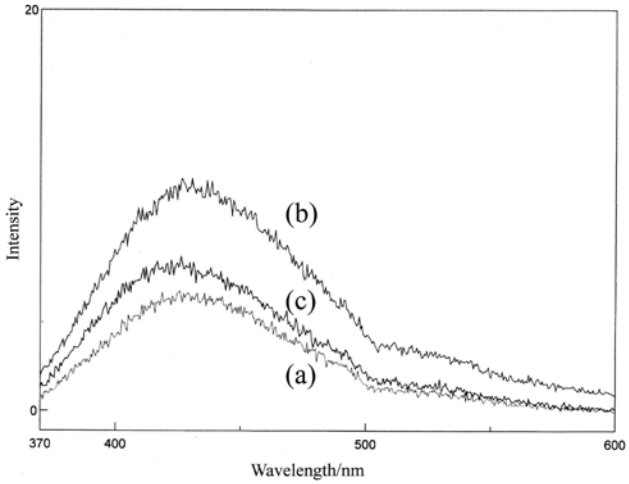

Fig. 4. Fluorescence spectra for aqueous extracts from PSU. Firing temperature of PSU: (a) 250, (b) 300, and (c) 350 °C

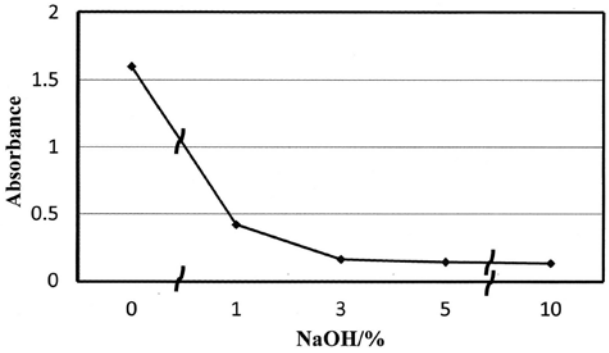

Fig. 6. Biuret test results for aqueous extracts from PSU after alkaline treatment.

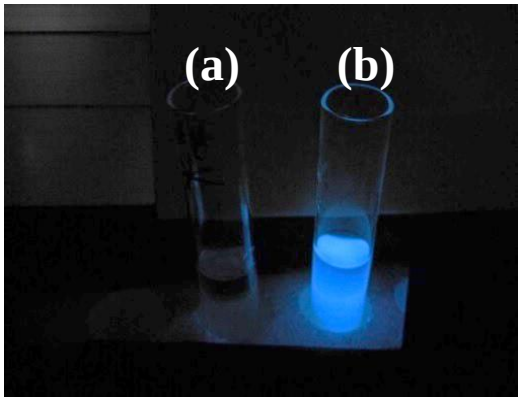

Fig. 5. Fluorescence of the extract under irradiation with a UV lamp (365 nm): (a) control (pure water), and (b) the aqueous extract.

Figure 6 shows the results of the biuret test. The changes in absorbance indicate that the organic substrates decrease with an increase of the sodium hydroxide concentration and the fluorescence intensities were also lowered with an increase of alkali concentration, as shown in Fig. 7. It was confirmed that no quenching of fluorescence occurs when the pH of the aqueous extract was over 9.0 (data not shown). This indicates that the organic substrate is involved in the fluorescent emission.

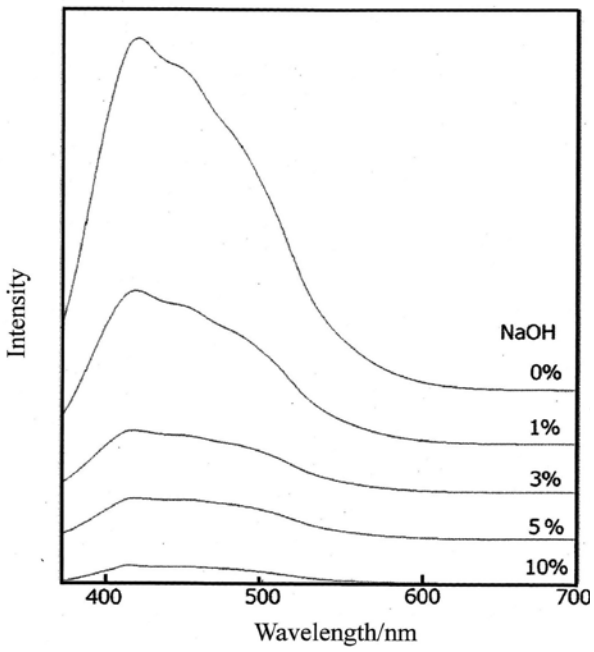

Fig. 7. Change in fluorescence intensity of aqueous extracts from PSU after alkaline treatment.

Figure 8 shows IR absorption bands for the PSU extract fired at 300 °C. The bands at 3360, 1408, 1652and 1572 cm^{-1} are attributed to amide bonds.

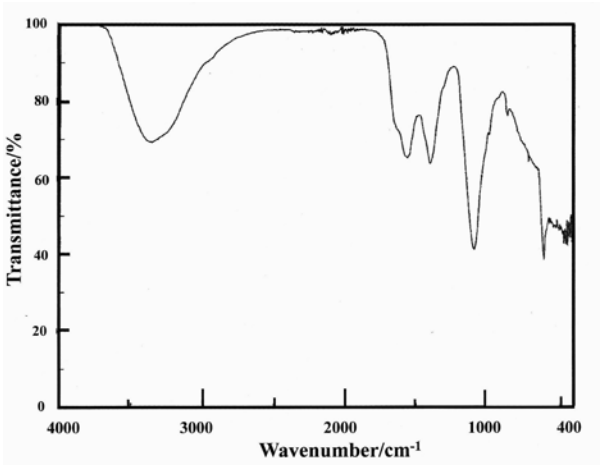

Fig. 8 IR spectrum for the aqueous extract from PSU.

The molecular weights of the fluorescent substances are small, according to GPC results using a Superdex 75 10/300GL column, UV-detector (215 nm) and standard protein for calibration. Further GPC was thus conducted using a Superdex™ Peptide 10/300GL column with a UV-detector (215 nm) or fluorescence-detector (λ_{ex}= 365 nm, λ_{em}= 440 nm). The chromatograms in Fig. 9 show that the molecular weights of the fluorescent substances in the aqueous extract from fired PSU were distributed over a wide range. The major peak at a retention time of 39.6 min corresponds to a molecular weight of ca. 914 according to the calibration standards. The substances in the extract are thus considered to be oligopeptides when also taking the IR spectrum into account.

In an aqueous extract from fired scallop shell, fluorescence was observed with a peak at 435 nm and a shoulder at 485 nm [5] under excitation at 370 nm, while a broad fluorescence spectrum with peaks at 420 nm (weak), 490 nm (medium) and 580 nm (strong) under excitation at 250 nm was observed for the fired scallop shell [2-4]. The luminescence centers were estimated to be Mn for 580 nm and Cu (copper protein) for 420 and 490 nm [4, 7].

Metals such as rare-earth elements were under the detection limit for semi-quantitative ICP-MS analysis of

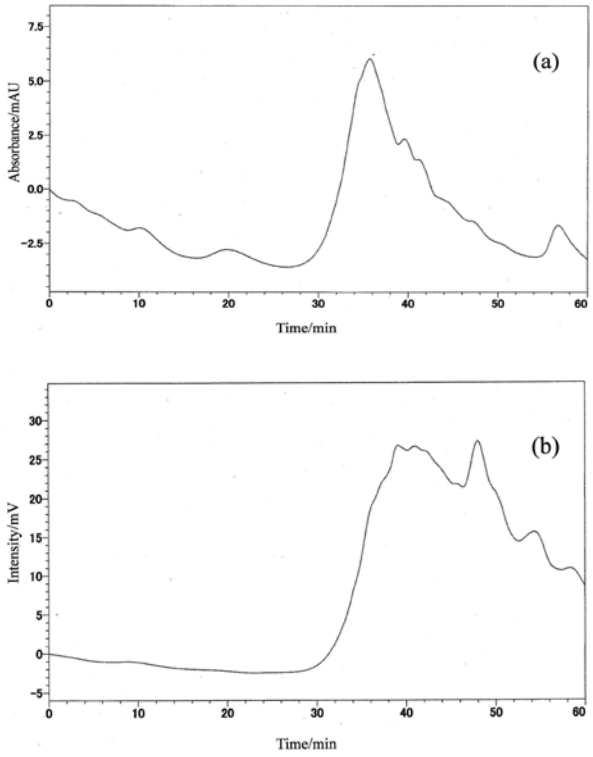

Fig. 9 GPC chromatograms for the aqueous extracts from PSU. Detection: (a) 215 nm, (b) λ_{ex}=365 , λ_{em}=440 nm.

10.0 mL aqueous extracts from 0.50 g of fired PSU. However, some transition metals were detected in the aqueous extracts, where the average concentrations were Cu (1.5 ± 0.9 ppb, n=7), Zn (1.4 ± 0.6 ppb, n=8), Ni (0.7 0 ± 0.54 ppb, n=8), Mo (0.4 0 ± 0.22 ppb, n=8), Pb (0.34 ± 0.21 ppb, n=6) and Fe (0.22 ± 0.03 ppb, n=8). It has been reported that mainly Cu and Zn are involved in the emission at 420 nm [4, 7] in the presence of Ca ions.

The major fluorescent substances in the extract obtained from PSU fired at 300 °C may be oligopeptide (ca. MW=910) and trace metals (Cu, Zn) based on the GPC, IR and ICP-MS analyses. These substances and calcium carbonate [6] are considered to be the light-emitting source of fluorescence. However, further investigations are required to clarify details such as complex formation between the metal ions and organic compounds.

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ムラサキウニ殻焼成物の水抽出物の蛍光特性

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要　旨

ムラサキウニ殻の焼成物を純水で抽出した溶液（抽出物）の蛍光特性について検討を行った。蛍光強度は 300℃焼成の抽出物で最も高く、励起波長 360nm で 435nm および 485nm（肩ピーク）を示した。
アルカリで前処理後に焼成した殻の抽出物では、蛍光が消失することから、殻に含まれる有機基質が蛍光に関与していると推察された。また、抽出物のＩＲ、ＧＰＣおよびＩＣＰ－ＭＳの分析結果から、蛍光には、$CaCO_3$および分子量が約 900 のオリゴペプチドならびに金属（Cu および Zn）が関与することが示唆された。

キーワード：ムラサキウニ、蛍光、水抽出物、ＩＲ、ＧＰＣ、ＩＣＰ－ＭＳ