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In vitro assessment of bioavailability of selenium from a processed Japanese anchovy, Niboshi

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ABSTRACT

Niboshi is a commonly used foodstuff that is processed from Japanese anchovy (*Engraulis japonicus*) in Japanese cuisine. It was previously demonstrated that Niboshi and its water extract contained highly bioavailable selenium for selenium deficient mice. In this study, we assessed the selenium bioavailability from the extract of the Niboshi, using cultured cells. The activity of selenium-dependent glutathione peroxidase (GPx) of rat dorsal ganglion cells and human cervical carcinoma cells incubated with selenium from the Niboshi extract was over 2 times of that of the extract-free control cells and comparable to that of cells incubated with selenious acid of the same selenium concentration. These results suggest that selenium from the Niboshi extract was utilized for synthesis of the selenoprotein. Such *in vitro* selenium bioavailability was consistent with our previous results of *in vivo* assessment in mice.

1. Introduction

Selenocysteine (SeCys) is the major chemical form of selenium species in organisms and is co-translationally incorporated into SeCys containing proteins (selenoproteins) (Labunskyy, Hatfield, & Gladyshev, 2014). Selenium in the environment is ingested in the food chain through plants and animals, and almost all selenium required for the selenoprotein synthesis in humans is provided from dietary food. Twenty-five human selenoproteins have been identified (Kryukov et al., 2003): glutathione peroxidases (GPxs) are the best-known selenoprotein family members, and are critical enzymes in antioxidative defense systems (Rayman, 2012). Because selenium is a micronutrient whose deficiency and toxic levels are close to each other, it is important to know its abundance and deficiency in foodstuffs. Additionally, foods with a high-selenium content are not necessarily better selenium sources, but we should consider the bioavailability of selenium from food. Although fish and shellfish contain relatively higher concentrations of selenium, the number of reports on the selenium bioavailability from seafood are fewer than that from meats, cereals, vegetables, *etc* (Dumont, Vanhaecke, & Cornelis, 2006; Finley, 2006; Moreda-Piñeiro & Moreda-Piñeiro, 2015; Yoshida, Haratake, Fuchigami, & Nakayama, 2011). This is because selenium in certain fish meat was reported to be less bioavailable due to the complex formation with heavy metal species, such as mercury (Afonso et al., 2015; Ralston & Raymond, 2010). The selenium bioavailability in several tuna products were compared to that in wheat products, and a significantly lower activity of GPx in the tissue of tuna product-fed rats was observed in contrast to that of wheat product-fed rats (Alexander, Whanger, & Miller, 1983). Meanwhile, several papers described that the bioavailability of selenium from fish and shellfish was higher than that of inorganic selenium and selenized yeast, commonly used as nutritional supplements. Fox et al. (2004) assessed the bioavailability of selenium from selenium-enriched trout fish and demonstrated that fish selenium was highly bioavailable in comparison to selenized yeast and sodium selenate. Hepatic cellular GPx activity of the mice fed with the diet supplemented by defatted dark muscle of tuna was higher than that of the mice fed with selenite-supplemented diet (Yoshida, Abe, Fukunaga, & Kikuchi, 2002). However, the bioavailability of selenium from fish and seafood materials still appears to remain controversial, which may be related to the species-specific chemical form of the selenium compounds.

Selenium-deficiency diseases and related pathologies have never been reported in Japan under normal nutritional conditions. This was thought to be attributed to dietary habit, as the Japanese population frequently have selenium-abundant fish and shellfish materials in their meals. Miyazaki et al. (2004) reported that fish and/or shellfish are the major dietary sources of selenium for the Japanese population (~60% of daily intake). In the Japanese diets, Niboshi (~1 μg Se/g) is a commonly used foodstuff that is processed from the Japanese anchovy (*Engraulis japonicus*), and its extract is used as a general base seasoning for a wide variety of Japanese cuisines, just like the *fumet de poisson*

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and/or *fond de veau* for the French cuisine. We have previously shown that selenium from the Niboshi and its extract could restore the activity of the hepatic cellular GPx in dietary selenium-deficient mice, and the ionic organic compound with a molecular mass of less than 5000 was the major selenium species in the Niboshi extract (Haratake, Takahashi, Ono, & Nakayama, 2007; Yoshida, Haratake, Fuchigami, & Nakayama, 2012). In this study, the authors addressed the *in vitro* assessment of the bioavailability of partially purified selenium species from the Niboshi extract using cultured cells.

The bioavailability of selenium from foodstuffs is usually assessed using dietary selenium-deficient animals. However, the assessments using experimental animals are time-consuming and tedious. In addition, a large amount of selenium compounds is required for the animal experiments. Some researchers used cultured cells to assess the absorption and utilization of selenium species. Zeng, Botnen and Johnson (2008) used selenium deficient human colon cancer Caco-2 cells as alternatives to selenium-deficient animals to assess the bioavailability of selenium species in selenized broccoli. Human hepatoma HepG2 cells are also used to evaluate the antioxidative effect of selenium compounds (Cuello et al., 2007; Marschall, Bornhorst, Kuehnelt, & Schwerdtle, 2016).

In this study, both primary-cultured dorsal root ganglion (DRG) cells and HeLa cells (human cancer cells) were used to investigate whether selenium from the Niboshi extract can be used for the synthesis of selenoproteins. Both cells were incubated with the partially purified selenium species in the Niboshi extract followed by determination of their cellular GPx activity.

2. Material and methods

2.1. Niboshi samples

Niboshi is a popular processed food product from Japanese anchovy. The size of 5–12 cm length of Japanese anchovy caught in the near shore of Japan were boiled in sea water for a few minutes immediately after unloading and dried in the sun or oven. Five hundred-gram packages of the Niboshi products were purchased at local grocery stores and stored at −20 °C until use without any treatments.

2.2. Preparation of the Niboshi extracts

Three pieces of almost size-matched Niboshi (~3 g) were placed in a non-woven fabric bag after cutting into ~ 10 pieces of almost equal size. These samples were placed in 30 ml of water, then heated and boiled for 10 min. The obtained solution was filtered through paper (particle size cutoff, 4 μm) and water was added to make a final volume of 20 ml, which was used as the Niboshi extract in this study. The concentrations of the selected substances in the Niboshi extracts were calculated by the following equation: = [(amount of substances in the extract used for analysis)/(volume of the extract used for analysis)] · [(Total volume of the extract prepared)/(weight of the Niboshi used for extraction)] (μg or mg/g-Niboshi).

2.3. Determination of selenium concentration

The selenium concentrations in the specimens were fluorometrically determined using 2,3-diaminonaphthalene (DAN, Tokyo Chemical Ind., Tokyo, Japan) after digestion with nitric acid and perchloric acid (Watkinson, 1966). After the reaction with the digested sample and 0.1% DAN-0.1 M HCl solution, the fluorescent intensity of the piaselenol generated from DAN (excitation wavelength: 375 nm, emission wavelength: 520 nm, working concentration range: 0.5–10000.0 ngSe/sample) was measured by an FP-6600 spectrofluorometer (JASCO, Tokyo, Japan). The selenium standard solution (Kanto Chemical Co., Inc., Tokyo, Japan) [1000 ppm as selenium (IV) dioxide in 0.1 M nitric acid] was used for preparation of the calibration curve.

2.4. Determination of selected component concentrations

After the digestion of Niboshi and its extract with nitric acid and perchloric acid, the specimens were diluted with nitric acid or water and adjusted to a 10 ml-volume in a volumetric flask. These solutions were subjected to elemental analysis after proper dilution. The sodium, magnesium, potassium and calcium contents in the specimens were determined using an AAnalyst 200 flame atomic absorption spectrometer (Perkin-Elmer, Inc., Waltham, MA). Atomic absorption spectrometry grade standard solutions of sodium, magnesium, potassium and calcium (Kanto Chemical Co., Inc., Tokyo, Japan) were used for preparation of the calibration curves. The determination of the phosphorus content was based on vanadium (V) that is capable of forming a yellow-colored complex with the phosphate ion. After the addition of 0.25% ammonium vanadate solution and 5% hexaammonium heptamolybdate tetrahydrate solution to digested samples, the color produced by the complexation was monitored at 440 nm by a V-660 UV–Visible spectrophotometer (JASCO, Tokyo, Japan) (Kiston & Mellon, 1944).

The chlorine content in the Niboshi extract was directly measured by the Mohr method. After a 4-fold dilution with deionized water, the Niboshi extract was mixed with potassium chromate and then titrated with 0.01 M silver nitrate solution.

The Niboshi extract was appropriately diluted with water (typically 50–100-fold), and the total free amino acid concentrations were spectrofluorometrically determined after the reaction with 100-fold volume of 1.8 mM *o*-phthalaldehyde and 6.9 mM dithiothreitol (DTT) in 0.02 M borate buffer (pH 9.5) to form a thio-substituted isoindole fluorophore (excitation wavelength: 340 nm, emission wavelength: 450 nm, working concentration range: 0.1–50 μM) (Jones, Owen, & Farrar, 2002). Glycine dissolved in water was used to make the calibration curve.

For the protein concentration determination, the sample was appropriately diluted with water, then directly measured by Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951). The diluted sample was mixed with a 5-fold volume of a 10:1:1 mixture of 2% Na_2CO_3-0.1 M NaOH, 1% $CuSO_4{\cdot}5H_2O$ and 2% sodium (+)-tartrate dihydrate and allowed to react for 10 min. A half volume sample of Folin-Ciocalteu's reagent solution diluted 2-fold with water was added and allowed to react for 30 min. The absorbance at 650 nm was monitored by a V-660 UV–Visible spectrophotometer (JASCO, Tokyo, Japan) and bovine serum albumin was used as the reference (working concentration range: 0.01–0.5 mg/ml).

2.5. Ion-pair extraction and mass spectrometry

Hexadecyltrimethylammonium chloride (HTAC, Tokyo Chemical Ind., Tokyo, Japan) dissolved in water was used for the ion-pair extraction. The HTAC solution was combined with the Niboshi extract to make its final concentration 1 mM, followed by extraction with chloroform. The obtained chloroform layer was concentrated *in vacuo* and subjected to fast atom bombardment mass spectrometry (FAB-MS) or matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF-MS). The mass spectra were acquired in the linear positive ion mode by a JMS-700 N (JEOL, Tokyo, Japan) using *m*-nitrobenzyl alcohol as a matrix and an Ultraflex (Bruker Daltonics, Inc., USA) using 2,5-dihydroxybenzoic acid as a matrix.

2.6. Cell culture

DRG cells were isolated from male 3–7 week old Wistar rats (weighing 35–280 g, specific pathogen free) from Clea Japan, Inc. (Tokyo, Japan). Rats were housed 1–3/cage (PC, 270 × 440 × 187 mm, with sawdust for animal experiments) on a 12 h light-12 h dark schedule at 23 ± 2 °C and 60% relative humidity. A regular breeding diet CE-2 (Clea Japan, nutritional composition of the diet can be seen at http://www.clea-japan.com/Feed/ce2.html) and