SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. High resolution MS/MS analysis of the selenium compound. The daughter ions of m/z 553 were recorded on a JMS-700.

FIGURE S2. **MS spectrum of the selenium compound.** The MS spectrum of the purified sample (A) of oxidized dimer of selenoneine was recorded on a Quattro II at m/z 552.9. After reduction in the presence of 10 mM glutathione at 25°C for 30 min, the monomer of selenoneine was detected at m/z 278.2 (B). The inserts show the enlarged spectra around the peak at m/z 278.

FIGURE S3. ¹H-NMR spectrum of the selenium-containing compound. The spectrum of the purified dimer of selenoneine was recorded on a 500-MHz spectrometer (ECA500, JEOL, Tokyo, Japan) in D₂O as the solvent.

FIGURE S4. UV spectrum of the selenium-containing compound. The spectrum was recorded at the concentration of 1.1 mM of the purified dimer of selenoneine in distilled water with a UV-visible spectrometer (DU640).

FIGURE S5. Speciation analysis of organic selenium in the various tissues of farm-raised bluefin tuna by LC-ICP-MS. An arrow shows the elution of selenoneine. A: serum, B: spleen, C: hepatopancreas, D: heart, E: red muscle, and F: white muscle. An asterisk indicates selenoproteins including GPx eluted near the void volume of the column. An arrow shows the elution of selenoneine. "x" denotes a peak of unidentified selenium compound.

FIGURE S6. Speciation analysis of organic selenium in the blood of fish by LC-ICP-MS. The blood of wild bluefin tuna (A), wild Pacific mackerel (B) and laboratory-reared tilapia (C) were examined, and selenoneine was detected in all of blood samples. In the tilapia blood, the serum (D) did not contained selenoneine, whereas the erythrocytes (E) washed with 50 volume of PBS contained selenoneine. An asterisk indicates selenoproteins including GPx eluted near the void volume of the column. An arrow shows the elution of selenoneine. "x" denotes a peak of unidentified selenium compound.

FIGURE S7. Speciation analysis of organic selenium in the animal tissues by LC-ICP-MS. Selenoneine was detected in the porcine kidney (A), chicken liver (B) and Japanese common squid (C) An asterisk indicates selenoproteins including GPx eluted near the void volume of the column. An arrow shows the elution of selenoneine. "x" denotes a peak of unidentified selenium compound.



Figure S1 Yamashita and Yamashita



Figure S2 Yamashita and Yamashita



Figure S3 Yamashita and Yamashita



Figure S4 Yamashita and Yamashita



Figure S5 Yamashita and Yamashita



Figure S6 Yamashita and Yamashita



Retention time (min)

