論文の内容の要旨

Determination of subnanomolar zinc and its speciation in seawater with improved clean sampling and analytical method

（クリーンな採水と分析法を用いた海水中の極微量亜鉛の濃度と存在状態に関する研究）

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Introduction

Zinc (Zn) is an essential micronutrient for bacteria and phytoplankton in the ocean. Laboratory studies examining the influence of Zn on phytoplankton growth showed that coastal species could be zinc-limited (Bruland et al., 1983) and that Zn was important for silicate uptake by diatoms (Rueter and Morel, 1981). It is also suggested that the Zn incorporated into the diatom frustules records the Zn speciation in the seawater at the time of growth and the resulting Zn:Si ratio of the diatom opal can be used for paleo interpretations of Zn speciation in the ocean (Elwood and Hunter, 1999). Reportedly, organic complexes of dissolved zinc in surface waters account for about 98% of the total dissolved zinc, and these organic forms, therefore, dominate the dissolved speciation of zinc in seawater shallower than 200m (Bruland, 1989). The organic complexation reduces the bioavailable fraction of zinc, the free metal ion (Zn^{2+}), to a level as low as 1 pmol/L (Bruland et al., 1991). Culture experiments have shown that at concentrations of less than 1 pmol/L, Zn^{2+} is limiting to the growth of some species of phytoplankton (Brand et al, 1983; Sunda and Huntsman, 1992, 1995). Nevertheless, biogeochemical cycles of Zn have not been fully revealed yet in the ocean since determination of Zn in seawater is very difficult because of contamination problems (Fitzwater et al., 1982). Cathodic strriping voltammetry (CSV) is sufficiently sensitive to determine low concentrations of trace metals in seawater such as Zn. Important advantages of voltammetry are that it can be used to determine the chemical speciation as well as the concentration of dissolved species. In this study, I have established a new reliable determination method and clean sampling technique for subnanomolar level of Zn in seawater. Using these methods, vertical distributions of Zn and its speciation were investigated.

Materials and procedures

1) Sample collection and storage

For comparing the performance of trace metal clean sampling methods, seawater samples were collected using three different sampling methods in which acid-cleaned Teflon-coated X-type Niskin bottles were (1) fixed on a conductivity-temperature-depth carousel multi-sampling system (CTD-CMS, SBE-

911plus and SBE-32 water sampler, Sea Bird Electronics, Inc.), with the Zn sacrificial anode being replaced with Al to avoid the possibility of Zn contamination from the frame; (2) attached bottle by bottle to a Kevlar wire (6 mm diameter, Hikari-kogyo); and (3) attached bottle by bottle to a Ti wire equipped in R/V Hakuho-maru. Seawater samples were filtered using acid-cleaned 0.2 μm pore-size Acropak filter cartridge (PALL Co.). Samples for total dissolved Zn concentration were then stored after being acidified to achieve a pH < 1.8 by using ultra pure HCl (Tamapure AA-100). Samples for Zn speciation analysis were also filtered and frozen immediately onboard the ship.

2) Determination of total dissolved Zn and its speciation in seawater

On the land-based laboratory, total dissolved Zn concentration was determined by using cathodic stripping voltammetry (CSV) after UV-digestion in Teflon beaker. Zn speciation was determined by titration using competitive ligand equilibrium / adsorptive cathodic stripping voltammetry (Donat and Bruland, 1990; Van den Berg, 1985). This involves the establishment of a competitive equilibrium between zinc-complexing ligands naturally present in the sample and a competing organic ligand, ammonium 1-pyrrolidinedithiocarbamate (APDC), added to the sample. Briefly, the complexation of zinc in seawater by a natural ligand (L) can be defined as

$$K'_{ZnL} = [ZnL] / ([Zn^{2+}][L']) \qquad (1)$$

where K'_{ZnL} is the conditional stability constant of the zinc complex in seawater. $[L']$ is the concentration of L not complexed by zinc, $[ZnL]$ is the concentration of zinc complexed with the ligand L, and $[Zn^{2+}]$ is the free zinc ion concentration. The total ligand concentration (C_L) is defined as

$$C_L = [ZnL] + [L'] \qquad (2)$$

Substitution for $[L']$ in Eq. (1) using Eq. (2) and rearranging gives

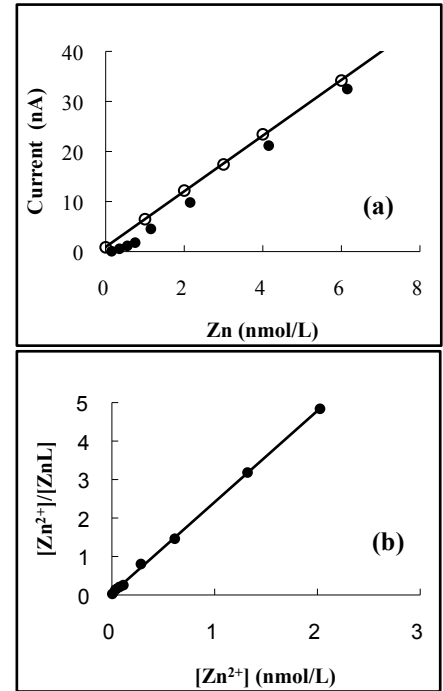$$[Zn^{2+}]/[ZnL] = [Zn^{2+}]/C_L + 1/(K'_{ZnL}C_L) \qquad (3)$$

Fig. 1. Example of Zn titration data. (a) The response of Zn peak current with increasing Zn additions. The line and blank circles indicate the response of UV-irradiated sample. (b) Linear relationship obtained by transforming the titration data, which a ligand concentration of 0.4 nmol/L and log K'_{ZnL} of 10.8 was calculated.

When values of $[Zn^{2+}]/[ZnL]$ are plotted against corresponding values of $[Zn^{2+}]$ a linear relationship is usually obtained with a slope equal to $1/C_L$ and with the intercept yielding $1/(K'_{ZnL}C_L)$ (Fig .1).

3) Study areas

To confirm and establish the clean sampling and analytical techniques for determining trace levels of Zn, seawater samples were collected in the subtropical North and South Pacific (December 2011 to January 2012) and subarctic North Pacific (August to October 2012) during the Hakuho-maru KH-11-10 and KH-12-4 R/V cruises, respectively. Seawater samples for investigating total dissolved Zn and its speciation in the western North Pacific and its marginal seas, the Sea of Okhotsk and the Sea of Japan (East Sea) were

collected during the R/V Hakuho-maru research cruise KH-10-2 (in June and July 2010, at stations CR-27, CR-30 and CR-47). Andaman Sea and the Bay of Bengal were also determined. Seawater samples for Andaman Sea and Bay of Bengal (July to August 2013) were collected during another R/V Hakuho-maru KH-13-4 cruise.

Result and Discussion

1) Procedural blank value, detection limit, and reference seawater for Zn analysis

To obtain a procedural blank for Zn analysis, surface seawater was passed through a chelating resin column (NOBIAS CHELATE-PA1, Hitachi High-Tec). By measuring the purified seawater repeatedly, the procedural blank value was calculated as 75 ± 3 pmol/L (n = 7). This obtained blank value was used for calculating the total dissolved Zn concentrations, which was subtracted from the measured values. Detection limit (calculated as three times the standard deviation of measurements of blank values for purified seawater) was 28 pmol/L. To confirm the precise Zn determination, reference seawater samples were determined. The results are in good agreement with consensus values. It indicates that precise seawater determination for Zn using CSV is accomplished.
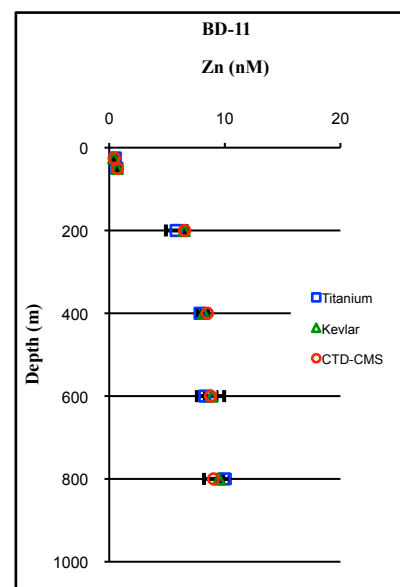

Fig. 2. Comparison of dissolved Zn concentrations in seawater collected with three different sampling methods, Niskin-X samplers with titanium wire hydrocasts, Kevlar wire hydrocasts and CTD-CMS

2) Comparing three sampling methods

Three different seawater sampling methods, Teflon-coated X-type Niskin samplers were 1) fixed on CTD-CMS, 2) attached to Kevlar wire and 3) attached to titanium wire were compared. Because Zn is used as sacrificial anode in the research vessel, especially in main propellers of the Hakuho-Maru, Zn contamination was observed during Kevlar wire hydrocasts that were performed from the stern of the vessel. By minimizing the influence from the propellers, almost the same Zn concentrations within analytical errors among those three different sampling methods have obtained (Fig. 2).

3) Distribution and speciation of dissolved zinc in the western North Pacific and its adjacent seas

Vertical distributions of Zn show nutrient-type vertical profiles, with a gradual increase in concentration from the surface to deep water. The dissolved Zn profiles are similar to those of Si in this study, which showed the same trends in the previous studies in the North Pacific Ocean (Bruland et al. 1978; Donat and Bruland 1990). In high latitude areas such as the subarctic North Pacific, the Sea of Okhotsk and the Sea of Japan (East Sea), Zn complexing ligands in most samples are already saturated because of the presence of high concentrations of total dissolved Zn. Relatively high total ligand concentrations have been obtained in the surface waters of the Sea of Okhotsk (2.6 nM) and the Sea of Japan (East Sea) (1.3 nM) compared with those in the open ocean (subtropical and subarctic North Pacific, 0.2–1.2 nM). In the western

North Pacific, positive relationships were obtained between total ligands and chlorophyll *a* concentrations (Fig. 3), suggesting that Zn complexing ligands in the surface water might be derived from bacteria and phytoplankton in the western North Pacific. However, the relationships were completely different in the Sea of Okhotsk and the Sea of Japan (East Sea) (Fig. 3), implying   that Zn complexed ligands in those marginal seas are derived from different sources.
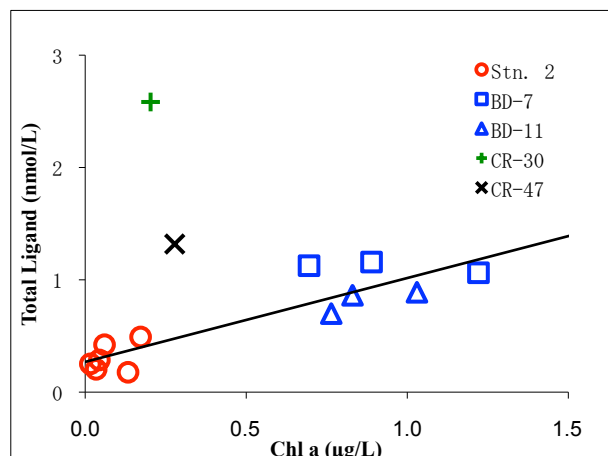

Fig. 3. Comparison of Chl *a* and total ligand concentrations in the western North Pacific (Stn. 2, BD-7 and BD-11), Sea of Okhotsk (CR-30) and Sea of Japan (East Sea)(CR-47). Black line indicates the relationship between Chl *a* and total ligand concentrations in the western North Pacific ($R^2 = 0.814$).

4) Distributions of total dissolved Zn and its speciation of the northeastern Indian Ocean and Andaman Sea

        In the Andaman Sea, results show that there are high influence of fluvial discharge from Irrawaddy and Salween rivers. In the northern Andaman Sea, where high Chl *a* content has obtained, total dissolved Zn were relatively lower than those of southern Andaman Sea, may indicate removal of total dissolved Zn in the northern Andaman Sea by biological uptake. Vertical distributions of total dissolved Zn in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 and MY-3) show almost identical concentrations from surface to 1000 m depth. Below 1000 m depth, total dissolved Zn was diverged, indicates the deep water in the Andaman Sea appears to be rapidly replaced by the incoming waters from the northeastern Indian Ocean across the sills and then homogenized by vertical mixing. In the northernmost station of the Andaman Sea (MY-6), total ligand concentrations are correlated with Chl *a* contens (Fig. 4), which supports that phytoplankton and bacteria-excreted organic substances were dominant source for Zn complexing ligands. Other stations in the Andaman Sea, however, poor correlation have obtained (Fig. 4), might suggest that different sources of Zn complexing ligands were dominant at those stations.

Fig. 4. Comparison of Chl *a* and total ligand concentrations in the northeastern Indian Ocean (NR-1) and Andaman Sea (MY-1 to MY-6).