

**Analysis of the requirement of selenium by coccolithophorids and its implication
for the control of a coccolithophorid bloom formation in the ocean**

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Unicellular calcifying algae, coccolithophorids, fix inorganic carbon mainly by two reactions, namely photosynthesis and intracellular calcification. The calcification reaction produces calcium carbonate crystals known as coccoliths, which are excreted and placed to cover the cell surface. The algae are known to form huge blooms in the ocean and is consequently expected to affect the air-sea interchange of CO₂. The growth of the algae and the production of coccoliths are affected by the status of nutrients, such as nitrogen and phosphorus. In addition to such major nutrients, microelements were also shown to affect the growth of coccolithophorids. Phytoplankton bloom appears as species-specific, area-specific and season-specific events. It is still unknown how such specificity is selected. We found that the addition of selenium is necessary for the rapid growth of coccolithophorids. The evidence suggests that such trace elements may be very effective to stimulate the growth of a certain selected phytoplankton in the ocean. In this study, we focused on studying on uptake and concentration of selenium by coccolithophorids and on identifying protein(s) which are specifically labeled with a radioactive selenium (⁷⁵Se).

When 3.2 nM ⁷⁵Se-selenite was added to the culture after the depletion of selenium, the intracellular concentration of selenium was increased to 8.6 μM. The concentration factor was 3000. The remaining selenite in the medium was 0.4 nM, suggesting that 80% of selenite ions in the medium were incorporated into cells. Uptake of selenite increased proportionally to the concentration of selenite in the medium, namely 0.09, 0.4, 1.0, 2.0 and 4.0 μM, and did not saturate even at 4 μM which is 400 times higher than that of the optimum concentration for growth. This evidence shows the presence of intracellular pool of selenium.

Intracellular Se-requiring proteins were identified. Since Se is an analogue of S, To distinguish specific Se-labeling from non-specific-labeling, a new method, named the double-label-method, was developed using radioactive ⁷⁵Se and ³⁵S in this study. Using the method, a novel selenoprotein with a molecular mass of 29 kDa on SDS-PAGE was found and the protein was purified partially. The protein is expected to be a key factor for the regulation of coccolithophorid growth.