Abstract

Iron (Fe) is the 4th-most abundant element in the Earth, and is one of the most important micronutrient for almost all organisms. To maintain cellular homeostasis, Fe plays a vital role in regulating the oxygen transport in blood, oxygen storage in muscle, as well as enzyme cofactor. The magnitude of Fe isotopic fractionation among the biological process can be large mainly due to changes in the strength of chemical bonding among the species through redox reaction (*i.e.*, from Fe^{2+} to Fe^{3+}, or *vice versa*). Thus, it is now well recognized that the Fe isotopic signature can be a sensitive indicator for the Fe absorption and Fe storage efficiencies. Moreover, systematic changes in the Fe isotope ratios ($^{56}Fe/^{54}Fe$) among the animals and plants can provide key information concerning the food-chain or bio-cycle of Fe in the various environment or animal habitat. Moreover, the $^{56}Fe/^{54}Fe$ ratio can change by reflecting the difference in the bioavailability of Fe among the terrestrial (~6 wt%), marine surface (~10^{-7} wt%), and deep-sea hydrothermal vent (~10 wt%) conditions. Another important feature to use the Fe isotope study is that the isotope signature is more robust than the abundance data. The abundance data is seriously dependent upon various conditions, such as water contents of the sample, sample preparation procedures, or nutritional status of the animals, whereas the Fe isotope signature should reflect inherent nutritional status of the plants or animals, and thus, further strict discussion for the Fe metabolism among the animals or plants can be made using the isotopes. To take a full advantage of the isotopes, we analyzed the Fe isotope ratios for marine animals of various trophic levels and biological availability of Fe. Hence, the Fe isotopic analysis for various soft tissue samples from marine animals (octopus, squid, tuna, billfish, melon-headed whale, *Crysomallon squamiferum*, *Gigantopelta aegis*) were subsidized to evaluate possible difference in the Fe metabolism under the different habitat.

In chapter II, the Fe isotope signature was employed as new proxy to decode the Fe biocycle in the open ocean. We have measured the Fe isotope ratios for soft tissues (i.e., blood, muscle, and liver) of high trophic level animals living in marine surface, namely octopus, squid, tuna, billfish, and melon-headed whale. To define the trophic

levels of the animals, the N isotopes ratios were also measured. The resulting Fe isotopic data showed no clear relationship between the trophic level and Fe isotope ratios. This implies that Fe metabolism could not be evaluated by the N isotopes, which can reflect the trophic levels of amino acids or proteins. The small Fe isotopic fractionation could occur in higher trophic level marine animals as well as terrestrial organisms, absorbing sufficient Fe from the diet. For marine mammals, the resulting for the Fe isotope ratios of female and male in blood samples indicated that no Fe excretion mechanism in the body reflect the similar δ^{56}Fe values in different gender, unlike humans. For migratory fish, namely tuna and billfish, have no significant differences between muscle and liver contrast to that for terrestrial organisms. No significant differences in the δ^{56}Fe values of soft tissues could be the unique feature for migratory fish: involving the mechanism of lower Fe storage efficiency which provide Fe to the functional Fe at the higher rate from the storage Fe.

In chapter III, we have determined the isotopic composition of Fe for two gastropods, *Crysomallon squamiferum* and *Gigantopelta aegis,* living side-by-side in the Longqi hydrothermal vent field located at 2,785 m in depth on the Southwest Indian Ridge, Indian Ocean, to investigate the difference in the Fe metabolism. Both species host endosymbiont bacteria in an enlarged 'trophosome' modified from oesophageal glands. Their external morphology is rather different: *C. squamiferum* have sclerites and shell covered by a layered of iron sulfide, while *G. aegis* lacks sclerites but instead form a thick layered Fe on its shell. In this study, Fe isotopic ratios of various components (muscle, ctenidium, blood, endosymbiont-containing oesophageal gland, shell, sclerites and Fe layer on shell) were measured. Large isotopic fractionation between soft tissues and seawater (i.e., mostly as ferrous Fe) was newly observed in the organisms inhabiting hydrothermal vent area, which have not been found in terrestrial and marine surface. For the oesophageal gland, there were no significant difference in the δ^{56}Fe values between *C. squamiferum* and *G. aegis*, whereas the δ^{56}Fe values for muscle, ctenidium, and blood from *C. squamiferum* were higher than those for *G. aegis* samples. More importantly, δ^{56}Fe values for the sclerites of *C. squamiferum* were lower than both soft tissues, as well as its shell and the layered Fe deposition on the shell of *G. aegis,* which were similar to that of average of hydrothermal fluids. This suggests that the iron sulfide on the shell and the sclerites could be produced by different

processes, and also the Fe in the sclerites were not the simple depositions precipitated from the hydrothermal fluids, probably explained by the bacterial origin or biomineralized origin. In contrast, the δ^{56}Fe values of the sclerites from Kairei vent field, Central Indian Ridge, did not vary significantly from that of the hydrothermal vent chimney. The difference in the Fe isotopic signature between the sclerites from Longqi and Kairei vent field were originating from the different formation sequence of the Fe-bearing components on the sclerites. Based on the Fe isotope ratios for the soft tissues of *C. squamiferum* sample, morphology differences between Longqi and Kairei samples and possibility of biomineralization in the Longqi sclerites were carried out together with the physicochemical properties through elemental compositions defined by SEM, elemental imaging using LA-ICPMS, and mineral composition analysis by XRD. The surface of the sclerites from the Kairei were covered with thick and well-crystallined iron sulfide with two layers, greigite (Fe_3S_4) and pyrite (FeS_2), whereas the Longqi has a very thin and poorly crystallined Fe-bearing layer, partially crystallized in pyrite and mostly amorphous phase. Moreover, the Ca and P bearing phases were present in the Longqi samples especially in the tip part of the sclerites as amorphous form, which were not found in the Kairei. We do not have any further data to discuss the formation sequence of the sclerites of *C. squamiferum*, this should remain as a possibility.

Through these studies, I clarified the new insights on unknown Fe metabolism using the Fe stable isotopic composition of marine animals of various habitat. In addition, this is the first study to demonstrate the important information about the possibility of biomineralization for generating iron sulfide on the sclerite or by *C. squamiferum*, which have been discussed for long years.