APPLICATION OF SIMS TO EXAMINATION OF COASTAL ORGANISMS

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The application of SIMS to the analysis of coastal organisms was successful using a silver membrane filter on which the samples were fixed. Although thin samples like gut tube had no electric charge, the same samples with thickness of 2 mm were charged. From the analysis of clay minerals and suspended flocculent mud of the Ariake Sea, and from the results of artificially raised artemia and killifish, we found that SIMS could be used to analyse clay minerals in the guts of coastal organims. From the ratio of Si/Al it was to determine whether coastal organisms or clay mineral.

1. INTRODUCTION

Because the detection of clay minerals taken into the guts of coastal organisms has so far been very difficult, the value of suspended flocculent mud as a food for these organisms is not well understood. In this study, We used SIMS to detect clay minerals in the guts of these organisms, because SIMS could be applied to micro region analyses with high sensitivity on very small samples.

In order to apply ion microanalysis to coastal organisms, it is necessary to prevent electric charging by the primary oxygen beam. To prevent charging, the organisms were fixed to a silver membrane filter in this study. Using this method, it was found that the guts of fish and zooplankton in a coastal region contained the inorganic flock with similar characteristics to the suspended flocculent mud in the same region.

2. EXPERIMENT

A Hitachi IMA−2 ion microanalyzer was used under the following experimental conditions: primary ions O_2^+ ,primary ion accelerating voltage 10 kV, primary ion current 1 ～ 5 μ A, secondary ion accelerating voltage 3 kV, and primary ion beam diameter 0.1 ～ 0.2 mm.

2.1 Samples

At the mouth of the Chikugo River in the Ariake Sea (having the greatest tide level difference in Japan and highest turbidity), we gathered water (containing suspended flocculent mud) and sea floor mud.

Killifish, which were used as the typical fresh−water fish, were fed artificial food containing added clay minerals and artemia salina. As natural samples we used phytoplankton, fish, shell fish and zooplankton gathered with planktonnet at the mouth of the Chikugo River. For coastal fish, small sardines were selected, their guts were burned in platinum crucibles, and the ash analyzed.

2.2 Method

After washing with distilled water, all the samples were fixed on silver membrane filters (SELAS Co.). The clay minerals and the gut of the sardine were pressed and fixed. Suspended flocculent mud and bottom mud were fixed after filtration.

Zooplankton, phytoplankton, the edge of the

guts of the fish and the shell fish were fixed. After drying in desicators, the fixed samples were put into the SIMS sample chamber for at least 12 hours.

These dried samples are insulators, in many cases they could be measured because the electric charge on the samples effected the irradiating positive ions. But we achieved direct observation without other treatment, because we succeeded in the direct observation of dust on the silver membrane filter with SEM.

We adopted clay with milk for the raising experiment, following the Shirota method[1]. We prepared clay fresh milk and fed a proper quantity by pipet every day to artemia and killifish. We compared non fed artemia with those fed 2 and 5 days after hatching. To excrete all food eaten before the experiment, we fed killifish collected from the pond milk−clay every day, until the excrement was completely white. After about a week we picked these fish from the breeding tank as samples. These fish samples were scanned repeatedly by SIMS from 23 to 58 m/z (1 cycle takes about 1 minute) .

3. RESULT

By fixing the samples on silver membrane filters it was possible to measure without electric charging. It was possible to obtain measurements when the beam size was enlarged; for thin guts the sample length was about 5 mm and the width was from 1 to 2 mm, zooplankton and thick guts had lengths from 1 to 2 mm, and widths of about 0.5 mm. Thick samples sometimes charged when the length was about 2 mm. Judging from the result of X−ray diffraction analysis, we estimated that bottom mud and suspended flocculent mud were clay minerals that included montmorillonite.

From the SIMS qualitative analysis, it was found that all samples had Al and Si as the main components and, compared with montmorillonite, the intensity of Al was higher than that for Si. This is because Al is more likely to ionize compared with Si and it might also indicate differences in the origin of the clay mineral. We thought that it might be possible to determine where an animal lived by examining the ratio of the Al and Si intensities. So we performed a preliminary breeding experiment using montmorillonite as an artificial breeding food.

After supplying one day old artemia salina with clay and milk or nothing for 24 hours, these fish were fixed with formalin and the gut from body surface to center was analyzed. We obtained the SIMS depth profile shown in Fig. 1.

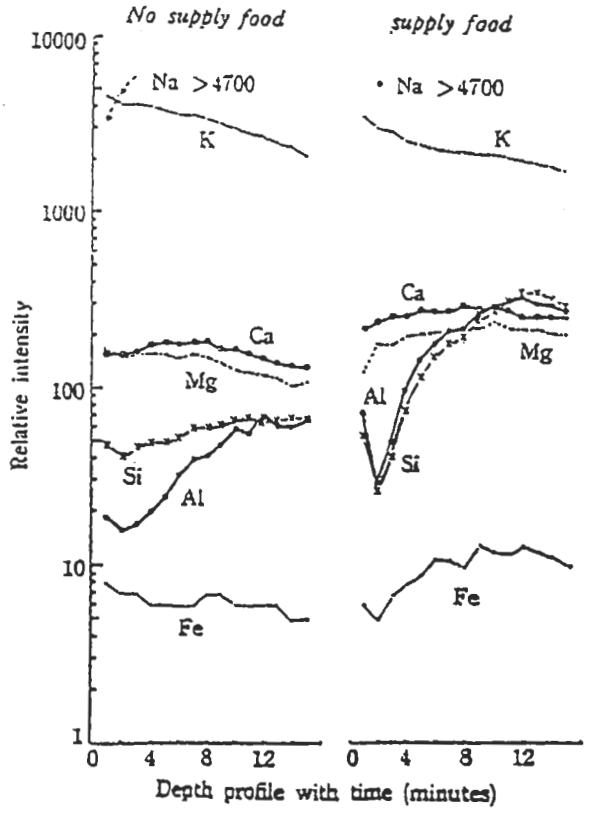

Fig. 1 Depth profile of Artemia raised for Experiment
(surface → interior of gut)

From the surface to the gut of the artemia,

the intensity of each element was similar regardless of feed. Ca, Mg and Fe of non feeding artemia changed little or showed a tendency to decrease with depth. On the other hand, in fed artemia, these elements tended to increase nearer the gut contents. We thought this was the effect of milk feeding. Potasium had the same distribution for both fed and non fed artemia. Si and Al had large differences between feeding and non feeding artemia. From this preliminary experiment we believe that Si and Al can be used for the detection of clay mineral in gut. We measured both elements in other samples. The results are shown in Fig. 2.

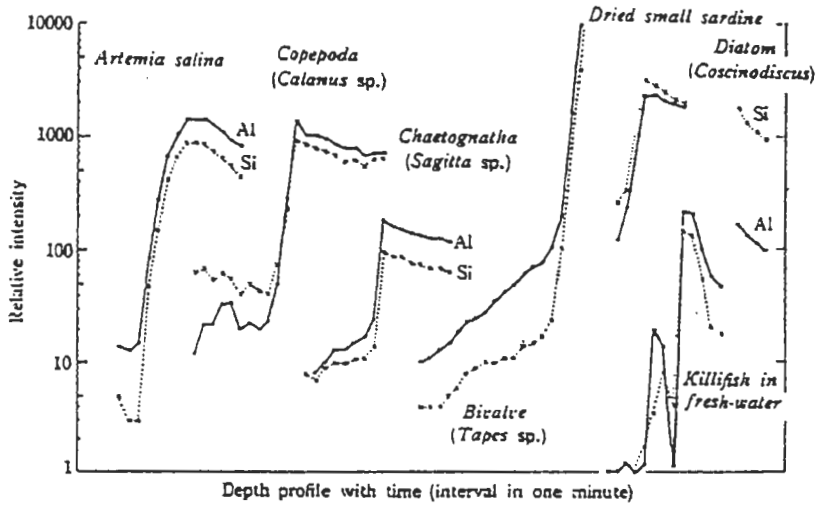

Fig. 2 Depth profile of interior of gut of coastal animals(Al, Si)

The SIMS result from the surface to gut from fifth day artemia salina, fed by clay with milk, showed an increasing difference in the secondary ion intensity of Si and Al in the surface and gut compared with the results from the second day artemia salina (Fig. 1). The relative intensities on the surface were below 20, but both elements in the gut changed in the range of 45 to 70 times the value. This indicates the existence of clay minerals in gut contents.

As for the gut of fed killifish, although Si and Al were almost non-existent in gut tube, the intensity of both elements was about 200 times higher in the gut. From the rapid increase of Si and Al with depth, the boundary between the tube and the inner part of gut could be determined.

The animals collected from the natural areas were typical coastal species. Similar results were obtained for the zooplankton: The tendency of Si and Al to increase rapidly with depth and the existence of clay minerals. The gut of the bivalve was thick, it took over 2 hours to sputter from the surface of the gut to the interior, where the secondary ion intensity of Si and Al increased rapidly. Bivalves live in bottom the analysis of clay minerals and suspended flocculent mud of the Ariake Sea, and from the results of artificially raised artemia and killifish, We found that SIMS could be used mud, have suspended flocculent mud and plankton and have a large quantity of clayminerals in their guts. In the internal organs of dried small sardines the same mineral was detected. The Si and Al intensity ratio of dried small sardines was different from other animals. The Si elemental intensity ratio was a little higher than Al. This is because sardines are omnivorous and eats diatoms of phytoplankton. We investigated the ratio of Si/Al in suspended flocculent mud, bottommud, montmorillonite, artemia, gut of killfish and dried sardine, copepoda, sagitta and gut of bivalves(Table 1). The values range from 0.5 to 1 compared with about 10 for the diatoms. Judging from the above results we found coastal organisms feed not on diatoms but on clay minerals.

4. SUMMARY

The application of SIMS to the analysis of coastal organisms was successful using a silver membrane filter on which the samples were fixed. Thin samples like gut tube had no electric charge. Same samples with thickness of 2 mm were charged. From the analysis of clay minerals and suspended flocculent mud of the Ariake Sea, and from the results of artificially raised artemia and killifish, we found that SIMS could be used to analyse clay minerals in the guts of coastal organisms and from the ratio of Si/Al it was to determine whether coastal organisms fed on diatoms or clay mineral.

Table 1 Secondary ion ratio of each sample
(M + /Al +)

SAMPLE	Si/Al	Mg/Al	K/Al	Ca/Al	Fe/Al
Suspended flocculent mud in the Ariake Sea	0.83	0.13	0.11	0.06	0.10
Bottom mud in the Ariake Sea	0.74	0.10	0.11	0.07	0.10
Pure clay (Montmorillonite)	1.39	0.18	0.08	0.06	0.03
Artemia (2 days after hatch)					
no supply of food	1.18	1.94	41.70	2.42	0.10
supply of food (clay with milk)	1.02	0.71	6.50	0.86	0.04
Artemia (5 days after hatch)					
supply of food (clay with milk)	0.64	0.44	2.00	0.20	0.02
Killifish Gut contents					
supply of food (clay with milk)	0.63	0.34	2.46	0.50	0.02
Diatom (Coscinodiscus sp.)	10.03	0.81	0.37	0.46	0.07
Dried small sardine Gut contents	1.13	3.01	9.96	3.08	0.27
Copepoda in the Ariake Sea Gut cont.	0.76	2.70	0.23	4.02	0.09
Sagitta in the Ariake Sea Gut cont.	0.54	1.13	0.41	2.01	0.07
Bivalve in the Ariake Sea. Gut cont. (Tapes sp.)	0.77	0.38	1.05?	1.08?	0.39

REFERENCE

1) A. Shirota: Fresh–Marine Organisms as Living Food for Fisheries. Koseisha Koseikaku, Tokyo, p358–364, p497–501 (1975).