Observation of iodine in oceanic plankton by scanning x-ray fluorescence micro-tomography

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Abstract. 3-dimensional distribution of iodine of the female oceanic zooplankton, Paracalanus parvus s.l., was observed by using scanning x-ray fluorescence micro-tomography. The distribution of iodine along the ventral border of the zooplankton was observed to be concentrated in seven regions between the head and the bases of the swimming legs. Then, 3-dimensional distributions of iron and zinc were also observed.

1. Introduction

There is widely supported hypothesis that life originated in the ocean [1] and the hypothesis was borne out experimentally [2]. The environment of the ocean, such as contained elements, could greatly affect development of the life. As one of the environmental features of the ocean, the ocean water is relatively rich in iodine (58 μg/kg [3]). Iodine is one of the essential trace elements for higher animals to maintain their life. Iodine is mainly accumulated in a thyroid gland to be secreted as thyroid hormone, which is generally known for a relationship with basal metabolism. In the process of evolution, the primitive life in the ocean, such as zooplankton, could accumulate iodine in their environment for preliminary stage of the thyroid gland. Therefore, the distribution of iodine may be a key to explain the evolution. Then, x-ray fluorescence computer tomography (XFCT) is a proper method to observe the distributions of the specific elements of a sample 3-dimensionally without any destructive process [4,5].

In this study, we noticed copepods as the typical oceanic zooplankton because the class of the copepods account for 70% of the oceanic zooplankton and distributes all over the world. The 3-dimensional distribution of iodine and the other elements of the calanoid copepod, Paracalanus parvus s.l., were observed by the XFCT to specify the preliminary organs or tissues of the thyroid gland.

2. Experiments
2.1. Sample

The oceanic zooplankton, *Paracalanus parvus* s.l. (female), was used as a sample. This calanoid copepod is widely distributed in epipelagic zone in coastal area throughout the world. In Japan, the copepod is distributed all coastal area [6].

The copepod sample was collected at Sagami bay (Kanagawa, Japan) and was immediately prefixed in 2% glutaraldehyde and 2.5% paraformaldehyde. After preservation for 1 week, the copepod was post-fixed in 1% osmium tetroxide solution. The fixed copepod was dehydrated through a graded ethanol series to 100% ethanol and was embedded in the Epon-812 resin.

2.2. Measurement

The sample was measured by using a scanning x-ray fluorescence microscope system at the BL37XU in the SPring-8 (Harima, Japan). The x-ray energy of 10 keV was used as an incident beam to avoid exciting osmium L-line. The x-ray fluorescence of calcium, iron, zinc and iodine (L-line) were detected. The spot size of the microbeam was 3.0 (H) × 1.5 (V) μm². The sample was scanned as follows: horizontal direction of 3 μm × 125 steps, the step of rotation angle of 2.4°, the helical pitch of 15 μm/rotation and 41 helical rotations, and the exposure time of 0.2 sec/point. In total, it took 71 hours to measure whole body of the copepod.

2.3. Reconstruction of x-ray fluorescence micro-tomography

Sinograms of the cross sections were extracted by using constant speed helical fullscan with interpolation method from the scanning data of each element [7]. The reconstructions were performed by simultaneous algebraic reconstruction technique (SART) with taking into account the absorption effects of the incident beam and the x-ray fluorescence inside the sample. In this experiment, distribution of linear absorption coefficient (LAC) of the incident beam (10 keV) was obtained by measuring I₀ and I, but those of the LACs of the measured x-ray fluorescence were not obtained. Therefore, the resin was regarded as main absorber of the sample to calculate the distribution of the LACs of the x-ray fluorescence by approximation. Thus, the modified distribution of the LAC of the incident beam, which was multiplied by the ratio of the LAC of the PMMA (C₅H₈O₂, ρ: 1.19 g/cm³) at the energies of the incident beam and the x-ray fluorescence, was used.

The median filter of 3×3 was used to reduce artifacts of the reconstructed FXCT images. 3-dimensional images were made from vertical stacks of the filtered FXCT images by using ImageJ (http://rsb.info.nih.gov/ij/).

3. Results

3-dimensional elemental distributions of iron, zinc and iodine are shown in figures 1. The reconstructed volume is 372×372×585 μm³ and the voxel size is 3×3×3 μm³. In these 3-dimensional images, the distribution of iron in figure 1(a) is like speckle and is relatively high concentration in the exoskeleton. The distribution of zinc in figure 1(b) clearly shows muscles. High concentration structure near the center of the image is an ovary (also shown in figure 2(a)). Iodine in figure 1(c) is mainly accumulated at 7 parts in the metasome. These distributions are along the ventral border. The longest one (upper right part of the image) is in the head. Judging from its position, this structure has

**Figures 1.** Lateral view of 3-dimensional distributions of (a) iron, (b) zinc and (c) iodine L-line. A head is on the upside and abdomen is toward right side in the image.
possibility to be a nerve cord [8]. The other 6 parts are seen at bases of swimming legs.

Cross section images of the distributions of zinc (shown in gray scale) and iodine (shown in dotted shapes) are shown in figures 2. Longitudinal positions of these cross section images are indicated by arrows in figure 1(c). In figure 2(a), the gut is seen at the centre of the cross section images like a hole. The ovary has high concentration and is observed around the gut. In figures 2(b), 2(c) and 2(d), the dense distributions of iodine coincide with those of zinc. On the other hand, in figure 2(a), the distributions of iodine and zinc do not coincide. These comparisons imply that the tissue which contains dense iodine in the head is different from the other parts.

Figures 2. The cross section images of the distributions of zinc (gray scale) and iodine (dotted shapes). Longitudinal positions of the cross section images correspond to arrows in figure 1(c).

4. Conclusion
The 3-dimensional distribution of iodine was observed at the ventral border of the head and at the bases of the swimming legs by using the FXCT. Then, the distributions of the other elements were helpful information to identify the anatomical structures. For further identification of the distribution of iodine and the organs or the structures, more anatomical investigation of the calanoid copepod is needed.

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