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TOF-SIMS analysis of magnetic materials in chum salmon head

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ABSTRACT

A piece of tissue extracted from a chum salmon *Oncorhynchus keta* head was measured with time-of-flight secondary ion mass spectrometry (TOF-SIMS) in order to evaluate the distribution and composition of magnetic materials in the tissue, which may concern with geomagnetic navigation of long-distance migrating salmon. Several depositions of iron compounds were detected in the tissue by TOF-SIMS analysis. Comparing with total ion images providing a topological tissue structure, specific distribution of iron ion in the tissue was clearly shown. Higher magnification TOF-SIMS analysis revealed the existence of the aggregations of iron particles. Iron oxide clusters comprising many submicron particles were also detected in the tissue using scanning electron microscopy and X-ray analysis, suggesting the common existence of submicron-scale iron oxides in salmon heads. These results suggest that TOF-SIMS analysis is a valid method to clarify detailed structures and chemical properties of candidate magnetoreceptors in fish heads.

1. Introduction

Some animal species are believed to use the earth's magnetic field to facilitate long-distance migration [1–5]. The magnetic sense of salmonids is an extensively studied subject: it has been elucidated through behavioral, electrophysiological, and anatomical studies [6–9]. Supporting the geomagnetic navigation hypothesis of salmonids, magnetic materials have been extracted from the salmon head [8–15] or lateral line [16]. Although magnetic materials must be related with the magnetic sense, magnetoreceptors have not been identified with certainty in any animal [17]. One reason for the difficulty in identifying magnetoreceptors is difficulty in observing and analyzing magnetic materials *in situ*. Magnetic materials *in situ* are too small for observing in detail by light microscopy and too rare for detecting by electron microscopy. For example, in previous TEM studies, the magnetic particles were gathered on a sample holder after they were isolated from tissues [10,11,13–16]. For those reasons, analytical methods with high sensitivity and chemical mapping are anticipated for use in identifying magnetic sensors *in situ* and for clarifying their function for magnetoreception. time-of-flight secondary ion mass spectrometry (TOF-SIMS) is capable of chemical imaging of raw samples and it is one of the most sensitive methods. Therefore, TOF-SIMS is inferred to be suitable for analyzing magnetic materials in salmon tissue.

In the present study, a piece of tissue extracted from a chum salmon *Oncorhynchus keta* head were measured with TOF-SIMS in order to evaluate the distribution and composition of magnetic materials in the fish head tissue.

2. Materials and methods

2.1. Sample preparation

An adult chum salmon *O. keta* (641 mm in fork length) was caught in the western North Pacific. The head of the salmon was used for the experiment. After the head was rinsed with distilled water, the skin, nose, mouth, eyes, and other surface tissues were removed from the salmon head using a ceramic knife. Subsequently, the head was rinsed again with distilled water and digested using a 5% sodium hypochlorite solution. Ether was added to extract fats from the digested salmon head. At this stage, most tissues had been dissolved, but the bulk of bone and tiny tissues remained undigested. The solution, including dissolved tissues and undigested miniscule tissues, was centrifuged. The solution was separated into two layers and the minutely disintegrated tissues were floated between the layers. When a magnet was brought near the interlayer, a tiny piece of tissue (ca. 0.8 mm) was attracted to the magnet, i.e. the tissue included magnetic materials. The tissue was moved into distilled water in a Petri dish and observed using a stereomicroscope. Several dark brown spots were observed in the tissue. The tissue was dehydrated using ethanol and put on an aluminum base for scanning electron microscopy (SEM, JSM-6100, JEOL) observation and X-ray analysis (Delta II, KEVEX Corp./ECON4, EDAX Inc.).

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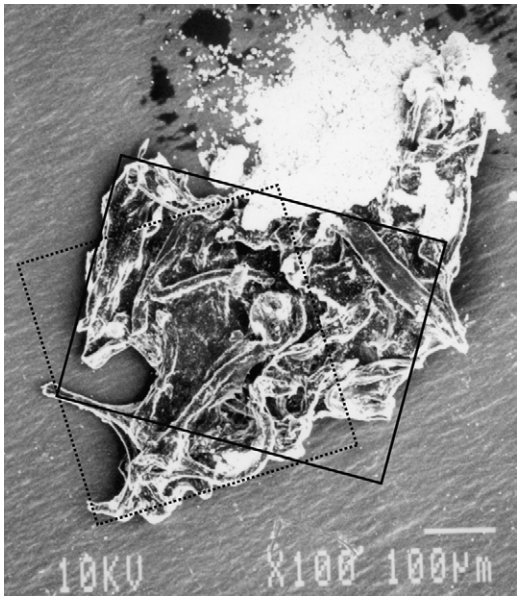

Fig. 1. SEM image of the tissue extracted from chum salmon head. The solid frame envelops the area for X-ray analysis shown in Fig. 2; the dotted frame envelops the area for TOF-SIMS analysis shown in Fig. 3.

2.2. TOF-SIMS measurement

The specimen of the tissue was etched with argon ion, and then it was measured by means of TOF-SIMS (TFS-2000, Physical Electronics) with the gallium ion after sputtering with continuous primary ion beam in order to eliminate contamination. The ion dose density used for sputter etching was approximately 10^{15} ions/cm^2. Positive and negative secondary ion spectra and secondary ion images were obtained. All the spectra, composed of positive ion TOF-SIMS spectra, were calibrated to the CH_3^+, $C_2H_5^+$, and $C_3H_5^+$ peaks.

3. Results and discussion

Fig. 1 shows a low-magnification SEM image of the tissue. Several iron depositions were detected in the tissue using X-ray analysis (Fig. 2, upper left). Several secondary ion peaks related to magnetic materials with iron were also observed in TOF-SIMS spectra. Fig. 3 shows TOF-SIMS secondary ion images of the tissue. The area indicated by an arrow in Fig. 3a corresponds to position A in Fig. 2. Comparing with total ion images providing a topological tissue structure, specific distribution of iron ion in the tissue is clearly shown in Fig. 3. The higher magnification TOF-SIMS image revealed the existence of smaller iron particles, suggesting the common existence of submicron-sized particles of iron compounds in salmon head.

Figs. 4 and 5, respectively, show SEM image and X-ray analysis results of iron deposition at position B in Fig. 2. The size of the iron deposition was approximately 50 μm. A mesh structure with submicron mesh size was observed through the cleavage of organic matters. The mesh structures were not observed with TOF-SIMS imaging, because they were damaged due to the etching. However, aggregated-particles of the iron deposition at submicron-scale were observed clearly as shown in Fig. 6. Iron and oxide were

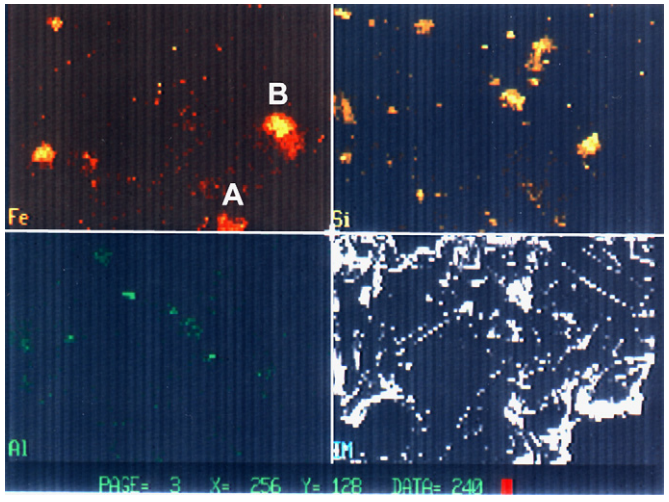

Fig. 2. Distributions of iron (upper left), silicon (upper right), aluminum (lower left), and a corresponding tissue image (lower right, the area corresponds to the solid frame in Fig. 1).

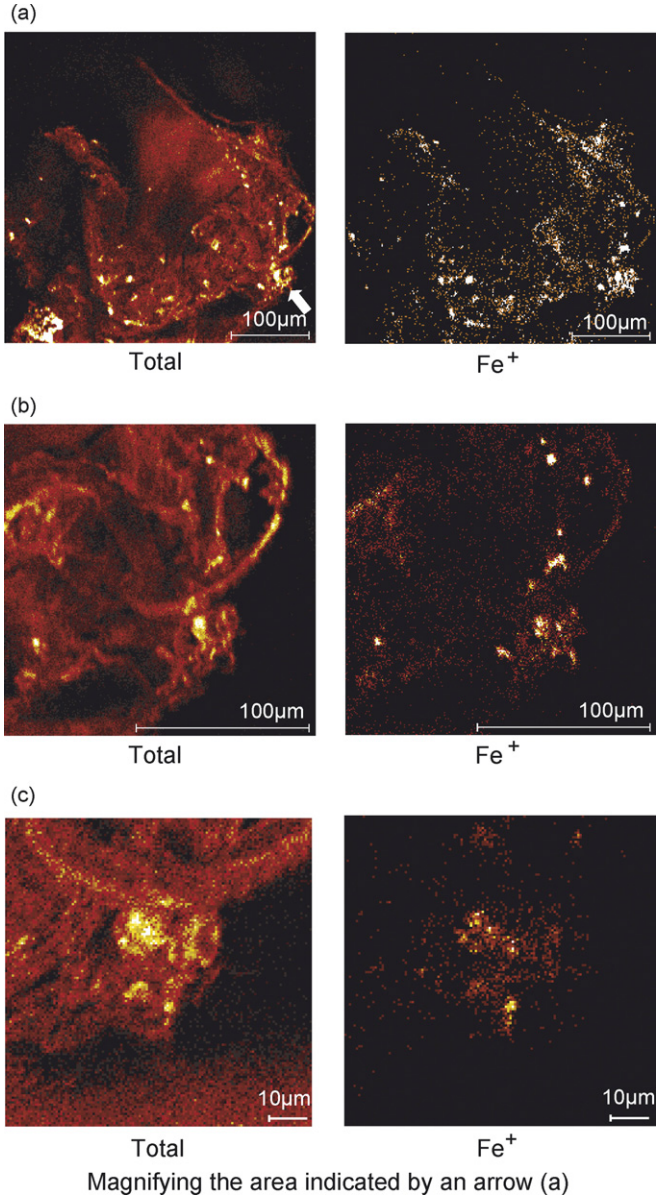

Fig. 3. Secondary ion images of the chum salmon head tissue. The TOF-SIMS image was obtained as a mirror image of SEM image of Fig. 1 (the area shown by the dotted frame). The area indicated by an arrow (a) corresponds to position A in Fig. 2. The fields of view in Fig. 3c is 80 μm × 80 μm with an image resolution of 256 × 256 pixels, the size bar = 10 μm.