Characterization of Microtubule Material Consisting of Iron Oxide Nanoparticles Produced by Bacteria

R. SAKUMA^{a)}, H. HASHIMOTO^{a)}, Y. KUSANO^{b)}, Y. IKEDA^{c)}, M. NAKANISHI^{a)}, T. FUJII^{a)}, M. TAKANO^{d)},
and J. TAKADA^{a,e)}

a) Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

b) Department of Applied Arts and Design, Kurashiki University of Science and the Arts, Kurashiki, Okayama 712-8505, Japan

c) Research Institute for Production Development, Sakyo-ku, Kyoto, 606-0805, Japan

d) Institute for Integrated Cell-Material Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8501 Japan

e) JST CREST, Okayama 700-8530, Japan

Abstract

Iron oxide microtubules produced by a species of iron-oxidizing bacteria, *Leptothrix ochracea*, have been expected to be used as unique multifunctional materials such as catalysts scaffolds, pigments, and magnetic materials. However, their structural characteristics and formation mechanism are still ambiguous. The detailed features of the microtubules were characterized through electron microscopies, X-ray diffractometry, and energy dispersive X-ray spectroscopy. We found that the microtubules have hierarchical microstructures with aggregates of amorphous iron oxide nanoparticles. Statistical analysis of distributions of the tubule diameters and the wall thicknesses suggested that the tubule layer could grow from outside to inside.

Key Words: Iron-oxidizing bacteria, Microtubules, Iron oxide nanoparticles, Amorphous

I. INTRODUCTION

There are various kinds of iron oxides produced by iron-oxidizing bacteria living in natural aquatic environments such as side ditches, irrigation channels, and even in deep-ocean hydrothermal vents [1]. Iron oxides with various shapes in micrometer scale are produced by different bacterial species and are formed everywhere as other precipitates [2–5]. They obtain energy by oxidizing aquatic Fe^{2+} into Fe^{3+} and precipitate extracellular iron oxides. The conventional studies on iron-oxidizing bacteria and their precipitates have been mainly conducted from microbiological and geochemical perspectives [6–8]. In contrast, we have investigated here them on the basis of solid-state chemistry and materials science viewpoints. We refer to such iron oxide precipitates as “biogenous iron oxides (BIOXs)”. The target BIOX in this study is the *L*-BIOX with a microtubule form produced by well-known iron-oxidizing bacteria, *Leptothrix ochracea* [9–11]. According to our recent studies, large potentials of *L*-BIOX for multifunctional materials such as catalysts scaffolds, pigments, and magnetic materials have been suggested [12–15]. However, their structural characteristics and formation mechanisms are still unclear. Here we studied the structural characteristics of *L*-BIOX in microscopic scale and proposed their formation mechanisms that are vitally important for future materials science research of this material.

II. EXPERIMENTS

Iron oxide precipitates containing *L*-BIOX were collected from groundwater purification tank at Joyo in Kyoto, Japan. The precipitates were washed several times with distilled water to remove contaminating impurities from groundwater and dried in vacuo. Morphology, chemical composition, and crystallographic structure of the samples were evaluated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), energy dispersive X-ray spectrometer (EDX), and X-ray diffractometry (XRD), respectively. The inner and outer diameters of 186 *L*-BIOX tubules were measured from the cross-sectional SEM images and statistical analysis of the distributions of their diameters and tubule layer thicknesses was performed.

III. RESULTS AND DISCUSSION

A typical SEM image of *L*-BIOX microtubule is shown in Fig. 1a. The tubule with $\sim 1\ \mu\text{m}$ -diameter was composed of two distinctive morphological structures with several tens of nanometers: (i) globular structures of inner layer and (ii) fibrillar structures of outer layer. The *L*-BIOX microtubules have a double-layered structure. Fig. 1b shows a typical

Fig. 1 (a) SEM and (b) TEM micrographs of *L*-BIOX. A red circle in (b) indicates primary particle.

high-power TEM image of an edge of *L*-BIOX microtubule. Both inner and outer layers having the globular and fibrillar structures, respectively, were primarily composed of extremely fine particles with several nanometers. Although it is very difficult to synthesize such complicated structures by artificial chemical processes, bacteria easily create such elaborate structures at eco-friendly conditions: ambient temperature, atmospheric pressure and neutral pH. According to EDX analysis the chemical composition of *L*-BIOX was roughly determined to be Fe:Si:P = 73:22:5 with the exception of C, O, and H. To clarify crystallographic structure of *L*-BIOX, XRD and high resolution TEM were applied. Two broad peaks were observed in XRD (Fig. 2a). This result showed a low crystalline feature of *L*-BIOX. No lattice fringe was observed in TEM images. A halo pattern in the two dimensional Fourier transformation image apparently proved the amorphous state of *L*-BIOX (Fig. 2b).

Fig. 2 (a) Typical XRD pattern and (b) HRTEM image of *L*-BIOX. Two dimensional Fourier transmission image of black square area is shown in the inset.

In order to clarify the formation mechanism of *L*-BIOX, especially the growth direction of the tubule layer, we performed statistical analysis of distributions of tubular diameters and layer thicknesses of various *L*-BIOX tubules. Fig. 3a shows the distribution of inner and outer diameters, and wall thicknesses. The average values of the inner and outer diameters were found to be 1.08 and 1.35 μm , respectively. Inner and outer diameters showed a normal distribution, while the thickness showed a log-normal distribution. These results suggest that the distribution of thickness could be related to the growth direction of tubule layers. Each diameter was plotted as a function of the layer thickness as shown in Fig. 3b. Although the outer diameter was nearly constant for the thickness, the inner diameter showed a negative correlation against it. Therefore, we concluded that the growth direction of tubule layers could be from outside to inside. This observation strongly supports our previous discussion [10]: the outer fibrillar structure may be initially constructed on bacterial secretions, whereas the inner globular structure may be subsequently constructed by abiotic Fe oxidation.

Fig. 3 (a) Distribution of inner (■) and outer diameters (▲), and tubular thickness (×) of 186 *L*-BIOX samples. (b) Inner (○) and outer diameters (□) are plotted as a function of the tubular thickness. Red and blue lines indicated the fitted lines for inner and outer diameters, respectively.

IV. CONCLUSION

L-BIOX tubule has a double-layered structure, where its inner and outer layers are composed of globular and fibrillar structures, respectively. The chemical composition of *L*-BIOX was roughly estimated to Fe: Si: P = 73: 22: 5. The crystallographic structure was amorphous. Statistical analysis of distributions of tubule diameters and layer thicknesses suggested that the growth direction of tubule layer could be from outside to inside.

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