Chemical modification of biogenous iron oxide to create an excellent enzyme scaffold†‡

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The biogenous iron oxide (BIO) from *Leptothrix ochracea* was transformed to an organic–inorganic hybrid support to prepare an excellent immobilized enzyme showing high catalytic performance.

Living things create fascinating materials with unique morphologies and nanostructures that cannot be synthesized artificially.[1] In particular, the extracellular materials produced by the iron-oxidizing bacteria, *Leptothrix ochracea*,[2,3] have attractive characteristics:[3] (i) these materials are hollow microtubes having an inner diameter of approximately 1 μm and various lengths of up to 200 μm; (ii) each sheath, with an average thickness of 0.15 μm, is composed of poorly crystalline nanoparticles of 10–40 nm in diameter, each of which is made up of finer particles; (iii) the elemental composition determined by energy dispersive X-ray (EDX) analysis is Fe:Si:P = 80:15:5. We refer to this microbial ceramic herein as "biogenous iron oxide (BIO)". A scanning electron microscopy (SEM) image of BIO is shown in Fig. 1.

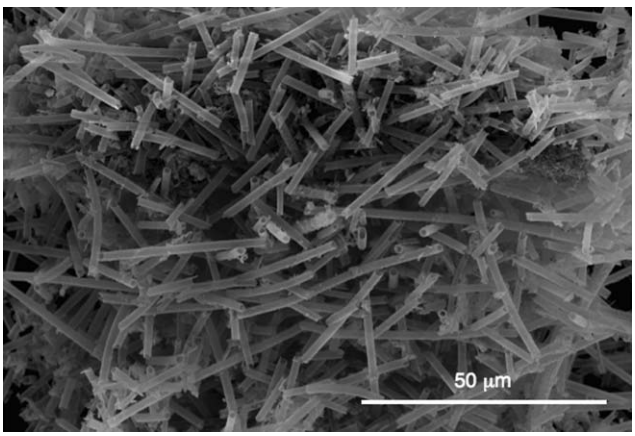
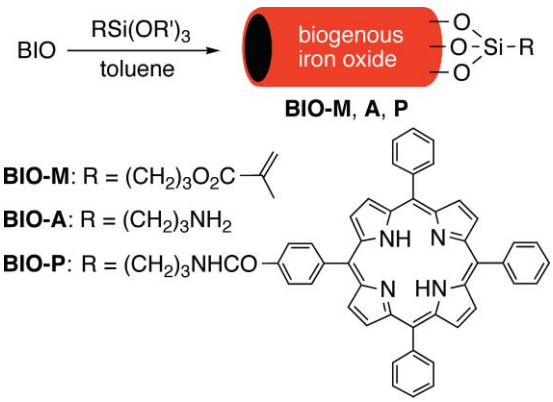


Fig. 1 SEM image of the unmodified BIO.

Although the biological and morphological aspects of BIO have been investigated,[2,3] no studies have been reported that explore the application of BIO to new functional materials. Because materials with a specific structure, size, shape, and chemical composition are the key to the creation of innovative functions, BIO is a promising candidate for advanced materials. Although large amounts of BIO sediment, which cause problems of pipe clogging and rusty water, have been considered to be waste, BIO is an environmentally benign, sustainable, and unused natural resource consisting of ubiquitous elements (*i.e.*, Fe, Si, and P). In addition, the laboratory synthesis of such a specific structure is currently impossible.[4–8] From these viewpoints, the functionalization of BIO is an important subject. Here we report the chemical modification of BIO, the immobilization of an enzyme on the modified BIO, and the resulting material's use as a highly active biocatalyst.

We employed silane coupling agents for the chemical modification of BIO. BIO, purified from sediment obtained at a water purification plant in Joyo City in Kyoto, Japan, was subjected to chemical modification with organotrialkoxysilane at 100 °C in dry toluene. BIOs modified with 3-methacryloxypropyltrimethoxysilane and with 3-aminopropyltriethoxysilane are designated herein as **BIO-M** and **BIO-A**, respectively (Scheme 1). Because the solid-state NMR (^{13}C CP/MAS NMR and ^{29}Si CP/MAS NMR) spectra of these samples exhibited no signals because of the paramagnetic Fe^{3+} in BIO, we used other methods to confirm the chemical modification of BIO. Elemental analysis indicated that the coverage (w/w) of the organic groups on BIO was 14.7% (0.94 mmol/g) for **BIO-M** and 15.0% (1.74 mmol/g) for **BIO-A**. The FT-IR spectrum of **BIO-M** exhibited a strong absorption band at 1717 cm^{-1}, which can be assigned to the C=O stretching vibration. These results strongly suggest that BIO has an OH group that can be grafted successfully with the organosilane moiety. Chemical modification of BIO was also confirmed by further derivatization with 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin. This compound was linked to the



Scheme 1 Chemical modification of BIO.

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amino group on the surface of **BIO-A** to yield **BIO-P** (Scheme 1), which was characterized by UV–vis spectroscopy and fluorescence microscopy (Fig. 2). The Soret band and the four Q bands of the porphyrin chromophore were observed around 400–450 and 500–650 nm, respectively, in the UV–vis spectrum of **BIO-P** (Fig. 2a). Red fluorescence, which is characteristic of the porphyrin chromophore, was emitted uniformly from **BIO-P** upon excitation at 530–550 nm (Fig. 2c), thus indicating the uniform distribution of the organic group on the BIO.

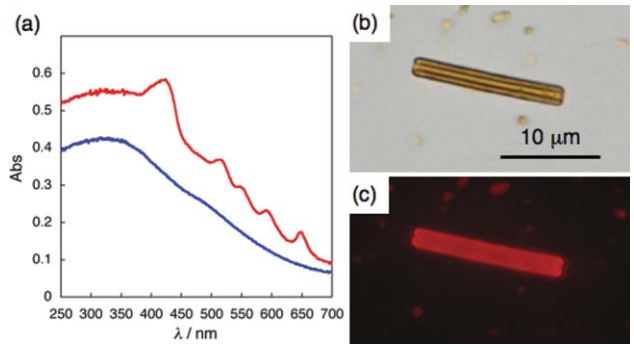


Fig. 2 Chemical modification of BIO with the porphyrin chromophore. (a) UV–vis absorption spectra of the unmodified BIO (blue line) and **BIO-P** (red line) (matrix: $BaSO_4$). (b) Optical microscope image of **BIO-P**. (c) Fluorescence microscope image of **BIO-P** with excitation at 530–550 nm.

We investigated the surface of **BIO-M** by means of electron microscopy. SEM indicated that **BIO-M** had the same tubular shape as the unmodified BIO (Fig. 3). Interestingly, the inherent morphology of BIO was retained even after the chemical modification, except the length of the sheath of the modified BIO was shorter than that of the unmodified BIO. Transmission electron microscopy (TEM) indicated that the primary and secondary particles forming the sheath of **BIO-M** were *ca.* 3 and 30 nm in diameter, respectively (Fig. 3); both of these sizes were close to those observed in the unmodified BIO. Both SEM and TEM images suggested that the sheath of **BIO-M** was porous. These characteristics prompted us to explore the function of the chemically modified BIOs.

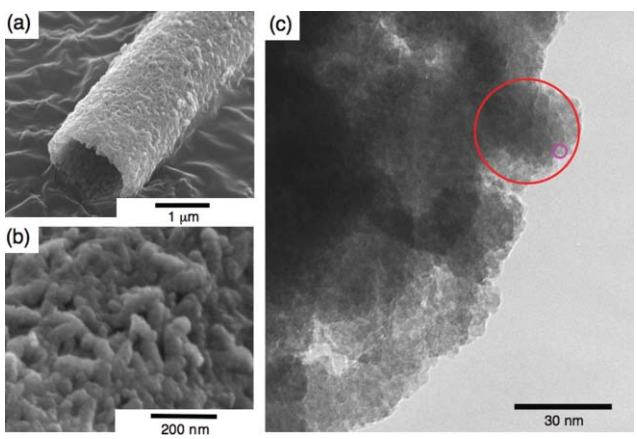
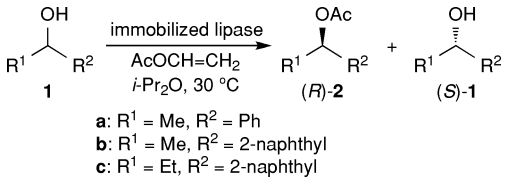


Fig. 3 (a), (b) SEM images of **BIO-M**. (c) TEM image of **BIO-M**. The primary and secondary particles are indicated by pink and red circles, respectively.

We expected that the amorphous and porous nanoparticles of BIO might create a surface suitable for immobilization of catalysts.[9] To substantiate this idea, we selected lipase as a catalyst and prepared immobilized enzymes.[10,11] Chemically modified BIO was added to a solution of *Burkholderia cepacia* lipase (BCL) in phosphate buffer (10 mM, pH 7.0), and the mixture was shaken at room temperature overnight. The mixture was filtered and dried in vacuo. The immobilized lipase preparations were used in the kinetic resolution of secondary alcohols **1a–c** in *i*-Pr_2O (Scheme 2). The enantiomeric purities (% ee) of **1** and **2** were determined by means of chiral GC or HPLC, and enantioselectivity was evaluated by the *E* value.[12] The results are summarized in Table 1.



Scheme 2 Kinetic resolution of secondary alcohols with BCL immobilized on chemically modified BIOs.

The amount of enzyme immobilized on the support reflects the maximal immobilization capacity of the support because we added the support to an excess amount of enzyme to prepare the immobilized enzyme. BCL was immobilized on **BIO-M** (4.6% (w/w)) more efficiently than on **BIO-A** (4.3% (w/w)), **BIO-P** (3.4% (w/w)), and the unmodified BIO (1.2% (w/w)). On the basis of these results, we concluded that a hydrogen bonding site with appropriate hydrophobicity on the surface of BIO is important for immobilization of the enzyme as we have previously proposed for Toyonite,[11b] which is a porous ceramic material prepared from kaolin minerals by a hydrothermal treatment under acidic conditions and is one of the best supports for BCL.[11a,b]

More importantly, BCL immobilized on **BIO-M** exhibited excellent catalytic performance (Table 1). The total turnover number (TTN), defined as the number of substrate molecules converted by one enzyme molecule, for BCL immobilized on **BIO-M** was calculated to be 33,000 for **1a** (entry 1), which is much larger than that reported for BCL immobilized on Toyonite-200M (TTN = 7,800 in 4.5 h for **1a**) or that reported for BCL immobilized on Celite toward other secondary alcohols

Table 1 Kinetic resolution of secondary alcohols with BCL immobilized on chemically modified BIOs[a]

Entry	Support	1	Time (h)	c^b	E^c	TTN[d]
1	**BIO-M**	**1a**	1	46	259	33, 000
2	**BIO-A**	**1a**	7	9	220	6, 500
3	**BIO-P**	**1a**	1.5	43	444	31, 000
4	**BIO-M**	**1b**	1	41	> 413	29, 000
5	**BIO-M**	**1c**	12	44	154	32, 000

[a] Conditions: immobilized lipase (10.0 mg of 4.6% (w/w) BCL/**BIO-M**, 10.6 mg of 4.3% (w/w) BCL/**BIO-A**, or 13.4 mg of 3.4% (w/w) BCL/**BIO-P**), **1** (1.00 mmol), vinyl acetate (2.00 mmol), molecular sieves 3Å (three pieces), dry *i*-Pr_2O (5 mL), 30 °C. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [d] Total turnover number of enzyme.

Table 2 Reusability of the immobilized lipase in the kinetic resolution[a]

Run	% ee[b] (*R*)-**2a**	% ee[b] (*S*)-**1a**	*c*[c]	*E*[d]	TTN
1st	98	93	49	340	35, 000
2nd	99	87	47	570	34, 000
3rd	99	84	46	532	33, 000
4th	99	79	44	483	32, 000
5th	99	71	42	425	30, 000

[a] Conditions: immobilized lipase (10.0 mg of 4.6% (w/w) BCL/**BIO-M**), **1a** (1.00 mmol), vinyl acetate (2.00 mmol), molecular sieves 3Å (three pieces), dry *i*-Pr_2O (5 mL), 1 h, 30 °C. [b] Determined by chiral GC. [c] See footnote *b* of Table 1. [d] See footnote *c* of Table 1.

(TTN = 5,000 in 3 h).[13] Notably, the TTN value reached 33,000 in just 1 h (entry 1), clearly indicating that the catalytic efficiency of BCL was improved by immobilization on **BIO-M**. Moreover, BCL immobilized on **BIO-M** could be recycled at least five times, during which enzymatic activity and enantioselectivity for **1a** remained high although the TTN value decreased gradually (Table 2).

BCL immobilized on **BIO-M** exhibited superior catalysis compared with BCL immobilized on **BIO-A** and **BIO-P** (Table 1, entries 1–3). Because it is well-known that BCL, which has a large lid capable of occluding the active site, can take both open and closed conformations,[14] we suppose that BCL takes different conformations on these supports. BCL is likely to mainly take a closed conformation at the hydrophilic surface of **BIO-A**, while BCL must take an open conformation at the hydrophobic surface of **BIO-M** and **BIO-P**. This difference in the conformation of BCL can rationalize well the difference in catalytic activity (Table 1, entries 1–3). In addition, we speculate that the specific shape, surface, and nanostructure of **BIO-M** enabled BCL to be immobilized with high accessibility to the substrate. We confirmed that the best enzyme preparation (BCL/**BIO-M**) was also effective for the kinetic resolution of **1b** and **1c** (Table 1, entries 4 and 5). The alcohol **1c**, having an ethyl group as a substituent, showed lower reactivity and enantioselectivity than **1b**, having a methyl group as a substituent, which is reasonable from the mechanistic viewpoint.[15] Even **1c** could be acylated with a TTN value of 32,000 in 12 h.

In summary, we have demonstrated that biogenous iron oxide (BIO) microtubes from the iron-oxidizing bacteria *Leptothrix ochracea* can be transformed into an organic–inorganic hybrid support suitable for enzyme immobilization. The immobilized lipase showed remarkably high catalytic activity. Further work is currently in progress to apply this new type of organic–inorganic hybrid support to the immobilization of other enzymes and catalysts.

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