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Enzymatic silicone oligomerization catalyzed by a lipid-coated lipase

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A lipid (1)-coated lipase can catalyze the oligomerization of diethoxydimethylsilane (DEDMS) in isooctane containing 2wt% water, where the polymerization occurs at the OH group of the coating lipid (1) in the enzyme cavity.

Industrial and geochemical production of silica or other polysiloxanes require extremes of pH, temperature, or both. In contrast, many living marine organisms synthesize large quantities of silica, biosilicification, under mild physiological conditions.[1,2] Recently, silica proteins that catalyze biochemical polysiloxane production have been investigated.[3–7] Morse and coworkers reported that an enzyme, silicatein α, has a serine protease-like active site and can produce silica or organically modified silica from tetraethoxysilane at neutral pH and moderate temperature.[5–7] This suggested to us that lipase, a stable serine esterase, would be a good candidate as an enzymatic catalyst for polysiloxane synthesis, as well as esterification or ring-opening polymerization of lactone using reverse hydrolysis reactions in organic media.[8–11]

We have reported a lipid-coated lipase system, in which hydrophilic head groups of the lipids interact with the enzyme surface and the two long lipophilic alkyl chains extend away from its surface to solubilize the enzyme in hydrophobic organic solvents.[12] Lipid-coated lipase, phospholipase, and catalytic antibodies[12] showed high catalytic activities such as enantioselective esterifications in dry isooctane or even in supercritical fluids.[13]

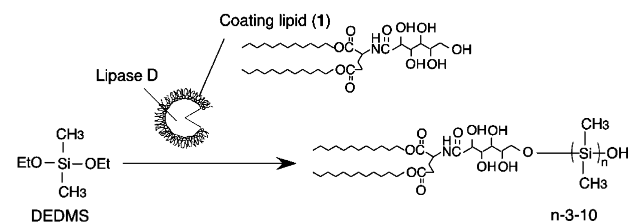
In this communication, we report that a lipid (**1**)-coated lipase can catalyze the oligomerization of diethoxydimethylsilane (DEDMS) in isooctane in the presence of 2 wt% of water. The polymerization is proposed to occur from the OH head group of the coating lipid (**1**). This is the first report of the lipase-catalyzed polysiloxane synthesis in organic solvents (Scheme 1).

A lipid-coated lipase D (from *Rhizopus delemar*) was prepared by mixing aqueous solutions of the enzyme and the lipid which contains many hydrophilic OH groups (**1**) in the same way as reported previously.[12,13] It was confirmed from elemental analysis, UV absorption, and gel chromatography in organic solvents that one enzyme is covered with about 300 ± 50 lipid molecules in a monolayer and that the protein content in the complex is 8 ± 2 wt%. The lipid-coated enzyme was soluble only in organic solvents such as isooctane (*ca.* 1 mg/10 mL), but not in aqueous solution.[12] Gel chromatography indicates that a small amount of free lipids (*ca.* 10% of the total lipids) exist in solution.

Fig. 1 shows typical time courses of polymerization of DEDMS (1 M) in isooctane (1 mL) containing 2 wt% of water catalyzed by a lipid (**1**)-coated lipase D (0.5 mg of protein/mL) at 40 °C. The reaction was followed by gel-permeation chromatography (TOSOH TSKgel G2000H, SC-8020). A new higher molecular weight peak than the free lipid (**1**) was apparent after 30 min of the start of the reaction start and the area of the new peak and of the lipid (**1**) increased and decreased with time, respectively (see the insert). The conversion of the lipids (**1**) reached 80% after 20 h in the presence of an excess amount of the DEDMS monomer. The average molecular weight (M_w) of the product, however, was constant at 1500 and the molecular weight distribution (M_w/M_n) was observed to be very narrow at 1.06. This indicates that a polymer with constant molecular weight was produced and that its yield increased with time.

Fig. 2 shows a MALDI-TOF MS spectrum of the isolated product (Shimadzu AX1MA CFR). Several peaks starting from the lipid (**1**, M_w = 664) and having a repeating unit of dimethylsiloxane (M_w = 74) indicate that the oligomerization is initiated from the hydrophilic OH head group of the lipid (**1**) and the degree of polymerization is 3 to 10 mer (average 5) and the total molecular weight is in the range of 800–1500. The chemical structure of the product was confirmed by ^{1}H- and ^{13}C-NMR spectra and elemental analysis. After the reaction proceeded, the isooctane solution became slightly turbid because of the precipitation of enzymes due to the silication of the coating lipids.

When the native lipase D was suspended with the lipid (**1**) in isooctane with 2% of water, instead of the lipid (**1**)-coated lipase, the reaction rate and the yield were low. When the reaction was carried out in the aqueous buffer solution or the isooctane/water two-phase solutions by using the native lipase, the monomer was hydrolyzed and polymerization scarcely occurred. When the lipase was coated with other lipids (**2**–**4**) shown in Scheme 2, the polymerization yield was largely depressed to 20% in isooctane with 2% of water. In the absence of lipase or in the presence of the serine-protease inhibitor



Scheme 1

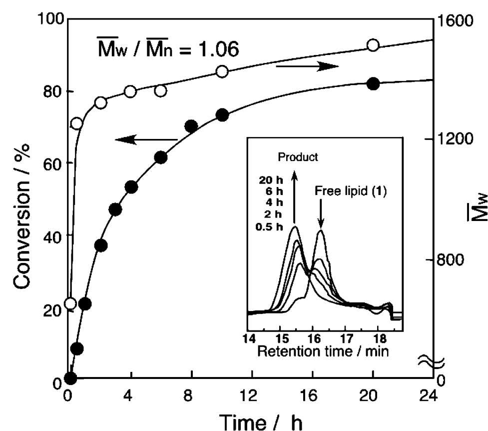


Fig. 1 Time courses of the conversion of the lipid (**1**) (●) and the average molecular weight (M_w) of the product (○) in enzymatic polymerization of diethoxydimethylsilane (DEDMS) analyzed by gel permeation chromatography (GPC). *Reaction conditions*: [DEDMS] = 1 M, [lipid-coated lipase D] = 0.5 mg of protein/mL, in isooctane (1 mL) with 2% H_2O, 40 °C. *GPC conditions*: eluent: tetrahydrofurane, detector: RI.

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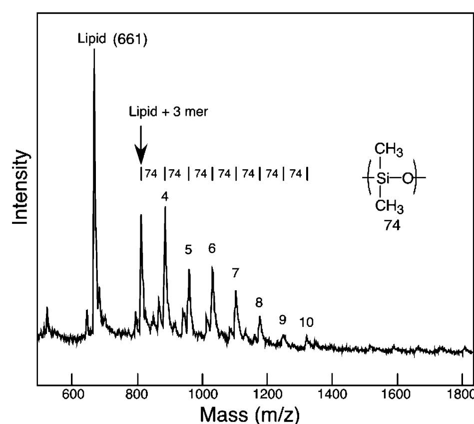
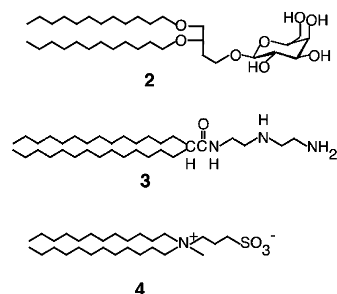


Fig. 2 MALDI-TOF MS of the isolated product of Fig. 1: matrix: 2,5-dihydroxybenzoic acid, instrument: Shimadzu AXIMA-CFR.

(phenylmethylsulfonyl fluoride) with the lipid (**1**)-coated lipase, polymerization scarcely occurred. When the reaction media was changed to the dry isooctane without 2% of water by using the lipid (**1**)-coated lipase, the reaction did not proceed. These results indicate that both the lipid (**1**)-coated lipase and monomers should be solubilized in the organic phase, and the free lipid (**1**) having OH groups and small amount of water are required to start the polymerization.

As shown in Fig. 3, when the DEDMS monomer concentration was changed (0.1–1.0 M) in the presence of the lipid (**1**)-coated lipase (0.5 mg of protein/mL) in 2% of water-containing isooctane (1 mL) at 40 °C, the initial rate of oligomerization showed Michaelis–Menten saturation behavior and the apparent K_m value could be obtained as 0.28 M. This shows that both substrates are incorporated in the enzyme and the oligomerization may proceed in the cavity of lipase leading to the narrow M_w/M_n value and the relatively low M_w of the polymers.

The estimated reaction mechanism is shown in Fig. 4. The siloxane intermediate is produced by the attack of the Ser–OH group to the DEDMS monomer and the activated monomer reacts with the incorporated lipid (**1**) in the lipophilic active site to form the Ser–O–Si–O-lipid linkage. Then the Ser–O–Si linkage is hydrolyzed with water to regenerate the active Ser–OH and the siloxane lipid. Continuous reactions occur to produce oligomers (n = 3–10) having a narrow M_w/M_n value in the cavity. Silica synthase, silicatein α, is found to have both Ser–OH and His-Im groups in the active site like serine protease, and to form Ser–O–Si linkages upon reaction with silicon alkoxides, followed by the hydrolysis to form active silanols.[7]



Scheme 2

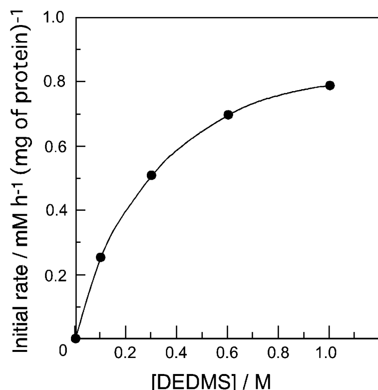


Fig. 3 Michaelis–Menten behavior when the DEDMS monomer concentration was changed. *Reaction conditions*: [DEDMS] = 0.1–1.0 M, [lipid-coated lipase D] = 0.5 mg of protein/mL, in isooctane (1 mL) with 2% H_2O, 40 °C.

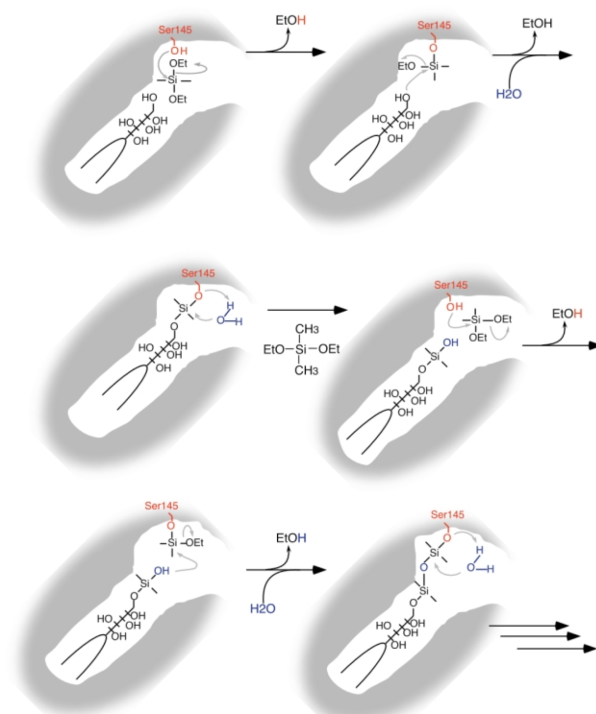


Fig. 4 Proposed mechanism of enzymatic oligomerization of diethoxydimethylsilane (DEDMS) catalyzed by a lipid (**1**)-coated lipase in organic solvents with a small amount of water.

In conclusion, this is the first report of enzymatic oligomerization of siloxane catalyzed by lipase in organic solvents. We believe that the lipid-coated lipase will become a good catalyst for the preparation of silicone-based polymers and materials under mild conditions.

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