SHORT COMMUNICATIONS

**Attaching of Poly(acrylic acid) to Inorganic Surface
and Its Application to Enzyme Immobilization**

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Covalent attaching of polymer chains is a useful technique of modifying inorganic materials to give new hybridized composites which have surface properties derived from the polymer chains attached.^{1–5} One can subject these composites to further organic modification by applying functional groups of attached polymer chains to incorporation of a variety of organic molecules. Telomers, as well as polymers, can be used for modification of inorganic solid surface as demonstrated by recent investigations,^{6,7} in which liquid chromatographic properties of the silica gel attached with lipid-analogous telomers were examined.

For the purpose of immobilizing enzymes on inorganic surface, the polymer-attaching technique is useful because a large number of functional groups of attached polymer chains can be applied to covalent immobilization. Polymerization of a vinyl monomer has been attempted in a redox system consisting of ceric ion and reducing groups introduced onto the surfaces of inorganic solid particles by the silane coupling, and it has been demonstrated that polymer chains can be attached to the surfaces.³ In this communication are reported the preliminary results of attaching of poly(acrylic acid) chains to the surfaces of magne-

tite and silica gel particles by the redox polymerization and immobilization of glucose oxidase (GOD) on the surfaces by the condensation reaction with carboxyl groups of the polymer chains attached.

EXPERIMENTAL

Magnetite and silica gel used were MAT-305 obtained from Toda Kogyo Corp. and Wakogel Q-63 from Wako Pure Chemical Industries, Ltd., respectively. The magnetite has an average particle size of 0.23 μm and a BET surface area of 7.2 $\text{m}^2 \text{g}^{-1}$. The silica gel has a particle size below 325 mesh and a BET surface area of 847 $\text{m}^2 \text{g}^{-1}$. GOD used (EC 1.1.3.4, grade II) was supplied by Toyobo Co., Ltd., which had an activity of 164 units mg^{-1} . Acrylic acid obtained from Wako Pure Chemical Industries, Ltd. was purified by distillation under reduced pressure prior to use. 3-Mercaptopropyltrimethoxysilane (MPS) from Kanto Chemical Co., Inc. was used without further purification. Other chemicals were guaranteed-reagent grade or analytical grade commercial materials and used without further purification.

Attaching of poly(acrylic acid) was carried out by following Scheme 1. The magnetite and the silica gel were treated with MPS to in-

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Scheme 1.

introduce mercapto groups onto their surfaces as described in a previous publication.³ Attaching of poly(acrylic acid) by the redox polymerization was carried out as follows: Into a flask, 0.5 g of the magnetite or the silica gel treated with MPS, 3.0 g of acrylic acid and 10.0 ml of distilled water were charged. After deaeration of the mixture, a solution of 0.2 mmol of ceric ammonium nitrate in 3.0 ml of 1 N nitric acid was added. The polymerization was conducted at 30°C with stirring under

nitrogen. After a given time, the polymerization was stopped with hydroquinone. The reaction mixture was diluted by addition of distilled water and centrifuged at 10^5 m s^{-2} until solid particles were precipitated completely. The solid particles precipitated were dispersed in distilled water and centrifuged once more. This procedure was repeated several times. The supernatant, on the other hand, was concentrated and solidified under reduced pressure.

The amount of attached poly(acrylic acid) was determined from the weight increase of the solid particles and confirmed by elementary analysis. The amount of free (unattached) polymer was estimated from the weight of the solidified supernatant by subtracting the weights of the ceric salt and hydroquinone added. The conversion was determined by the following equation:

Conversion (%)

$$= \frac{\text{Attached polymer (g)} + \text{Free polymer (g)}}{\text{Acrylic acid used (g)}} \times 100$$

IR spectra of the solids attached with poly(acrylic acid) and the untreated solids were recorded on a Shimadzu FTIR-8100M spectrometer.

The GOD immobilization was carried out in 0.1 M phosphate buffer solution (pH 6.5) at room temperature by using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate as a condensing agent according to the method of Mosbach.⁸ The amount of the immobilized GOD was estimated with the Folin phenol reagent after alkaline copper treatment according to the directions of Lowry.⁹ The activity of the GOD was measured by the colorimetric method based on the procedure of Trinder.¹⁰

Figure 1. Polymerization of acrylic acid in the redox system consisting of ceric ion and mercapto groups introduced onto the surface of magnetite, ○; silica gel, □.

RESULTS AND DISCUSSION

It was determined by elemental analysis that the amounts of mercapto groups introduced onto the surfaces of magnetite and silica gel particles were 0.12 and 0.91 mmol g⁻¹, respectively. Figure 1 shows the results of polymerization of acrylic acid in the redox system consisting of ceric ion and the mercapto groups on the surfaces of the magnetite and the silica gel. A considerable rate of the polymerization was observed in each of the systems containing the magnetite and the silica gel, where the initial molar ratios of the mercapto groups to the monomer were 0.14:100 and 0.99:100, respectively.

IR spectra of the magnetite and the silica gel attached with poly(acrylic acid) by the redox polymerization are compared with those of the

Figure 2. IR spectra of untreated magnetite, Fe₃O₄; the magnetite attached with poly(acrylic acid), Fe₃O₄-pAA; untreated silica gel, SiO₂; the silica gel attached with the polymer, SiO₂-pAA.

Table I. Amount and activity of immobilized GOD

Solid ^a	Polymer attached ^b	GOD immobilized	Apparent activity	Specific activity
	mg g-solid ⁻¹	mg g-solid ⁻¹	U g-product ⁻¹	U mg-GOD immobilized ⁻¹
Magnetite	56	2.8	226	81
Silica gel	40	2.6	103	40

^a Mercapto groups were introduced by the treatment with MPS. ^b The polymerization of acrylic acid was carried out for 60 min in the presence of the magnetite and for 90 min in the presence of the silica gel. The values were determined from the weight increase of the solids, which were in good agreement with the following results obtained from elemental analysis: 59 mg g-magnetite⁻¹; 40 mg g-silica gel⁻¹.

untreated solids in Figure 2. In the IR spectra of the solids attached with the polymer, an absorption in the vicinity of 1730 cm⁻¹, corresponding to stretching of C=O bond of carboxyl groups, was observed. Although direct evaluation of molecular weight of the attached polymer has not been accomplished yet, GPC analysis using a TSK-gel-GMPWXL column showed that the weight-average molecular weight (M_w) and the number-average molecular weight (M_n) of the unattached polymer were 1.4×10^5 and 2.4×10^4 , respectively ($M_w/M_n = 5.8$).

The amount and the activity of GOD immobilized on each surface of magnetite and silica gel particles, together with the amount of poly(acrylic acid) attached to each surface of the solids, are given in Table I. The polymerization of acrylic acid was not initiated in the presence of untreated magnetite or silica gel, and no polymer was attached to the surface of each solid. In the presence of the solids treated with MPS, however, poly(acrylic acid) was attached to the surface of each solid, and GOD was immobilized on the surface by succeeding condensation reaction of carboxyl groups of the polymer with the enzyme. The immobilized enzyme had a specific activity of 40–80 units mg⁻¹ (25–50% of the free enzyme's).

It has been demonstrated in this communication that a polymer containing carboxyl groups can be attached to the surfaces of inorganic materials, and the functional groups can be

applied to immobilization of an enzyme on the surfaces. The immobilization on the surfaces of such magnetizable materials as magnetite enables one to carry out transport of the enzyme into a reactor or recovery from reaction mixture magnetically. On the other hand, if the immobilizing technique is applied to introduction of an antibody onto the surface of a piezoelectric crystal, detection of the antigen will be performed by the piezoelectric immunosensing system.^{11,12} These applications are under investigation.

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