Adsorption of Amphiphilic Block Copolymer-Catalase Aggregate at Silica-Gel

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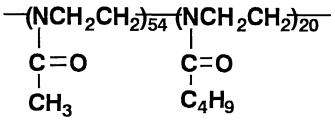
The amount of adsorbed catalase at silica-gel in aqueous solution at pH 7.0 was significantly increased in a presence of the polymer aggregate which was prepared from an amphiphilic block copolymer (**1**) consisting of poly[(N-pentanoylimino)ethylene] and poly[(*N*-acethylimino)ethylene].

There has been tremendous interest in the adsorption of proteins from aqueous solution on solid surfaces due to their practical applications.[1-3] For preparation of bioreactor, immobilization of enzymes by physical interaction is considered to be the easiest way. However, the immobilization essentially involves some disadvantages, such as limited adsorbed amount of enzymes and weak interactions between enzymes and adsorbents. In the past decade polymer adsorption phenomena have been studied intensively to gain understanding of interactions between polymer chains and adsorbents. Much of the early work concentrated on homopolymers, but the past several years have seen an increasing use of block copolymers.[4] Their main utility is a steric stabilizer of a dispersion. Due to their amphiphilic nature, block copolymers often associate and form micelle-like aggregates.[5] These polymer aggregates could work as hosts not only for various small organic molecules but for large biopolymers such as enzymes. We have reported that an amphiphilic block copolymer containing poly[(*N*-acetylimino)ethylene] and poly[(*N*-pentanoylimino)ethylene] forms micelle-like aggregate in water and it has a capability of incorporating enzymes such as horseradish peroxidase (HRP) or Lipases.[6] We now report a first observation of enzyme adsorbed on silica surface with the polymer aggregate in a phosphate buffer solution (pH 7.0), whereas the amount of adsorbed enzyme is extremely low without the polymer aggregate under the same condition.



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An amphiphilic block copolymer (**1**) containing poly[(N-pentanoylimino)ethylene] (hydrophobic chain) and poly[(*N*-acethylimino)ethylene] (hydrophilic chain) was prepared as previously reported.[6b] The number molecular weight of **1** was estimated to be 7140 and the unit ratio of **1** was 54:20 by ^{1}H NMR. Silica-gel (Wakogel C-300, with a diameter range of 45-75 μm and a specific surface area of 450 m^2/g) was obtained from Wako Pure Chemical. Bovin liver catalase was obtained from Sigma Chemical Co. and purified as follows. Catalase suspension was diluted with 0.05 M phosphate buffer (pH7.0) and a clear supernatant solution was obtained by a centrifugation at 3000 r.p.m. for 5 min. The amount of catalase was determined by the strong absorption peak of heme at 405 nm.[7]

First of all, we studied an adsorption phenomena of the block copolymer (**1**) from aqueous solution (0.05 M phosphate buffer, pH 7.0) to silica surface. Desired amount of **1** was added to the silica-gel suspension containing a phosphate buffer and incubated for 3 h at room temperature. Then the solid was separated by centrifugation and the adsorbed amount of **1** was determined by IR, in which the polymer content in silica-gel was evaluated from absorbance at 1640 cm^{-1} (C=O stretching band of **1**) and 1100 cm^{-1} (Si-O stretching band of silica-gel) by using a calibration curve obtained from mixtures of silica-gel with **1**. Fig. 1 shows a plot of the adsorbed amounts of **1** for silica-gel as a function of the initial concentration of **1**. As can be seen in Fig. 1, the catalase adsorption increases with an increase in the initial concentration and reaches a plateau value at 2 mg/mL of the initial concentration of **1**. The saturation value of the adsorbed amount is about 600 mg/g of silica-gel. The result confirms that a large amount of **1** adsorbed on silica surface. The main interaction of **1** and silica surface may be a hydrogen bonding between the -SiOH groups of the silica surface and the hydrogen acceptors of poly[(*N*-acylimino)ethylene].[2,8]

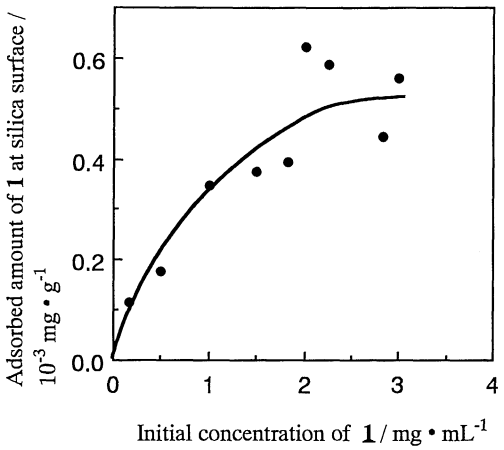

Figure 1. Adsorbed amount of **1** at silica surface as a function of the initial concentration of **1** in 0.05 M phosphate buffer (pH 7.0).

We have found that the polymer aggregate of **1** incorporates proteins such as horseradish peroxidase (HRP), lipases, glucose oxidase, or catalase.[4] Therefore we assumed that these proteins could be adsorbed on silica surface with the polymer aggregate of **1**. Here we used Bovine liver catalase as a model protein for this hypothesis. Catalase stock solution was diluted with 0.05 M phosphate buffer (pH 7.0) to make desired concentrations. A fixed amount of silica-gel (6 mg) and **1** (10 mg) was placed in a test tube and then mixed with 6 mL of the catalase solution. The sample was gently stirred at room temperature for 3 h. The adsorbed amounts of catalase were determined from difference in the concentrations between the dosage and the supernatant (Cp). A clear supernatant solution was obtained by a centrifugation at 4500 r.p.m. for 5 min. We also studied adsorption phenomena of

catalase at silica-gel in the absence of **1**. It is known that an affinity of a silica surface for catalase in aqueous solutions decreases almost three order of magnitude when pH in the solutions is raised over its isoelectric point (pI=5.5) due to their electrostatic repulsion.[1] Therefore, the adsorbed amount of catalase at pH 7.0 can be expected to be low. The adsorption isotherms of catalase with and without **1** are shown in Fig. 2. The both adsorption isotherms showed plateaus. The plateau values of the adsorption isotherms were significantly affected by the presence of **1**. In the absence of **1** in solution, the adsorbed amount of catalase was saturated below $1X10^{-5}$ mmol of heme/g of silica-gel which was corresponded to 0.6 mg(catalase)/g of silica-gel. In the presence of **1**, however, catalase was largely immobilized on silica surface. The maximum adsorbed amount of $7X10^{-5}$ mmol of heme/g of silica-gel was corresponded to 4.3 mg(catalase)/g of silica-gel. These observations lead to a conclusion that the adsorbed amount of catalase on silica surface with the polymer aggregate of **1** in a phosphate buffer solution (pH 7.0) is more than 7 times larger than that of in the absence of **1**.

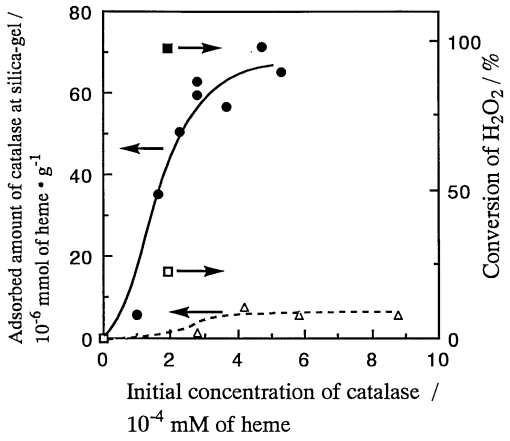

Figure 2. Adsorbed amount of catalase at silica surface as a function of the initial concentration of catalase in 0.05 M phosphate buffer (pH 7.0) with **1** (●) and without **1** (Δ). Conversion of H_2O_2 catalased by catalase immobilized silica-gel with **1** (■) and without **1** (□) after 2 min incubation in 0.05 M phosphte buffer (pH 7.0) containing 20 mM of H_2O_2.

The decomposition of H_2O_2 catalyzed by catalase-immobilized silica-gels which were prepared in the presence of **1** and in the absence of **1** under the same initial catalase concentration were measured in phosphate buffer solution (pH 7.0) containing 20 mM of H_2O_2. The consumption of H_2O_2 was calorimetrically determined.[9] We found that the catalase-immobilized silica-gel prepared with **1** completely decomposed H_2O_2 within 2 mins at 30 °C, whereas catalase-immobilized silica-gel prepared without **1** decomposed H_2O_2 only 22 % after 2 mins incubation at 30 °C (Fig. 2). This result means that catalytic activity of catalase was not interfered by the immobilization to a silica-gel with **1**. To clarify the activity and stability of this catalase-immobilized silica-gel, further detail study is now in progress.

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