Efficient enzyme encapsulation inside sol-gel silica sheets prepared by poly-L-lysine as a catalyst

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ABSTRACT
High catalytic activities of enzymes are necessary for the enzyme immobilizing technology for the development of better biocatalysts and biosensors. Basic polypeptide (poly-L-lysine)-templated precipitation of silica synthesized by sol-gel chemistry produced a composite material that allows high enzyme activity. This study investigates the structural properties of the composite material that allow retaining the glucose oxidase (GOx) activity. Scanning (SEM) and transmission (TEM) electron microscopies reveal that the composite has plate- or sheet-type morphologies composed of hexagonal structures that are 0.2–2 µm in diameter. After the encapsulation in a sol-gel silica matrix, encapsulated GOx retained high activity (over 85% of oxidation activity compared with that of free GOx). The relative activity of GOx-encapsulated in SiO$_2$@pLys remained approximately at 50–60% after eight cycles; in addition, the catalytic stability of encapsulated GOx improved under high temperature (60°C) and in several solvents (e.g., HCl, urea, and acetone). Moreover, D-glucose detection was performed in the linear range of 1–400 µM using SiO$_2$@pLys-GOx composites. The obtained results show that SiO$_2$@pLys-GOx composites can be used for enzyme encapsulation with high activity and stability and as biosensor materials with high sensitivity.

1. Introduction
Recently, the a sol-gel matrix (especially silica) has been immobilization of biomolecules via encapsulation inside studied [1–5]. Encapsulation restricts the biomolecules, such as proteins and antibodies, can prevent denaturation in harsh conditions related to organic solvent, temperature, and pH. Therefore, many biomolecules have been encapsulated inside sol-gel silica, and this research has led to important applications of these materials for biosensors and biocatalysts [6–11]. However, this method sometimes decreases the activity of encapsulated enzymes because the preparation of sol-gel silica gel needs an acid or a base as a catalyst. To avoid the reduction in the activity of the encapsulated enzymes, a method for preparing silica gel under mild conditions (e.g., ambient temperate and neutral pH) is needed [12,13].

The key point in the investigation of immobilization procedure of these biomolecules is in the use of biomineralization [14–19]. In this field, the inorganic materials that use biomineralization approaches have become an important area of the research [20–25]. One extensively study is the biomineralization of highly ordered biosilica from alkoxysilane such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) using biomolecules (e.g., peptides, proteins, and nucleic acids); this system offers precise control the nanostructure of the obtained inorganic material [26–30]. In addition, polyamines and polypeptides (like poly-L-lysine) can catalyze silicic acid deposition [31–35]. Interestingly, Tomczak et al. [36] determined that
poly-L-lysine with an α-helix conformation precipitated to form hexagonal silica platelets.

We previously described [37] that urease-catalyzed precipitation of silica synthesized by sol-gel chemistry produces a composite material, which allowed to achieve high urease activity. We also reported [38] the mesoporous silica (MPS) sheet (the thickness of < 100 nm) synthesized using a novel approach for the immobilization of enzymes. Enzyme immobilized on the MPS sheet exhibited a remarkably higher catalytic oxidation activity than free native enzyme. The above-mentioned results show that sheet morphology considerably influences the activity of immobilized enzyme.

Enzyme-based biosensors are used in an increasing number of clinical, environmental, and biochemical applications [39–45]. Redox enzymes, such as glucose oxidase (GOx), do not exchange electrons with simple metal electrodes [46–51]. Historically, electrical communication between enzyme and electrode has been achieved using diffusing mediators. The first mediator employed for flavin adenine dinucleotide (FAD)-enzyme electrodes was the natural substrate of flavin-linked oxidases, O_2 [52,53]. To produce the highly sensitive and selective glucose biosensors and other biosensing devices, it is essential to develop the immobilization method of enzymes using simple procedures.

In this study, we present the details of our investigation on the immobilization of GOx in a sol-gel silica matrix using poly-L-lysine-assisted direct condensation reactions of silicon oxide, tetramethoxysilane. The resulting GOx-silica composites with hexagonal sheet morphologies were thoroughly characterized in terms of morphology, size, and surface structure. The activity and stability (e.g., thermal, solvent, and recyclability) of the immobilized GOx were also investigated in details. To our knowledge, the formation and characterization of enzyme-silica sheet-type composites by direct poly-peptide-assisted reaction have not yet been reported.

2. Experimental
2.1. Materials
GOx, from Aspergillus niger, Cat. NO. G7141, molecular weight = 160 KDa; pI = 4.2) and peroxidase (POD, from horseradish, Cat. NO. P8375, molecular weight = 44 KDa; pI = 3.0–9.0), five poly-L-lysine with different molecular weights (molecular weight = 500–2000, 2KDa: 1,000–5,000, 5KDa: 4,000–15,000, 15KDa: 15,000–30,000, 30 KDa: 30,000–70,000, 70 KDa: 70,000–150,000, 150 KDa), and L-lysine were obtained from Sigma–Aldrich (St. Louis, MO). Uricase (UOx, from Yeast, Cat. NO. 46686003, molecular weight = 34 KDa; pI = 6.0–9.5) were purchased from Oriental Yeast Co., Tokyo, Japan. Phenol, 4-aminoantipyrine, hydrogen peroxide, D-glucose, and uric acid were acquired from Wako Pure Chemicals, Tokyo, Japan. Tetramethoxysilane (TMOS) was obtained from Shin-Etsu Chemical Co., Tokyo, Japan. All materials were of analytical grade and used as received without further purification.

2.2. Immobilization of GOx on a silica sheet using poly-L-lysine
The synthesis of GOx immobilized on silica sheet was carried out using poly-L-lysine as a catalyst for sol-gel reactions. The GOx solution (30 µL, 1 mg/mL, 100 units/mg GOx) was added to the mixture of 100 µL of poly-L-lysine (1 mg/mL) and 10 mM phosphate buffer of pH 7.0 (820 µL). After being stirred for 10 min at 4°C, 50 µL of tetramethoxysilane (TMOS) was added, and the mixture was stirred for another 5 min at 4°C. Upon gel formation, the suspension was centrifuged to collect the enzyme-silica composites. The obtained solid was washed two times by the same buffer, and the composites were refrigerated before use in activity measurements and material characterization.

2.3. Characterization of GOx-silica sheet composites
The morphology of the products was characterized using field-emission scanning electron microscope (FE-SEM, Hitachi S-4700, Hitachi, Tokyo, Japan). For transmission electron microscopy (TEM) studies, a small aliquot was taken from the suspension of methanol and placed in a lacey carbon-coated TEM grid that was pulled through the suspension and allowed to dry in air. The resulting sample was examined with a JEOL JEM 2010 microscope (JOEL Co., Tokyo, Japan) operated at 200 kV. Fourier transform infrared spectroscopy (FT-IR) spectra were obtained using MFT-2000, JASCO, Tokyo, Japan. For FT-IR analyzes, the samples were pelletized with KBr (sample/KBr = 1:100) using a hydraulic press. Thermo gravimetry and differential thermal analysis (TG and DTA, respectively) were performed on SSC-S200, SEIKO Instrument, Tokyo, Japan. The data were collected from room temperature to 1050°C (heating rate = 10°C/min). Furthermore, the Brunauer–Emmett–Teller (BET) specific surface area and pore volume of the samples were determined using N2 adsorption–desorption isotherms, and the pore size distribution curves were obtained by the Barret–Joyner–Halenda (BJH) method using a Micromeritics analyzer TriStar 3000 (Shimadzu Co., Tokyo, Japan). The particle diameter and zeta potential in the buffer solution were measured by ELSZ-2 (Otsuka Electronics Co., Tokyo, Japan).

2.4. Catalytic activity of GOx immobilized on the silica sheet
The potential for the application of silica sheet prepared by poly-L-lysine as biocatalyst and biosensor based on catalytic activity was investigated using
GOx. The oxidase activity of GOx was measured using D-glucose as a substrate. Two enzymatic reactions are involved in the system described here. First, GOx catalyzes the oxidation of D-glucose by dioxygen to give D-gluconolactone and hydrogen peroxide. Then, horse-radish peroxidase (POD) catalyzes the reactions of dye precursors with hydrogen peroxide to produce colored dyes. This procedure is based on commonly employed methods for the quantitation of glucose in solution. Two different dye precursors were used. Using 4-aminoantipyrine and phenol as the precursors, a quinone imine dye with an absorption maximum at 500 nm was formed. The rate of formation of colored dyes is indicative of the degree of activity of the enzymes and can be monitored by optical spectroscopy. A sample of free (30 µg, 10 units) or immobilized GOx on silica sheet was added to the mixture containing 80 µL of 4 mM D-glucose as the substrate and a 10 mM phosphate buffer solution (pH 7.0, 750 µL). This mixture was stirred at room temperature for 3 min to generate hydrogen peroxide through the enzymatic reaction. Then, the supernatant was added to a mixture of 30 µL of horseradish peroxidase (POD) solution (1 mg/mL, 300 units/mg POD), 20 µL of 1.76% of 4-aminoantipyrine, and 20 µL of 6% phenol, which was reacted at room temperature for another 3 min. Then, the solid was separated by centrifugation (12000 rpm) at 4°C for 2 min, and the absorbance of supernatant was measured with a UV-vis spectrophotometer at the fixed wavelength of 500 nm (V-560, JASCO Co., Tokyo, Japan). The activity of unimmobilized enzyme (native) was measured as a positive control. The recyclability of GOx in silica sheet was also determined using a similar procedure. No oxidative reaction occurred on only silica sheet (without GOx) under the above-mentioned reaction conditions. Each reported value is the mean of the results of at least three experiments.

2.5. Conformational changes of GOx immobilized on a silica sheet

The conformational changes of GOx immobilized on a silica sheet were evaluated using 8-anilino-1-naphthalenesulfonic acid (ANS), which is a popular fluorescence probe that is typically used to investigate the conformational changes of proteins [50]. For analysis, the samples were mixed at the volume ratio of 1/1 (v/v) with a GOx solution in both composite and native states (40 µg/500 mL)/ANS solution (40 µg/500 mL). After stirring in the dark at 25°C for 3 h, the fluorescence emission spectra of ANS-bound GOx were recorded at the excitation and fluorescence wavelengths of 387 nm and 400–600 nm, respectively.

2.6. Solvent stability of GOx-silica sheet composites

Hydrochloric acid (HCl, 10 mM), urea (5 M), or acetone (50%) solutions (600 µL) were added to the GOx-silica sheet composite mixture in the phosphate buffer (500 µL). The resulting suspension was kept at 4°C for 15 h for urea, 24 h for acetone, and 3 d for HCl. Then, the solid was washed with a 10 mM phosphate buffer (pH 7.4), and the remaining activity was determined by the same procedure described in Section 2.4. Before immobilization, 570 µL of each solvent was added to the free GOx solution (30 µg/200 mL), which was kept for each period depending on the solvent. GOx was concentrated by centrifugation at 6000 rpm at 4°C in Vivaspin 6 (Sartorius Stedim Biotech, Gottingen, Germany) and was subsequently analyzed using the same procedure, as described earlier in the evaluation of catalytic activity.

3. Results and discussion

3.1. Encapsulation of GOx in silica sheets and their characterization

In this study, we applied the hydrolysis and condensation catalyst “poly-L-lysine (pLys)” as a nanoreactor for the room temperature synthesis of silica particles. The advantage of this biomolecular template is that only the surrounding the enzyme particle can be controlled by the hydrolysis and condensation of tetramethoxysilane (TMOS) (Figure 1). GOx, which is an acidic protein (pI = 4.2), was coated by a basic peptide (poly-L-lysine) after the silica-formation reaction occurred on the
surface of pLys-GOx composites obtained by mixing pLys and GOx in a phosphate buffer at 4°C for 30 min. Surprisingly, the hydrolysis and condensation reaction of TMOS proceeded smoothly at 4°C within 5 min, and the silica particles encapsulated pLys-GOx composites were obtained by centrifugation. First, preliminary experiments established the molecular weight of pLys required for the acceptable biomineralization yields of the SiO$_2$@pLys-GOx composites. Three pLys (molecular weight = 15,000–30,000, 30 kDa; 30,000–70,000, 70 kDa; 70,000–150,000, 150 kDa) can catalyze the silica formation reactions, and a yellowish powder appeared; the precipitates were easily collected by centrifugation. Longer reaction period (e.g., 30 min at 4°C) led to the gelation of 1 mL of the entire solution. No condensation reaction occurred by the other three pLys (molecular weight = 500–2000, 2kDa; 1,000–5,000, 5kDa; 4,000–15,000, 15kDa) with a lower molecular weight within 5 min. These results indicated that the molecular weight of the basic peptide was important for catalyzing the hydrolysis and condensation of TMOS.

Table 1 shows the zeta potential of the free GOx, pLys-GOx, and SiO$_2$@pLys-GOx composites prepared from pLys 150 kDa and TMOS. According to the technical sheet from the supplier, the isoelectric point of free GOx is approximately pl 4.2, and the value of the zeta potential of free GOx in a phosphate buffer at pH 7.0 was ~−16.5. However, basic polypeptide (pLys 150 kDa, approximately pl = +13.1) showed the value of +17.3. On the basis of the value of pLys-GOx (+12.3), the adsorption of pLys on the surface of GOx was confirmed. Different values for GOx inside the silica network (+11.2, SiO$_2$@pLys-GOx) and silica particles prepared from TMOS without an enzyme (~−15.8, SiO$_2$@pLys) were obtained, which suggested that the pLys molecules were encapsulated inside the silica networks. However, a part of pLys molecules may be appeared on silica particles by covering on the surface on the enzyme.

The morphology of SiO$_2$@pLys and SiO$_2$@pLys-GOx samples was characterized using FE-SEM (Figure 2(a−f)) and TEM (Figure 3(a−f)). The FE-SEM observations revealed that aggregated structures with plates or sheets and particle sizes of 100 nm to 2 μm were formed (Figure 2(a−e)). It is clear from the image in Figure 2(a) that the SiO$_2$@pLys 30 K particles had a spherical shape and an approximately 100-nm size. By increasing the molecular weight of pLys to the larger size of 70 kDa and 150 kDa, the particle morphologies dramatically changed, and the silica sheets with the dimensions of several μm were obtained (Figure 2(b,c)). Moreover, SiO$_2$@pLys 30 K-GOx particles were composed of the plate-like morphologies with a diameter of approximately 200 nm (Figure 2(d)), which indicated that GOx molecules affected silica formation and morphologies. Moreover, the analysis of SiO$_2$@pLys 70 K-GOx and SiO$_2$@pLys 150 K-GOx by FE-SEM revealed that they had a larger size (several μm) with sheet-type structures that were similar to those of the corresponding composites excluding enzyme GOx (Figure 2(e,f)). To corroborate the high degree of structural characteristics, the TEM analysis of the SiO$_2$@pLys-GOx series was carried out (Figure 3(a−f)). SiO$_2$@pLys 30 K-GOx had a plate-like morphology, and the particle diameters and thickness were approximately 200 nm and 50 nm, respectively (Figure 3(a,d)). The hexagonal morphologies with a 3 ~ 5 μm diameter and thinner thickness (~30 nm) were formed in the case of SiO$_2$@pLys 30 K-GOx (Figure 3(b,e)) and SiO$_2$@pLys 150 K-GOx (Figure 3(c,f)). We believe that the higher molecular weight of pLys worked as the better catalysts for the condensation process of TMOS and also as organic templates for the formation of uniformly hexagonal silica sheets. In addition, Fig. S1 shows the FE-SEM image of SiO$_2$@pLys 30 K-GOx (sample in Figure 2(d)) after calcination at 600°C for 1 h. The morphological change from plate-like to spherical can be attributed to the fact that organic templates such as pLys and GOx formed silica frameworks and preserved the structural and interfacial stabilization.

The pLys 30 K-GOx and SiO$_2$@pLys 30 K-GOx particles were measured by the distribution of particle size, and the results are listed in Fig. S2, which shows the particle size distribution curve before and after silica formation. The mean particle sizes show a tendency to increase from 120 nm to 190 nm when coating the silica layer on pLys 30 K-GOx. Specifically, a 70-nm thickness of silica layer on the surface of pLys 30 K-GOx composites was obtained by the peptide-catalyzed condensation reaction. The aggregation of pLys 30 K-GOx and its silica coating subproducts were also observed, which changed from 4400 nm to 7700 nm after the sol-gel process.

The pLys 30 K-GOx particles encapsulated by silica coating were also monitored by FT-IR spectroscopy (Fig. S3). The characteristic peak of silica was observed at 1060 cm$^{-1}$, which corresponded to silica vibrations (Si−O−Si). The absorption bands observed at 3000−3500 cm$^{-1}$ were assigned to the OH vibrational mode of silanol groups. The FT-IR spectra showed N−H deformation vibration bands at 1650 cm$^{-1}$ and 1500 cm$^{-1}$ for amide I and amide II, respectively, and

| Samples         | Zeta-potential$^a$ (mV) |
|-----------------|------------------------|
| GOx             | ~16.5                  |
| pLys            | +17.3                  |
| pLys-GOx        | +12.3                  |
| SiO$_2$@pLys    | ~15.8                  |
| SiO$_2$@pLys-GOx| +11.2                  |

$^a$The zeta-potential of composites was obtained using the electrophoretic light scattering method. The particles were prepared by dispersion in a 10 mM phosphate buffer (pH 7.0) with sonication for 3 min.
showed a peak for C–H stretching at 2910 cm\(^{-1}\), which corresponded to CH\(_2\) of peptides and proteins and suggested the encapsulation of pLys-GOx in silica gel.

The TG-DTA spectra of SiO\(_2\)@pLys 30 K and SiO\(_2\)@pLys 70 K and their GOx-encapsulated composites are shown in Fig. S4. The TG curves of the composites show clear weight loss stages. The weight loss between 300°C and 600°C was assigned to the decomposition of peptide and protein. The largest weight loss (~50 wt%) was observed in the composite prepared from SiO\(_2\)@pLys 30 K-GOx, and the smallest weight loss (~35 wt%) was obtained from SiO\(_2\)@pLys 30 K (Fig. S4A), which indicated that the composite was encapsulated with a small amount of silica. The weight loss of SiO\(_2\)@pLys 150 K and its GOx composites (Fig. S4B) was similar (~40 wt%). The above-mentioned results showed that the higher molecular weight of pLys was catalyzed more actively for TMOS, and consequently, a thicker silica layer was obtained.

The BET surface area and pore volume for the composite SiO\(_2\)@pLys 30 K-GOx (total powder weight; 1.8 mg) are 76.8 m\(^2\)/g and 0.22 cm\(^3\)/g, respectively (Table 2). In addition, the values for the other two composites were 51.4 m\(^2\)/g and 0.16 cm\(^3\)/g for SiO\(_2\)@pLys 70 K-GOx (2.6 mg) and 46.5 m\(^2\)/g and 0.17 cm\(^3\)/g for SiO\(_2\)@pLys 150 K-GOx (2.9 mg), respectively; thus, smaller values were obtained with an increase in the particles size of the composites.

### 3.2. Catalytic performance of SiO\(_2\)@pLys-GOx composites

The reaction mechanism of GOx used in this study is shown in Fig. S5. First, D-\(\alpha\)-glucose was oxidized to glucono-\(\delta\)-lactone by GOx; then, the formed hydrogen peroxide (\(\text{H}_2\text{O}_2\)) reacted with phenol and 4-aminoantipyrine by a different enzyme, horseradish peroxidase. The absorbance of the final product (a red quinoneimine dye) was measured by UV-vis spectroscopy for the determination of the GOx relative activity. Figure 4 shows the catalytic activities of GOx (30 µg of free enzyme and all of encapsulated composites containing 30 µg enzyme). The relative activities of three GOx-encapsulated on SiO\(_2\)@pLys exhibited high and comparable values, which were slightly lower than those of free GOx. Thus, the oxidation activity of GOx-encapsulated on these composites was higher than 85% compared to that of native GOx (considered to be 100%). No enzymatic reaction occurred from the separated supernatant solution after the removal of the composites by centrifugation, which indicated

| SiO\(_2\)@pLys-GOx | Weight (mg) | Pore volume\(^a\) (cm\(^3\)/g) | Specific surface area\(^a\) (m\(^2\)/g) |
|-----------------|------------|-------------------------------|---------------------------------|
| 30K             | 1.9        | 0.22                          | 76.8                           |
| 70K             | 2.6        | 0.16                          | 51.4                           |
| 150K            | 2.9        | 0.17                          | 46.5                           |

\(^a\)The specific surface area and pore volume of SiO\(_2\)@pLys-GOx composites were calculated on the basis of nitrogen adsorption–desorption isotherms by the Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods.
that all molecules of enzymes were encapsulated inside the silica layer through sol-gel reactions by pLys.

Fig. S6 shows the Lineweaver–Burk plot, and Table 3 summarizes the values of $K_m$, $V_{max}$, and $K_{cat}/K_m$. The values of $K_m$ and $V_{max}$ indicate the enzyme’s affinity toward the substrate and the ability of how many times enzymes can catalyze a reaction per minute, respectively. The ratio of $K_{cat}/K_m$ indicates the catalytic efficiency, and the values for GOx-encapsulated on silica layers (i.e., 11.25 for SiO$_2$@pLys 30 K-GOx, 16.00 for SiO$_2$@pLys 70 K-GOx, 16.42 for SiO$_2$@pLys 150 K-GOx) were slightly lower than those of free GOx (17.53). The above-mentioned results showed that GOx-encapsulated composites had high ability for enzyme catalysts.

8-Anilino-1-naphthalenesulfonic acid (ANS) is an excellent fluorescence probe for binding to the hydrophobic domains of the protein. Therefore, ANS was bound to GOx to investigate the interactions between GOx and silica composites. Figure 5(a) indicates the fluorescence spectra of ANS-binding GOx coated by pLys 150 K and GOx-encapsulated silica layers. The three-dimensional structure of GOx was determined from the Protein Data Bank; ID: 1NPF (Figure 5(b)). Figure 5(a) shows that similar intensity and maximum peak wavelength were observed for both free GOx and pLys-GOx, which suggested that no conformational change occurred by pLys binding to enzyme molecules. However, the enhancement of the maximum emission intensity in the spectra and its blueshift were observed for encapsulated GOx bound with ANS. These results showed that the conformational change in GOx occurred owing to the GOx-encapsulated inside silica materials. The interaction between the side chains of amino acids in enzyme surface and silanol sites in silica caused a conformational change of enzyme. It can be assumed that the above-mentioned results led to lower enzyme relative activities (Table 3).

### 3.3. Stability of SiO$_2$@pLys-GOx composites under various conditions

Moreover, cycling test was carried out, and the remaining activity was measured after each cycle. Figure 6 shows that the relative activity of three SiO$_2$@pLys-GOx composites was higher than 55% of the initial activity after eight cycles. After the first reaction, the relative

![Figure 4](image)

**Figure 4.** Catalytic activity of three SiO$_2$@pLys-GOx composites and free GOx.

![Figure 5](image)

**Figure 5.** Conformational changes of free GOx, pLys 150K-GOx, and SiO$_2$@pLys 150K-GOx. (a) Conformational changes of the tertiary structure of GOx determined by the 8-anilino-1-naphthalenesulfonic acid (ANS)-binding fluorescence spectra. (b) Three-dimensional structure of GOx obtained from the Protein Data Bank; ID: 1NPF. Red balls represent tryptophan; blue balls represent hydrophobic amino acids.

### Table 3. Apparent $K_m$, $V_{max}$, and $K_{cat}/K_m$ values for three SiO$_2$@pLys-GOx composites and free GOx shown in Fig. S6 calculated using the Michaelis–Menten equation.

| SiO$_2$@pLys-GOx    | $K_m$ ($\text{mM}$) | $V_{max}$ (µmol min$^{-1}$ mg$^{-1}$) | $K_{cat}/K_m$ ($\text{mM}^{-1}$ min$^{-1}$) |
|---------------------|---------------------|--------------------------------------|-----------------------------------------------|
| Free                | 16.8                | 0.074                                 | 17.53                                         |
| 30 k                | 319.8               | 0.903                                 | 11.25                                         |
| 70 k                | 17.6                | 0.071                                 | 16.00                                         |
| 150 k               | 12.7                | 0.052                                 | 16.42                                         |

* $K_m$ is the Michaelis constant. $V_{max}$ is the maximum velocity. $K_{cat}/K_m$ is the specificity constant.
activity reduced to approximately 70%; however, from second to eight times, the relative activity remained similar. These results confirmed that the SiO$_2$@pLys-GOx composites can be better candidates for immobilizing carriers of enzymes with high stability.

Figure 6. Residual activity of SiO$_2$@pLys-GOx composites as a function of the catalyst recycle number.

Figure 7. Solvent stability of free GOx and three SiO$_2$@pLys-GOx composites in (a) 10 mM HCl, (b) 5 M urea, and (c) 50% acetone solution. The residual activities were determined by the same procedure mentioned in Figure 4.
Next, we examined the activity of immobilized GOx in 10 mM HCl, 5 M urea, and 50% acetone (Figure 7). When the phosphate buffer (pH 7.0) was used, the catalytic activity of free GOx was taken to be 100%. All encapsulated GOx proteins showed high activity compared with those observed with free GOx under the solvents. When the encapsulated GOx was incubated in 10 mM HCl (Figure 7(a)), it showed a relatively high residual activity (45 – 65%) compared to that of free GOx (39%). For the 5 M urea solution, the relative activity of encapsulated GOx remained higher than 95% for all SiO$_2$@pLys-GOx composites (Figure 7(b)). However, a sharp decrease in activity was observed when free GOx (48%) was incubated under the same conditions. In addition, a similar stabilization was also observed in 50% acetone for the SiO$_2$@pLys-GOx composite (Figure 7(c)).

High temperature strongly affects the catalytic stability of the encapsulated GOx system. Therefore, we studied the thermal stability of GOx by measuring the residual activity after the incubation of free and encapsulated GOx at 40 – 90°C for 30 min. Without the thermal treatment, the catalytic activity of GOx was assumed to be 100%. The thermal stabilities of free and three SiO$_2$@pLys-GOx composites were analyzed. As seen in Figure 8(a), more than 80% of its activity was observed after incubation at 60°C on SiO$_2$@pLys 30 K-GOx and 70 K-GOx; however, 40% of its activity was retained after incubation at the same temperature on free and SiO$_2$@pLys 150 K-GOx. SiO$_2$@pLys 150 K-GOx had a larger hexagonal morphology and silica thinner layer judgment from TEM images (Figure 3). Therefore, SiO$_2$@pLys 150 K-GOx may reduce its activity. The above-mentioned results show that the solvent and thermal stabilities of GOx were increased by encapsulation inside peptide-silica layers. These strategies can be applied to the efficient encapsulation and stabilization of enzyme GOx.

To reveal the glucose-sensitivity for encapsulating GOx, these responses were measured in the range of glucose concentration of 1–1000 µM, as shown in Figure 9. In the case of SiO$_2$@pLys 30 K-GOx, the glucose response curves show a linear range from 1 µM to 400 µM of glucose. This range in the encapsulated samples was also obtained using the free GOx solution. Specifically, both enzymes provided the comparable sensitivity at the lower glucose concentration of < 100 µM. This result showed that the as-synthesized SiO$_2$@pLys-GOx could be utilized as glucose sensing materials with high sensitivity.

3.4. Adaptation of the SiO$_2$@pLys encapsulation method to another enzyme

To estimate the effectiveness for this encapsulation system, uricase (UOx) was selected and prepared under a similar reaction conditions using pLys-catalyzed sol-gel systems in TMOS. Similar to GOx, the UOx encapsulation reaction proceeded smoothly and solid materials, with UOx encapsulated inside silica gel, was obtained within 5 min. The catalytic activity of the collected composites was tested using a similar procedure to GOx except for uric acid as an enzyme substrate. Figure 10 shows the relative activities of SiO$_2$@pLys-UOx composites. All composites explored comparative or better relative activity than that of free UOx, which indicated that these methodologies (i.e., polypeptide-catalyzed sol-gel coatings on the enzyme surface) can be applicable to other enzyme and encapsulation.

4. Conclusion

The results of this study provide important insights into the structural organization of the composite materials composed of enzymes and silica and synthesized thorough the sol-gel methods. The hierarchical structure for the composite material can be proposed based on the FE-SEM, TEM, BET, TG-DTA, DLS, and $\zeta$-potential analyses. The key factor for preparing SiO$_2$@pLys-GOx with plate-or sheet-type structures uses a higher molecular weight of pLys as a catalyst for TMOS sol-gel reactions. The particle
sizes and morphologies of SiO$_2$@pLys-GOx composites were successfully controlled by varying the reaction conditions. The SiO$_2$@pLys-GOx composites exhibited a robust catalytic activity during D-glucose, which is similar to the oxidation of free GOx. These results provide information not only for the encapsulation and stabilization of enzymes in silica matrices but also for the potential industrial applications of silica-enzyme composites such as biosensing tools to determine D-glucose, uric acid, and other compounds in blood. The precise control and further extension of the assembled structure and other enzyme activity/stability in the basic peptide-silica matrix are currently being studied and will be reported elsewhere. These encouraging findings open novel and interesting prospects for the immobilization methodologies of enzymes and a new potential for biocatalysts and biosensors of enzyme-silica composites.

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