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Catalysis of Gold Nanoparticles within Lysozyme Single Crystals

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Bio–nano hybrid materials have been the focus of numerous studies because they combine the merits of both biomolecules and nanomaterials, with potential for a wide range of applications in catalysis, electronic devices, energy conversion and storage, drug delivery, imaging, and sensing.[1–7] Because of the promise, many biological molecules, such as DNA, proteins, carbohydrates, bacteria, and viruses, have been used to prepare these bio–nano hybrid materials.[6,8–29] Among them, proteins have received special attention due to their nanometer sizes, which are comparable to many nanomaterials, and their diverse structures and functionalities, which allow selective recognition for directed assembly.

To date, most studies in this area have focused mainly on synthesis of biomolecules and nanomaterials individually before conjugating them together. Taking advantage of protein single crystals as highly ordered 3D assembled structures, we and others have developed a new approach to synthesis of nanoparticle-in-protein crystals via controllable growth of metal nanoparticles within a protein single crystal.[30–33] By using the chicken egg white lysozyme as a model system,[34–36] gold nanoparticles (AuNPs) within lysozyme single crystals (shorten as AuNPs@Lys) have been prepared and carefully characterized (Figure 1 a, b).[32] While the work offered insights into AuNP growth in the presence of proteins, the functional properties of the system have not been explored.

AuNPs are among the most useful nanomaterials due to their unique optical and catalytic properties, high chemical stability, and wide range of applications.[37] A particularly exciting area of research is the application of AuNPs in catalysis.[38,39] However, detailed understanding of the mechanism of the AuNP catalysis and fine control of the activity has not been achieved. Herein we demonstrate that this new type of nanoparticle-in-protein crystal can be used for catalytic reduction of *p*-nitrophenol to *p*-aminophenol by $NaBH_4$ (Figure 1 c).[40] The ability of the protein single crystals to control the size of AuNPs within the crystals made it possible for us to find correlations between the size of the particles and catalytic activity. The catalytic activity could be further modulated by tuning AuNPs@Lys growth with external chemicals.

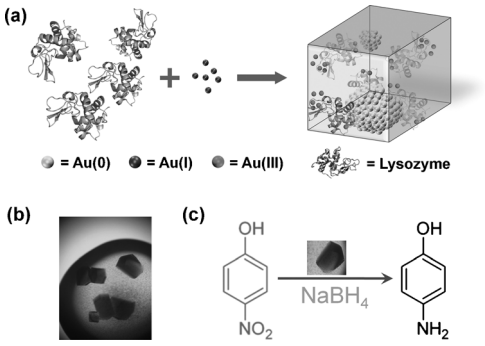

Figure 1. a) Formation of AuNPs@Lys crystals and b) the corresponding optical image after 20 days of growth. c) The catalytic reaction within AuNPs@Lys crystals. Note: Parts (a) and (b) were adapted from previous results.[32]

AuNPs@Lys crystals were prepared by in situ growth of crystals from lysozyme and $[ClAuS(CH_2CH_2OH)_2]$ solutions, as reported.[32] The crystals were collected at different growth stages and used for catalytic reactions. When $NaBH_4$ was added to the solution of *p*-nitrophenol and AuNPs@Lys, UV/Vis spectra were recorded to monitor the reaction. Figure 2 a shows a typical time-dependent evolution of UV/Vis spectra of the reaction catalyzed by AuNPs@Lys after 10 days of growth. Over time, the intensity of the characteristic absorption peak of *p*-nitrophenol at 400 nm decreased, thus indicating the successful reduction of *p*-nitrophenol to *p*-aminophenol. To further confirm the catalytic activity of the AuNPs@Lys, the same reaction was performed in the absence of the AuNPs@Lys catalysts (Figure S1 in the Supporting Information). The reaction solution remained yellow, and the intensity of the absorption peak did not decrease after overnight incubation, thus indicating that *p*-nitrophenol was not reduced without the catalyst.

In the reaction system, $NaBH_4$ was in approximately 100-fold excess, which meant that the reaction rate would follow pseudo-first-order kinetics. The linear regression of the logarithm of absorbance of *p*-nitrophenol at 400 nm ($\ln(A_{400})$) versus reaction time confirmed the pseudo-first-order kinetics. As shown in Figure 2 b, the apparent rate constant (k) of the reaction could be calculated from the slope of $\ln(A_{400})$ versus time, which was $-0.039 \pm 0.002\ min^{-1}$.

Because the sizes of the AuNPs grown within the single crystals of lysozyme can be precisely controlled,[32] AuNPs@Lys crystals grown at different stages were tested to eluci-

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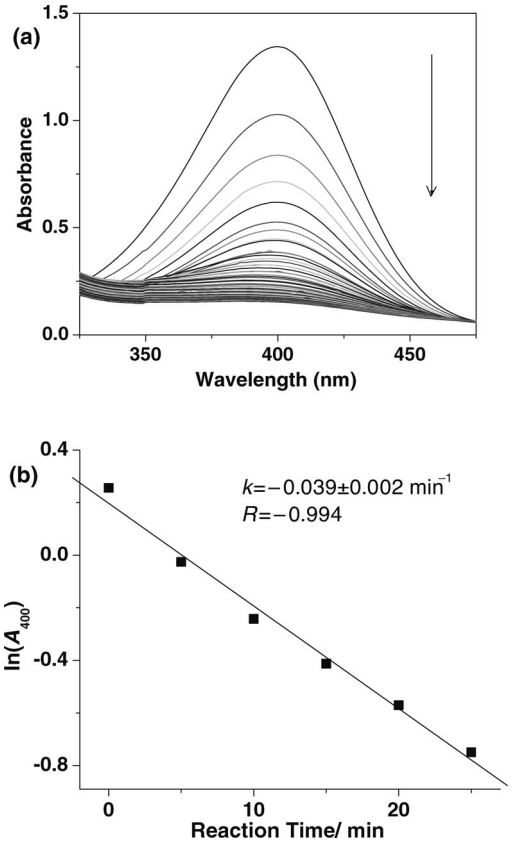

Figure 2. a) A typical time-dependent evolution of UV/Vis spectra, showing the catalytic reduction of *p*-nitrophenol to *p*-aminophenol by $NaBH_4$ using AuNPs@Lys after 10 days of growth. b) Plot of ln(A_{400}) versus reaction time.

date the relationship between the sizes of AuNPs@Lys and catalytic activity. As shown in Figure 3b and Figure S2 in the Supporting Information, AuNPs@Lys crystals at early growth stages (i.e., two, three, and five days) showed higher catalytic activity than the ones at late growth stages (i.e., 10 and 20 days). AuNPs@Lys crystals obtained after three days showed highest activity, while AuNPs@Lys crystals obtained after 20 days showed lowest activity. The AuNPs sizes were measured to find the correlation between the AuNPs sizes and the catalytic activity (Figure 3a).[32] As shown in Figure 3b, the catalytic activity increased initially with increased size of AuNPs until the size reached 7.4 nm on the third day, after which the activity decreased with increasing sizes of the AuNPs. The activity decrease with increasing sizes of the AuNPs from 3 to 20 days was expected, since the larger nanoparticles had less surface-exposed atoms as catalytic sites. However, it is interesting that the AuNPs@Lys obtained after two days showed lower activity when compared with the AuNPs@Lys after three days. In the early stage of the AuNP growth, the sizes of the AuNPs were small and comparable to the dimension of the lysozyme (ca. $3.0\times3.0\times4.5$ nm^3).[41] The active sites on the surface of the AuNPs can thus be blocked by the protein, resulting in lower activity.

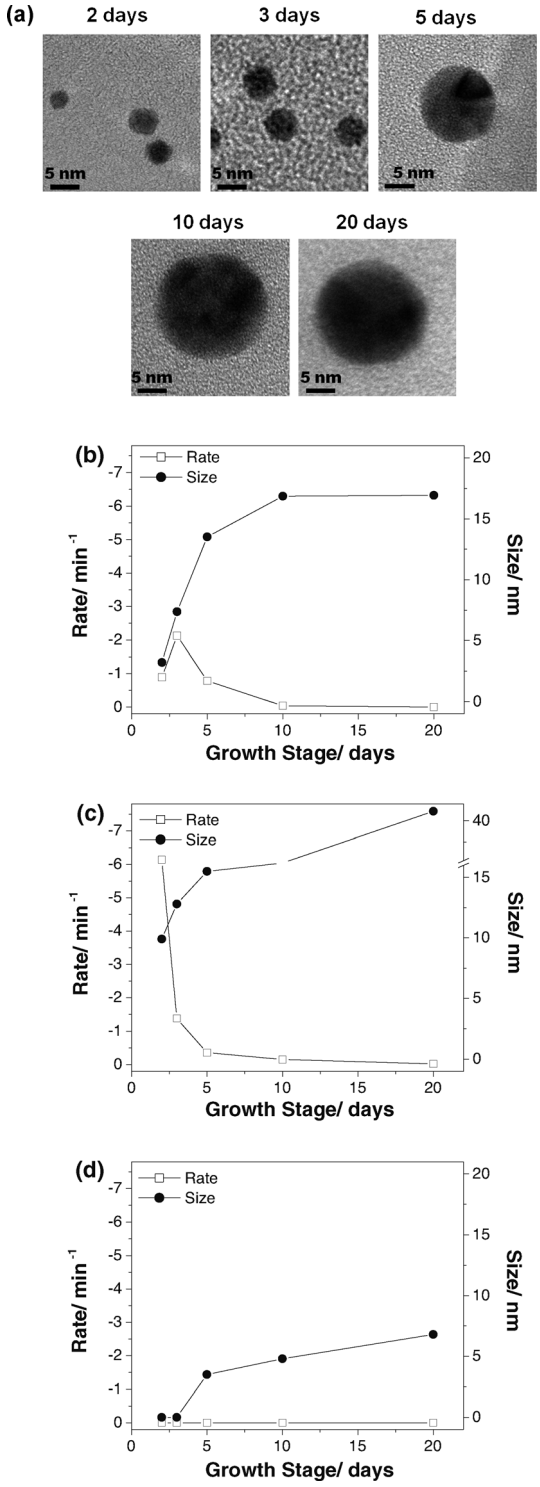

Figure 3. a) TEM images of AuNPs@Lys at different growth stages. b–d) Plots of apparent rate constant and AuNP size versus crystals growth stages. b) AuNPs@Lys, c) AuNPs@Lys-Hg, and d) AuNPs@Lys-TCEP. Note: Part (a) was adapted from previous results.[32]

Since the growth of AuNPs within lysozyme single crystals could be tuned by adding external chemicals,[32] we explored further modulation of the catalytic reactivity by adding ex-

ternal chemicals. As shown in Figure 3c and Figure S2 in the Supporting Information, the longer the growth of the crystals and the larger the AuNPs were, the lower the catalytic activities of the reduction. This result agrees well with size-dependent catalytic activity of AuNPs. Interestingly, in comparison with AuNPs@Lys as the catalyst (Figure 3b), the catalytic activities of AuNPs@Lys-Hg crystals obtained after two days is much higher ($-6.12\ min^{-1}$ for AuNPs@Lys-Hg vs. $-0.89\ min^{-1}$ for AuNPs@Lys, see Figure 3c and Figure S2 in the Supporting Information). To understand the relationship between the sizes of AuNPs and catalytic activity, we plotted apparent rate constant versus size of AuNPs from both AuNPs@Lys and AuNPs@Lys-Hg. As shown in Figure S5 in the Supporting Information, the activity increased initially with the increasing sizes of AuNPs until the size reached 9.9 nm (AuNPs@Lys-Hg after 2 days), after which the activity decreased with increasing sizes of the AuNPs. This trend is similar to that in Figure 3b, and thus the results can be explained by the same reasons as discussed above.

It has also been reported that addition of tris(2-carboxyethyl)phosphine (TCEP) could inhibit the AuNPs growth within the lysozyme crystals. As shown in Figure 3d and Figure S2 in the Supporting Information, the catalytic reduction of *p*-nitrophenol was almost completely inhibited when AuNPs@Lys-TCEP crystals were used as catalyst. This result is consistent with inhibited growth of AuNPs within the lysozyme crystals and thus lack of many AuNPs for catalytic reactions. Moreover, TCEP might also adsorb onto the AuNP surface and thus block the active sites of AuNPs.

In summary, we have demonstrated that AuNPs grown in situ within a lysozyme protein crystal could be used as efficient catalyst to catalyze the reduction of *p*-nitrophenol by $NaBH_4$. The precise control of the AuNP sizes by the single crystals of lysozyme allowed us to elucidate the relationship between the catalytic activity and the size of the crystals. Furthermore, we showed that the catalytic activity of the AuNPs@Lys could be modulated by fine-tuning the AuNPs growth with additional chemicals to either accelerate or inhibit the AuNP growth. This work provides insights into the development of new highly efficient catalysts by incorporating inorganic materials within protein crystals.

Experimental Section

Materials

Chloroauric acid, 2,2′-thiodiethanol, *p*-nitrophenol, tris(2-carboxyethyl)-phosphine, sodium borohydride, and lysozyme lyophilized powder (from chicken egg white) were purchased from Sigma–Aldrich Chemical Co. All other chemicals were from Fisher Scientific Inc. The [ClAuS-$(CH_2CH_2OH)_2$] (Au^I) in 0.1 M sodium acetate (pH 4.5) was freshly prepared before use according to a method reported previously.[42] Specifically, 2,2′-thiodiethanol (0.7 μL) was added into 35 mM $HAuCl_4$ (100 μL) in 0.1 M pH 4.5 sodium acetate solution (molar ratio of 2,2′-thiodiethanol to $HAuCl_4$ was 2:1). After incubation for a few minutes at room temperature, the color of the solution changed from yellow to colorless, indicating the formation of Au^I. The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Growth of AuNPs within a Lysozyme Crystal

The AuNPs within lysozyme single crystals were grown according to our reported method.[32] Specifically, lysozyme (75 mg mL^{-1}, 5 μL) in 0.1 M sodium acetate (pH 4.5) were mixed with 35 mM Au^I (3 μL) in 0.1 M sodium acetate (pH 4.5) at room temperature for 5 min. Then the crystal trays were set up by mixing the as-prepared lysozyme–Au^I complex (7 μL) and the well solution (3 μL). The well solution was 6.5% NaCl (w/v) in 0.1 M sodium acetate (pH 4.5). The well of the crystal tray was filled with 800 mL of the same solution. Colorless crystals were observed after one day of growth. The crystals changed from colorless to pink then to red as the growth time increased. Crystals at different stages of growth were washed with the well solution and collected for further catalytic reactions.

To fine-tune the growth rate of AuNPs within the lysozyme crystals, 1 mM Hg^{2+} or 400 mM TCEP (each ca. 0.5 μL) were added to the crystal drop solution after one day of growth.

Catalytic Reaction

The catalytic reduction of *p*-nitrophenol by $NaBH_4$ was chosen as a model reaction to test the catalytic activity of the AuNPs@Lys. For a typical reaction carried out at room temperature, 10 mM *p*-nitrophenol (20 μL) and AuNPs@Lys (2.4 mg) were added into H_2O (2780 μL) first, and then 100 mM $NaBH_4$ (200 μL) was added to initiate the reduction reaction. The catalytic reaction was monitored by recording the UV/Vis spectra from 325 to 475 nm every 5 min.

Instrumentation

Absorption spectra were obtained on a Cary 5000 spectrophotometer (Varian, USA). TEM images were obtained on a JEOL 2010 LaB6 transmission electron microscope at an acceleration voltage of 200 kV. TEM samples were prepared by grinding the crystals into a fine powder with a mortar and pestle, then transferring the crystal powder onto copper TEM grids.

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Keywords: gold · heterogeneous catalysis · hybrid materials · nanoparticles · proteins

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