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Stabilization and decomposition of organic matters by nano-porous metals

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Abstract

Nanoporous (np-) metals affect chemical stability of various organic matters much more than their bulk counterparts. Self-assembly monolayers (SAMs) of 4-aminothiophenol are more stable on np-Au fabricated by dealloying Ag-Au alloy than on flat Au, which were elucidated by cyclic voltammetry (CV). The first-principles calculations indicate that atomic defect of Au surface, which is characteristic of ligament surface of np-Au, reduce the binding energy of thiol molecule.

The stabilized SAMs on np-Au are also effective for enzyme immobilization. When laccase was immobilized on np-Au surface, its thermal stability was improved compared with nonfixed laccase. The higher stability of immobilized laccase is due to the synergistic effect of reduced conformational flexibility of the enzyme in nanopores and SAMs stability. CV with the working electrode of enzyme-immobilized np-Au revealed that the electron transfer between enzyme and electrode is successfully enhanced by SAMs. These aspects of enzyme-immobilized and SAMs-decorated np-Au can be applied to the electrodes of biofuel cell.

Np-metals such as np-Au, np-Pd and np-Ni, on the other hand, remarkably decompose methyl orange (MO, a typical stable azo dye in the textile industry) in an aqueous solution, while MO is highly stable in aqueous solutions which include their bulk counterparts. Ultraviolet-visible light spectroscopy and high-performance liquid chromatography demonstrated that np-Au breaks the azo bond ($-N=N-$) in MO molecules. Defective surface of ligaments in np-metals plays an important role in this catalytic decomposition of MO.

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1. Introduction

Nanoporous (np-) metals fabricated by dealloying have various interesting properties including mechanical, catalytic, piezoelectrical ones (Ding and Chen (2009)). The three-dimensional np-structure induces surface lattice disorders such as lattice expansion/contraction and atomic defects. Here we demonstrate that np-metals have different influences from their bulk counterpart on surrounding organics (Hakamada et al. (2011), Hakamada et al. (2011), Hakamada et al. (2012), Hakamada et al. (2012)).

2. Experimental Methods

2.1. Self-assembled monolayers (SAMs)

Detailed experimental methods are described in the published paper (Hakamada et al. (2011)). In brief, Np-Au was synthesized by dealloying of the $\text{Au}_{0.35}\text{Ag}_{0.65}$ alloy (free corrosion) at 253 K for 15 h in 70 mass% HNO_3 . Some of the sample was freely corroded at room temperature and annealed at 423 K for 15 min under Ar atmosphere to coarsen the porous structure. The 4-aminothiophenol (4-ATP) thiolate monolayers were self-assembled on both dealloyed and coarsened samples. For the preparation of SAMs on np-Au, the samples were soaked in a 20 mmol/L ethanolic solution of 4-ATP for 65 h at room temperature.

Cyclic voltammetry (CV) was carried out at room temperature to determine the stability of the adsorbed SAMs on np-Au. A three-electrode electrochemical cell with a platinum wire as a counter electrode, the np-Au sample decorated with SAMs as a working electrode and the saturated calomel electrode (SCE) as a reference electrode were used. For comparison, smooth Au surface (polished Au plate) decorated with 4-ATP SAM is also used as a working electrode. The electrolyte was 0.1 mol/L NaOH and the scan rate was 5 mV/s from 0 to -1.2 V. Curves for the first scan are used for consideration.

2.2. Enzymes

Detailed experimental methods are described in the published papers (Hakamada et al. (2011), Hakamada et al. (2012)). In brief, np-Au samples (with and without a 4-ATP SAM) were stored quiescently in 2 mL of 7 mg/mL laccase solution (diluted by 0.1 mol/L citrate–0.2 mol/L phosphate buffer solution (PBS), pH=5.0) at 4 °C for 24 h. Np-Au samples were then rinsed five times with 10 mL of buffer solution to remove any excess or only weakly adsorbed enzyme. An appropriate amount of laccase was stirred with 3 mL of buffer solution and 300 μL of 10mmol/L 2,6-dimethoxyphenol (DMP) (Qiu et al. (2008)), and the activities of free and immobilized laccase were spectrophotometrically determined on the basis of the absorbance change at 470 nm. The effect of incubation time was examined by incubating free laccase and laccase-immobilized np-Au in buffer solution (pH=5.0) at 50 °C. At given time intervals, 5 μL of free enzyme solution or 20 mg of laccase-immobilized np-Au was removed and left to stand in the buffer solution for 1 h at room temperature. The residual activity was then assayed at 35 °C.

Cyclic voltammetry (CV) was carried out at room temperature to elucidate the electrochemical properties and stability of the enzyme-immobilized samples. A three-electrode electrochemical cell with Pt wire as a counter electrode, the laccase-immobilized np-Au electrode as a working electrode, and saturated calomel electrode (SCE) as a reference electrode was used. Potentials were documented vs standard hydrogen electrode (SHE) unless otherwise stated in this section. For the laccase-immobilized electrode, the electrolyte of 0.1 mol/L citrate-0.2 mol/L PBS was air-saturated with dry air bubbling for 1 h. CV was conducted at 100 mV/s from -0.15 to 0.7 V.

2.3. Azo dyes

Detailed experimental methods are described in the published paper (Hakamada et al. (2012)). In brief, methyl orange (MO), a typical azo dye used in the textile industry, is very stable and is commonly used as the probe for evaluating photocatalysts (Yen et al. (2009)) thus the catalytic activities of the np-Au for degradation of the MO was assessed at room temperature (≈ 298 K). Different samples (with nominal exposed areas of 2 cm^2) were immersed into 5 mL of MO solution (2×10^{-5} mol/L). The experiments were conducted under dark conditions to distinguish

the MO decrease from photocatalysis. The concentration of MO in the solution as a function of soaking time was measured by monitoring the absorbance of the MO at its absorption maximum wavelength using a UV-Vis spectrometer.

3. Results and Discussion

3.1. Self-assembled monolayers (SAMs)

The scanning electron microscopy (SEM) shown in Fig. 1 confirmed the formation of as-dealloyed and coarsened np-Au (Hakamada et al. (2011)). The average pore sizes were 6 and 40 nm for as-dealloyed and coarsened samples, respectively. Typical CV curves (Hakamada et al. (2011)) for as-dealloyed and coarsened np-Au after SAM decoration are shown in Fig. 2. CV curves for a smooth Au surface are also included in the figure. The CV curves for np-Au exhibited a clear reductive peak during cathodic scan, which shows that both np-Au samples are successfully decorated with SAMs. The cathodic potential scan of both curves shows major current peaks below -1.0 V. The major peak (indicated by arrows) corresponds to the reductive desorption of 4-ATP SAMs from Au surfaces (Valério et al. (2008), Komura et al. (2004)). The peak potential ($= -1.15$ V) of as-dealloyed np-Au decorated with 4-ATP SAMs was lower than that ($= -1.05$ V) of the coarsened one. On the other hand, only a broad peak around -0.84 V is observed in the cathodic CV curve for the smooth Au surface.

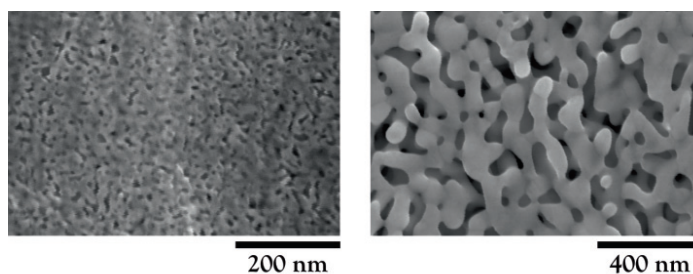


Figure 1: Scanning electron microscopy images of (left) as-dealloyed and (right) coarsened np-Au samples.

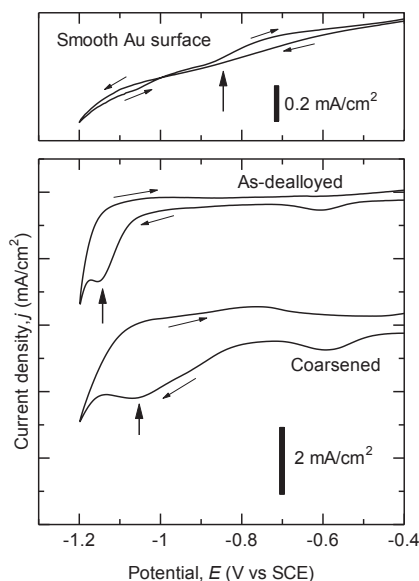


Figure 2: Cyclic voltammetric curves of as-dealloyed and coarsened np-Au samples decorated with 4-ATP SAMs. The electrolyte used was 0.1 M NaOH and the scan rate was 5 mV s^{-1} . The curve for smooth Au surface with 4-ATP SAMs is also shown.

It has been reported that the more negative the potential of the desorption peak, the stronger the bonding between SAMs and the Au surface (Cortés et al. (2009), Kawasaki and Iino (2006)). Hence, the bonding between 4-ATP SAMs and the surface of as-dealloyed np-Au is stronger than that between SAMs and the surface of the coarsened sample. Thus, the as-dealloyed np-Au provides a higher electrochemical stability of SAMs of 4-ATP than the coarsened one.

Ab initio calculations (Hakamada et al. (2011)) suggested that the atomic defects at the Au surface of np-Au markedly contribute to the SAM stabilization. Therefore, np-Au is an attractive material as a substrate for thiolate-based devices.

3.2. Enzymes

The effect of incubation time at 50 °C on the activity of free and immobilized laccase is shown in Fig. 3 (Hakamada et al. (2011)). The activity of free laccase and laccase-immobilized np-Au without SAMs was found to decrease with increasing incubation time. After 30 min, free laccase had lost approximately 70% of its activity, compared with laccase-immobilized np-Au in the absence of a SAM which had lost only 20% of its activity. Furthermore, laccase-immobilized np-Au with SAM showed far higher thermal stability than the other samples. Little degradation in activity (10%) was observed even after a 2 h incubation at 50 °C.

Immobilized enzymes have frequently been shown to exhibit higher thermal stability than the corresponding free enzyme, because of the reduction in conformational flexibility (Hudson et al. (2008)). The enhancing effect of SAMs on the function of adsorbed enzymes has been repeatedly demonstrated (Gupta et al. (2003)), and the improvement in SAM stability on defective Au surfaces has been also reported (Cortés et al. (2009)). Np-metals possess lattice disorder including changes in lattice spacing and atomic vacancy at the ligament surface (Hakamada et al. (2010)). As a consequence, SAMs by themselves exhibited a stabilization of the np-Au. The synergy between np-structure and the enhanced stability and promoting effect of the SAM resulted in an increased thermal stability of laccase, which protected the enzyme from thermally-induced conformational changes.

Figure 4 shows CV curves for the laccase-immobilized np-Au electrodes (Hakamada et al. (2012)). As shown in Fig. 4a, the laccase-immobilized np-Au electrode without SAM exhibited one reversible broad peak pair with a midpoint potential of 0.13 V, similar to a previous study (Cortés et al. (2009)). Figure 4a also shows no apparent degradation in the laccase-immobilized np-Au electrode without SAM after 100 cycles of measurement. In contrast, laccase-immobilized np-Au with SAM showed two sets of reduction-oxidation (redox) peaks in the CV curves (Fig. 4b). The first and second peak pairs had midpoint potentials of +0.26 and +0.55 V, respectively. The double peak pair indicated electron transfer via different copper centers (T2 and T1 for the lower and higher potential peaks, respectively) in *T. versicolor* laccase (Frasconi et al. (2010)). Thus, the SAM modification of np-Au promoted the redox electron transfer at the T1 copper center of immobilized laccase.

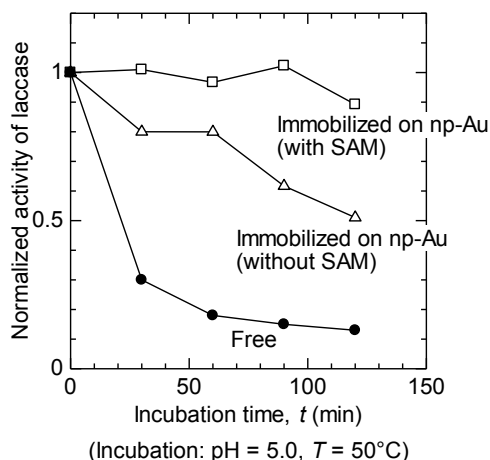


Figure 3: Effect of incubation time on laccase activity for free and immobilized laccase.

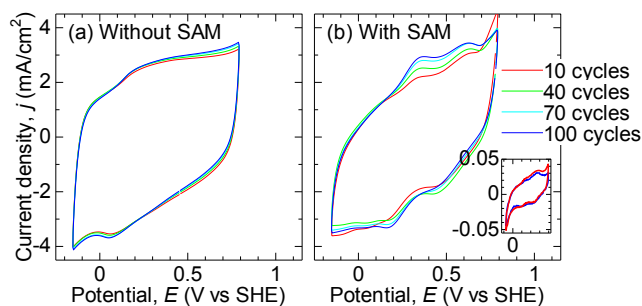


Figure 4: Cyclic voltammetry curves for laccase-immobilized np-Au working electrodes with (a) and without (b) a self-assembled monolayer (SAM) of 4-aminothiophenol. Inset in b shows CV curve for laccase-immobilized smooth surface of Au with the SAM.

To utilize the improved activities of the enzymes immobilized on SAM-modified np-Au, a glucose/O₂ biofuel cell consisting of glucose oxidase- and laccase-immobilized np-Au electrodes with SAM was preliminarily fabricated. The maximum power density delivered by the biofuel cell was 52 $\mu\text{W}/\text{cm}^2$ at 0.21 V. The present results suggest that np-Au is a promising material for biofuel cells.

3.3. Azo dyes

Time variation of the MO concentration after sample immersion is shown in Fig. 5a (Hakamada et al. (2012)). Np-Au (samples 1–3) significantly reduced MO concentration, whereas bulk Au, bulk Au_{0.3}Ag_{0.7}, and bulk Au_{0.7}Ag_{0.3} had no effect on the MO concentration. After 30 h immersion of the np-Au with ligaments and a pore size of less than 10 nm (samples 1 and 3), the MO concentration became negligible. This is also demonstrated in Fig. 5b, which shows the appearance of the MO solution after the 30 h immersion (Hakamada et al. (2012)).

It is indicated that the defective and strained surface of the ligaments in np-Au can increase the reactivity of the Au surfaces, and decrease the concentration of the MO solution. In the past, many reports have shown that nanostructured Au has a wide variety of catalytic properties (Min and Friend (2007)). However, there have been no reports on unsupported np-Au exhibiting the catalytic degradation of complicated organics such as azo dyes. The present results also add the information on bond-breaking capacity of gold (Freyschlag and Madix (2011)).

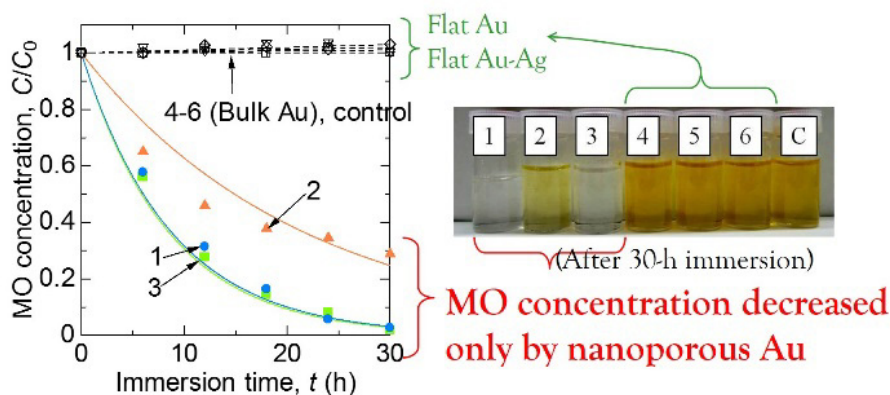


Figure 5: (a) Time variation of MO concentration after the immersion of nanoporous Au and bulk Au samples. (b) Appearance of MO solution after the 30 h immersion of samples. The numbers indicate the sample no. presented in Table 1 in Ref. 5. “C” means control experiment with no sample immersion.

4. Conclusions

As described above, np-metals have critical effects on the stabilization and decomposition of surrounding organic matters. These effects seem to be attributed to the atomic defects on the ligament surface of np-metals.

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