Ionic Liquid as an Efficient Modulator on Artificial Enzyme System: Toward the Realization of High-Temperature Catalytic Reactions

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S Supporting Information

ABSTRACT: Herein, with the aid of ionic liquid, we demonstrate for the first time that highly stable Au/SiO_2 hetero-nanocomposites can serve as a robust and recyclable peroxidase mimic for realizing high-temperature catalytic reactions. Our findings pave the way to use nanomaterials for the design and development of efficient biomimetic catalysts and, more significantly, to apply ionic liquid as a positive modulator in catalytic reactions.

Natural enzymes, which possess high activity and high substrate specificity under mild reaction conditions, have been a constant source of inspiration for chemists in their efforts to engineer synthetic structures that mimic their complexities and functions.[1] Very recently, the merging of nanotechnology with biology has also ignited research efforts for designing functional nanomaterials with enzyme-like catalytic activities.[2] For instance, magnetic nanoparticles, metallic nanocomposites, and carbon nanomaterials have been discovered to possess unique peroxidase-mimic activity and shown promising potential in environmental detection and biomedical applications.[1d,2b,3] Although they usually exhibit superior thermal stability to natural enzyme, none of them can realize high-temperature catalytic reactions mainly because of the thermally induced instability of enzymatic product.[1d,3d,4]

Ionic liquids (ILs) are attractive, environmentally acceptable solvents because of their near-zero vapor pressure, thermal stability, and widely tunable properties through appropriate modification of the cations and anions.[5] Recently, ILs have been actively explored for a variety of applications including as media for catalytic reactions,[6] sensors,[7] biomolecules stabilization and biopreservation.[8] For instance, some proteins and DNA have been found that they can be stabilized at higher temperatures in IL media as compared to aqueous solution.[8] Additionally, in some cases, the reactive catalytic species or reaction intermediates can also be stabilized in ILs, which may significantly improve the catalytic performance or even make it possible to achieve catalytic reactions that are not possible to conduct in common solvents.[9]

Inspired by these unique features, herein, we propose a conceptually new approach: by utilizing IL as an efficient modulator, novel highly stable Au/SiO_2 hetero-nanocomposites with remained surface accessibility can serve as a robust and reusable peroxidase mimic for realizing high-temperature reactions (Scheme 1). Porous silica (SiNP) as the skeleton

Scheme 1. (a) Scheme Describing the Synthesis of Au/SiO_2 Catalyst; (b) Schematic Illustration for the Realization of High-Temperature Catalytic Reaction by Utilizing Thermally Stable Au/SiO_2 Catalyst as a Peroxidase Mimic and Ionic Liquid as a Modulator
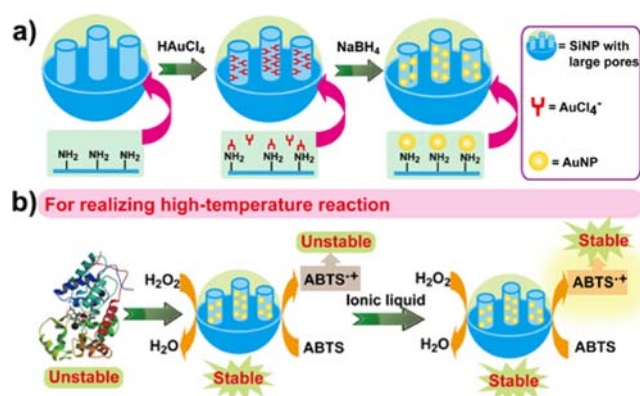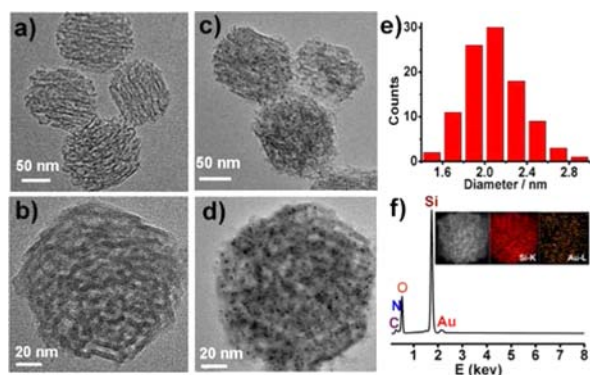

played a key role in the design and development of our new biomimetic catalyst. Initially, the encapsulation of AuNPs in SiNP provided a high density of very small and homogeneously dispersed AuNPs. Meanwhile, it was able to hinder the aggregation of neighboring particles and allow the diffusion of small active molecules in and out of the nanospheres. Here, the Au/SiO_2 nanocatalyst exhibited high peroxidase-like activity that could catalyze the reaction of peroxidase substrate ABTS in the presence of H_2O_2 to produce a colored product $ABTS^{·+}$. In contrast, commonly used AuNPs stabilized by charge or steric repulsion were inactive in catalytic reactions. Furthermore, the heterogeneous nanostructures-based artificial enzyme offered a new opportunity to achieve catalysis with high operational stability and reusability. More significantly, ILs could act as a stabilizing agent for enhancing the thermal stability of enzymatic product, thus enabling high-temperature catalytic reactions. Such unexpected promotion of catalytic activity at high temperature may eventually be used in practical catalysis. To the best of our knowledge, the present work demonstrates for the first time that peroxidase mimics can realize efficient high-temperature reactions.
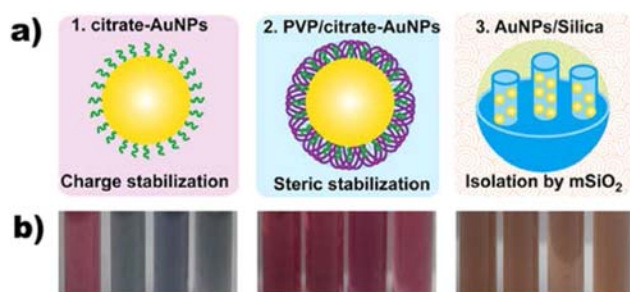
Scheme 1a illustrates the rather simple procedure of using SiNP with large pores as porous support to encapsulate AuNPs. Recently, ordered mesoporous materials with large pores are in urgent demand for applications in the encapsulation of functional nanoparticles without the possibility of causing pore blocking.[10] In this work, SiNP with large pores was first synthesized according to a swelling agent incorporation method.[11] The degree of pore size expansion could be easily controlled by adjusting the synthesis conditions such as reaction temperature and time. Here, mesopores were expanded to about 3.1 times the size (8.7 nm, mean pore size) of the original one (2.8 nm), according to nitrogen sorption data (Figure S1a,b). The obtained large-pore SiNP was studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), indicating that the prepared SiNP had outer diameter of about 100 nm, while retaining spherical morphology and monodispersity (Figure 1a,b, Figure S2). Its surface was then functionalized with amine

Figure 2. (a) Schematic illustration for different types of Au catalysts for mimicking peroxidase. (b) The corresponding stability of these catalysts in the presence of various solutes: (1) control; (2) 1 M NaCl; (3) 50% DMSO; (4) 20% [ch][dhp].

Figure 1. TEM images of large pore silica nanoparticles (a, b) and Au/SiO$_2$ catalyst (c, d). (e) Size distribution histogram of AuNPs in Au/SiO$_2$ catalyst. (f) EDX pattern of Au/SiO$_2$ catalyst. Inset shows the dark-field TEM image, and corresponding TEM elemental mappings.

groups by treatment with APTES,[12] and these groups present on the wall structure can act as a stabilizing agent by providing an anchoring surface.[13] The next step is to adsorb AuCl$_4^-$ to the NH$_2$-group-rich surface via electronic interactions. Finally, AuNPs were immobilized onto the SiNP surface by in situ reduction of auric chloride ions. TEM images and the corresponding size distribution histogram revealed that a high density of dispersed AuNPs with an average size of 2.1 nm supported on the SiNP surface(Figure 1c−e), which were expected to exhibit much higher catalytic activity.[14] The formation of AuNPs was also verified by the energy-dispersive X-ray (EDX) spectra and the X-ray powder diffraction (XRD) pattern (Figure 1f, Figure S3). Meanwhile, the corresponding TEM elemental mappings indicated the uniform distribution of Si and Au elements in the same particle.

Next, the stability of Au/SiO$_2$ hetero-nanocomposites was tested under different conditions, compared with that of commonly used AuNPs stabilized by charge or steric repulsion (Figure 2a). Figure 2b and Figure S4 show the behavior of citrate-capped AuNPs, PVP/citrate-AuNPs and Au/SiO$_2$ hetero-nanocomposites in the presence of salt, organic solvents, and ILs. Aggregation of citrate-AuNPs can be easily observed via a red-to-blue color change, whereas aggregation of PVP/citrate-AuNPs and Au/SiO$_2$ nanocatalyst does not occur under these relatively hash conditions. These results indicate that citrate-AuNPs via charge stabilization is sensitive to environ-

mental conditions, such as salt and ILs. Although PVP/citrate-AuNPs via steric stabilization are stable, direct application of these nanoparticles as catalysts also faces significant challenges because the coated polymers will block the particle surface from adsorbing other molecules.[15] Interestingly, the immobilization of AuNPs on the mesoporous support is able to hinder the interaction between NPs and keep these gold catalysts stable, while in the meantime, allow the diffusion of small active molecules in and out of the nanospheres.[10b]

The peroxidase-mimicking activity of different Au nanomaterials was then evaluated in the IL. Although ILs have been widely explored in various bioapplications in recent years, surprisingly, no attempt to apply ILs to artificial enzyme system has been reported so far. In this study, we carefully investigated the interactions between ILs and artificial enzyme, which might allow for broader applications. A representative IL, choline dihydrogen phosphate ([ch][dhp]), was used as a model system to provide the "proof-of-principle" verification of the concept. The catalytic activities were characterized in the ABTS-H$_2$O$_2$ reaction system. As shown in Figure 3 and Figure
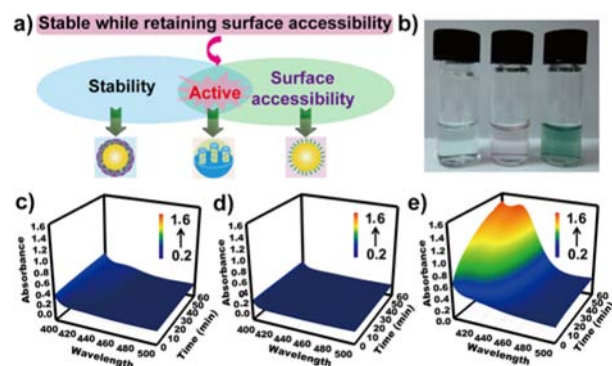
Figure 3. (a) Effect of stability and surface accessibility on the peroxidase-mimicking activity of Au catalysts. Visual color changes (b) and the corresponding time-dependent absorption spectra of different Au catalysts (c−e) in the presence of 1 mM ABTS, 60 mM H$_2$O$_2$ and 20% [ch][dhp].

S5, the oxidation of ABTS produced a green color with major absorbance peaks at 417 nm, indicating that our Au/SiO$_2$ hetero-nanocomposites exhibit peroxidase-like catalytic activity in the IL.[1d,3d] Control studies indicated the enzymatic reactions did not ensue in the absence of H$_2$O$_2$ or catalyst (Figure S6). Meanwhile, the catalytic activity is dependent on catalyst concentration and pH (Figure S6, S7). Additionally, the reaction rate decreased gradually with increasing concentrations

of [ch][dhp] (Figure S8), which is probably associated their viscosity[6a,d] and their impact on catalyst activity[8b] (Figure S9). Above results demonstrate that Au/SiO$_2$ catalyst can be served as an effective artificial enzyme in ILs. In contrast, neither citrate-capped AuNPs nor PVP/citrate-AuNPs could efficiently oxidize ABTS (Figure 3b−d), further demonstrating that the enzyme-like activity of AuNPs is highly dependent on their colloidal stability and surface accessibility.[15a] Our finding could offer a facile and efficient strategy for tuning catalysts from inert to active in ILs.

One of the most important advantages for heterogeneous catalyst is that this catalyst can be recovered and reused.[16] To test its reusability, we compared the fresh and recovered Au/SiO$_2$ catalyst during the 5-min peroxidization of ABTS in a solution of 20% [ch][dhp]. The result shows that the amount of product in the eighth run reaches 71% of that in the first run (Figure S10). Another notable feature is its high thermal stability, compared with natural enzyme (HRP). The activity of natural enzyme is completely inhibited under heating (85 °C, 5 min), whereas the activity of Au/SiO$_2$ catalyst after heating almost remains the same as before (Figure S11). Therefore, these attractive properties are particularly useful for industrial applications.

After have demonstrated that our Au/SiO$_2$ nanocatalyst exhibits high peroxidase-mimicking catalytic activity in the IL, we then address the possibility of applying thermal stable artificial enzyme to high-temperature reactions with the aid of IL (Scheme 1b, Figure 4). Initially, we explore the possibility of using our enzyme mimic to catalyze the substrate oxidation reaction in the absence of IL since it possesses high thermal stability. Unfortunately, similar to HRP and other peroxidase mimic (e.g., Fe$_3$O$_4$, graphene oxid),[1d,3d] the catalytic perform-

ance of Au/SiO$_2$ catalyst was far below expectations, which attained its highest catalytic activity at ~55 °C in buffer and deactivated at ~65 °C (Figure 4b). Previous work revealed that enzymatic product (ABTS$^{·+}$) is relatively unstable, and decays to a colorless product through disproportionation.[4] We speculated that its insufficient stability might seriously restrict further high-temperature applications. As seen in Figure 4c and Figure S12, ABTS$^{·+}$ is unstable and very rapidly degraded in buffer when handled at relatively high temperature (demonstrated by the color change and the fast decreasing of its UV−vis signal). Therefore, the thermal stability of product plays an important role in high-temperature catalysis, which has been largely ignored. Although regulation of enzyme-catalyzed reaction has always attracted great attention,[17] the exploring of efficient modulator that make peroxidase mimics possible to achieve high-temperature reactions still remains a big challenge. Inspired by the unique properties of ILs,[8,9] we reasoned that IL could serve as a stabilizing agent for improving thermal stability of ABTS$^{·+}$, and subsequently enabling high-temperature reaction that are not working efficiently in buffer. As expected, ABTS$^{·+}$ are tremendously stabilized upon addition of 20% [ch][dhp] under heating (Figure 4c). More importantly, the activity of nanocomposites in the presence of [ch][dhp] was much greater than that obtained in the buffer at high temperature (Figure 4b), suggesting that IL imparts substantial thermostability to ABTS$^{·+}$. To further demonstrate the ability of IL to inhibit ABTS$^{·+}$ disproportionation, another experiment was designed (Figure 4d), in which [ch][dhp] was added to Au/SiO$_2$ catalyst catalyzed ABTS/H$_2$O$_2$ system after the reaction was performed in buffer for 10 min. Initially, the absorption signal gradually intensified during the first 125 s, whereas, after then, the decrease in absorption signal was observed, which could be attributed to the disproportionation of ABTS$^{·+}$. Interestingly, when [ch][dhp] was added, no color fade was observed; on the contrary, the absorption signal increased again, demonstrating that ABTS$^{·+}$ disproportionation was efficiently inhibited by IL. In addition, control studies indicated that another IL (20% [BMIm]BF$_4$) could also have positive effect on nanocatalyst activity and ABTS$^{·+}$ stability at high temperature (Figure 4e). Similarly, another peroxidase substrate, TMB, also had high activity in these ILs compared with that in the buffer (Figure S13). Significant enhancement of the activities suggests that ILs play an important role in catalysis, and their positive effect is possibly connected with their greater solvation power (Figure S14),[8e] a large number of cations and anions, and their weak interactions with substrate and product. Taken together, although the detailed mechanism remains unclear, these observations demonstrated that IL can act as a stabilizing agent for enhancing thermostability, which offers a strategy for realizing high-temperature catalysis (Figure S15).

In summary, we have demonstrated the first example that AuNPs encapsulated in porous support could serve as a robust and efficient peroxidase mimic at high temperature by utilizing ILs as an efficient modulator. Porous silica as the skeleton assisted the synthesis of a high density of very small and stable AuNPs and facilitated the catalytic reaction by providing porous diffusion channels. Furthermore, our new artificial enzyme exhibits high thermal stability and excellent reusability. More importantly, the positive effect of ILs on the artificial enzyme system may eventually be utilized advantageously in practical catalysis. Our findings pave the way to use hetero-nano-composites for the design and development of highly stable
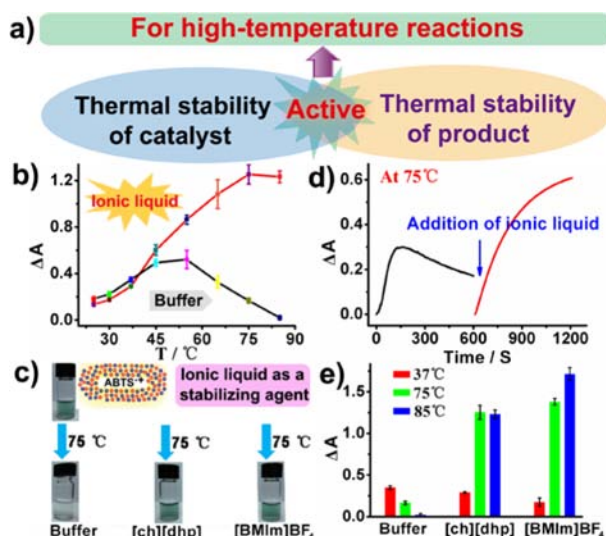
Figure 4. (a) Critical factors for realizing high-temperature reactions. (b) Catalytic activities of Au/SiO$_2$ catalyst as a function of incubation temperature in buffer or 20% [ch][dhp]. (c) Thermal stability of enzymatic product in different fluids at 75 °C; inset shows IL can act as a stabilizing agent. (Product results from the oxidation of ABTS by Au/SiO$_2$/H$_2$O$_2$ at 37 °C.) (d) Time-dependent absorbance changes at 417 nm as a result of the catalyzed oxidation of ABTS at 75 °C: (Black) absorption changes in buffer; (red) absorption changes after addition of 20% [ch][dhp]. (e) The temperature-dependent catalytic activities of Au/SiO$_2$ catalyst in buffer; 20% [ch][dhp]; 20% [BMIm]BF$_4$.

biomimetic catalysts with remained surface accessibility, and offer a strategy for understanding the role of ILs in artificial enzyme system.

ASSOCIATED CONTENT

● Supporting Information

Detailed experimental procedures and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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