DOI: 10.1002/cplu.201200051

Fast and Sensitive Colorimetric Detection of H_2O_2 and Glucose: A Strategy Based on Polyoxometalate Clusters

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Natural enzymes have been intensively studied for a long time and play central roles in biochemistry.[1,2] Natural enzymes as biological catalysts possess remarkable advantages such as high substrate specificities and high efficiency under mild conditions and have significant practical applications in medicine, the chemical industry, food processing, and agriculture. Unfortunately, natural enzymes are proteins and inherently bear some serious disadvantages, such as easy denaturation by environmental changes, digestion by proteases, as well as time-consuming and expensive preparation and purification.[3] Therefore, much effort has been put into developing artificial enzyme mimics.[2,4–8]

The accurate determination of H_2O_2 is of great practical importance in many fields such as food, pharmaceutical, clinical, industrial and environment protection.[9] It is also important for minimizing diabetic complications to maintain blood glucose concentrations within the normal physiological range for control of diabetes mellitus. Peroxidase has great potential for practical application and can be used as a diagnostic kit for H_2O_2 and glucose.[10] From the point view of artificial enzyme, it is highly desired to develop peroxidase mimetics for practical applications. Indeed, several compounds, including hemin,[11] hematin, hemoglobin, cyclodextrin, and porphyrin, and a wealth of nanostructures, such as Prussian blue nanoparticles, nanostructured FeS,[12] V_2O_5 nanowires,[13] bimetallic alloy nanoparticles,[14] positively-charged gold nanoparticles,[15] Fe_3O_4 nanoparticles,[16,20] polymer-coated CeO_2 nanoparticles,[17] carbon dots,[18] single-wall carbon nanotubes,[19] and graphene oxide (GO),[10] have been used as peroxidase-like catalysts for colorimetric detection of H_2O_2 and (or) glucose.[21] More recently, carboxyl-functionalized mesoporous polymers,[22] iron-substituted SBA-15 microparticles,[23] and photoluminescent carbon nitride dots,[24] and iron(III)-based coordination polymer nanoparticles[25] have also been applied by our research group in this assay. All of the above-mentioned nano/microstructure-based systems, however, suffer from drawbacks, such as relatively long reaction times and relatively high detection limit. It pushes us to explore new peroxidase mimetics capable of fast and sensitive detection of H_2O_2 and glucose.

Polyoxometalates (POMs) are nanometer-sized metal oxide cluster compounds, which have attracted considerable attention in the fields of catalysis, medicine, surface science, and materials science owing to their nanosize and tunable acid/base, redox, magnetic, catalytic, and photochemical properties.[26,27] It is well-documented that POMs can catalyze H_2O_2-based epoxidation and oxidation of organic substrates by O_2 and H_2O_2 by multistep electron-transfer processes.[28–30] Given the general concept used in colorimetric detection of H_2O_2 lies in the fact that H_2O_2 oxidizes peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of catalysts to produce a blue color. It is rational to believe that POMs can be used as effective inorganic molecular catalysts for the oxidation of TMB using H_2O_2. Thus, a novel colorimetric detection system for H_2O_2 and glucose was subsequently developed. It is common knowledge that reactions take place at the surface of catalysts, rather than in the bulk of the catalyst. Small-sized catalysts have a higher surface-to-volume ratio and hence are superior to bigger catalysts in terms of catalytic performance. Given POMs molecular clusters are much smaller in size than other nano/microstrcutures-based peroxidase mimetics, a faster reaction kinetics (shorter detection time) and a lower detection limit may be achieved with such systems based on POMs. To the best of our knowledge, however, such a concept has never been proven before.

Herein, we demonstrates the proof-of-concept of using silicotungstic acid ($H_4SiW_{12}O_{40}$, denoted as HSiW), a typical POMs with the size about 1.1 nm[31] as a peroxidase mimetic to catalyze H_2O_2-based oxidation of various peroxidase substrates including TMB, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS), and *o*-phenylenediamine (OPD) to produce colored solutions. We further demonstrates a simple, fast, sensitive, and selective colorimetric assay to detect H_2O_2, with the linear range and detection limit estimated to be from 1 to 20 μM and 0.4 μM, respectively. The application of this colorimetric assay to glucose detection both in buffer solution and diluted serum was also demonstrated successfully. This glucose sensor exhibits excellent performance with a linear range from 1 to 10 μM and detection limit at about 0.5 μM.

We present proof-of-concept to demonstrate the feasibility of using HSiW as a model system for colorimetric detection of H_2O_2. Figure 1 shows the photographs of solutions of TMB, TMB + H_2O_2, TMB + HSiW + H_2O_2, TMB + HSiW, and HSiW in

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cplu.201200051.

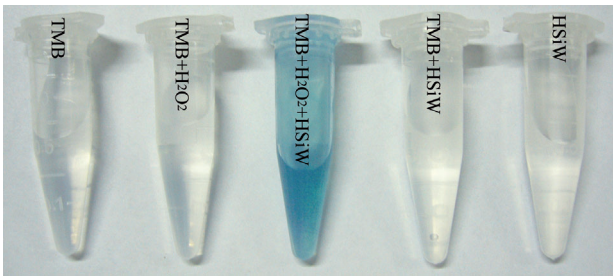

Figure 1. Photographs of solutions of TMB, TMB + H_2O_2, TMB + HSiW + H_2O_2, TMB + HSiW, and HSiW in pH 4.0 NaAc buffer ([TMB] = 0.1 mM, [H_2O_2] = 44 mM, [HSiW, $H_4SiW_{12}O_{40}$] = 1.5 mM).

pH 4.0 NaAc buffer. It is seen that no color change is observed for the solution of TMB in the presence of H_2O_2, indicating that no oxidation reaction has occurred.[22] Furthermore, no color change was observed for both the HSiW and TMB + HSiW solutions in the absence of H_2O_2. In contrast, the TMB solution changed to a blue color after addition of HSiW solution in the presence of H_2O_2. All these observations clearly show that HSiW indeed catalyzes the oxidation of TMB by H_2O_2.

The UV/Vis spectra of all above samples are shown in Figure 2 further reveal the differences between them. It is seen that both solutions of TMB in the absence and presence of

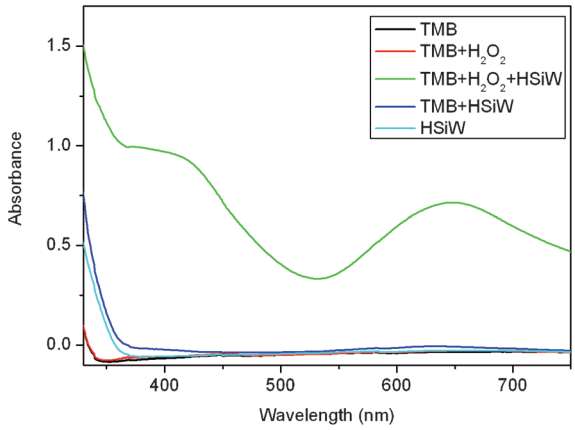

Figure 2. UV/Vis spectra of solutions of TMB, TMB + H_2O_2, TMB + HSiW + H_2O_2, TMB + HSiW, and HSiW in pH 4.0 NaAc buffer ([TMB] = 0.1 mM, [H_2O_2] = 44 mM, [HSiW, $H_4SiW_{12}O_{40}$] = 1.5 mM).

H_2O_2 exhibit no strong absorption bands ranging from 330 to 800 nm. However, after addition of HSiW, the TMB + H_2O_2 solution exhibits two strong absorption peaks centered at 456 and 651 nm, respectively, and indicate the fact that HSiW catalyzes the oxidation of TMB by H_2O_2.[16] All these observations further confirm that HSiW possesses peroxidase-like activity for the oxidation of TMB by H_2O_2 and hence can be used for colorimetric detection of H_2O_2. It should be noted that only 5 minutes is required to complete the oxidation of TMB by H_2O_2 in our present system. This timeframe is much faster than previously reported systems based on Fe_3O_4 nanoparticles (10 min),[20] gold nanoparticles (10 min),[15] and carbon dots (30 min).[18]

The peroxidase-like activity of HSiW was also examined by the catalytic oxidation of other peroxidase substrates such as ABTS, and OPD, by H_2O_2. An absorption peak at 415 nm and a blue solution attributed to the oxidation product of ABTS were observed upon addition of HSiW into a solution of ABTS in the presence of H_2O_2 (Figure S1a in the Supporting Information). Furthermore, an absorption peak at 450 nm and a yellow solution attributed to the oxidation product of OPD were also observed by addition of HSiW into a solution of OPD in the presence of H_2O_2 (Figure S1b). All these observations further confirm the peroxidase-like activity of HSiW.

We further examined the effects of pH value, temperature, and concentration of H_2O_2 on the catalytic activity of both HSiW and natural horseradish peroxidase (HRP) toward the oxidation of TMB by H_2O_2 (Figure S2–S4). It is seen that HSiW shows pH-, temperature-, and H_2O_2 concentration-dependent catalytic activity, and the maximum catalytic activity was obtained under the following optimal conditions: pH 4.0, 40 °C, and 200 mM H_2O_2. It should be mentioned that HSiW exhibits good catalytic stability and is more stable than HRP at relatively high temperature and requires a high concentration of H_2O_2 (200 mM) to reach the maximum level of peroxidase-like activity. Thus, HSiW-based H_2O_2 detection can be carried out over a wide range of temperature and H_2O_2 concentration.

As demonstrated above, the color variation of TMB oxidation catalyzed by HSiW was H_2O_2 concentration-dependent, and therefore, we designed a colorimetric method for detection of H_2O_2. The inset of Figure 3 shows the dependence of the ab-

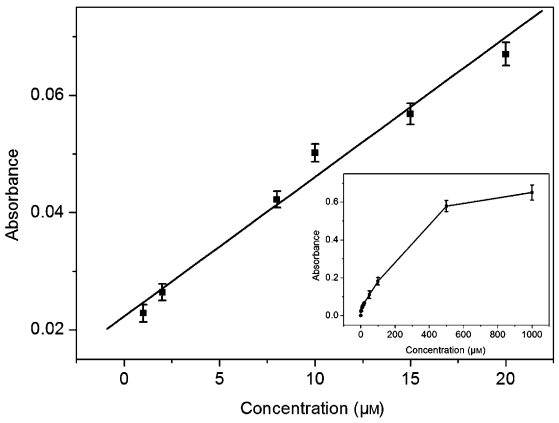

Figure 3. The linear calibration plot of the absorbance at 651 nm on the concentration of H_2O_2 from 1 to 20 μM. The inset shows dependence of the absorbance at 651 nm on the concentration of H_2O_2 in the range from 0 to 1 mM.

sorbance at 651 nm on the concentration of H_2O_2, thus revealing that the intensity of this absorption peak increases with increased H_2O_2 concentration from 0 to 1 mM. Figure 3 shows the corresponding calibration plot. The linear detection range is estimated to be from 1 to 20 μM (r = 0.985) and the detection limit is estimated to be 0.4 μM. It is worthwhile mentioning that our HSiW-based sensing system gives lower detection limit than those based on Fe_3O_4 nanoparticles (3 μM)[20] and

gold nanoparticles (0.5 μM),[15] and could be attributed to the particularly small particle size of HSiW molecular clusters.

We also designed a colorimetric method for detection of glucose using this HSiW-based sensing system. It is well known that glucose oxidase (GOx) catalyzes the oxidation of glucose to gluconolactone and this property is exploited in the fabrication of amperometric sensors with high sensitivity and selectivity. Owing to the fact that GOx would be denatured in pH 4.0 buffer, a two-step reaction was carried out: 1) GOx catalyzes oxidation of glucose to gluconic acid in a pH 7.0 buffer solution, while in the meantime, the substrate O_2 is converted into H_2O_2, and 2) the resultant H_2O_2 catalyzes oxidation of TMB in the presence of HSiW to produce a blue solution. The inset of Figure 4a shows the dependence of the absorbance at 651 nm

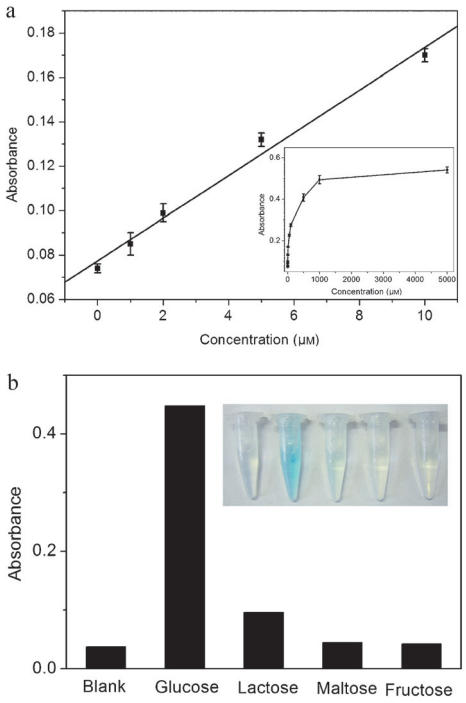

Figure 4. a) The linear calibration plot of the absorbance at 651 nm on the concentration of glucose from 0 to 10 μM. The inset shows dependence of the absorbance at 651 nm on the concentration of glucose in the range from 0 to 5 mM. b) Determination of the specificity of GOx for glucose detection with 5 mM lactose, 5 mM maltose, 5 mM fructose, and 1 mM glucose. The inset shows color change with the different solutions.

on the concentration of glucose from 0 to 5 mM, thus indicating that the intensity of this absorption peak increases with increased glucose concentration. The corresponding calibration plot is shown in Figure 4a. The linear detection range is estimated to be from 1 to 10 μM ($r = 0.994$). The detection limit is estimated to be 0.5 μM, which is lower than those of previous reports based on GO (1 μM),[10] Fe_3O_4 nanoparticles (30 μM),[20] and gold nanoparticles (4 μM).[15]

To further examine the specificity of GOx toward glucose, detection experiments were performed in the absence of glucose and in the presence of glucose, lactose, maltose, and fructose. A blue solution was obtained in the presence of glucose, however, no obvious blue color was observed for other compounds even though their concentration was five-fold that of glucose (Figure 4b inset). Figure 4b shows the corresponding intensity of absorbance at 651 nm for all samples measured. Taken together, these observations indicate our sensing system can be used for the detection of glucose.

In further experiments, the potential application of this sensing platform for real sample analysis was challenged with samples of human blood serum by detection of glucose in the diluted blood serum. According to the calibration curve, the concentration of glucose is about 5.1 mM—the general range of blood glucose concentration in healthy people is about 3 to 8 mM. The concentration of glucose in another blood samples (from another person) is about 6.4 mM, which is similar to the result provided by The First Hospital of Jilin University (6.36 mM; Table S1). The relative standard deviation (RSD) of the response to 6.4 μM of glucose is 5.7% for three successive measurements. Table S1 shows the results of glucose detection in diluted blood by addition of various amounts of glucose. Figure S4 shows the results of detection of glucose in diluted blood without addition of glucose and addition of glucose, lactose, maltose, and fructose, respectively. All these observations indicate that, upon further development, the present assay based on our HSiW colorimetric method might to be capable of practical use for glucose detection.

The mechanism for detection of H_2O_2 in our present study is proposed as follow: The tungstate in HSiW was oxidized to a peroxotungstate such as $[W_2O_3(O_2)_4(H_2O)_2]^{2-}$ by H_2O_2 first[28–30] and then the resultant peroxo species, which exhibits strong oxidation ability, subsequently catalyze the oxidation of TMB to produce a blue solution. Because H_2O_2 is a main product of GOx-catalyzed reactions,[31–34] colorimetric detection of glucose can also be realized. Figure 5 illustrates the colorimetric detection of H_2O_2 and glucose using HSiW as a peroxidase-like catalyst. It should be noted that our sensing system exhibits some advantages as compared to the hemin/G-quadruplex catalyst, such as high stability and low cost.

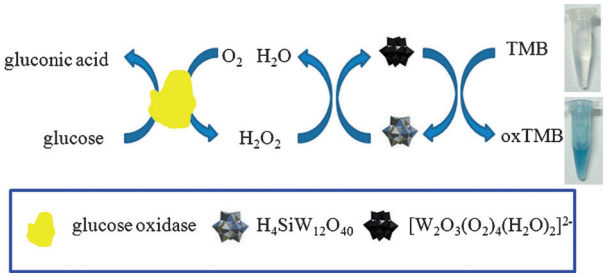

Figure 5. An illustration (not to scale) of the colorimetric detection of H_2O_2 and glucose using HSiW as a peroxidase-like catalyst.

In summary, a novel strategy based on POM molecular clusters for the colorimetric detection of H_2O_2 and glucose has been developed for the first time. This detection system is simple, fast, sensitive, and selective. Our present observations are important because it provides us a novel peroxidase mimetic for biosensing applications.

Experimental Section

Materials

Acetic acid, TMB, ABTS, OPD, and HSiW were purchased from Aladin Ltd. (Shanghai, China). NaH_2PO_4, Na_2HPO_4, sodium acetate (NaAc), H_2O_2 (30%), glucose, fructose, lactose, and maltose were purchased from Beijing Chemical Corp. Horseradish peroxidase (HRP) and glucose oxidase (GOx) were purchased from Aldrich Corp. All the chemicals were used as received without further purification. The water used throughout all experiments was purified through a Millipore system. UV/Vis spectra were measured on a UV5800 spectrophotometer.

Detection of H_2O_2 and glucose

For detection of H_2O_2, kinetic measurements were carried out by monitoring the absorbance change at 651 nm. In a typical run, 15 μL of HSiW solution (0.1 M) was added into 800 μL of NaAc buffer solution (pH = 4.0), followed by the addition of 200 μL of TMB solution (1 mM in ethanol) and 5 μL of H_2O_2. The UV/Vis spectra were recorded after reaction for 5 min at 40 °C. Glucose detection was performed as follows: 1) 100 μL of 1 mg mL^{-1} GOx, 100 μL of glucose of different concentrations, and 200 μL of 10 mM Na_2HPO_4 buffer (pH 7.0) were incubated at 37 °C for 60 min. 2) 200 μL of TMB (1 mM in ethanol), 15 μL of HSiW solution, and 800 μL of NaAc solution were added into above glucose reaction solution. 3) The mixed solution was incubated at 40 °C for 60 min to measure the concentrations of glucose.

Acknowledgement

This study was supported by the National Basic Research Program of China (No. 2011CB935800) and the National Natural Science Foundation of China (No. 21175129).

Keywords: colorimetric detection · glucose · peroxidase-like catalysts · polyoxometalates · sensors

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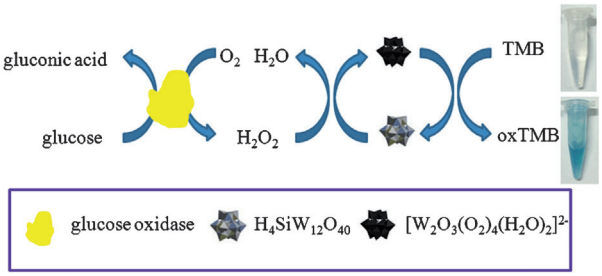
Received: March 7, 2012
Published online on ■■ ▮▮, 0000



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Fast and Sensitive Colorimetric Detection of H_2O_2 and Glucose: A Strategy Based on Polyoxometalate Clusters

Proof-of-concept: A polyoxometalate cluster has been used as a highly effective peroxidase mimetic to catalyze H_2O_2 oxidation of various peroxidase substrates to give colored solutions, leading to a simple, fast, sensitive, and selective assay for H_2O_2 with a linear range and detection limit of 1–20 and 0.4 μM. This assay was also used for glucose detection both in buffer solution and diluted serum with a linear range and detection limit of 1–10 and 0.5 μM.

