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Characterization of glucose oxidation by gold nanoparticles using nanoceria

Nathan J. Lang, Biwu Liu, Juewen Liu *

Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

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ABSTRACT

Gold nanoparticles (AuNPs) can oxidize glucose, producing hydrogen peroxide and gluconic acid, which are the same products as those generated by glucose oxidase (GOx). In this regard, AuNPs are a nanozyme. Herein, a new colorimetric method is developed to understand the surface chemistry of gold nanoparticles for this oxidation reaction. The color of nanoceria is changed to yellow by the hydrogen peroxide generated during glucose oxidation. Using this assay, we find that adsorption of small molecules such as citrate does not deactivate AuNPs, while adsorption of polymers including serum proteins and high molecular weight polyethylene glycol inhibits glucose oxidation. In addition to glucose, AuNPs can also oxidize galactose. Therefore, this reaction is unlikely to be directly useful for glucose detection for biomedical applications. On the other hand, AuNPs might serve as a general oxidase for a broad range of substrates. The glucose oxidation reaction is slower at lower pH. Since the reaction generates an acid product, glucose oxidation becomes slower as the reaction proceeds. The effects of temperature, AuNP size, and reaction kinetics have been systematically studied. This work provides new insights regarding the surface chemistry of AuNPs as a nanozyme.

1. Introduction

Nanozymes are nanoparticles with catalytic activity [1–3]. In the past decade, gold nanoparticles (AuNPs) [4–7], magnetic iron oxide NPs [8], and cerium oxide NPs (nanoceria) have been reported to mimic various enzymes [9–16]. Oxidation of glucose by AuNPs was first reported by Comotti et al. in 2004 [4]. In this reaction, oxygen and glucose are consumed to produce gluconic acid and hydrogen peroxide (H_2O_2) [4–6,17,18], which are the same products as those generated by glucose oxidase (GOx). Therefore, AuNPs are a mimic of GOx. The enzyme properties of these two have been systematically compared and the k_{cat} and K_m values are reported to be quite similar [6].

Recently, analytical chemists picked up this reaction for biosensor development and many interesting observations were made. For example, the H_2O_2 generated during glucose oxidation was used as a reducing agent to react with $HAuCl_4$ [19]. The newly reduced gold is deposited on the original particle surface to produce enlarged AuNPs. The growth of AuNP size is monitored by the shift of the surface plasmon peak or color change [5,6]. With a short single-stranded DNA, glucose oxidation by AuNPs is impeded, which is attributed to the adsorption of DNA onto the gold surface. In addition, it was noticed that AuNP nanozymes seem to be deactivated in the reaction process (so called self-limiting reaction). In other words, glucose conversion becomes progressively slower as the reaction proceeds. This gives a relatively small turnover number, which may compromise its application. This self-limiting behavior was attributed to the capping of AuNP surface by the gluconate product [4–6]. Both DNA adsorption and gluconate adsorption indicate the importance of the surface chemistry of AuNPs for catalysis.

Despite these progresses, a lot remains to be learned to fully understand AuNP nanozymes. First, we are intrigued by the surface chemistry aspect of this reaction. In particular, we aim to compare the activity of AuNPs as a function of the binding affinity and size of surface ligands. Second, we study the buffer conditions, from which we suggest an alternative explanation for the self-limiting reaction. Finally, protein enzymes have excellent substrate specificity. It is unclear whether AuNPs are specific for glucose; a few other sugar molecules have been tested in this work as well.

2. Materials and methods

2.1. Chemicals

AuNPs (5, 10, 20, 30, and 50 and 100 nm) were purchased from Ted Pella Inc. AuNPs (5, 13, and 50 nm) were prepared by $NaBH_4$ or

* Corresponding author.
E-mail address: liujw@uwaterloo.ca (J. Liu).

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citrate reduction in our own lab. $HAuCl_4$, nanoceria, glucose, sodium gluconate, galactose, fructose, bovine serum, and hydrogen peroxide were purchased from Sigma–Aldrich. Trisodium citrate was from Mandel Scientific Inc. (Guelph, Ontario, Canada). Sucrose and all the PEG samples were from VWR. Milli-Q water was used for preparing all the solutions. The original 20% stock solution of nanoceria has a particle concentration of 860 μM. We typically dilute it first 47.3 times and then 32.26 times to reach a final particle concentration of ~564 nM in the final assay tube.

2.2. Preparing AuNPs

13 nm AuNPs were prepared by the standard citrate reduction method (particle concentration 10 nM) [20]. 5 nm AuNPs were prepared by mixing 125 μM $HAuCl_4$ and 1 mM $NaHCO_3$ with a freshly prepared 100 mM solution of $NaBH_4$, added in 10 μL drops. The initial volume was 40 mL. The solution was stirred and cooled with ice throughout the synthesis. The addition was stopped after adding ~240 μL of $NaBH_4$. Note that the color stops changing after adding roughly 40 μL. The molar concentration of the as-prepared 5 nm AuNP is ~4.4 nM.

2.3. UV–vis spectroscopy

In a typical assay, ~2.2 nM 5 nm AuNPs were mixed with 10 mM phosphate buffer (pH 8) and 5 mM glucose. After 45 min, the samples were treated with 10 mM KCN to dissolve the AuNPs, and then mixed with 564 nM (nanoparticle concentration) CeO_2. After CeO_2 addition, exposure to light was minimized, as this bleaches the yellow color produced by Ce reacting with H_2O_2. The samples were scanned using a UV–vis spectrometer (Agilent 8453A). The extinction ratio of 400 nm/290 nm was used to quantify the amount of H_2O_2 produced by the AuNP and glucose reaction. It is important to note that fresh nanoceria may need to be prepared occasionally as we found that the spectrum of the nanoceria by itself changed after a few days. It is also important to keep the pH buffered at roughly 8 for the AuNPs to work properly. To test the effect of AuNP size, 5, 10, 20, 30, 50, and 100 nm diameter AuNPs were mixed with 5 mM glucose and 10 mM phosphate buffer (pH 8).

2.4. Varying reaction conditions

To study the effect of PEG adsorption, AuNP samples were mixed with 1 mM of either PEG 200, 400, 2 k, 20 k or 35 k before the glucose addition. To test sensor specificity, AuNPs were mixed with 5 mM of either sucrose, fructose, galactose, ethylene glycol, or glycerol before glucose was added. To test the effect of citrate, 3 mM trisodium citrate was mixed with the $NaBH_4$ reduced 5 nm AuNPs before adding glucose. To test the sensor at different pH levels, a 10 mM citrate buffer of either pH 4, 6, or 8 was substituted for the 10 mM phosphate buffer used otherwise. pH 10 and 12 were achieved by adding NaOH to pH 8 phosphate buffer. To observe the effect of temperature, samples with 5 mM glucose and 5 nm AuNPs with phosphate buffer were incubated for 45 min at the appropriate temperature. For these experiments, the sample lids were shut to avoid evaporation of H_2O_2 or water.

2.5. Monitoring pH change

A 5 mL sample of 5 nm AuNPs were prepared without buffer. pH was monitored using a pH meter (UltraBasic, Denver Instrument) for 4 h, mixing with a final concentration of 1.5 mM NaOH approximately at the end of each hour.

3. Results and discussion

3.1. Visual detection

One method to monitor the glucose oxidation reaction is to add $HAuCl_4$ to produce new gold surfaces [6,21]. However, this will change the original gold surface chemistry, introducing artifacts for our mechanistic studies. To solve this problem, we developed a new method using nanoceria. Nanoceria normally has a light yellow color at high concentration. At low concentration, it is almost colorless. In the presence of H_2O_2, an intense yellow/orange color is generated [22]. The detailed reaction mechanism between nanoceria and H_2O_2 is quite complex, since H_2O_2 can act as an oxidizing agent, a reducing agent and a ligand [23]. This color change and the related redox reactions have been applied to design various sensors [9,22,24–26].

Our reaction scheme is presented in Fig. 1A. AuNPs convert glucose into gluconic acid, which dissociates into gluconate and proton. Hydrogen peroxide is also produced in the same process. We employ nanoceria as the colorimetric reporter for H_2O_2. Our AuNPs were prepared by reducing $HAuCl_4$ with $NaBH_4$, yielding an average particle diameter of ~5 nm as characterized by dynamic light scattering (DLS, Fig. 1B, red trace). Transmission electron microscopy (TEM) shows that these AuNPs are spherical (Fig. S1, Electronic Supplementary Information, ESI). Our nanoceria has a diameter of ~5 nm as characterized by TEM (Fig. 1D), and DLS showed a similar average size of but a broader size distribution (Fig. 1B, blue trace). With a concentration of 0.02% nanoceria (860 nM particle concentration), almost no color is initially perceived by the naked human eye; however adding H_2O_2 produces a bright yellow color (Fig. 1A). The UV–vis spectrum of fresh nanoceria has a peak at 290 nm. In the presence of H_2O_2, this peak disappears while the absorption at ~400 nm increases. By subtracting the original nanoceria spectrum (black trace, Fig. 1A) from the one after adding H_2O_2 (normalized at 290 nm), a difference spectrum with a peak at 400 nm was obtained (red dashed spectrum). This new species explains the yellow color. We estimated the extinction coefficient of 5 nm nanoceria (after H_2O_2 treatment) to be $10 \times 10^6\ M^{-1}\ cm^{-1}$ at 400 nm, which is comparable with AuNPs of similar size (e.g. $9 \times 10^6\ M^{-1}\ cm^{-1}$ for 4.6 nm AuNPs) [27]. Such a high extinction coefficient makes it possible to achieve sensitive visual detection. For subsequent quantitative studies, the ratio of absorbance at 400 nm over 290 nm was used to quantify the color change and thus of the amount of hydrogen peroxide produced in a sample. Such ratiometric methods are convenient for quantification and have been used to quantify the color change of AuNPs [28]. Using nanoceria to detect H_2O_2 is highly sensitive [22], and in our system we can easily detect 3 parts-per-million H_2O_2 (Fig. S2).

3.2. Effect of buffer conditions

To understand the kinetics of the reaction described in Fig. 1A, we mixed glucose with AuNPs and then added nanoceria at designated time points. To minimize the background color interference from AuNPs, KCN was added to dissolve the AuNPs before adding nanoceria. Control experiments showed that KCN does not interfere with the reaction between nanoceria and H_2O_2 (Fig. S3). Without KCN, the surface plasmon peak of AuNPs can be observed at 520 nm (Fig. S4). As expected, higher ratio of absorption at 400 nm over 290 nm is observed by using longer incubation time and the reaction reaches a plateau in 30 min (Fig. 2A), where the absorbance ratio is ~0.08. There is still a lot of room for nanoceria to further change its color, since the ratio could reach ~0.4 based on the UV–vis spectra in Fig. 1C. Since the amount of glucose