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A Chromatic Sensor to Detect Free Radicals Using H_2O_2 as an Analyte with DTT and Au-NPs as Sensing Agents Microscopy

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Abstract

In this study, a simple chromatic biosensor for free radicals, which accumulate inside human bodies and can be identified as an essential sign of various diseases, is presented. Hydrogen peroxide (H_2O_2) was selected as a detection target as it is one of the main species of free radicals and owns longer life cycle than other species. Previous studies also showed that the cancer and cardiovascular disease were strongly correlated with the concentration of free radicals in urine greater than 10^{-4} M. Different concentrations of H_2O_2 were added into 1,4-dithiothreitol (DTT) solution for detection. The DTT could not only act as a common reductant reacting with H_2O_2 through redox reaction, but also cause the aggregation of gold-nanoparticles (Au-NPs), which resulted in color change of Au-NPs attributed to the effect of surface plasmon resonance (SPR). The H_2O_2 concentration is therefore can be detected from its correlation with the color change of H_2O_2/DTT/Au-NPs solutions, as more H_2O_2 in solution will lead to more redox reaction and consequently less DTT to cause Au-NPs aggregation. Results show that the H_2O_2 concentrations ranged between 10^{-1} M to 10^{-6} M can be detected by naked eyes from color change. In additions, ultraviolet-visible (UV-Vis) absorption spectra were measured to further verify the correlation. Furthermore, liquid transmission electron microscopy (liquid-TEM) was also used to confirm the aggregation of Au-NPs in solutions. From above mentioned methods, the feasibility of using a chromatic biosensing system for free radical detection was demonstrated, showing the potential for future disease detection.

Keywords: Chromatic biosensing system; H_2O_2; Free radical; 1,4-dithiothreitol (DTT); Gold nanoparticles (Au-NPs); UV-Vis; Liquid transmission electron microscopy (liquid-TEM)

Introduction

Chromatic biosensors utilizing the correlation between the concentration of substances and the color of readout have drawn dramatic attention in medical and clinical treatments [1]. The most outstanding advantage of this kind of techniques is that they are quite simple, inexpensive, and portable during investigating process. They are also extremely convenient since recognition of the results from this device can be achieved with naked eyes.

Studies have been conducted to use such chromatic means to measure substances, such as DNA, proteins and ions [2-4]. Among these studies, gold-nanoparticles (Au-NPs) have been extensively applied to chromatic biosensors attributed to its high biocompatibility and strong surface plasmon resonance (SPR) effect [5], which may consequently cause a red-to-purple color change and a red-shift absorption peak on the UV-Vis spectrum. For instance, Lim et al. used mannose-stabilized Au-NPs to detect glucose in human serum and found its detection limit at about 2 x 10^{-3} M [6]. Mirkin et al. used the Au-NPs modified with single-stranded DNA to detect certain species of polynucleotide [7]. However, even this strategy has been developed for years; few of them put the focus on free radical detection [8,9]. Therefore, this study is aimed at developing an innovative chromatic biosensor for free radical detection.

In recent years, there is growing attention toward the generation of free radicals inside human bodies. Free radicals are atoms, molecules or ions with unpaired electrons, which make them extremely active and tend to scavenge other electrons from human bodies, causing aging. In addition to aging, free radicals are also associated with cancer, cardiovascular diseases, and even mental diseases [10,11]. According to Long et al. [12], when the concentration of free radicals in urine exceeds 100 μM, it may significantly increase the incidence of these diseases. Therefore, the extent of free radicals accumulating inside bodies is believed to be an important index to determine health condition and thus developing a new kind of chromatic biosensor to monitor the concentration of free radicals is considered as an essential issue. In this study, H_2O_2 was selected as the detection target since it is one of the main species of free radicals and owns longer life cycle than other species.

Materials and Method

For the design of the chromatic biosensor, H_2O_2, 1,4-dithiothreitol (DTT) and citrate-stabilized Au-NPs were utilized in this work. As shown in Figure 1a, the DTT powder (7.713 mg) was dissolved in 500 mL de-ionized water to form 10^{-4} M DTT solution. Five beakers containing 166 μL of DTT solution individually were prepared. In order to oxidize DTT in various degrees, five different concentrations of H_2O_2 solutions including 10^{-1}, 10^{-4}, 10^{-5}, 10^{-6}, and 0 M (without H_2O_2 as a reference sample) were prepared, as the H_2O_2 concentration exceeding 10^{-4} M may imply the unhealthy condition of immune system as reported by Long et al. previously. After that, five different concentrations of H_2O_2 solutions were mixed with five previously prepared DTT solutions, respectively. The mixed DTT/H_2O_2 solutions were stirred with ultrasonic for 10 min to go through reduction-oxidation reaction. Finally, 1 mL citrate-stabilized Au-NPs (dispersed, color red) was added into each previous solution for continuous reaction under ultrasonic for 2 hr. The color change of the Au-NPs in solution was then observed as the readout to quantify the concentration of H_2O_2.

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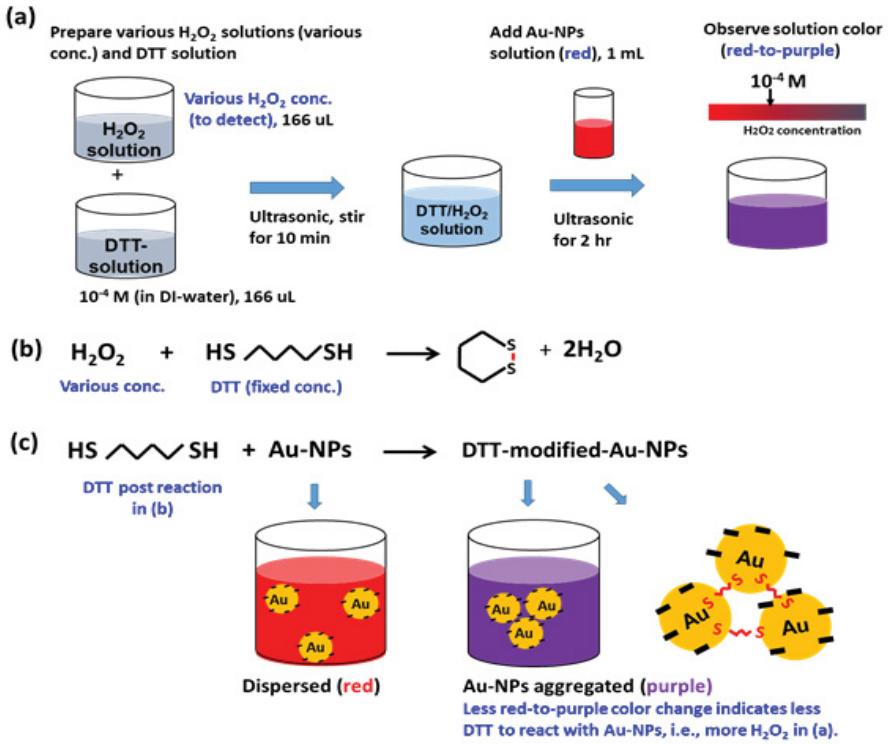

Figure 1: (a) The process flow and design concept of the simple chromatic biosensing system developed in this work, (b) the reduction-oxidation reaction of various concentrations of H_2O_2 with fixed concentration of DTT solution. (c) The residual DTT to reaction with dispersed Au-NPs (red) for the degree of color change to purple (aggregated Au-NPs) so as to correlate with H_2O_2 solution (i.e., the less color change, the more H_2O_2).

Basic concepts of simple chromatic biosensing system

The concept of above chromatic biosensor design is proposed as following. The DTT is a compact and small molecule with two thiol groups (-SH) at each two ends [13,14], as shown in Figure 1b. Since thiol groups can form strong S-Au covalent bonding with gold, DTT is believed to have high tendency to penetrate through the citrate corona and attach onto the surface of Au-NPs [15]. Assume as the concentration of DTT is high enough, some of the DTT molecules can attach on two different Au-NPs and act as a bridge between them. Therefore, the distance between Au-NPs can be shortened, causing the effect of Au-NPs aggregation. During this process, Au-NPs exhibit surface plasmaon resonance (SPR) effect, leading to a color change from wine-red to purple, as shown in Figure 1c. On the other hand, DTT can also be treated as a common organic reductant to react with H_2O_2 through reduction-oxidation reaction, transferring into the oxidized form of 6-membered ring with disulfide-bonding (S-S), which consumes thiol groups. In other words, the H_2O_2 with various concentrations may inhibit the ability of DTT to aggregate Au-NPs, which give rise to the color change from SPR effect as a readout, in varying degrees. In brief, it is expected that more reduction-oxidation between H_2O_2 and DTT in solution in Figure 1b will occur at a higher concentration of H_2O_2 concentration. It is therefore to inhibit more of DTT to react with Au-NPs (i.e. less DTT-modified-Au-NPs formed for Au-NPs aggregation/conjugation), consequently less degree of red-to-purple color change in solution. In this work, ultraviolet-visible (UV-Vis) absorption spectra were measured on the solutions with Au-NPs for the quantified analyses of red-shift to further confirm the correlation in addition to naked eyes observation. The liquid transmission electron microscopy (liquid-TEM) developed earlier in this group utilizing a disposable K-kit [16], now produced by Bio MA-Tek, for sample loading was also used for the observation of H_2O_2/DTT/Au-NPs solution to confirm the aggregation of Au-NPs.

Results and Discussion

Naked eye observation on the color of the H_2O_2/DTT/Au-NPs solutions

The color of H_2O_2/DTT/Au-NPs solutions with various H_2O_2 concentrations was observed by naked eyes. As shown in Figure 2a, the difference on the color of the solutions with 10^{-1} M to 10^{-6} M H_2O_2 and that without H_2O_2 (0 M) can be observed. It shows less degree of color-change to purple for the solution with 10^{-1}M compared to that of 10^{-6} M H_2O_2. The color for the solution with a concentration 10^{-1} M shows wine-red and as the concentration of H_2O_2 gradually decrease down to 10^{-6} M and 0 M (without H_2O_2), it shows a slight change to dark-purple.

As mentioned, this is likely because higher concentration of H_2O_2 will result in more reduction-oxidation reaction between H_2O_2 and DTT, which will inhibit more of DTT to react Au-NPs (less Au-NPs aggregation) and consequently less degree of color-change to purple.

UV-Vis analyses of H_2O_2/DTTH_2O_2/Au-NPs solutions

The aggregated Au-NPs were analyzed with ultraviolet-visible (UV-Vis) to confirm the relationship between the concentration of H_2O_2 and the color change of Au-NPs. The absorption spectra of UV-Vis are shown in Figure 2b. As can be observed, the characteristic peak of absorption located in the wavelength range of visible light (approximately from 380 to 780 nm) is at about 520 nm. It is worth noting that as the concentration of H_2O_2 decreases from 10^{-1} M to 10^{-6}

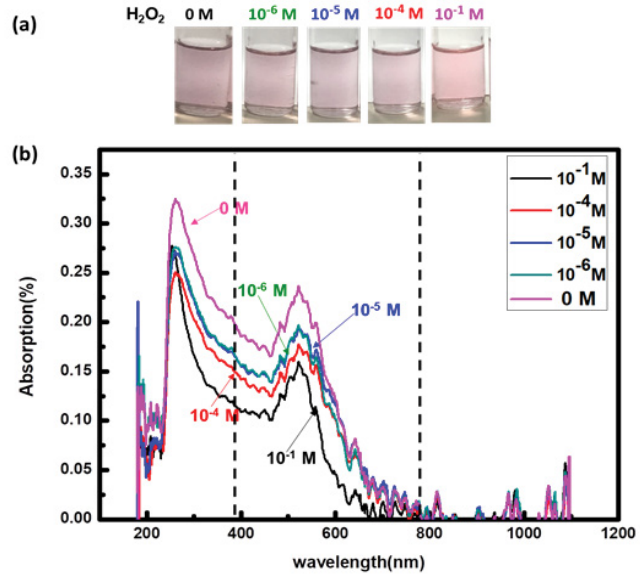

Figure 2: (a) The color of the H_2O_2/DTT/Au-NPs solutions with different H_2O_2 concentrations, showing more degree of color-change to purple for the solution with the concentration of 10^{-4} M or lower compared to that of 10^{-1} M H_2O_2. (b) The UV-Vis absorption spectra measured on the DTT /H_2O_2/Au-NPs solution with various concentrations of H_2O_2, showing more absorption on the 520 nm characteristic peaks for the solutions at higher H_2O_2 concentrations (i.e., more degree of wine-to-purple color change observed from naked eyes).

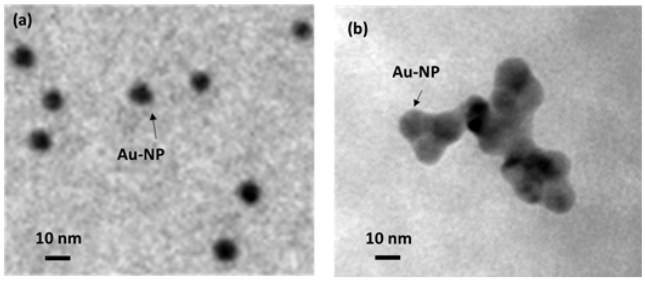

Figure 3: The liquid-TEM image of Au-NPs in H_2O_2/DTT/Au-NPs solutions with 10^{-4} M H_2O_2, showing the (a) original ~10 nm Au-NPs and (b) aggregated Au-NPs forming a cluster with a size of tens of nanometers.

M even 0 M (without H_2O_2, the reference sample), the intensity of the characteristic peak increases gradually. Meanwhile, the color of the sample varied slightly from wine-red to dark purple. As mentioned, it is possible that as the concentration of H_2O_2 decreases, DTT is more likely to remain in reducing form, which may increase its probability to attach on the surface of Au-NPs and contribute to the aggregation of Au-NPs. Therefore, the intensity of absorption increases and the color of H_2O_2/DTT/Au-NPs solution approach dark-purple as observed by naked eyes.

Liquid TEM analyses of Au-NPs in H_2O_2/DTT/Au-NPs solutions

The liquid transmission electron microscopy (liquid-TEM) developed in this group and utilizing a disposable K-kit [16], which was transferred to Bio MA-Tek for production, for sample loading was also conducted to inspect the H_2O_2/DTT/Au-NPs solution. The liquid TEM images of original An-NPs solution and the H_2O_2/DTT/Au-NPs solutions with 10^{-4} M H_2O_2 are shown in Figure 3a and Figure 3b, respectively. As can be observed, the original dispersed Au-NPs with 10 nm in diameter for original An-NPs solution is shown in Figure 3a. However, the Au-NPs in the solutions with 10^{-4} M H_2O_2 were aggregated to form a cluster with a larger size in terms of nanometers as shown in Figure 3b, which confirms that the color change of Au-NPs is likely related to the aggregation of Au-NPs.

Conclusion

An easy approach to detect the concentration of H_2O_2 via a chromatic biosensor has been developed in this work. By utilizing the combination of DTT and Au-NPs, the concentration of H_2O_2 can be detected from 0.1 M down to 10^{-6} M by naked eyes, which can benefit the detection of cancer and cardiovascular disease since when the concentration of free radicals exceeds 10^{-4} M may imply the existence of these diseases. As the concentration of H_2O_2 decreased from 0.1 M to 10^{-6} M, the color of Au NPs solution changed from wine-red to purple. Moreover, the UV-Vis and liquid-TEM results also verified the assumption of this study, the color change of Au-NPs and the reaction of DTT with Au-NPs (i.e., forming DTT-modified-Au-NPs) that cause the conjugation and aggregation of DTT-modified-Au-NPs, especially. In conclusion, by this strategy, the biosensor could be applied to detect numerous diseases simply through direct observation of color change. With further investigation on actual serum samples, interference, and stability in the future, this biosensor will be feasible for daily disease detection.

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