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Preparation of silver nanoprism-dye complex for fluorescent detection of dhydrogenperoxide in milk

C.-F. Peng, C.-L.Liu and Z.-J.Xie

Introduction

Hydrogen peroxide (H$_2$O$_2$) involves in various biochemical processes of cells and organisms. It has been widely applied in many fields including foods, pharmaceuticals, dental products, textiles and environmental protection.¹ H$_2$O$_2$ can be utilized as a food additive of controlling the growth of microorganisms, or bleaching in food processing in many countries. It has a long-established history of using H$_2$O$_2$ as a preservative in stored milk before cheese-making.², ³

However, high concentration of H$_2$O$_2$ is extremely toxic to cells.⁴ Administration with 0.1% (w/w) H$_2$O$_2$ can cause cancer in the duodenum of mouse.⁵ H$_2$O$_2$ also shows short-term genotoxicity.⁶

H$_2$O$_2$ residues are not permitted in food under European Union, Japanese and Taiwanese regulations due to health concerns.⁷ A regulation of Food and Drug Administration (FDA) currently limits residual H$_2$O$_2$ to 0.5 ppm, leached into distilled water, in finished food packages.⁸ It is also important to control and inspect H$_2$O$_2$ residue in aseptically packaged beverages which are often utilized as disinfectant during processing.⁹ Therefore, accurate detection of H$_2$O$_2$ in food industry is very important for food safety.²

Numerous methods have been developed to detect H$_2$O$_2$ such as spectrophotometry, luminescence, and electrochemistry.¹⁰ Several methods have also been proposed for the determination of H$_2$O$_2$ in milk.¹¹, ¹² The SPR absorption of Ag NPs is extremely sensitive to their size, shape, and distance, based on which various sensors can be fabricated.¹³ Anisotropic Ag NPs such as triangular prisms and plate-like nanostructures are extensively investigated due to their outstanding plasmonic features across visible-NIR regions. Since the edges/tips of Ag nanoparticles are highly reactive, they can be etched into round nanostructure generally referred to as nanodiscs by some compounds such as hydrogen peroxide and iodide.¹⁴ Due to the strongly tip sharpness and aspect ratio dependent SPR absorption, these interesting phenomena have been introduced to build colorimetric sensors for the determination of mercury ions, iodide, cysteine, glucose and DNA, etc.¹⁵ However, it is still difficult to apply colorimetric sensors based on Ag nanoparticles to determine analyte in complex media such as milk. Recently, nanoparticle-based fluorescent sensors were paid comprehensive attention on account of high sensitivity and relative versatility. For example, fluorescein isothiocyanate (FITC) functionalized magnetic core–shell Fe$_3$O$_4$/Ag hybrid nanoparticles was utilized to detect biotriols sensitively.¹⁶ A novel fluorescent detection of copper ion was designed based on FITC functionalized gold nanoparticles (FITC-AuNPs).¹⁷ In this communication, we prepared an Ag nanoprisms-dye complex (FDSNP) which was applied to detect H$_2$O$_2$ in milk. The novel method provided a sensitive and convenient detection of H$_2$O$_2$ in milk.

Experimental

Hydrogen peroxide (H$_2$O$_2$, 30 wt-%) was purchased from Sinopharm. Fluorescein isothiocyanate (FITC, 99+%) was purchased from Suzhou Cyto Bio-technology. Silver nitrate (AgNO$_3$, 99+%) was obtained from J&K. Sodium borohydride (NaBH$_4$, 99%), sodium citrate tribasic...
dehydrate (TSC, 99%) and bovine serum albumin (BSA, 98%) were bought from Sigma-Aldrich. All chemicals were used as received without further treatment. All solutions were prepared with double-deionized water.

The morphology and microstructure of the Ag nanoprisms were characterized by electron microscope (TEM, JEM-2100, JEOL, Japan) operating at a 200 kV accelerating voltage. Fluorescence spectrometer (F-7000, Hitachi Co. Ltd., Japan) with a Xenon lamp excitation source was employed to record fluorescence spectra. The excitation wavelength was set at 490 nm and the emission was monitored from 490 nm to 600 nm.

Ag nanoprisms were prepared following the method reported before.\(^2\)\(^0\) The Ag nanoprisms obtained were centrifuged at 8000 rpm for 10 minutes. Then 200 μL of BSA (10 mg/mL) aqueous solution was added into the above-prepared Ag nanoprisms and incubated for one night. The FITC solution in DMSO was added to the mixture and incubated for varied hours. The Ag nanoprisms–FITC complex obtained was resuspended in 4.0 mL of sodium citrate solution (15 mM) after centrifuged at 8000 rpm for 10 minutes. To 40 μL of prepared FITC–Ag nanoprisms, 40 μL of H\(_2\)O\(_2\) standard solutions with different concentrations were added and mixed, respectively. After the mixtures reacted for 20 min, the fluorescence intensity was measured at 520 nm.

Milk samples were spiked with 0, 0.5 and 5.0 μM H\(_2\)O\(_2\) min, the fluorescence intensity was measured at 520 nm.

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### Results and discussion

Scheme 1 outlines the proposed sensing mechanism. Initially, the Ag nanoprisms were covered by BSA. Then the added FITC was conjugated with the free amino of the protein molecule. After bound with BSA on the Ag nanoprisms surface, the fluorescence of FITC was quenched to some extent by the Ag nanoprisms due to fluorescence resonance energy transfer (FRET) between FITC and Ag nanoprisms. Since Ag nanoprisms can be etched to round nanodiscs by H\(_2\)O\(_2\), the etching will result in the FITC–BSA molecules release from the Ag nanoprisms surface. As a result, the fluorescence of FITC–BSA will recover to some extent.

Here, Ag nanoprisms were prepared under the condition without polyvinylpyrrolidone (PVP) macromolecules,\(^7\) which facilitates the absorption of BSA onto the surface of Ag nanoprisms and the labeling of FITC on the Ag nanoprisms surface. The surface plasmon resonance (SPR) absorption of the prepared Ag nanoprisms was at 750 nm (Fig 1) and the products are mainly triangular nanoparticles (Fig 2). After BSA was absorbed onto the Ag nanoprisms surface, almost the same SPR absorption spectrum was observed (Fig 1).

Initial experiment showed high concentration of FITC can slowly degrade the BSA–Ag nanoprisms and resulted in blue-shift of SPR spectrum, which is possibly due to similar dissolution of cyanide ions against silver nanoparticles.\(^2\)\(^1\) Recent works have demonstrated that the high surface energy vertices of Ag nanoprisms are readily degraded by halide ions, thiols, and acid.\(^2\)\(^2\) Nevertheless, the BSA molecules were absorbed onto the surface of Ag nanoprisms and passivated the Ag nanoprisms surface to some extent, which can protect Ag nanoprisms from low concentration of FITC and the above halide ions (data not shown).

Considering the above effects, varied concentrations of FITC were incubated with BSA–Ag nanoprisms for 12 hours. It was found that higher concentration of FITC can improve the fluorescence detection signal of H\(_2\)O\(_2\) in the range of 2.5–7.5 μg/mL, but excess amount of FITC (10 μg/mL) will impair the fluorescence detection of H\(_2\)O\(_2\) (Fig. S1). Then 7.5 μg/mL FITC was incubated with BSA–Ag nanoprisms for different time. It was found that the highest fluorescence intensity recovered by 5 μmol/L H\(_2\)O\(_2\) was obtained after FITC was incubated with BSA–Ag nanoprisms for 4 hours (Fig. S2). In the optimized condition, the obtained FITC–BSA–Ag nanoprisms (FDSNP) showed a very small blue-shift of SPR absorption (λ\(_{\text{abs}}\)=750 nm) compared with BSA–Ag nanoprisms (Fig 1). In addition, TEM images of the Ag nanoprisms and the FDSNP showed negligible difference between them (Fig 2). Therefore, this highest fluorescence signal to H\(_2\)O\(_2\) was due to that the FDSNP were kept undamaged. However, high concentration of H\(_2\)O\(_2\) can etch the sharp tips of the FDSNP into nanodiscs (Fig 2), which resulted in blue-shift spectra of FITC–BSA–Ag nanoprisms (Fig 1). After the FDSNP is etched completely by H\(_2\)O\(_2\), the biggest fluorescence change of the FDNSNP will happen. It was found that the FDSNP can be etched rapidly by H\(_2\)O\(_2\) in 20 min when the changed fluorescence intensity reached a plateau (Fig. S3).

The fluorescence signal of reaction solutions were measured at varied concentration of H\(_2\)O\(_2\). It was found that the emission intensity at 525 nm increased as the H\(_2\)O\(_2\) concentration ranged from 20.0 nM to 5.0 μM (the inset in Fig 4, R\(^2\)=0.99) and from 5.0 μM to 100 μM (the inset in Fig 4, R\(^2\)=0.998). The detection limit was evaluated as 4.0 nM, which was calculated by a signal-to-noise ratio of 3 (S/σ). Here, S means the slope of the linear equation and σ means the standard deviation of blank measurements.

To investigate the selectivity of the fluorescent detection method, possible interfering substances in milk were interrogated under the selected optimal conditions in the presence of 5.0 μM H\(_2\)O\(_2\). It was found that the specific fluorescence signal decreased by less than 5% in the presence of following compounds, CaCl\(_2\) (5.0 mM), NaCl (5.0 mM), KI (0.002 mM), NaNO\(_2\) (5.0 mM), FeSO\(_4\) (0.05 mM), ZnSO\(_4\) (0.1 mM), CuSO\(_4\) (0.1 mM), vitamin C (0.05 mM), and C\(_6\)H\(_{12}\)COONa (0.1 mM). This interference should be mainly due to the concomitant halide ions, vitamin C and acid.\(^7\) It should be noted that this matrix effects can be further decreased by simple dilution since the milk samples will be diluted before fluorescence determination.

The comparison of analytical performances between the methods of H\(_2\)O\(_2\) detection (Table S1) shows that our developed fluorescent method is one of most sensitive methods.

To evaluate the potential application in real samples, the newly established method has been used to determine H\(_2\)O\(_2\) in three milk samples. Table 1 showed the determination results. Trace H\(_2\)O\(_2\) (0.56, 0.64, and 0.93 μM) in three milk samples have been detected. After three different concentrations of H\(_2\)O\(_2\) (0, 0.5 and 1.0 μM) were spiked into the three milk samples respectively, the milk samples were measured again. The recoveries of H\(_2\)O\(_2\) ranged from 82.1% to 92.8% have been obtained with relative standard derivation (RSD) less than 9.3%. These results demonstrated the highly practical potential of this fluorescent detection of H\(_2\)O\(_2\).

### Conclusions

In summary, we have developed a fluorescent detection of H\(_2\)O\(_2\) in milk based on H\(_2\)O\(_2\) induced morphology transition of the FDSNP. It offers many advantages such as simple, rapid, high sensitivity and excellent selectivity for the detection of H\(_2\)O\(_2\) in milk. This new FDSNP-based H\(_2\)O\(_2\) sensor holds...
promising application potential for H$_2$O$_2$ detection in milk. In addition, this detection format for H$_2$O$_2$ detection may be improved further through binding fluorescent nanoparticles, such as quantum dots, to the Ag nanoprism surface.

Table 1 Results of the measurements of H$_2$O$_2$ in three milk samples (n=4)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<td>89.6</td>
<td>9.2</td>
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</table>

Notes and references

‡Supplementary information includes effect of FITC concentration on H$_2$O$_2$ detection, effect of conjugation time on H$_2$O$_2$ detection and effect of etching time on H$_2$O$_2$ detection.

Legend of figures

Scheme 1. Schematic illustration of the preparation of the Ag nanoprism-FITC complex and the application of H$_2$O$_2$ sensing.

Fig 1. (A) UV-vis absorption spectra and (B) Fluorescence spectra of (a) Ag nanoprisms, (b) BSA-Ag nanoprisms, (c) FITC-BSA- Ag nanoprisms and (d) FITC- BSA-Ag nanoprisms after incubated with 50µM H$_2$O$_2$ for 10 minutes. The inset shows a photograph of corresponding solutions.

Fig 2. TEM images of (a) Ag nanoprism, (b)FITC-BSA-Ag nanoprism and (c) FITC-BSA-Ag nanoprism after etched by 50 µM H$_2$O$_2$.

Fig 3. The emission spectra of reaction solutions after the FITC-BSA-Ag nanoprism complex was etched by H$_2$O$_2$.

Fig 4. The plots of the increased fluorescence intensity at 525 nm versus different concentration of H$_2$O$_2$. The insets show the plots of the increased fluorescence intensity at 525 nm versus H$_2$O$_2$ concentration ranges from 20 nM to 5.0 µM and from 5.0 µM to 100 µM.
Fig. 3

Fluorescence intensity / Arb. Unit

Wavelength (nm)

0 0.01µM 0.02µM 0.5µM 5µM 30µM 50µM 100µM