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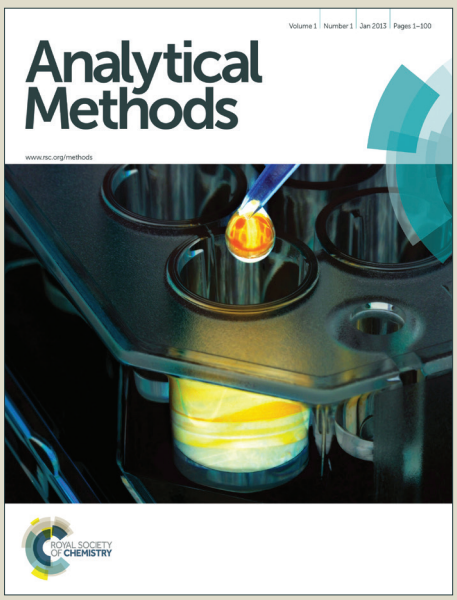

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COMMUNICATION

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

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Asimple and sensitive"turn-on"fluorescent detection of H_2O_2 in milk was developed based on H_2O_2induced morphology transition of fluorescent dye-protein complex-capped silver nanoprisms (FDSNP). The rounding of FDSNP accompanies desorption of the fluorescent dye-protein complex, which resulted in recovery of the fluorescent intensity. In a 4 order of magnitude dynamic range from 10 nM to 100 μM, the fluorescent intensity of the FDSNP sensitively increased with H_2O_2concenrtration increasing. The limit of detectionfor H_2O_2 was 4.0 nM. Furthermore, the developed method was applied for detection H_2O_2 in milk with satisfying recoveries over 80% after milk was simply pretreated.

Introduction

Hydrogen peroxide (H_2O_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_2O_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_2O_2 as a preservative in stored milk before cheese-making. [2, 3]

However, high concentration of H_2O_2 is extremely toxic to cells.[4] Administration with 0.1% (w/w) H_2O_2 can cause cancer in the duodenum of mouse. H_2O_2 also shows short-term genotoxicity.[5] H_2O_2 residues are not permitted in food under European Union, Japanese and Taiwanese regulations due to health concerns.[6] A regulation of Food and Drug Administration (FDA) currently limits residual H_2O_2 to 0.5 ppm, leached into distilled water, in finished food packages.[7] It is also important to control and inspect H_2O_2 residue in aseptically packaged beverages which are often utilized as disinfectant during processing.[8] Therefore, accurate detection of H_2O_2 in food industry is very important for food safety.[2]

Numerous methods have been developed to detect H_2O_2such as spectrophotometry, luminescence, and electrochemistry.[9] Several methods have also been proposed for the determination of H_2O_2 in milk.[10], such as oxygen electrode method, flow injection analysis, FT-IR method and amperometric biosensor.[11, 12]

Silver nanoparticles (Ag NPs) exhibit intensive surface plasmon resonance (SPR) bands in the wavelength range of 300–900 nm and have now widely applied in biological, chemical, and environmental fields.[13, 14] The SPR absorption of Ag NPs is extremely sensitive to their size, shape, and distance, based on which various sensors can be fabricated.[14] 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 nanoprisms are highly reactive, they can be etched into round nanostructure generally referred to as nanodiscs by some compounds such as hydrogen peroxide and iodide.[15] 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.[13-17] However, it is still difficult to apply colorimetric sensors based on Ag nanoprisms 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_3O_4/Ag hybrid nanoparticles was utilized to detect biothiols sensitively.[18]A novel fluorescent detection of copper ion was designed based on FITC functionalized gold nanoparticles (FITC-AuNPs).[19]In this communication, we prepared an Ag nanoprism-dye complex (FDSNP) which was applied to detect H_2O_2 in milk. The novel method provided a sensitive and convenient detection of H_2O_2 in milk.

Experimental

Hydrogen peroxide (H_2O_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

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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 to600 nm.

Ag nanoprisms were prepared following the method reported before.[20] The Ag nanoprisms obtained were centrifuged at 8000 rpm for 10 minutes. Then 200μL 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 nanoprism-FITC complex obtained was resuspended in 4.0 mL of sodium citrate solution (15mM) after centrifuged at 8000 rpm for 10 minutes. To 40μL of prepared FITC-Ag nanoprisms, 40 μL of H_2O_2standard 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 μMH_2O_2, and then 50-fold diluted by ultrapure water.

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_2O_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) macromeluecules[18], 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.[21] Recent works have demonstrated that the high surface energy vertices of Ag nanoprisms are readily degraded by halide ions, thiols, and acid.[22] 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_2O_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_2O_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 $H_2O_2$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_{SPR}=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_2O_2 was due to that the FDSNP were kept undamaged. However, high concentration of H_2O_2 can etched 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_2O_2, the biggest fluorescence change of the FDNP will happen. It was found that the FDSNP can be etched rapidly by H_2O_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_2O_2.It was found that the emission intensity at 525 nm increased as the H_2O_2concentration increased from 10 nM to 100 μM (Fig.3 and Fig. 4).The increased fluorescence intensities at 525 nm were well linearly correlated with the H_2O_2 concentrations ranged from 20.0 nM to 5.0 μM (the inset in Fig 4, R^2=0.99) and from 5.0 μM to 100μM (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 (3σ/S). 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 5.0μM H_2O_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), $ZnSO_4$ (1.0 mM), KH_2PO_4 (1.0 mM), $MnCl_2$ (0.5 mM), $Al_2(SO4)_3$ (0.5 mM), $CuSO_4$(0.1 mM), vitamin C (0.05 mM), and C_6H_5COONa (0.1 mM). This interference should be mainly due to the concomitant halide ions, vitamin C and acid.[3]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_2O_2 detection (TableS1) 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_2O_2 in three milk samples. Table 1 showed the determination results. Trace H_2O_2 (0.56, 064, and 0.93 μM) in three milk samples have been detected. After three different concentrations of H_2O_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_2O_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_2O_2.

Conclusions

In summary, we have developed a fluorescent detection of H_2O_2 in milk based on H_2O_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_2O_2 in milk. This new FDSNP-based H_2O_2 senor holds

promising application potential for H_2O_2 detection in milk. In addition, this detection format for H_2O_2 detection may be improved further through binding fluorescent nanoparticles, such as quantum dots, to the Ag nanoprisms surface.

Table 1 Results of the measurements of H_2O_2 in three milk samples (n=4)

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
No. 1	0	0.56	-	6.9
	0.5	1.02	82.1	9.3
	5.0	5.17	92.2	8.5
No. 2	0	0.64	-	7.1
	0.5	1.04	80.0	6.9
	5.0	5.28	92.8	8.6
No. 3	0	0.93	-	7.0
	0.5	1.35	84.0	8.5
	5.0	5.41	89.6	9.2

Notes and references

‡Supplementary informationincludes effect of FITC concentration on H_2O_2 detection, effect of conjugation time on H_2O_2 detection and effect of etching time on H_2O_2 detection.

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Legend of figures

Scheme 1. Schematic illustration of the preparation of the Ag nanoprism-FITC complex and the application of H_2O_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_2O_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_2O_2.

Fig 3. The emission spectra of reaction solutions after the FITC-BSA-Ag nanoprism complex was etched by H_2O_2.

Fig 4. The plots of the increased fluorescence intensity at 525 nm versus different concentration of H_2O_2. The insets show the plots of the increased fluorescence intensity at 525 nm versus H_2O_2 concentration ranges from 20 nM to 5.0 μM and from5.0 μM to 100 μM.

Scheme 1

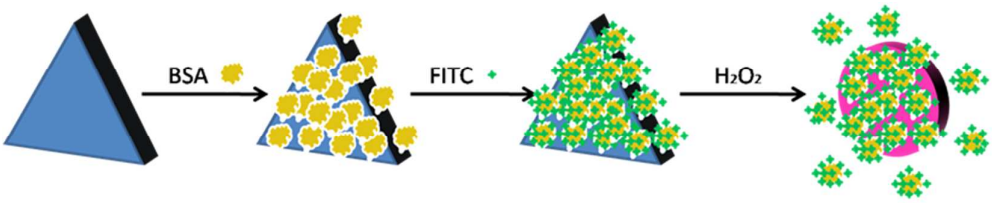


Fig. 1

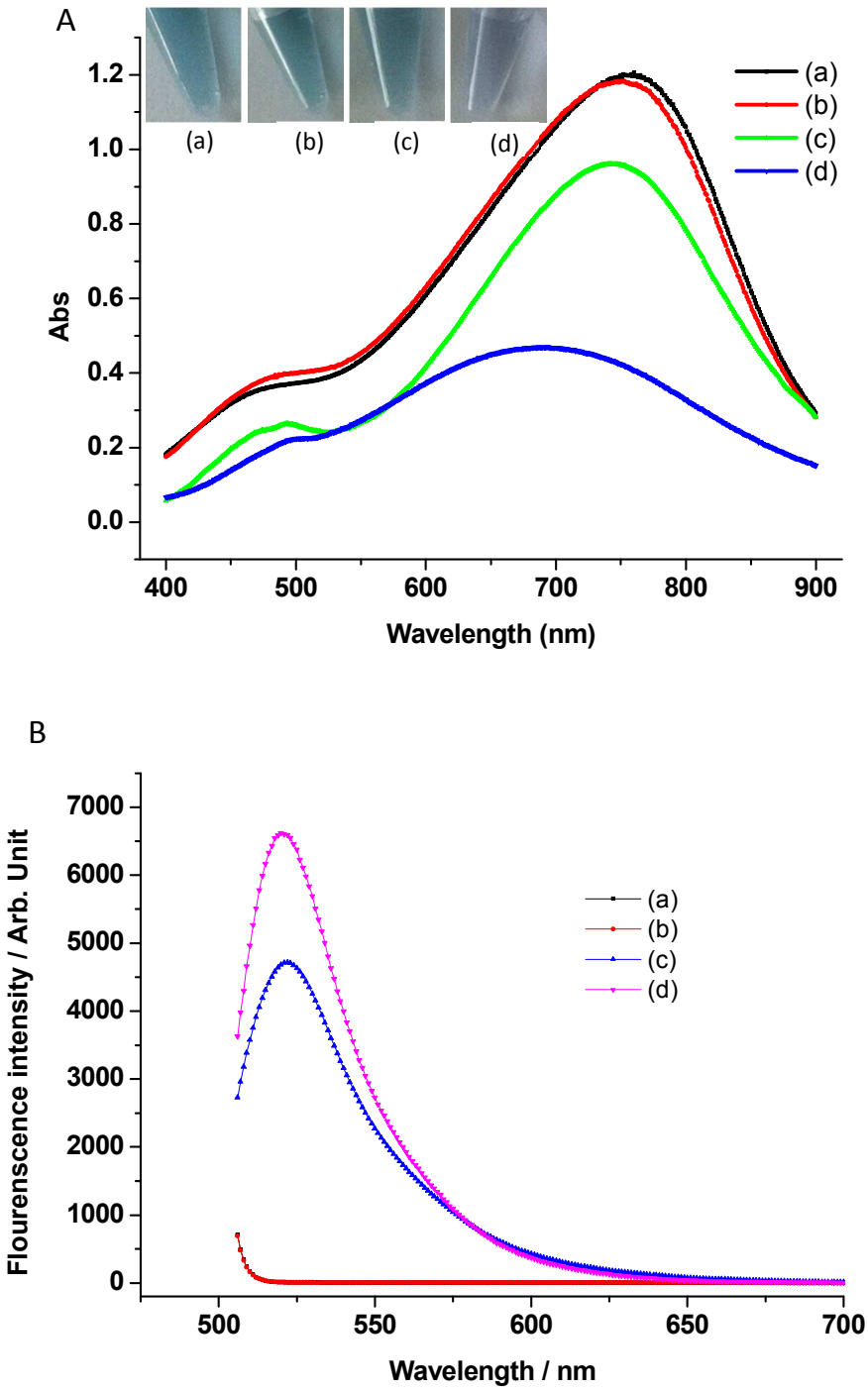
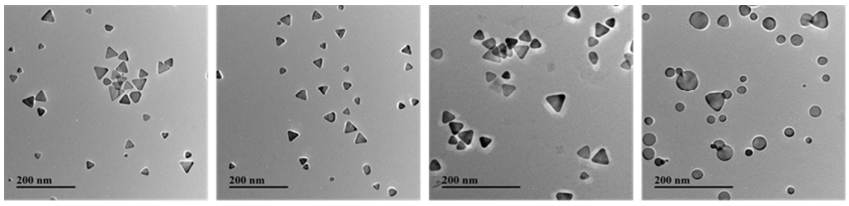


Fig. 2



(a) (b) (c) (d)

Fig. 3

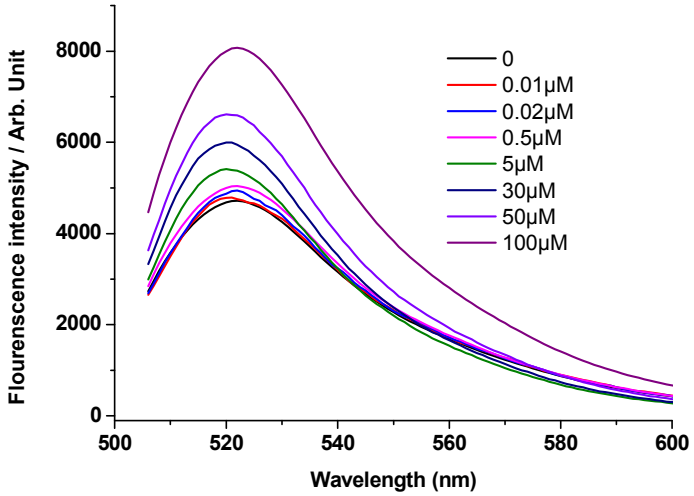


Fig. 4