Macromolecules
COMMUNICATION TO THE EDITOR

Macromolecules **2011**, *44*, 429–431 **429**
DOI: 10.1021/ma102608a

Stimuli-Triggered Off/On Switchable Complexation between a Novel Type of Charge-Generation Polymer (CGP) and Gold Nanoparticles for the Sensitive Colorimetric Detection of Hydrogen Peroxide and Glucose

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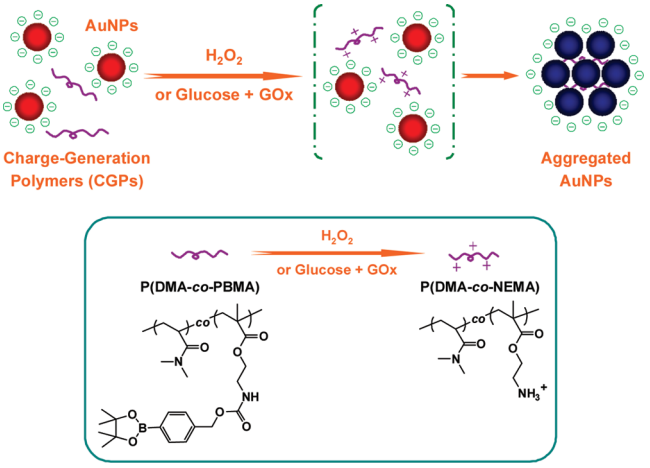
Received November 16, 2010
Revised Manuscript Received January 9, 2011

Hydrogen peroxide (H_2O_2) is a key oxygen metabolite which has been considered as a "necessary evil" owing to its "double-faced" physiological and pathological effects in living organisms.[1] Besides acting as a messenger in normal cellular signal transduction processes and a killing agent released by immune cells,[2] the abnormal production of H_2O_2 also leads to oxidative stress and damage events associated with aging and severe pathologies such as cancer and Parkinson's and Alzheimer's diseases.[3] Moreover, H_2O_2 is also involved in many environmental and industrial food processes.[4] Thus, the highly sensitive and selective detection of H_2O_2 is quite crucial. Although in recent years a variety of designing strategies based on chemiluminescence, fluorescence, and electrochemical techniques have been developed,[5] it is still a challenging task to explore a simple, reliable, cost-effective, and highly sensitive detection system for H_2O_2.

Colorimetric approaches based on gold nanoparticles (AuNPs) have been widely employed to probe various target species such as oligonucleotides,[6] protein,[7] small bioactive molecules,[8] explosives,[9] metal ions,[10] and other hazardous molecules[11] by taking advantage of their facile surface functionalization and unique distance-dependent surface plasmon absorption properties.[12] Target species mediated or triggered aggregation and disintegration of AuNPs typically leads to discernible colorimetric changes, which can be checked by the naked eye or more quantitatively by UV−vis spectroscopy. It is worthy of noting that nearly all the above colorimetric sensing systems are fabricated via small molecule or complementary oligonucleotide sequence-based molecular recognition. On the other hand, it has been well-established that charged inorganic nanoparticles are subjected to aggregation and assembly in the presence of oppositely charged (block) polyelectrolytes via the formation of electrostatic hybrid complexes.[13] However, to the best of our knowledge, polyelectrolyte-induced AuNP aggregation has not been utilized to design practicable sensing systems, mainly due to the lack of suitable analyte-triggerable polyelectrolyte/AuNP complexation systems.

The above two aspects have prompted us to design a special type of water-soluble synthetic polymers which can undergo stimuli-triggered transition from the initially uncharged state to the charged one, so that a colorimetric detection system for H_2O_2 and in a further step for glucose (in the presence of glucose oxidase, GOx) based on the triggered aggregation of AuNPs can be constructed. To differentiate from the recently emerged pH-induced charge-reversal polymers[14] and photoinduced charge-bearing polymers,[15] we have termed these novel types of polymers as stimuli-triggered charge-generation polymers (CGPs). Herein, we report a proof-of-concept example for the fabrication of colorimetric detection system for H_2O_2 and glucose by engineering negatively charged AuNPs and a water-soluble CGP exhibiting selective H_2O_2-triggered transformation into a cationic polyelectrolyte. Electrostatic complexation between AuNPs and the newly generated positively charged polymer can then lead to prominent colorimetric transitions due to the aggregation of AuNPs (Scheme 1).

Scheme 1. Schematic Illustration of the Construction of Colorimetric Sensing System for H_2O_2 and Glucose (in the Presence of GOx) via the Combination of Negatively Charged AuNPs with a Water-Soluble Charge-Generation Polymer (CGP) Exhibiting Selective H_2O_2-Triggered Transition from the Initially Uncharged State to a Cationic Polyelectrolyte



On the basis of the above proposed strategy (Scheme 1), we first designed and synthesized a new polymerizable monomer, PBMA, via the reaction of boronate ester-functionalized benzyl alcohol derivative with 2-isocyanatoethyl methacrylate (Scheme S1 and Figure S1 in the Supporting Information). It should be noted that in literature reports the aryl boronate motif has rendered the construction of a series of small molecule H_2O_2-selective fluorescent probes and in vivo imaging agents.[5b−d] As PBMA monomer lacks sufficient water solubility, we then opted to synthesize PBMA-containing water-soluble polymers via the free radical copolymerization of PBMA with *N*,*N*-dimethylacrylamide (DMA). The obtained P(DMA-*co*-PBMA) copolymer was subjected to further fractionation to afford relatively narrow-disperse samples. ^{1}H NMR (Figure S2a) and gel permeation chromatography (GPC, Figure S3) analysis revealed an overall degree of polymerization (DP) of 620, an M_w/M_n of 1.27, and a PBMA molar content of 11%. As expected, the initially uncharged $P(DMA_{0.89}$-*co*-$PBMA_{0.11})_{620}$ transforms into a positively charged polyelectrolyte in the presence of H_2O_2, as evidenced by ^{1}H NMR analysis from the complete disappearance of resonance signals characteristic of boronate ester moieties after treating with H_2O_2 (Scheme 1 and Figure S2). It is worthy of noting that pendent aliphatic amine moieties typically possess a pK_a of ∼9,[16] and under neutral pH conditions the newly generated amine residues triggered by H_2O_2 are in the protonated state, which can effectively eliminate any amine-involved side reactions such as the amidation of ester moieties.

Citrate-stabilized AuNPs with an average diameter of ∼13 nm were synthesized according to literature procedures (Figure S4).[17]

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Published on Web 01/12/2011

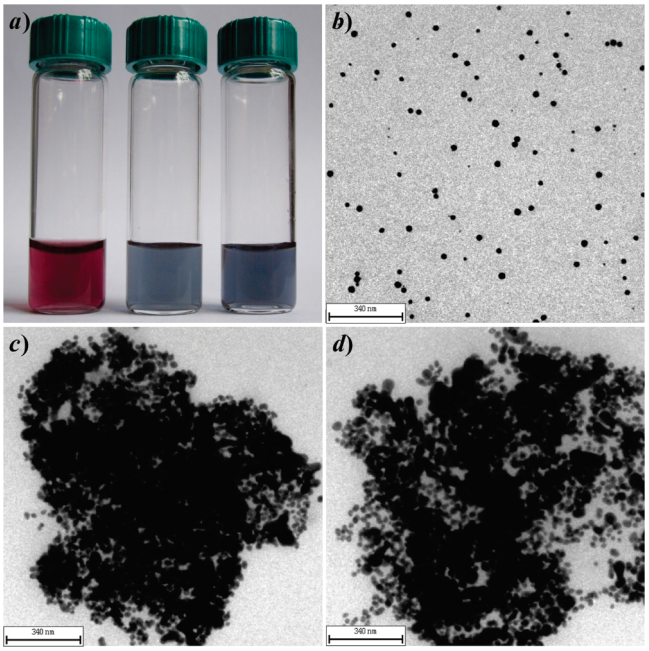

Figure 1. (a) Optical visualization of the colorimetric assay (4.5 nM of AuNPs and 1.8×10^{-3} g/L of CGP with [PBMA] = 1.5 μM) in the absence of H_2O_2 (left) and 1 h after the addition of the aqueous solution of (middle) H_2O_2 (120 equiv relative to PBMA moieties) or (right) the glucose oxidization mixture (300 equiv glucose and 0.2 g/L of GOx) at pH 7.4 and 25 °C. (b−d) The corresponding TEM images (scale bar is 340 nm) obtained by drying the three aqueous dispersions shown in (a).

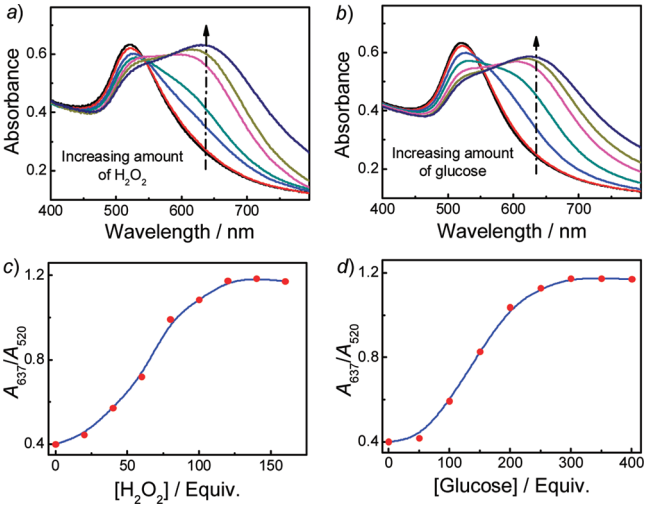

Figure 2. (a, b) UV−vis spectra and (c, d) absorbance intensity ratios, A_{637}/A_{520}, recorded for the aqueous colorimetric assay (4.5 nM of AuNPs and 1.8×10^{-3} g/L of CGP with [PBMA] = 1.5 μM) upon adding varying amount of (a, c) H_2O_2 (0−160 equiv relative to PBMA moieties) and (b, d) the glucose oxidization mixture (0−400 equiv glucose and 0.2 g/L of GOx were employed for the enzyme-catalyzed reaction) at pH 7.4 and 25 °C. All measurements were conducted 1 h after the addition of analytes.

The negatively charged wine red AuNP dispersion exhibits a characteristic surface plasmon resonance (SPR) band at ~520 nm, which is almost unchanged upon the sole addition of H_2O_2 (Figure S5), indicating that citrate-stabilized AuNPs do not react with H_2O_2. The aqueous colorimetric assay system for H_2O_2 was then optimized to consist of negatively charged AuNPs (4.5 nM) and the CGP, P(DMA$_{0.89}$-*co*-PBMA$_{0.11}$)$_{620}$ copolymer (1.8×10^{-3} g/L, [PBMA] = 1.5 μM) at pH 7.4. Initially, AuNPs remain well-dispersed in the aqueous solution of CGP, as revealed by the characteristic wine red color (Figure 1a), UV−vis absorption band at 520 nm (Figure 2a), and TEM analysis (Figure 1b). This is reasonable considering that there does not exist specific interactions between negatively charged AuNPs and the neutral P(DMA$_{0.89}$-*co*-PBMA$_{0.11}$)$_{620}$. For H_2O_2 detection, its aqueous solution with the volume being half of original aqueous assay mixture was used. Upon addition of the aqueous solution of H_2O_2 (120 equiv relative to PBMA residues), the dispersion gradually turns from wine red to purple blue within ~0.5 h. This apparently confirmed our design strategy as shown in Scheme 1. As expected, TEM analysis of the purple blue dispersion revealed the presence of aggregated AuNPs (Figure 1c). The presence of H_2O_2 can trigger the transformation of uncharged P(DMA$_{0.89}$-*co*-PBMA$_{0.11}$)$_{620}$ into a cationic polyelectrolyte (Figure S2). As ~68 positively charged amine moieties are present per newly generated polyelectrolyte chain if we assume 100% functional group transformation, it can act as an effective interparticle cross-linker for negatively charged AuNPs, and the dispersion containing aggregated AuNPs exhibits the characteristic colorimetric transitions.

H_2O_2-triggered aggregation of AuNPs can also be quantitatively determined by changes in the SPR bands. UV−vis spectroscopy analysis was then performed to evaluate the sensitivity of colorimetric H_2O_2 assay. The time duration needed for the colorimetric changes to reach the final stable state was determined at first (Figure S6). Upon addition of 120 equiv of H_2O_2 (relative to PBMA moieties), we can observe that the SPR band at 520 nm gradually decreases and exhibits a slight red shift with time and then stabilizes out after ~40 min, which is also accompanied by the appearance and the dramatic increase in intensity of a new absorption band at around ~637 nm originating from the interparticle coupled plasmon absorbance.[10d,12a,18] It is worthy of noting that the appearance of a new absorption band at ca. 640 nm has been typically observed for highly aggregated gold nanoparticles with diameters of ~13 nm.[10d,20b] To maintain the consistency of H_2O_2 assays, in subsequent experiments all aqueous mixtures were incubated for 1 h after the addition of H_2O_2. On the basis of the results shown in Figure 2a, we can tell that gradually increasing the amount of H_2O_2 led to more prominent red shift of the absorption spectrum, which can be qualitatively expressed as the absorbance intensity ratio, A_{637}/A_{520} (Figure 2c). An almost linear H_2O_2 concentration dependence of the absorbance intensity ratio was observed at $[H_2O_2] < \sim 120\ \mu$M, which matches quite well with the physiologically relevant concentration of H_2O_2 (~10−100 μM).[19] If we arbitrarily define the H_2O_2 detection limit as the concentration at which a 10% increase of A_{637}/A_{520} ratio can be achieved, the H_2O_2 detection limit was then determined to be ~20 μM (~0.68 mg/L) under the current setup conditions of H_2O_2 colorimetric assay, which is quite comparable with those of small molecule fluorescent probes based on aryl boronates.[5b−d] On the basis of the above results, we have established that highly sensitive colorimetric H_2O_2 detection system can be successfully constructed by combining negatively charged AuNPs with a novel type of CGP exhibiting H_2O_2-triggerable transformation from the uncharged state to a cationic polyelectrolyte. Moreover, it has been well-documented that H_2O_2 is quite selective over other reactive oxygen species (ROS) toward the deprotection of aryl boronate moieties.[5b,d] Thus, the reported H_2O_2 assay system possesses combined advantages such as high sensitivity, selectivity, and ease of fabrication. Apart from this, the current colorimetric assay can provide a facile and cost-effective readout of low concentrations of H_2O_2 even with the naked eye. This is excellent as compared to previously reported H_2O_2 assay protocols employing fluorometric or electrochemical and techniques.[5]

Since glucose oxidase (GOx) can specifically catalyze the oxidation of glucose to produce H_2O_2,[20] we then employed the above

H_2O_2 assay kit for the colorimetric detection of glucose. First, the mixture of glucose and GOx was incubated at 37 °C under an O_2 atmosphere for 10 min; then the oxidation product was analyzed by using the above H_2O_2 colorimetric assay at pH 7.4 and 25 °C. As shown in Figure 1a, upon addition of the glucose oxidation product, a gradual colorimetric transition of the assay mixture from wine red to purple blue can be observed (Figure 1a). This indicated that the H_2O_2 assay protocol established in the previous section can also be successfully employed for glucose detection, again via the aggregation of AuNPs induced by the CGP triggered by H_2O_2 (Figure 1d). In the control experiments, pure GOx or glucose was also separately added, none of them can induce the aggregation of AuNPs, and a colorimetric transition was not observed. Figure 2b,d further indicates that by increasing the amount of glucose used in GOx-catalyzed reaction, its oxidized product can induce more dramatic shift in the SPR band of AuNPs and concomitantly the more prominent changes in absorbance intensity ratios (A_{637}/A_{520}). On the basis of Figure 2d, we can determine that the glucose detection limit can be down to ~50 μM, which is quite comparable with those previously reported for the colorimetric assay based on ssDNA/AuNPs[20b] and fluorescent glucose probes based on aryl boronates.[20c] It has been well-known that the normal blood glucose concentration is in the range 3.5−6.1 mM, and abnormal glucose levels can reach as high as ~20 mM. Thus, the reported colorimetric H_2O_2 assay protocol based on AuNPs and the CGP can be facilely applied for the highly sensitive detection of glucose.

In summary, we presented an effective strategy for the quantitative and sensitive colorimetric detection of H_2O_2 and glucose by combining a novel concept of stimuli-triggered charge-generation polymer (termed as CGP) with AuNPs. The presence of H_2O_2 can trigger the transformation of the CGP from the initially uncharged state into a cationic polyelectrolyte via the deprotection of carbamate-based amine protecting moieties, which can then induce the aggregation of negatively charged AuNPs and result in a dramatic red shift of the SPR band and apparent colorimetric transitions. We expect that this proof-of-concept example can be further generalized to the design of a variety of other sensing systems by employing alternate trigger-specific charge-generation building moieties.[21]

Acknowledgment. The financial support from National Natural Scientific Foundation of China (NNSFC) Projects (20874092, 91027026, and 51033005), Fundamental Research Funds for the Central Universities, and Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP) is gratefully acknowledged.

Supporting Information Available: Experimental details and spectroscopic/analytical data of ^{1}H NMR, GPC, TEM, and UV−vis measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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