Cerebral Glucose Detection

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Colorimetric Detection of Glucose in Rat Brain Using Gold Nanoparticles**

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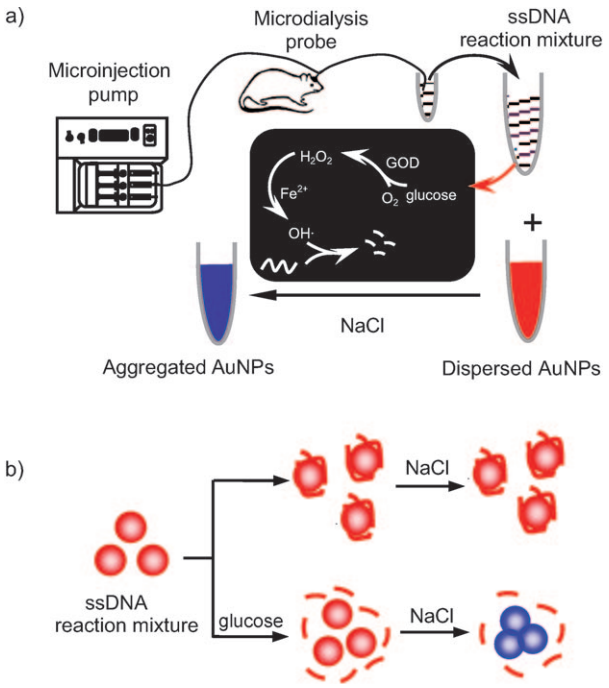
Direct, selective, and sensitive determination of the physiologically important species involved in brain function has drawn much attention because understanding the chemical nature in such physiological and pathological events affords a platform for, for example, neurotransmission and the diagnosis and therapy of diseases.[1] Although a number of methods have been developed to accomplish such a purpose,[2] the ever-increasing interest in the interface between chemistry and physiology presents a pressing need to establish theoretically simple, less technically demanding methods that are more readily adaptable to physiological conditions to probe the chemical systems involved in brain functions.

Gold nanoparticle (AuNP)-based colorimetric assays have been applied in a variety of research fields owing to the efficient integration of the unique optical properties of gold nanoparticles and the excellent surface/interface recognition ability afforded from the rational design of the surface chemistry of AuNPs.[3] For instance, rational design of the surface chemistry of AuNPs promotes specific interactions between the receptors and analytes and, as a result, makes the measurements highly selective.[4] Furthermore, the high extinction coefficient of AuNPs, particularly in relation to those of common organic chromophores, enables the colorimetric assay to be very sensitive.[3a,f,g,5] Moreover, colorimetric assays have advantages in their simplicity, both in the instrumentation used and in their operation. For example, detection is possible with the naked eye or can be concisely performed with UV/Vis spectrometry, without the requirements for either complicated instrumentation or much knowledge of the electron or energy transfer involved in the electrochemical or fluorescent systems. Whilst these advantageous properties make the colorimetric assays particularly attractive for the effective detection of physiologically important species in cerebral systems, such systems have not been reported so far.

Herein, we describe a colorimetric assay for the detection of cerebral glucose. In cerebral systems, glucose not only represents the primary energy source for the brain, but also plays important roles in synaptic transmission.[6] Whilst some methods, typically electrochemical, have been developed for the detection of glucose,[7] the complexity of cerebral systems presents a great challenge to these existing methods to provide glucose detection in cerebral systems with theoretical and technical simplicity. The rationale for the colorimetric assay of cerebral glucose is essentially based on in vivo microdialysis, and the aggregation of AuNPs induced by glucose through cascade reactions involving glucose, H_2O_2, and $^{\bullet}OH$ (Scheme 1). To the best of our knowledge, this is the first example in which a AuNP-based colorimetric assay has been utilized to detect physiologically important species in a cerebral system. This capability is envisaged to be applicable to monitoring brain chemistry in a simple fashion.

This assay utilizes the aggregation-induced changes in the color and UV/Vis spectrometry associated with 13 nm gold nanoparticles. Initially, AuNPs that were synthesized with



Scheme 1. a) Colorimetric detection of glucose in rat brain using gold nanoparticles; b) Glucose-induced color change of gold nanoparticles.

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citrate as the stabilizer were red in color and exhibited an absorption at 520 nm (not shown); both features are characteristic of well-dispersed 13 nm AuNPs. The addition of 25 μL NaCl (0.4 M) to a 180 μL AuNP dispersion (10 nM) resulted in the aggregation of the AuNPs, which was reflected by changes in both the color (from red to purple-blue) and in the UV/Vis spectrum of the AuNPs, with the decrease in absorption at 520 nm and the production of a new absorption peak at 650 nm (not shown). The salt-induced aggregation of AuNPs was considered to be a consequence of the suppressive effect of the salt against the coulombic repulsive interaction between citrate-stabilized AuNPs. This aggregation was efficiently retarded with the prior addition of ssDNA to a dispersion of AuNPs, which was verified by the almost unchanged color and absorption spectrum of a AuNP dispersion after the addition of NaCl[8] (Supporting Information, Figure S1, curve, vial 1). The protective effect of ssDNA toward the salt-induced aggregation of AuNPs was attributed to the high density charge in the backbone of ssDNA adsorbed onto the AuNPs that greatly eliminated the salt-induced AuNP aggregation. Importantly, the protective effect of ssDNA toward the salt-induced aggregation of AuNPs was suppressed after sequential addition of Fe^{2+} (in the form of [Fe(edta)]; edta = ethylenediaminetetraacetic acid) and H_2O_2 to the ssDNA solution (Supporting Information, Figure S1, red curve, vial 4). As control experiments, we found that sole addition of Fe^{2+} (Supporting Information, Figure S1, green curve, vial 2) or H_2O_2 to the ssDNA solution (Supporting Information, Figure S1, blue curve, vial 3) did not lead to aggregation of AuNPs or any changes in the color or spectra of the resulting AuNP dispersions, after subsequent addition of NaCl. The H_2O_2-induced aggregation of AuNPs was understood to be a consequence of the cleavage of the ssDNA stabilizer into small fragments by hydroxyl radicals (•OH) generated from the Fe^{2+}-catalyzed Fenton reaction of H_2O_2[9] and also of the low ability of the as-formed fragments to protect the salt-induced aggregation of AuNPs.

The colorimetric detection of glucose was essentially based on the H_2O_2-induced aggregation of AuNPs through cascade reactions using GOD, as the recognition element for glucose, to produce H_2O_2 in situ (Scheme 1). As displayed in Figure 1, the concomitant addition of glucose and GOD to a reaction mixture consisting of ssDNA and Fe^{2+}, and then mixing the reaction mixture into the AuNP dispersion, eventually led to aggregation of the AuNPs, after the subsequent addition of NaCl. This aggregation was reflected by the changes in both the color and the UV/Vis spectrum of the AuNP dispersion (Figure 1, red curve, vial 3). The sole addition of glucose or GOD in the ssDNA reaction mixture did not result in such changes (Figure 1, black and blue curves, vials 1 and 2), thus suggesting that the presence of glucose induced the aggregation of AuNPs (see the Supporting Information, Figure S2). These observations revealed that the in situ generation of H_2O_2, from the GOD-catalyzed oxidation of glucose, allows the occurrence of a Fenton reaction to cleave the ssDNA stabilizer under the joint reaction with Fe^{2+}. Such a property eventually resulted in a change in the agglomeration state of AuNPs from a well-dispersed state to an aggregated one, which essentially forms

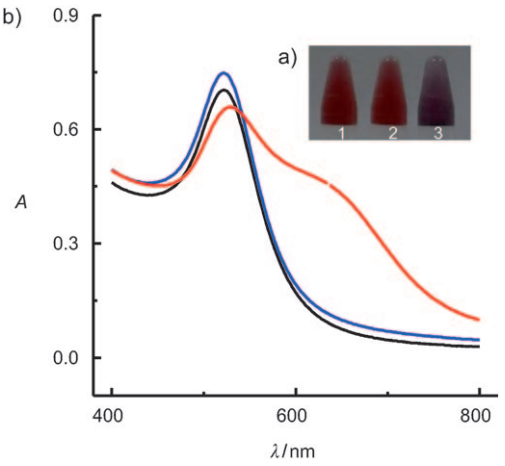

Figure 1. Glucose-induced aggregation of AuNPs. a) Photographs and b) UV/Vis spectra of AuNP dispersions, prepared first by addition of 15 μL glucose (40 mM; black curve, vial 1), 2 μL GOD (0.3 mg mL^{-1}; blue curve, vial 2), or 2 μL GOD (0.3 mg mL^{-1}) + 15 μL glucose (40 mM; red curve, vial 3) to the ssDNA reaction mixture. After being incubated for 2 h at 37 °C, each of the resulting mixtures was added to 180.0 μL AuNPs (10 nM). After 2 min, 25 μL NaCl (0.4 M) was added to the AuNP dispersions. GOD = glucose oxidase.

the basis for the colorimetric detection of glucose, as discussed below.

To evaluate the sensitivity of the assay, different concentrations of glucose were added to individual ssDNA reaction mixtures containing GOD, and the resultant mixtures were mixed into AuNP dispersions, followed by the final addition of NaCl salt (Figure 2). When an ssDNA reaction mixture containing no glucose was added to the AuNP dispersion, the subsequent addition of NaCl did not afford any change in the UV/Vis spectrum or of the color of the AuNP dispersion (Figure 2, black curve). However, the addition of various concentrations of glucose to ssDNA reaction mixtures containing GOD clearly led to changes in the AuNP dispersion, both in terms of the color (from red to purple-blue) and in the UV/Vis spectrum, with a decreased absorption at 520 nm (A_{520}) and an increased absorption at 650 nm (A_{650}). The ratio of A_{650}/A_{520} increased with the concentration of glucose in the ssDNA reaction mixture (Figure 2b, inset) and shows a linear relationship for glucose concentrations within the concentration range 0–5.0 mM ($A_{650}/A_{520} = 0.033\ C_{glu}\,\text{mM} + 0.170$, $R^2 = 0.98$). As has been previously reported,[7g,h,10] the basal level of glucose in cerebral systems was on the millimolar level, which falls within the linear range of the colorimetric assay demonstrated in this study, thus validating the assay for the measurement of glucose in these cerebral systems.

The selectivity of the colorimetric assay was evaluated by testing the response of the assay towards other species that coexist in the cerebral systems, of which H_2O_2 was first taken into account since the colorimetric assay for the cerebral glucose is essentially based on the oxidative cleavage of ssDNA with •OH radicals produced from H_2O_2. As one of the most reactive oxygen species, H_2O_2 is present in the cerebral system in only micromolar levels.[11] This level becomes further diluted after in vivo microdialysis sampling. There-

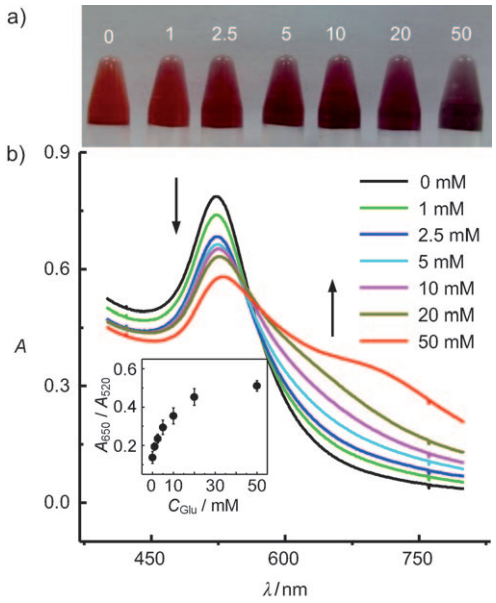

Figure 2. Sensitivity tests of the colorimetric detection of glucose. a) Photographs and b) UV/Vis spectra of AuNP dispersions prepared by addition of various concentrations of glucose to ssDNA reaction mixtures that contain 2 μL GOD (0.3 mg mL^{-1}). After incubation for 2 h at 37 °C, the resulting mixtures were added to individual vials containing 180.0 μL AuNPs (10 nM). After 2 min, 25 μL NaCl (0.4 M) was added to the mixtures. Glucose concentrations: 0, 1, 2.5, 5, 10, 20, and 50 mM. Inset in (b): Plot of A_{650}/A_{520} against C_{Glu} for the quantitative detection of glucose. Each point reflects the average of four independent experiments. Error bars indicate standard deviations.

fore, we used 0.5 μM H_2O_2 to study its potential interference toward the detection of glucose. For this purpose, 15 μL H_2O_2 (0.5 μM) was added to the ssDNA reaction mixture and the mixture was then incubated for 2 hours at 37 °C before being added to a AuNP dispersion. We found that the subsequent addition of NaCl salt to the AuNP mixture did not lead to changes in either the color or the spectrum of the AuNP dispersion (not shown), thus revealing that the physiological level of H_2O_2 did not interfere with the colorimetric detection of glucose.

On the other hand, as one of the most physiologically important small molecules, ascorbic acid (AA) is present in high levels (around the millimolar level) in these cerebral systems and has been demonstrated to be involved in many physiological and pathological processes.[2e,12] AA has an opposite effect toward ·OH production than H_2O_2: it may effectively scavenge radicals and enhance metal-ion-catalyzed ·OH generation in the presence of oxygen.[9f,12b,c,13] Although these two opposing effects may potentially interfere with the colorimetric detection of glucose, the addition of AA to the ssDNA reaction mixture did not lead to changes in either the color or in the spectrum of the AuNP dispersion (see the Supporting Information, Figure S3), thus indicating that the colorimetric assay is free from AA interference. Moreover, we have also found that this assay method is unaffected by other species that commonly exist in the cerebral systems, including DOPAC, DA, UA, 5-HT, and lactate (see the Supporting Information, Figure S3). This property further validates this colorimetric assay technique for the detection of cerebral glucose.

In general, assay methods for the detection of physiologically important species with the naked eye without resorting to any complicated instrumentation or operation theory are particularly convenient. For this reason, an assay that could effectively detect cerebral glucose, on the basis of cascade reactions initialized by glucose, would be of great interest; at present, there are no colorimetric assays that provide this capability. The use of AuNPs for this purpose was demonstrated in Figure 3. To determine glucose in the rat brain, the brain microdialysate (15 μL) was added to an ssDNA reaction mixture containing 2 μL GOD (0.3 mg mL^{-1}). After being incubating for 2 hours at 37 °C, the resulting mixture was added to 180 μL AuNPs (10 nM), followed by the addition of 25 μL NaCl (0.4 M). As shown in Figure 3 a, after the addition of the brain microdialysate to the GOD-containing ssDNA reaction mixture, the subsequent addition of NaCl led to a slight change in the color of the AuNP dispersion from red to somewhat purple (Figure 3 a, vial 2), compared with that with the addition of the same volume of the pure aCSF (i.e., without glucose; aCSF = artificial cerebrospinal fluid) to the GOD-containing ssDNA reaction mixture (Figure 3 a, vial 1), thus suggesting the presence of glucose in the brain microdialysate. To ensure that the color change had originally resulted from the generation of H_2O_2 from the GOD-catalyzed oxidation of glucose in the brain microdialysate, and to further confirm the selectivity of the colorimetric assay demonstrated here, 2.0 μL catalase (0.2 mg mL^{-1}), an enzyme that catalyzes H_2O_2 disproportionation, was added to an ssDNA reaction mixture containing GOD prior to the

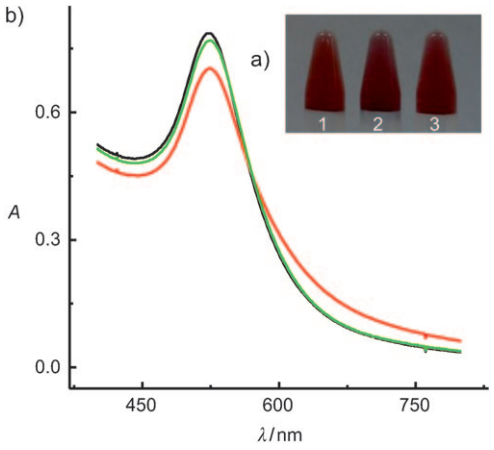

Figure 3. Colorimetric detection of glucose in the brain microdialysate. a) Photographs and b) UV/Vis spectra of AuNPs prepared without (black curve, vial 1) and with (red curve, vial 2) the addition of 15 μL brain microdialysate to the ssDNA reaction mixture containing 2 μL GOD (0.3 mg mL^{-1}). The green curve and vial 3 represent the UV/Vis spectrum and photograph of the dispersion of AuNPs after the first addition of 2 μL catalase (0.2 mg mL^{-1}) and then 15 μL brain microdialysate to the ssDNA reaction mixture containing 2 μL GOD (0.3 mg mL^{-1}). After being incubated for 2 h at 37 °C, each of the resulting mixtures was separately added to 180.0 μL AuNPs (10 nM). After 2 min, 25 μL NaCl (0.4 M) was added to the mixtures. The perfusion rate for in vivo microdialysis was 0.5 μL min^{-1}.

addition of the brain microdialysate. Unlike the results observed without the prior addition of catalase to the ssDNA reaction mixture (Figure 3a, vial 2), the first addition of catalase and then addition of brain microdialysate to the ssDNA reaction mixture did not lead to the color change of AuNPs after the subsequent addition of NaCl (Figure 3a, vial 3). The UV/Vis spectrum of AuNPs allowed a more precise detection of cerebral glucose (Figure 3b): the addition of brain microdialysate to the ssDNA reaction mixture resulted in a decrease in the absorption of AuNPs at 520 nm and an increase in the absorption at 650 nm (Figure 3b; red curve). According to the calibration curve described above, the basal level of glucose in the microdialysate from the rat brain was 2.4 mM, which was almost consistent with the reported values.[7g,h,10] Similarly, the addition of brain microdialysate to a ssDNA reaction mixture containing catalase did not lead to a change in the spectrum of AuNPs (Figure 3b, green curve), again suggesting that the colorimetric assay demonstrated here is very selective for the detection of glucose in the cerebral systems.

In summary, by taking advantage of the unique optical properties of AuNPs and the cascade reactions of glucose oxidase catalyzed oxidation of glucose and the Fenton reaction of H_2O_2, as well as the oxidative cleavage of ssDNA with •OH radicals, we have successfully demonstrated the application of a AuNP-based colorimetric assay for the simple but effective detection of glucose in the rat brain. The assay has a number of advantages over conventional systems for probing brain chemistry both in the simplicity of operation and in the instrumentation. The simplicity of this assay should make it more convenient and more readily adopted by physiologists than the existing systems, and it should thus find broad application in physiological and pathological investigations. This study not only provides a new colorimetric assay for the simple detection of glucose in the cerebral systems, but also potentially offers a new analytical platform for understanding the molecular basis of physiological and pathological events.

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