Colorimetric Visualization

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Sensitive and Selective Colorimetric Visualization of Cerebral Dopamine Based on Double Molecular Recognition**

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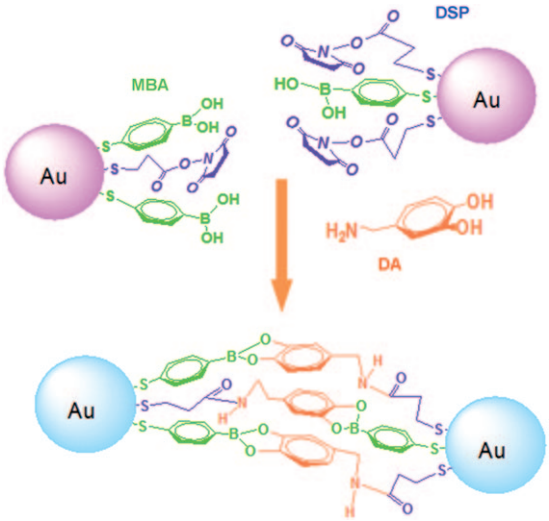
The development of direct, selective, and sensitive assay methods for the detection of physiologically important species involved in the brain function has drawn much attention, because the analytical methods are still the bottleneck for progress in understanding the role of neurotransmitters on neural activity, animal behavior, and so on.[1] Dopamine (DA) is one of the most important neurotransmitters, because it plays a central role in brain functions such as reward-related behavior, movement, and mood.[2] Alternations in neuronal release and uptake of DA have been implicated in psychiatric disorders, addiction, and Parkinson's disease.[3] In the past 30 years, a large number of elegant methods, including electrochemical strategies, spectroscopic approaches, chromatography, and so on, have been employed for DA detection.[1c,3b,4,5] However, the complexity of cerebral systems presents a great challenge to these existing methods to provide theoretically and technically simple DA detection in cerebral systems.

Herein, we first report a direct, selective, and sensitive strategy for the colorimetric visualization of cerebral DA at nanomolar levels using gold nanoparticles (AuNPs), with theoretical simplicity and low technical demands. Colorimetric methods, which take advantage of the color change that arises from the interparticle plasmon coupling during the aggregation of AuNPs or the redispersion of AuNP aggregates, have been applied for the detection of a large variety of targets, including nucleic acids, proteins, small molecules, and metal ions.[6]

Boronic acids form stable boronate complexes with diols, and the resulting complexes were employed to develop optical and electrochemical or electronic sensors for DA.[7] Although the benefits of these biosensors are already apparent, the selectivity of the DA sensors over biomolecules such as glucose and lactate with same diols are still limited. We first designed and functionalized the AuNPs with both 4-mercaptophenylboronic acid (MBA) and dithiobis(succinimidylpropionate) (DSP; MBA-DSP-AuNPs). These two molecules not only act as stabilizers for AuNPs but also interact with diols and amine functional groups, respectively, in DA to doubly recognize DA with high specificity. As shown in Scheme 1, boronic acids in MBA form stable boronate complexes with diols, while the amine-reactive succinimidyl residue in DSP reacts with the amine groups of DA. Double interactions between the functionalized AuNPs and DA triggered AuNP aggregation, thus resulting in a color change from wine red to blue and to direct colorimetric visualization of DA. Because such a color change can be seen visually, this method is very simple and does not require much instrumentation. Moreover, the double molecular recognition, together with the amplifying properties of AuNPs, was important to enable a colorimetric visualization of cerebral DA at nanomolar levels with high sensitivity and selectivity. To our knowledge, this is the first report in which a AuNP-based colorimetric assay has been utilized to detect neurotransmitters in a cerebral system.



Scheme 1. Colorimetric detection of dopamine using functionalized gold nanoparticles (the MBA-DSP-AuNPs probe).

As a proof of concept, AuNPs were first synthesized with citrate as the stabilizer. The prepared AuNPs were wine red in color and showed an absorption peak at 519 nm (black curve and vial 1 in Figure 1), which was ascribed to the surface plasmon resonance of the AuNPs. The addition of MBA and DSP with the optimized molar ratio of 1:2 to the initially prepared AuNPs essentially generates the MBA-DSP-AuNPs because of the formation of strong Au–S covalent bonds.

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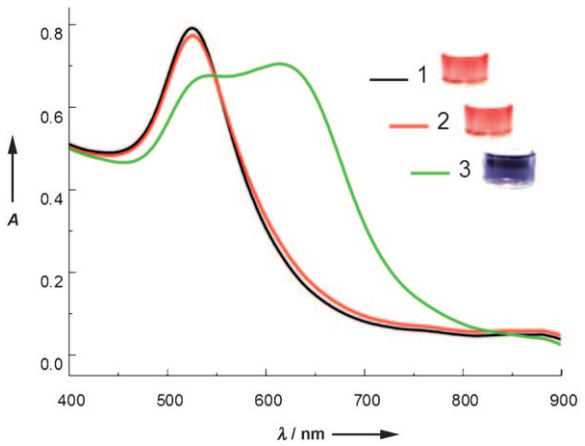

Figure 1. UV/Vis absorption spectra of AuNPs (black), MBA-DSP-AuNPs (red), and MBA-DSP-AuNPs with the addition of 265 nM dopamine (green). Inset: Direct observation of the corresponding color changes of AuNPs.

(see the Supporting Information, Figure S1). The modification of AuNPs with MBA and DSP was confirmed by X-ray photoelectron spectroscopy (XPS, see the Supporting Information, Figure S2). No obvious change was observed for the MBA-DSP-functionalized AuNPs in the color or in the characteristic peak at 519 nm (red curve and vial 2 in Figure 1). However, when DA was added into an aqueous suspension of the MBA-DSP-AuNPs probe, the color obviously changed from wine red to violet blue (vial 3 in Figure 1), thus indicating the aggregation of the AuNPs. The plasmon peak of the monodispersed AuNPs decreased in intensity, and a new, strong absorption peak appeared at 650 nm as the AuNP clusters formed (green curve in Figure 1). Such a change in the spectrum, which coincides with the change in the solution color, could be well explained by the DA-induced aggregation of the MBA-DSP-AuNPs through double molecular recognition. Under the conditions employed, the reactions were complete within approximately 5 min. AuNP aggregation was also confirmed by transmission electron microscopy (TEM; see the Supporting Information, Figure S3). The AuNP clusters were stable once formed, as the ratio of the absorbance at 519 nm to that at 650 nm remained constant for over 2 h (at room temperature in a 2 mL tube). The DA-induced assembly of the MBA-DSP-AuNPs was reproducible from batch to batch.

To further evaluate the analytical performance of the developed method for DA detection based on the concept demonstrated above, different amounts of DA were added to the MBA-DSP-AuNPs probes. As presented in Figure 2a, upon addition of increasing concentrations of DA, the color of the functionalized AuNPs gradually changed from initially wine red to purple and finally to blue. These results were

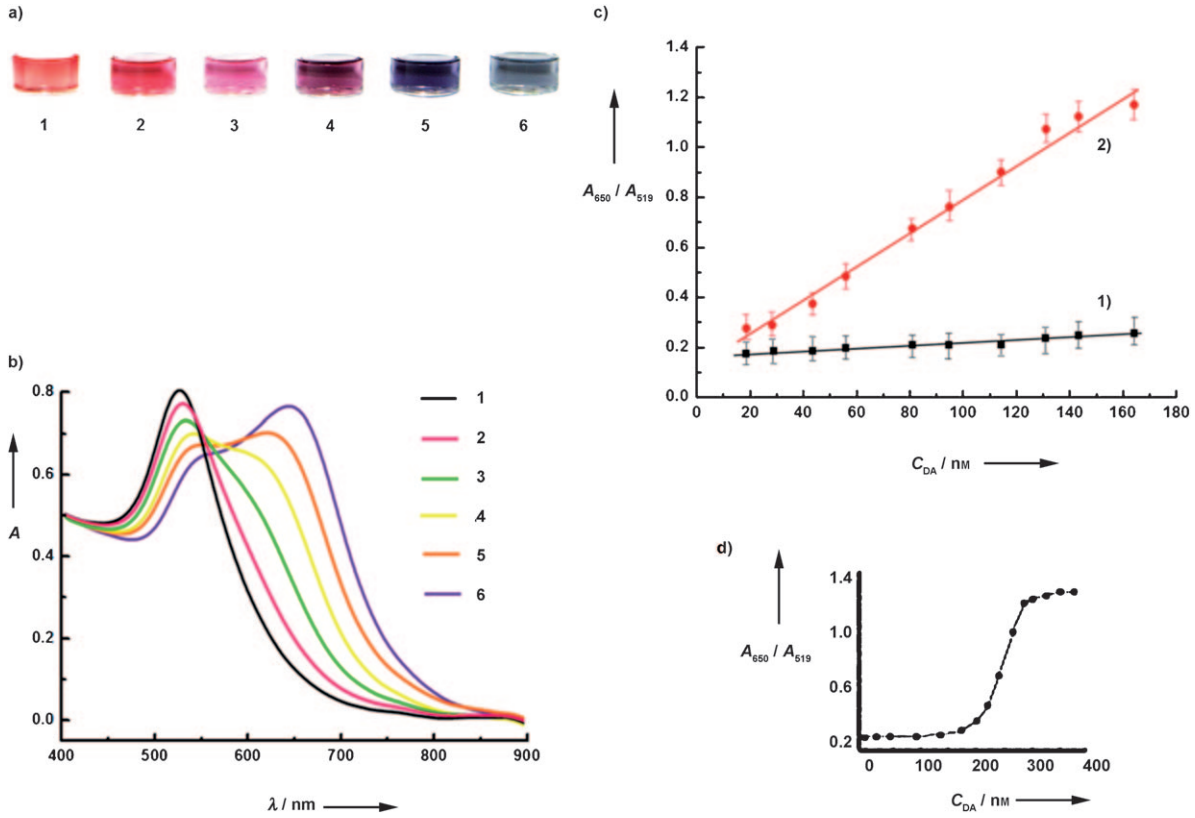

Figure 2. a) Colorimetric visualization and b) UV/Vis spectra of the MBA-DSP-AuNPs probe with the addition of DA at concentrations of 0, 185, 235, 255, 275, and 305 nM for vials (a) and curves (b) 1–6, respectively. c) Linear plots of A_{650}/A_{519} versus DA concentration obtained with 1) the MBA-DAP-AuNPs probe and 2) the optimized DA probe. d) The plot of A_{650}/A_{519} versus DA concentration (5–380 nM) obtained with the MBA-DAP-AuNPs probe.

further confirmed by UV/Vis spectroscopy. As shown in Figure 2b, with the addition of an increasing concentration of DA to the suspension of functionalized AuNPs, an obvious decrease in the absorption peak at 519 nm (A_{519}) and a strong increase in the absorption peak at 650 nm (A_{650}) were clearly detected. The ratio A_{650}/A_{519} increased with increasing concentration of DA in the low-concentration range (below 175 nM). However, a much more significant intensity increase was observed in the concentration range above 175 nM (Figure 2d). This observation reveals that the sensitivity for DA detection can be greatly improved by adding a certain amount of DA to the stock solution of MBA-DSP-AuNPs probes before use. To improve the probe sensitivity, 175 nM DA was added to the MBA-DSP-AuNPs solution in advance. This probe is denoted as the optimized DA probe hereafter. It is clear that the optimized sensitivity (curve 2 in Figure 2c) is approximately 35 times larger than before (curve 1 in Figure 2c). The detection limit for DA was determined to be approximately 0.5 nM with a relative standard deviation of 1.0% ($n=5$). Under the optimized conditions, a good linear relationship ($r=0.999$) can be found between the ratio A_{650}/A_{519} and concentration of the added DA between 5 and 180 nM. The basal level of DA in cerebral systems is on the nanomolar scale, which just falls within the linear range of the present optimized analytical method,[2d,5a,8] thus confirming that the strategy developed in this study is able to realize the colorimetric detection of cerebral DA.

The complexity of cerebral systems presents a great challenge to the analytical methods for DA detection not only in the detection limit and sensitivity but, more importantly, in selectivity. The selectivity of the present approach was evaluated by monitoring the extinction ratio (A_{650}/A_{519}) response in the presence of other biomolecules that may coexist in cerebral systems. The possible interferences that commonly exist in the cerebral systems, such as ascorbic acid (AA), epinephrine (E), norepinephrine (NE), glucose, lactate, and 3,4-dihydroxyphenylacetic acid (DOPAC), were also examined. Low responses were observed after the addition of these interferences in physiological concentrations, compared with that of DA (see the Supporting Information, Figure S4).[1d,9,10] Moreover, the effects of all these interferences on the signal of DA were also investigated in detail. No obvious signal changes were observed when all the above compounds were added in the presence of DA, thus suggesting high selectivity of the present method.

As demonstrated above, the present colorimetric method for DA detection at nanomolar levels with high selectivity provided a simple and direct platform for assaying DA in the rat brain based on in vivo microdialysis. As shown in Figure 3a, after the addition of the brain microdialysates (10 μL) into the optimized DA probe (vial 1 in Figure 3a), the color changed from wine red to somewhat purple (vial 2 in Figure 3a), although the color had no observable changes with the addition of the same volume of pure aCSF (i.e., without DA, aCSF = artificial cerebrospinal fluid). These results suggest the presence of DA in the brain microdialysates. Furthermore, the color of the optimized DA probe changed to purple (vial 3 in Figure 3a) with the addition of the brain dialysates 10 min after the injection of

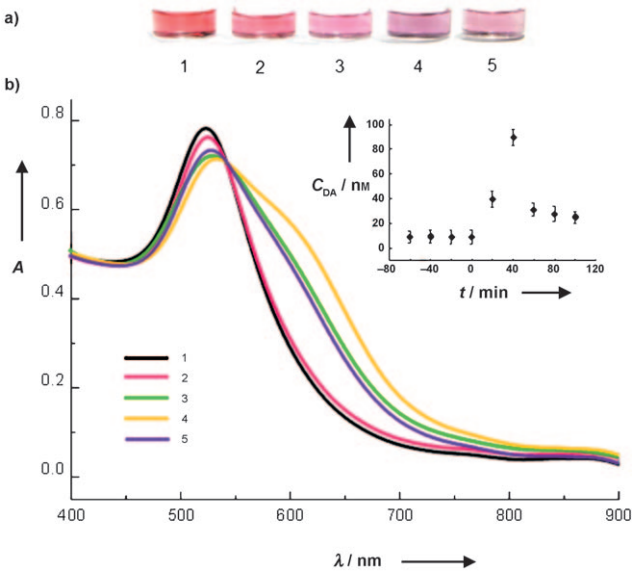

Figure 3. Colorimetric detection of DA in rat brain dialysates by the optimized DA probe. a) Direct observation of the color changes and b) UV/Vis spectra of the optimized DA probe: 1) without any additives and 2–5) with the addition of the brain dialysates under the basal level (2) and 10 (3), 30 (4), and 50 min (5) after the injection of nomifensine in the rat brain striatum (injection time of nomifensine is marked as time 0). Inset: plot of DA concentration changes in the brain dialysates after injection of nomifensine.

nomifensine, a DA reuptake blocker, and then changed to violet (vial 4 in Figure 3a) after 30 min, thus indicating the obvious increased concentration of DA. Then the color returned to purple (vial 5 in Figure 3a) 50 min after the injection of nomifensine. UV/Vis spectroscopy provides a more precise approach for the detection of cerebral DA. As shown in Figure 3b, the ratio A_{650}/A_{519} increased markedly with the addition of the brain microdialysates collected after the injection of nomifensine. Concentration changes of DA in the brain dialysates different periods of time after injection of nomifensine are plotted in the inset of Figure 3b. According to the calibration curve (curve 2 in Figure 2c), the basal level of DA in the brain dialysates was estimated to be approximately 10 nM, which was located in the range of literature values for basal microdialysate concentration of DA (5–15 nM).[5,8] The concentration is not corrected for in vitro probe recovery. Moreover, the DA level increased to approximately 82.4 nM 30 min after nomifensine was injected and then gradually relaxed to a value (ca. 36.3 nM) still higher than the basal level of DA after 50 min, which also agreed well with the literature.[5] Furthermore, the concentration changes of DA in the rat brain before and after the local infusion of tetrodotoxin (TTX, a dopamine synthesis inhibitor) were also investigated. Local infusion of TTX caused a significant decrease of DA concentration in the microdialysate samples (see the Supporting Information, Figure S5). To further confirm the selectivity, high-performance liquid chromatography with an electrochemical detector (HPLC-EC) was employed to determine the basal level of DA in the brain microdialysate samples. The basal DA concentration in the brain dialysates assayed by the conventional HPLC-EC

method was in a good agreement with that obtained by the optimized DA probe (see the Supporting Information, Table S1).

In summary, we have first designed the MBA-DSP-AuNPs probe, which can interact with diols and amine functional groups in DA to doubly recognize DA. Based on the double molecular recognition, together with the amplifying properties of AuNPs and the optimized probe, we have developed a direct, selective, and sensitive strategy for the colorimetric visualization of DA at nanomolar levels. The developed method, which is theoretically simple and has low technical and instrumental demands, has been successfully applied for the reliable detection of DA in the rat brain. Although multiple analytes cannot be simultaneously detected by the optimized probe, the simplicity in operation and instrumentation of this method should help it to find broad applications in biochemical investigations. This work provides a methodology to design AuNP-based colorimetric assays with high specificity and sensitivity, and it establishes a simple and reliable approach for monitoring cerebral species in brain chemistry, which may be related to physiological and pathological events.

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