Supplementary information

Simple and rapid detection of L-Dopa decarboxylase activity using gold nanoparticles

So Young Park, Dohyoung Kwon, Hyejung Mok and Bong Hyun Chung

a BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), 305-806, Republic of Korea.
b School of Engineering, University of Science and Technology (UST), 305-600, Republic of Korea
c Department of Bioscience and Biotechnology, Konkuk University, 143-701, Republic of Korea

E-mail : bhchung@kribb.re.kr
Experimental

Materials

L-Dopa decarboxylase (DDC) was purchased from R&D Systems (USA). 3,4-Dihydroxy-L-phenylalanine (L-Dopa), dopamine (DA), L-tyrosine, L-tryptophan, tryptamine, L-phenylalanine, phenylethylamine, glucose, ascorbic acid, 3,4-Dihydroxyphenylacetic acid (DOPAC), epinephrine, human serum, pyridoxal 5′-phosphate hydrate (PLP), carbidopa, gold chloride hydrate (HAuCl₄), and trisodium citrate dihydrate were purchased from Sigma (USA). Sodium dodecyl sulphate (SDS) solution (20 %) was purchased from LPS solution (Korea). Centrifugal filters (30 kDa) were obtained from Millipore (USA).

Synthesis of AuNPs

All glassware was thoroughly cleaned with aqua regia (HCl:HNO₃ = 3:1), rinsed in distilled water and dried in an oven prior to use. Citrate-coated AuNPs with a diameter of 13 nm were prepared as reported previously. Fifty millilitres of 1 mM HAuCl₄ was boiled, and 5 mL of 38.8 mM trisodium citrate was rapidly added to the solution. The solution was stirred vigorously until a color change from pale yellow to red was observed. After the solution cooled to room temperature, the AuNP solution was filtered through a 0.22-μm pore-size filter. Then, 1.6 mL of 20% sodium dodecyl sulphate (SDS) solution diluted 30 times was added to 50 mL of the AuNP solution. The size of the AuNPs was determined using UV/Vis spectroscopy (DU 800, Beckman coulter), dynamic light scattering (DLS, Otsuka Electronics, Japan) and transmission electron microscopy (TEM, 200 kV, Phillips). The concentration of the AuNPs was estimated using the Beer-Lambert law with an extinction coefficient of ~ 2.7×10⁸ M⁻¹cm⁻¹ at λ 520 nm for 13 nm particles.

Study of AuNP aggregation in the presence of L-Dopa, DA and the DDC reaction mixture

L-Dopa and DA were dissolved in deionised H₂O at a total concentration of 5 mM. These solutions were diluted 25 times with HEPES buffer (50 mM, pH 7.2) containing NaCl (100 mM). L-Dopa or DA solution (final concentration, 100 μM) was added to 300 μL of the AuNP solution (11 nM). The color and absorption spectra of the AuNPs were observed with naked eye and confirmed by UV/Vis spectroscopy.

Development of a calibration curve for determining the percentage conversion of L-Dopa

Various concentrations of DA (0 ~ 200 μM) and L-Dopa were mixed at a total concentration of 200 μM in HEPES buffer solution. The mixtures with different ratios of DA to L-Dopa were incubated with AuNP solutions (11 nM, 300 μL), and the spectra were recorded after 3 min. The calibration curve for the detection of DDC activity was constructed using the ratio of the absorbance at 519 nm and the absorbance at 650 nm as a function of the DA concentration.
Assay for sensing of DDC activity

The DDC reaction solution contained various amounts of DDC (from 37.5 nM to 150 nM), 1 mM L-Dopa, and 0.1 mM PLP. The reaction was carried out in 50 mM HEPES buffer (pH 7.2, 100 mM NaCl) at 37 °C and was stopped by inactivating the enzyme in boiling water for 2 min. The reaction mixture was diluted five times by HEPES buffer and filtered at 13,000 rpm for 5 min using a 30 kDa centrifuge filter to remove the enzyme. The filtered sample was added to 300 μL of AuNPs, and the UV/Vis spectra were measured 1 min after mixing, as shown in figure Fig. 3(a).

Enzyme inhibition assay

DDC (60 nM) was incubated with 2μM of carbidopa in HEPES buffer for 10 min at room temperature. L-Dopa (1 mM) and PLP (0.1 mM) was then added to the mixture, and the reaction mixture was incubated at 37 °C for 30 min. The enzymatic reaction was performed by incubating the solution as previously described.

References

Fig. S1. (a) DLS data for AuNP solutions after the addition of L-Dopa and DA. (b) TEM images of AuNP solutions after the addition of L-Dopa (left) and DA (right).
Fig. S2. (a) UV-Vis spectra of AuNP solutions in the presence of various concentrations of DA. (b) The A650/A519 ratios are plotted as a function of the concentration of DA. (fig.S2 (a) was corrected for light scattering contribution)
Fig. S3. The A700/A519 ratios of AuNPs after incubation with various substrates and products of DDC and other interferences at a concentration of 150 μM. (1; L-tyrosine, 2; Tyramine, 3; L-Tryptophan, 4; Tryptamine, 5; L-Phenylalanine, 6; Phenylethylamine, 7; Glucose, 8; human serum, 9; Ascorbic acid, 10; DOPAC, 11; Epinephrine, 12; L-Dopa, 13; Dopamine)