ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2015, 51, 13752

Received 20th July 2015,
Accepted 23rd July 2015

DOI: 10.1039/c5cc06055a

www.rsc.org/chemcomm

Dendrimer-encapsulated Pt nanoparticles with peroxidase-mimetic activity as biocatalytic labels for sensitive colorimetric analyses†

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We demonstrate the feasible use of Pt nanoparticles encapsulated inside amine-terminated fourth-generation polyamidoamine dendrimers as peroxidase-mimetic labels for sensitive colorimetric assays. This was performed by utilizing intrinsic dual functionalities of the dendrimer-encapsulated Pt nanoparticles, *i.e.* peroxidase-like activity and multiple conjugation sites.

In this communication, we report on the feasibility of utilizing dendrimer-encapsulated Pt nanoparticles (Pt DENs) as peroxidase-mimetic labels for amplified signal transduction events in colorimetric assays. We synthesized uniform Pt DENs having well-defined sizes with sub-nanometer accuracy, and found peroxidase-like activity (comparable to that of natural peroxidases such as horseradish peroxidase (HRP)) of the synthesized Pt DENs toward typical peroxidase substrates. Furthermore, we demonstrated the versatility of the Pt DENs as biocatalytic labels by utilizing their unique dual functionalities for a sensitive and selective colorimetric analysis of glucose as a model analyte: the Pt nanoparticles within the interior of the dendrimer and the high number of functional groups on the surface of the dendrimer. These dual functionalities offer intrinsic peroxidase-like activity and multiple conjugation sites, respectively, for simple but sensitive colorimetric assays. The significance of this study arises from the feasible use of Pt DENs as versatile peroxidase-mimetic labels capable of overcoming the inherent limitations of natural enzyme peroxidases commonly used in a variety of colorimetric assays including the glucose assay.

Natural peroxidases including HRP have been extensively used as biocatalytic labels in a wide range of colorimetric analyses in biomedical, pharmaceutical, agricultural, biochemical, and environmental applications.[1] Peroxidases exhibit high catalytic activity and substrate specificity in the conversion of chromogenic substrates to colored products in the presence of H_2O_2.[2] However, natural peroxidases also have inherent limitations such as easy loss of catalytic activity and substrate specificity due to their denaturation susceptible to environmental changes. Furthermore, their preparation, purification, and storage requirements are time-consuming and expensive. Therefore, considerable efforts have been made to discover and design efficient enzyme mimics. For example, ferromagnetic nanoparticles have been found to possess intrinsic peroxidase-like activity even with additional advantages of inorganic nanoparticles such as high stability and controllable preparation at relatively low cost.[3] Other unique nanoscaled materials, such as metal or metal-oxide nanomaterials, carbon nanotubes, carbon nanodots, and graphene oxides, have also been reported as enzyme mimics.[4] Recently, Cheng and co-workers used acetylated ninth-generation polyamidoamine (Ac-G9 PAMAM) dendrimers (which have a similar molecular size and globular shape to catalase (~10 nm)) to synthesize Pt nanoparticles (Ac-G9/Pt NP) as efficient catalase mimics, showing catalytic activity approaching that of natural catalases.[5] However, they were not concerned with the feasibility of the Pt nanoparticles as enzyme-mimetic labels in colorimetric assays. To the best of our knowledge, the feasible use of DENs for overcoming the limitations of natural enzyme labels in colorimetric assays has been rarely explored; however, it provides unique ways to design new enzyme-mimetic labels utilized in various colorimetric analyses. In contrast to the previous studies, we report here the feasibility of DENs as peroxidase-mimetic labels for sensitive colorimetric assays. Specifically, we synthesized Pt DENs (diameter 1.25 ± 0.27 nm) containing an average of 55 Pt atoms using amine-terminated fourth-generation polyamidoamine (PAMAM) dendrimers, which we denote as G4-NH_2(Pt_{55}) (experimental details are provided in the ESI†). The synthesized Pt DENs exhibited peroxidase-like activity, superior or at least comparable to that of natural HRP and other peroxidase-mimetic nanomaterials, toward typical peroxidase substrates including 3,3′,5,5′-tetramethylbenzidine (TMB), 3,3′-diaminobenzidine (DAB), *o*-phenylenediamine (OPD) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). In addition, the peroxidase-mimetic Pt DENs allowed the facile conjugation of

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† Electronic supplementary information (ESI) available: Experimental section and the additional data described in the text. See DOI: 10.1039/c5cc06055a

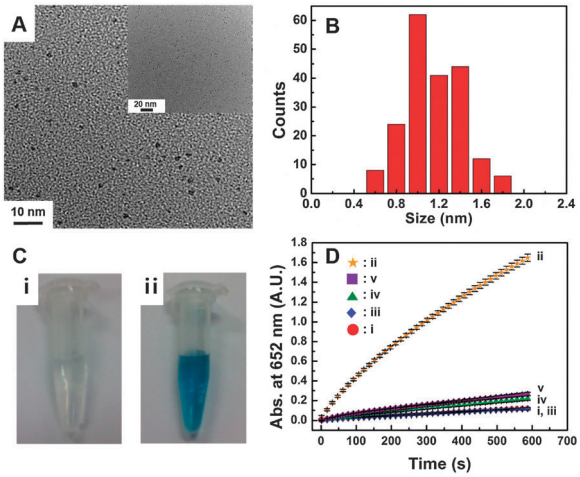

Fig. 1 (A) TEM images and (B) particle size distribution of as-synthesized G4-NH_2(Pt_{55}) DENs. The inset in (A) shows a TEM image having a large field of view. (C) Photographs of 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) containing 100 mM H_2O_2 (i) in the absence or (ii) in the presence of 30 nM G4-NH_2(Pt_{55}) DENs. Incubation time: 10 min. (D) Time-dependent absorbance changes at 652 nm of 800 μM TMB solutions (200 mM NaAc buffer, pH 4.0) containing 100 mM H_2O_2 (i) in the absence of 30 nM G4-NH_2(Pt_{55}) DENs, (ii) in the presence of 30 nM G4-NH_2(Pt_{55}) DENs, (iii) 30 nM G4-NH_2 dendrimers, (iv) 30 nM G4-NH_2–Pt precursor ion complexes, or (v) 30 nM catalases. The error bars represent the standard deviations derived from independent measurements (n = 3).

glucose oxidase (GOx) due to the intrinsic multiple amine groups on the surface of the dendrimers, which led to sensitive colorimetric analysis of glucose as a model analyte with the Pt DEN–GOx conjugates.

Fig. 1A shows representative TEM images of the as-synthesized Pt DENs, *i.e.* G4-NH_2(Pt_{55}) DENs, which were spherical in shape. It is important to note that the Pt DENs were nearly monodisperse in size and rarely aggregated due to the stabilization of the Pt nanoparticles inside the dendrimers.[6] As shown in Fig. 1B, the measured average diameter of the Pt DENs was 1.25 ± 0.27 nm, which is consistent with the theoretical value (1.17 nm) for nanoparticles containing 55 atoms with sub-nanometer accuracy.[7] To investigate the peroxidase-like activity of the synthesized Pt DENs, we then performed catalytic oxidation of TMB (one of the most common chromogenic substrates of peroxidase) in the presence of H_2O_2. As shown in Fig. 1C, we observed a significant change in the blue color upon the addition of the Pt DENs to the peroxidase substrate TMB solution containing H_2O_2; on the other hand, the color change was negligible without the addition of the Pt DENs. The change in the blue color was attributed to the catalytic formation of blue color products with maximum absorbance at 370 nm and 652 nm (ESI,† Fig. S1).[2] It is worth noting that the added Pt DEN solution itself was colorless (ESI,† Fig. S2). We also observed the catalytic oxidation process of TMB by monitoring the time-dependent absorbance changes of the TMB reaction solutions containing H_2O_2. Fig. 1D shows the time-dependent absorbance changes at 652 nm. Upon the addition of the Pt DENs, the absorbance at 652 nm increased markedly ((ii) in Fig. 1D) during the reaction process, while the absorbance changed negligibly in the absence of the Pt DENs ((i) in Fig. 1D). However, the addition of only dendrimers (without the encapsulated Pt nanoparticles) resulted in no meaningful increase in the absorbance at 652 nm ((iii) in Fig. 1D), which excluded the possibility that the observed catalytic oxidation of TMB is caused by the dendrimer itself. Interestingly, we observed a small increase in the absorbance at 652 nm upon the addition of dendrimer-Pt precursor ion complexes to the TMB reaction solutions ((iv) in Fig. 1D), indicating slight catalytic activity of the dendrimer-Pt ion complexes, although much smaller activity compared to that of Pt DENs for the oxidation of the TMB substrate in the presence of H_2O_2. As an additional control experiment, we also observed the oxidation process of TMB upon the addition of catalase enzymes to the TMB reaction solutions. We observed a slightly larger increase in the absorbance at 652 nm over time upon the addition of the catalases ((v) in Fig. 1D) than the increase upon the addition of the dendrimer-Pt precursor ion complexes ((iv) in Fig. 1D), which suggests the oxidation of TMB by oxygen generated by the catalases in the presence of H_2O_2. However, the increase in the absorbance at 652 nm in the presence of the catalases ((v) in Fig. 1D) is significantly smaller than the increase in the presence of the Pt DENs ((ii) in Fig. 1D), which confirms that the catalytic oxidation of TMB in the presence of the Pt DENs is due to the peroxidase-mimetic activity of Pt DENs and not the catalase-mimetic activity of Pt DENs. We also found that the oxidation of TMB by oxygen, which could be generated by catalytic decomposition of H_2O_2 possibly due to catalase-mimetic activity of Pt DENs, is much negligible compared to the catalytic oxidation of TMB in the presence of the Pt DENs (ESI,† Fig. S3), which again confirms the peroxidase-mimetic activity of Pt DENs. In addition, the time-dependent absorbance changes against the concentration of Pt DENs added to the TMB reaction solutions were observed. The changes exhibited a significant increase in the reaction rates of catalytic TMB oxidation upon increasing the Pt DENs concentration (ESI,† Fig. S4). These results clearly confirm that the observed catalytic activity for the oxidation of the TMB substrate in the presence of H_2O_2 (*i.e.* peroxidase-like activity) originates from the Pt nanoparticles encapsulated inside the dendrimers. In addition to TMB, we also tested other typical peroxidase substrates including DAB, OPD, and ABTS, which confirmed the catalytic oxidation of the chromogenic substrates upon the addition of the Pt DENs, giving a brown product, an orange product, and a green product, respectively (ESI,† Fig. S5). On the basis of the above results, we conclude that the synthesized Pt DENs exhibited intrinsic peroxidase-like activity toward typical chromogenic peroxidase substrates.

We found that the peroxidase-like activity of Pt DENs is dependent on pH and H_2O_2 concentration, which is in agreement with previously reported peroxidase-mimetic nanomaterials and natural peroxidases (ESI,† Fig. S6).[3,4d,e] The optimal pH value was found to be 4.0, which is very close to the values of other peroxidase mimics and natural HRP.[3,4c,e] The Pt DENs also required higher concentration of H_2O_2 than natural HRP in order to approach the maximal level of peroxidase-like activity as that of

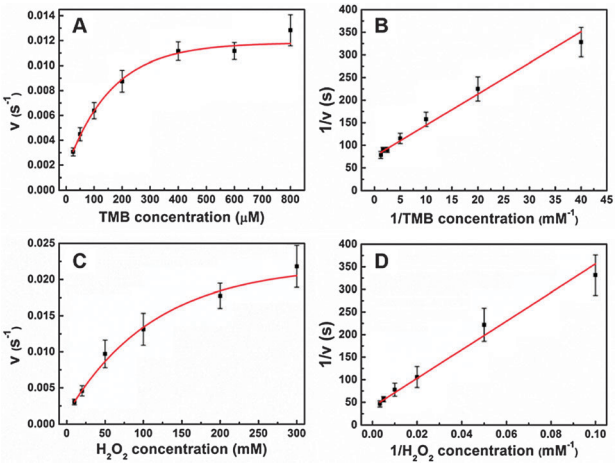

Fig. 2 Steady-state kinetic assays of G4-NH_2(Pt_{55}) DENs. (A) Michaelis–Menten and (B) Lineweaver–Burk plots for the TMB substrate. (C) Michaelis–Menten and (D) Lineweaver–Burk plots for the H_2O_2 substrate. Experimental details are provided in the ESI.† The error bars represent the standard deviations derived from independent measurements (n = 3).

other previously reported peroxidase mimics.[3,4d,e] Under the optimized conditions, we investigated apparent steady-state kinetics for the TMB oxidation reaction to quantify the peroxidase-like activity of Pt DENs in comparison with that of natural HRP. As shown in Fig. 2A, a typical Michaelis–Menten curve was obtained for the Pt DENs in a certain range of TMB concentrations. Using a Lineweaver–Burk plot (Fig. 2B), we obtained the Michaelis constant (K_m), indicating the enzymatic binding affinity of Pt DENs to TMB. The apparent K_m value of Pt DENs with TMB was found to be 0.091 mM, which is superior or at least comparable to that of natural HRP enzyme and other peroxidase-mimetic nanomaterials (ESI,† Table S1).[3,8] More importantly, we also observed little change in the apparent K_m value of Pt DENs, even after harsh treatment of the Pt DENs at 95 °C for 30 min (ESI,† Fig. S7). This clearly indicates the excellent preservation of the peroxidase-like activity of Pt DENs after exposure to the harsh heat treatment (ESI,† Fig. S8). Additionally, we found that the Pt DENs exhibited a typical Michaelis–Menten behavior for the H_2O_2 substrate with an apparent K_m value of 80.25 mM, which is inferior to that of natural HRP enzyme but is in good agreement with those of other peroxidase-mimetic nanomaterials (Fig. 2C and D).[3,9] This observation is consistent with that of the higher H_2O_2 concentration that was required to achieve the maximal peroxidase-like activity of the Pt DENs as discussed above.

On the basis of the intrinsic peroxidase-like activity of Pt DENs, we designed a sensitive colorimetric analysis for the detection of glucose (an important indicator for the diagnosis of diabetes mellitus) as a model analyte. Specifically, we conjugated streptavidin-modified GOx onto the terminal amine groups of Pt DENs using amine-reactive *N*-hydroxysuccinimide (NHS) esters of biotin (see the ESI† for experimental details). Since the Pt DEN itself provides a large number of amine groups at the dendrimer terminals as conjugation sites, it is viable to conjugate the GOx to the Pt DENs, even without the

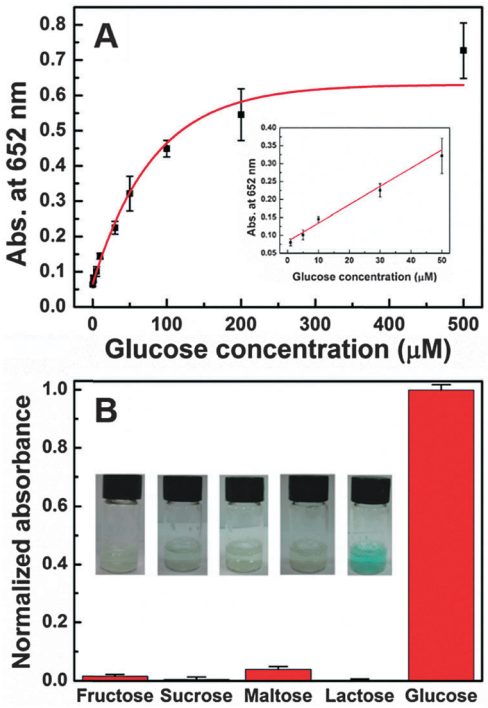

Fig. 3 (A) Calibration curve obtained with Pt DEN–GOx conjugates for colorimetric analysis of glucose in 200 mM NaAc buffer (pH 4.0) containing 800 μM TMB. (B) Normalized absorbance at 652 nm obtained with Pt DEN–GOx conjugates in 200 mM NaAc buffer (pH 4.0) containing 800 μM TMB after adding 16.67 μM glucose or 166.7 μM glucose analogues. The inset shows photographs of the corresponding samples after the colorimetric analysis. The error bars represent the standard deviations derived from independent measurements (n = 3).

additional formation of conjugation sites. It is worth noting that many peroxidase-mimetic nanomaterials often required additional formation of coating layers as conjugation sites using different compounds including silanes, poly(styrenesulfate) (PSS), polyethylene glycol (PEG), and dextran.[3,4a] The resulting Pt DEN–GOx conjugates catalysed both oxidation of the glucose while producing H_2O_2 and subsequent oxidation of the TMB chromogenic substrate to generate blue color products in the presence of the liberated H_2O_2 *via* the biocatalyses of GOx and Pt DEN, respectively. As shown in Fig. 3A, we obtained a calibration curve with the Pt DEN–GOx conjugates, utilizing the Pt DENs as peroxidase-mimetic catalytic labels that amplify biorecognition events of GOx for the sensitive colorimetric analysis of glucose. The calibration curve represents a good linear relationship between the absorbance at 652 nm and the glucose concentration ranging from 1 to 50 μM with a correlation coefficient of 0.946, which allows the detection of glucose as low as 1 μM. This is comparable to other state of the art glucose sensors, exhibiting great potential of the Pt DENs for the simple but sensitive colorimetric analysis of glucose (ESI,† Table S2). In addition, the Pt DEN–GOx conjugates exhibited high selectivity for colorimetric detection of glucose among glucose analogues such as fructose, sucrose, maltose, and lactose, even at 10 times higher concentration than that of glucose (Fig. 3B). These results clearly demonstrated the feasibility of the Pt DENs as versatile labels by utilizing the unique dual functionalities of Pt

DENs, *i.e.* the intrinsic peroxidase-like activity of the Pt nanoparticle encapsulated inside the dendrimers and the multiple conjugation sites of dendrimers.

In summary, we reported Pt nanoparticles encapsulated inside amine-terminated fourth-generation PAMAM dendrimers, which exhibited intrinsic abilities as versatile peroxidase-mimetic labels for sensitive colorimetric analyses. The Pt DENs (diameter 1.25 ± 0.27 nm) were uniform in size with sub-nanometer accuracy, and showed peroxidase-like activity toward typical chromogenic substrates (including TMB, DAB, OPD, and ABTS) of peroxidases. In addition, the peroxidase-mimetic Pt DENs provided a high density of amine groups at the dendrimer terminals, which allowed easy conjugation of GOx without the additional formation of coating layers on the Pt DENs. This therefore led to the sensitive and selective colorimetric assay of glucose as a model analyte with the Pt DEN–GOx conjugates. These results clearly demonstrated the unique abilities of the Pt DENs as versatile peroxidase-mimetic labels for colorimetric analyses, *i.e.* peroxidase-like activity and the multiple conjugation sites. We envision that the peroxidase-mimetic activity of Pt DENs can be tailored further since the size, composition, and structure of Pt nanoparticles inside dendrimers (which are expected to affect the peroxidase-like activity of Pt DENs) can be precisely controlled.[10]

This work was financially supported by the National Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning (NRF-2014S1A2A2028540 and NRF-2014R1A1A2058218), and the Agency for Defense Development through Chemical and Biological Defense Research Center.

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