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Nanozyme activity of platinum nanoparticles prepared by pulsed laser ablation

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Abstract: Platinum nanoparticles (Pt-NPs) were generated by pulsed laser ablation in liquid and proved to be stable in aqueous dispersion for at least six months when stabilized by small amount of sodium citrate. Their nanozyme activity was tested photometrically with tetramethylbenzidine (TMB) in the absence and presence of hydrogen peroxide (H_2O_2) at 22°C, which lead to specific activities (SA) of 1.9U/mg Pt-NPs and 375U/mg Pt-NPs and limits of detection (LOD) of 1.86pmol/L and 0.026pmol/L, respectively. In comparison, a SA of 289U/mg and a LOD of 36pmol/L were determined for a recombinant horseradish peroxidase in presence of H_2O_2 at 22°C. In particular, the lower detection limit of Pt-NPs by a factor of 1384 compared to the recombinant horseradish peroxidase indicates their great potential for future bioassays.

Keywords: platinum, nanoparticles, nanozyme, horseradish peroxidase, specific activity

Introduction

Nanoparticles (NP) with enzyme-like catalytic activities, also referred to as nanozymes, are promising for the use in bioassays, as they have several advantages over natural enzymes such as higher stability, resistance to external conditions, and strong catalytic performance [1]. Furthermore, they can be tailored for specific applications (e.g., sample preparation, purification, labelling) [2] by bioconjugation of the metal surface to biomolecules. In this study, ultrapure Pt-NPs, prepared by pulsed laser ablation in liquid (PLAL) are investigated on their nanozyme activity (peroxidase and oxidase capability) and compared in their activity to recombinant horseradish peroxidase (HRP).

Materials and Methods

HRP was purchased from Assay Genie. Hydrogen peroxide (H_2O_2) (31wt%), tetramethylbenzidine (TMB), sodium acetate trihydrate and acetic acid were purchased from Sigma Aldrich. Ultrapure, spherical Pt-NPs were synthesized in 0.5mM sodium citrate aqueous solution by PLAL to a final NP concentration of 0.97 mg/mL [3]. Storage stability of at least six months was observed by visual inspection. The mean diameter of the Pt-NPs was measured with a Zeiss Gemini Scattering electron microscope (SEM) using the secondary electron detector and 5kV accelerating voltage. A dilution of Pt-NPs in 0.5mM citrate solution containing 25µg/mL bovine serum albumin were drop casted on a cleaned silica wafer piece and allowed to dry at room temperature. SEM image analysis for mean diameter (D) determination was performed with ImageJ open-source software. A measurement protocol was adapted from Jiang et al. [4] to measure specific peroxidase activities for the enzyme and nanozyme. 0-12.88ng/171.8µl Pt-NPs or HRP in acetate buffer (200mM, pH: 3.6) were mixed in a well of a 96 well-plate with 8.6µL TMB in dimethyl sulfoxide (10mg/mL) and 19.7µL H_2O_2. For measuring oxidase activity, H_2O_2 was replaced with acetate buffer. Absorbance was measured at a wavelength of λ=652nm and at 22°C in triplicates on a Perkin Elmer Enspire Plate Reader (60 repeats, interval 10s). The specific activity *SA* (U/mg) was calculated via the equation:

$$SA = (V/(\varepsilon \times l) \times (\Delta A/\Delta t))/W, \qquad (1)$$

where V is the total reaction volume (200µL), ε the extinction coefficient of TMB at a wavelength of 652nm (39000M^{-1} cm^{-1}), l the optical path length (0.56cm), $\Delta A/\Delta t$ the initial change in absorbance per minute (derived from a linear fit over the first 100s), and W the weight per well of enzyme or Pt-NP in mg. The limit of detection (LOD) was determined by the intersection of the linear fit of the absorbance values (after 100s) with the blank value plus three times the standard deviation (SD). The molar concentration of Pt-NPs was calculated from W, the Pt density (21.45g/cm^3) and the volume of the Pt-NPs, calculated from the mean diameter derived by SEM [5].

Results and Discussion

The mean diameter of the Pt-NPs amounted to 10.9±4.5nm. Crucial for the activity measurement was the stability in the acetate buffer at low pH of 3.6. Under these conditions, 63µg/ml Pt-NPs kept their slightly yellowish color for at least 30min, and thus, activity measurements with and without H_2O_2 were performed in this time frame. Recombinant HRP was characterized as reference with the TMB/H_2O_2 assay. For the TMB/H_2O_2 assay a SA for HRP of 289U/mg and a SA for Pt-NPs of 375U/mg could be calculated. In absence of H_2O_2, a SA for Pt-NPs of 1.9U/mg Pt-NPs could be observed. In literature, SAs of NPs with other core materials such as gold, carbon, magnetite, are reported for 37°C with H_2O_2 ranging from 1.6-5.1U/mg [4]. Citrate-capped Pt-NPs (diameter of 2.5nm), which are chemically synthesized are reported with similar SAs of 323.7U/mg at 55°C with H_2O_2 [6].

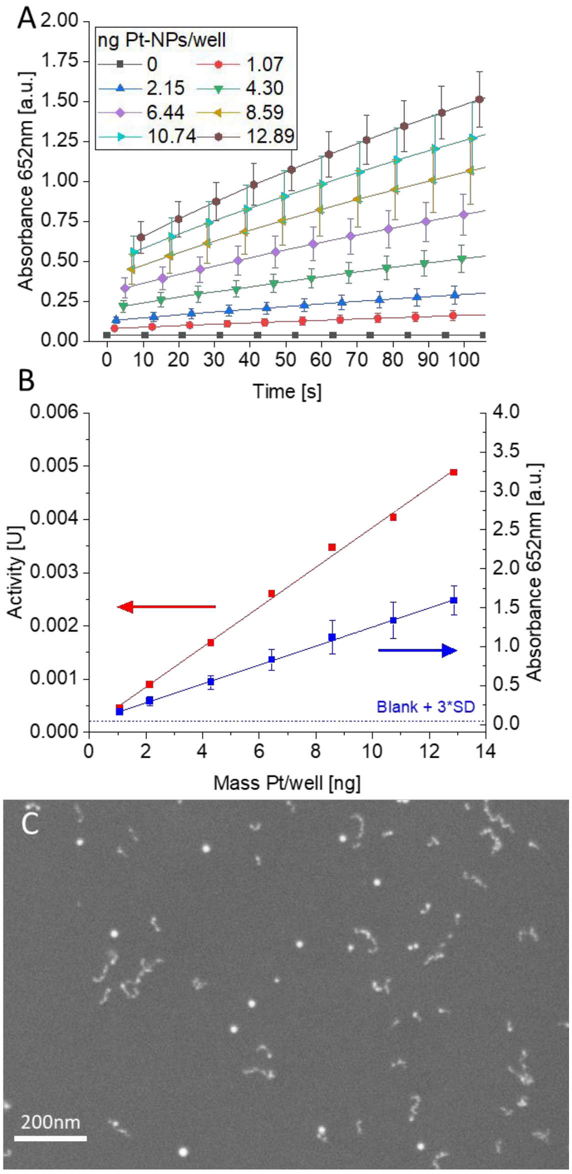

Fig. 1. A) 100s absorbance measurement of Pt-NPs with TMB/H_2O_2, B) activity (left y-axis) and absorbance at λ=652nm after 100s (right y-axis) vs mass Pt-NPs/well, C) SEM image of Pt-NPs, 200k× magnification.

Tab. 1: Overview of SAs and LODs

Enzyme/ nanozyme	Specific activity in U/mg (correlation R^2)	LOD in ng/ml (pmol/L)
Peroxidase activity (H_2O_2 present)		
HRP	289.0 ± 6.5 (0.9975)	1.6 (36)
Pt-NPs	374.8 ± 8.9 (0.9972)	0.231 (0.026)
Oxidase activity (no H_2O_2 present)		
Pt-NPs	1.86 ± 0.06 (0.9940)	16.7 (1.91)

Analyzing the Pt-NPs of this study, a value of 1.86pmol/L without H_2O_2 and 0.026pmol/L with H_2O_2 is found for the LOD, which is significantly below the value of 36pmol/L measured for HRP using the TMB/H_2O_2 assay (see Tab.1).
In summary, the Pt-NPs exhibit excellent peroxidase-specific activity and a low LOD at 22°C, even in the absence of H_2O_2. This makes the PLAL-produced Pt-NPs promising nanozymes for use in bioassays.
Future studies need to investigate the influence of dissolved oxygen in the measurement buffer to understand the oxidase activity of Pt-NPs.

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