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(Electro)catalytic and Sensing Properties of Redox-Active Nanoparticles with Peroxidase-Like Activity †

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Abstract

Herein we first attempt to compare catalytic and electrocatalytic properties of the most commonly used peroxidase mimicking nanozymes based on transition metal ions, including magnetite, cerium oxide, and Prussian Blue. For the nanomaterials under consideration, the catalytic rate constant for reducing substrate increases upon decreasing its redox potential and reaches the ultimate values for Prussian Blue in the presence of ferrocyanide (k_{cat} = 3.8 s^{-1} per single redox-active site). In addition to the highest k_{cat}, Prussian Blue nanoparticles in electrochemical sensors exhibit sensitivity to H_2O_2 by more than 3 orders of magnitude higher than other nanomaterials. Sensing properties of the electrodes modified with Prussian Blue nanoparticles appear to be dependent on their diameter; particles with diameter of 140 nm provide optimal sensitivity and lifespan of the corresponding sensor. The achieved exceptional (electro)catalytic properties of Prussian Blue nanoparticles open the prospects for their application as universal labels for personal analyzers with either optical or electrochemical readout.

Keywords: nanozyme; Prussian Blue; peroxidase; electrocatalysis; hydrogen peroxide

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1. Introduction

Nanozymes (nanoparticles with enzyme-like activity) favorably differ from their natural counterparts by combining high stability with low cost. These advantages make them attractive for the elaboration of personalized medical devices. Since most of enzymatic sensing systems utilize peroxidases as labels, the nanozyme-related research is mainly devoted to the development of peroxidase-mimicking particles. To date, peroxidase-like activity has been demonstrated for a large number of materials, including metal oxides, carbon-based structures, and noble metal nanoparticles [1]. Probably the most studied nanozymes are magnetite nanoparticles, for which peroxidase-like activity was first demonstrated [2], and CeO_2 nanoparticles, generally applied for therapeutic applications [3,4]. Such nanozymes are able to successfully replace the enzyme labels in a number of colorimetric assays [5–7]. However, in terms of personalized sensing devices, the challenge of precise optical reading limits the use of nanozymes, which appear to be suitable only for immunochromatographic test strips. In this regard, electrochemical analysis is attractive, combining high accuracy with the simplicity of readout, though requiring the

use of electrocatalytic labels. Despite the promised advantages of electrochemical biosensing, the electrocatalytic properties of even the most common nanozymes are rarely discussed with the only exception of Prussian Blue-based nanostructured catalysts. The latter are recognized as the most efficient electrocatalysts of H_2O_2 reduction displaying high selectivity and sensitivity [8,9]. Such nanozymes are applicable for the development of electrochemical DNA/RNA sensors [10].

With this study, we intend to compare the catalytic and electrocatalytic activity of a set of electroactive nanomaterials to reveal the most promising candidates for the elaboration of universal labels for further biosensing applications.

2. Materials and Methods

2.1. Materials

Hydrogen peroxide (30% solution), KCl, K_2HPO_4, KH_2PO_4, and citric acid were purchased from Reachim (Russia). $FeCl_3 \cdot 6H_2O$, $K_3[Fe(CN)_6]$, catechol, o-phenylenediamine, 3,3′,5,5′-tetramethylbenzidine (TMB), $Ce(NO_3)_3 \cdot 6H_2O$, ethylene glycol, ammonia solution (25–28%) were purchased from Sigma Aldrich (USA). Iron (III) acetylacetonate from Acros Organics (USA), benzyl alcohol from Alfa Aesar (USA) were used as received. $LiFePO_4$ with an average size of nanoparticles 8 nm was purchased from AME Energy Co. (China).

2.2. Instrumentation

The UV/Vis absorption measurements in transmission mode were carried out using a Lambda 950 Spectrophotometer (PerkinElmer, USA). Electrochemical measurements were carried out using PalmSens 4 (PalmSens, The Netherlands) and planar screen-printed structures with a graphite working electrode (d = 1.8 mm; Rusens, Russia).

2.3. Methods

2.3.1. Synthesis

Catalytic synthesis of Prussian Blue nanoparticles with different sizes (roughly 30–300 nm (Figure S1a,b)) was carried out as we reported earlier in Ref. [11]. Hydrogen peroxide (10–100 mM) was added to an equimolar mixture of $FeCl_3$ and $K_3[Fe(CN)_6]$ (5–100 mM) in 0.1 M KCl/HCl upon ultrasonication. After centrifugation, the resulting nanoparticles were redispersed in 0.1 M KCl/HCl solution, and the washing cycle was repeated 5–7 times.

Using the solvothermal method, Fe_3O_4 and CeO_2 nanoparticles with an average size of 6 nm and 4 nm (Figure S1c,d) respectively, were synthesized. A detailed synthesis technique is described in the Supporting Information.

2.3.2. Electrochemical Investigations of the Prussian Blue Modified Electrodes

For the electrode modification, 2 μL of nanozyme colloids with a bulk concentration of Prussian Blue from 0.1 to 4 mM was drop-cast onto its surface and annealed at 100 °C for 20 min.

The sensitivity of the nanoparticle-based sensors was investigated in chronoamperometry mode (E_{DC} = 0.0 V) by correlating the current response to an increase in the H_2O_2 concentration in batch mode upon vigorous stirring.

To study the operational stability of the electrode coatings based on Prussian Blue nanoparticles with different sizes, a continuous detection of 0.1 mM H_2O_2 monitoring was carried out. All electrochemical studies were carried out in a phosphate buffer (pH = 6.0).

2.3.3. Investigation of Peroxidase-Like Activity

Steady-state kinetics measurements were carried out at room temperature in phosphate-citrate buffer (pH = 5.0), containing 2 mM of H_2O_2, from 0.5 μM to 50 mM of reducing substrate and 0.2–200 μM of electroactive material. The concentration of the oxidized substrate was monitored spectrophotometrically.

3. Results and Discussion

In this article, we consider probably the most widely used peroxidase mimetics based on Fe_3O_4, CeO_2, and Prussian Blue nanoparticles. Along with the listed materials, $LiFePO_4$ nanoparticles displaying high intrinsic electroactivity were studied. One can assume that similarly to peroxidases, catalysis with such electroactive nanoparticles occurs upon redox transformations of the transition metal atoms. Accordingly, it is expected that both the intrinsic electroactivity and the substrate reducing ability will significantly affect the catalytic properties of nanozymes.

The kinetics of hydrogen peroxide reduction reaction catalyzed by the nanozymes was studied in the presence of the substrates with different electron-donor ability: $K_4[Fe(CN)_6]$ ($E^{0\prime}$ = 0.21 V), o-phenylenediamine ($E^{0\prime}$ = 0.29 V), catechol ($E^{0\prime}$ = 0.36 V), o-dianisidine ($E^{0\prime}$ = 0.43 V), and TMB ($E^{0\prime}$ = 0.50 V). The reaction rate was determined spectrophotometrically by registration of the substrate oxidized form under conditions, which are optimal for the peroxidases ($[H_2O_2]_0$ = 2 mM, pH = 5.0). The initial reaction rate depends on the reducing substrate concentration hyperbolically, while it is proportional to the nanozyme concentration (Figure S2), which allows its formal analysis using the Michaelis-Menten equation and estimation of the catalytic constants (k_{cat}) values. Catalytic constants per single transition metal atom, which can be involved in the redox reaction, were considered.

As shown, k_{cat} strongly depends on the substrate: a decrease of redox potential by 300 mV leads to practically 100-fold increase in the k_{cat} (Figure 1). Regardless of the substrate used, the catalytically synthesized Prussian Blue nanoparticles exhibit at least an order of magnitude higher k_{cat} values compared to the other nanomaterials (Figure 1). The ultimate k_{cat} value (3.8 s^{-1}) was achieved for Prussian Blue-based nanozymes in the presence of $K_4[Fe(CN)_6]$.

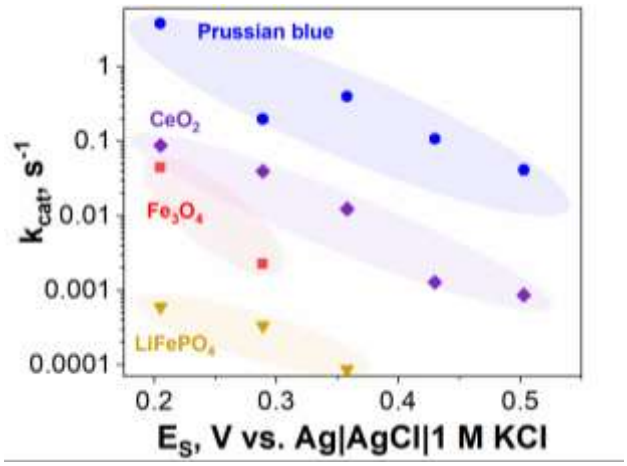
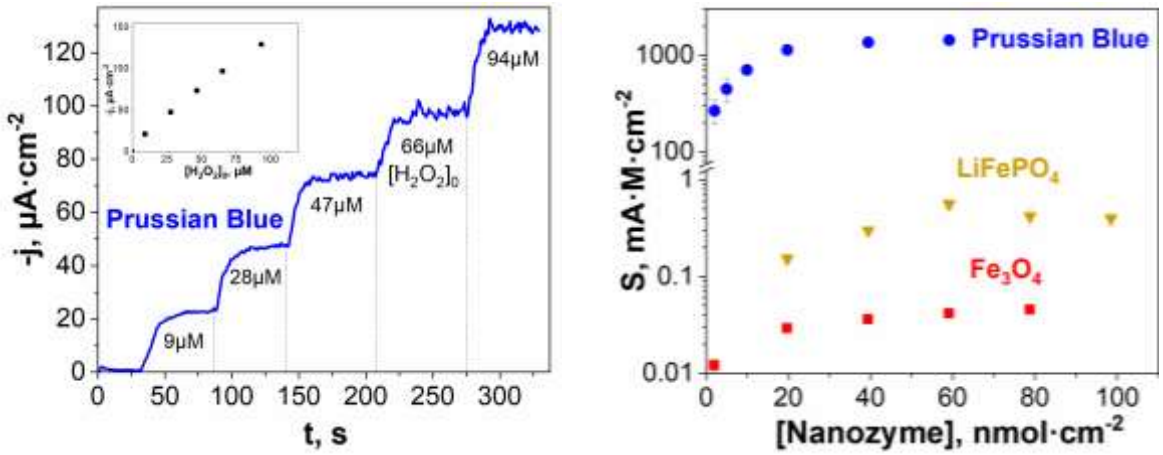


Figure 1. Catalytic rate constants (k_{cat}) per single potentially electroactive site of different nanozymes (catalytically synthesized Prussian Blue, CeO_2, Fe_3O_4, $LiFePO_4$) depending on the substrates redox potential; $[H_2O_2]_0$ = 2.0 mM, citrate-phosphate buffer, pH = 5.0.

By simple drop-casting of nanozymes' suspensions, the electrodes can be modified with different amounts of electroactive nanomaterials. The sensitivity to H_2O_2 of the

resulting nanozyme-based electrodes was investigated at E_{DC} = 0.0 V (Figure 2a), which allows to achieve the limiting electrocatalytic current values for all the studied nanomaterials ($E^{0\prime}$(Prussian Blue/Prussian White) = 0.14 V, $E^{0\prime}(Fe(OH)_2^{+}@Fe_3O_4/Fe^{2+}_{(s)}@Fe_3O_4)$ = 0.12 V, $E^{0\prime}(FePO_4/LiFePO_4)$ = 0.05 V) [12].

As shown, the sensitivity grows with the surface concentration of immobilized nanozymes increase, reaching a plateau at high surface concentrations: 1.4±0.1, (4±1)·10^{-4}, and (4±1)·10^{-5} A·M·cm^{-1} for electrodes based on Prussian Blue, $LiFePO_4$, and Fe_3O_4 nanoparticles, respectively (Figure 2b). Such significant difference is probably related to different electroactivity of immobilized nanomaterials: while the electroactive fraction of immobilized Prussian Blue reaches 60% [13], it is about several percent for $LiFePO_4$ and below tenth of a percent for Fe_3O_4 (Figure S3). Despite its high catalytic activity, the electrocatalytic activity of CeO_2 turned out to be too low for sensor applications. This may probably be due to its low conductivity, as estimated by means of impedance spectroscopy (Figure S4): the charge transfer resistance for electrodes based on CeO_2 is about 870 Ohm·cm^2. This value is 1000-fold higher than for Prussian Blue [14], as a result, the fraction of electroactive material in case of CeO_2 cannot be determined reliably.



(**a**) (**b**)

Figure 2. (**a**) The dependencies of the current density on the added concentration of H_2O_2 for the electrode with immobilized Prussian Blue nanoparticles (d = 30 nm); surface concentration 79 nmol·cm^{-2}. (**b**) Sensitivity of the nanozyme-based electrodes to hydrogen peroxide depending on the surface concentrations of the immobilized material; phosphate buffer pH = 6.0, E_{DC}= 0.0 V.

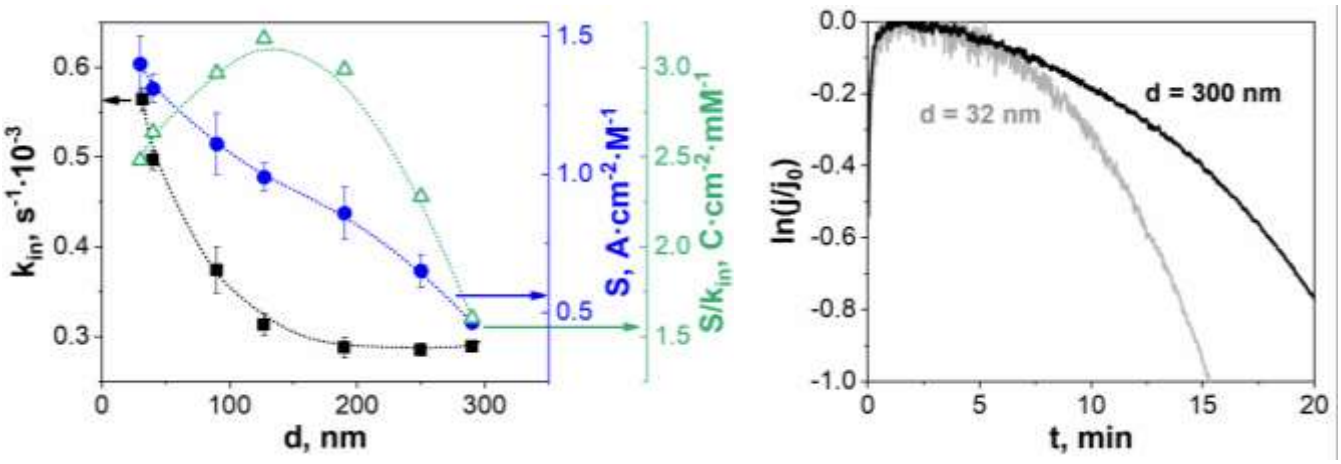
For the Prussian Blue-based nanozymes the size-effects of their electrocatalytic properties were investigated. For this aim the carbon electrodes were modified with equal amounts (79 nmol·cm^{-2}) of Prussian Blue consisted of nanoparticles sizing from 30 to 300 nm.

As shown, in the case of the smallest nanozymes a 3-fold higher sensitivity can be achieved compared to the electrodes with 300 nm particles (Figure 3a). This is probably due to insufficient contact area for the larger nanoparticles between themselves and the electrode surface. In turn, for smaller nanoparticles the contact area is higher, while enhanced porosity of the resulting sensing coating facilitates diffusion of H_2O_2 in its bulk.

The operational stability of the electrodes modified with the Prussian Blue nanoparticles was investigated in the course of continuous monitoring of 0.1 mM H_2O_2 (Figure 3b). The gradual current decrease, generally associated with Prussian Blue solubilization, can be fit to the first-order reaction equation, which allows evaluating the inactivation constant (k_{in}):

$$\frac{j}{j_0} = exp(-k_{in}t). \quad (1)$$

In contrary to the sensitivity, the operational stability of the larger nanozymes is expectedly higher. As a result, as the size of the nanoparticles composing the electrode coating increases from 32 to 300 nm, k_{in} is decreased down to 2 times, reaching the value of ~$3 \cdot 10^{-4}$ s^{-1}.



(**a**) (**b**)

Figure 3. (**a**) Dependencies of the inactivation constant (k_{in}, ■), sensor sensitivity (S, ●), and their ratio (S/k_{in}, △) on the size of the catalytically synthesized Prussian Blue nanoparticles. (**b**) Logarithm of the relative change in the current response of sensors based on Prussian Blue nanoparticles of different sizes (32 and 300 nm) to $[H_2O_2]_0$ = 0.1 mM as a function of time.

Since both high sensitivity and operational stability are important for practical use, which depend on size in the opposite way, we considered their ratio (S/k_{in}) as optimization parameter. As shown, S/k_{in} dependence on the size of nanozyme reaches a maximum for the particles of about 140 nm in diameter.

To sum up, the demonstrated high (electro)catalytic activity of Prussian Blue-based nanozymes makes them promising nanomaterials for analytical applications. High catalytic activity of nanozymes can be used to produce sensors with an optical readout. At the same time, the outstanding electrocatalytic properties would allow applying Prussian Blue nanoparticles as labels for personalized electrochemical test-systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1: TEM images of nanozymes; Figure S2: examples of initial reaction rate dependence on the substrate and nanozymes concentrations; Figure S3: electroactive versus immobilized amounts of nanozymes; Figure S4: impedance spectra on Nyquist plots for a glassy carbon electrode modified with CeO_2-based nanozymes.

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Abbreviations

The following abbreviations are used in this manuscript:

TMB	3,3′,5,5′-Tetramethylbenzidine
DC	Direct current.

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