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Revisiting catechol derivatives as robust chromogenic hydrogen donors working in alkaline media for peroxidase mimetics

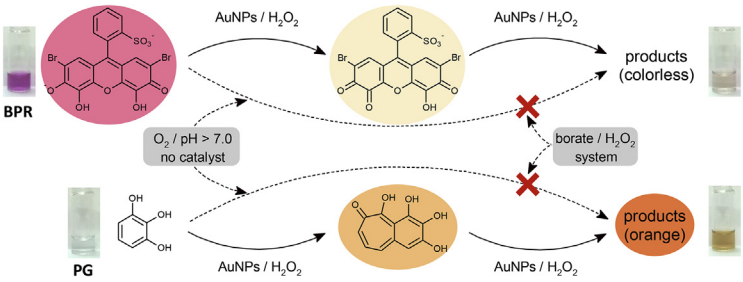
Marcin Drozd [a], Mariusz Pietrzak [a, *], Jakub Pytlos [a], Elżbieta Malinowska [a, b]

[a] Department of Microbioanalytics, Institute of Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland
[b] Biotechnology Platform, CEZAMAT, Polna 50, 00-644, Warsaw, Poland

HIGHLIGHTS

- Gold nanoparticles exhibit activity towards peroxidation of pyrogallol and bromopyrogallol red.
- Catechol derivatives work as hydrogen donors in a peroxidase-like activity assays.
- Complexation of PG and BPR by borate enables their application in alkaline media.
- AuNPs-mediated peroxidation is governed by a mechanism of a heterogeneous catalysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Colloidal noble metal-based nanoparticles are able to catalyze oxidation of chromogenic substrates by H_2O_2, similarly to peroxidases, even in basic media. However, lack of robust chromogens, which work in high pH impedes their real applications. Herein we demonstrate the applicability of selected catechol derivatives: bromopyrogallol red (BPR) and pyrogallol (PG) as chromogenic substrates for peroxidase-like activity assays, which are capable of working over wide range of pH, covering also basic values. Hyperbranched polyglycidol-stabilized gold nanoparticles (HBPG@AuNPs) were used as model enzyme mimetics. Efficiency of several methods of improving stability of substrates in alkaline media by means of selective suppression of their autoxidation by molecular oxygen was evaluated. In a framework of presented studies the impact of borate anion, applied as complexing agent for PG and BPR, on their stability and reactivity towards oxidation mediated by catalytic AuNPs was investigated. The key role of high concentration of hydrogen peroxide in elimination of non-catalytic oxidation of PG and improvement of optical properties of BPR in alkaline media containing borate was underlined. Described methods of peroxidase-like activity characterization with the use of BPR and PG can become universal tools for characterization of nanozymes, which gain various applications, among others, they are used as catalytic labels in bioassays and biosensors.

1. Introduction

Catalytic oxidation of selected substrates by hydrogen peroxide has attracted a great interest in analytical chemistry for several decades. Enzymatic reaction catalyzed by horseradish peroxidase

Abbreviations: BPR, bromopyrogallol red; PG, pyrogallol; HBPG@AuNPs, hyperbranched polyglycidol-stabilized gold nanoparticles; HRP, horseradish peroxidase; DTPA, diethylenetriaminepentaacetic acid; HBPG, hyperbranched polyglycidol.

* Corresponding author.
E-mail address: mariusz@ch.pw.edu.pl (M. Pietrzak).

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(HRP) enables determination of hydrogen peroxide and species of biological origin such as glucose and cholesterol in various samples [1,2]. Native peroxidase and its mimetics can be also employed as catalytic labels in biosensors and assays after conjugation with bioreceptors [3,4].

Recently, a variety of novel catalysts acting as enzyme mimetics, including inorganic nanoparticles made of noble metals (Au, Pt, Pd) and their alloys [5–9] or metal oxides (MnO_2, V_2O_5, Co_3O_4, Fe_3O_4) have been reported [10–13]. Numerous advantages of noble metal-based nanocatalysts (often called "nanozymes") over native enzymes, with respect to improved stability, low cost, simplicity of preparation and functionalization together with a variety of available activities (peroxidase [14,15], oxidase [6], catalase [8], polyphenol oxidase [16]) make them an alternative to natural enzymes in bioanalytical enzyme-related applications.

To date, nanozymes have been mainly employed in analytical chemistry as catalysts for direct detection of H_2O_2 [15,17] and as catalytic labels in colorimetric bioassays. For example, Gao et al. reported immunoassay of immunoglobulin G from rabbit serum [18]. Recently, Wan et al. described optical assay for detection of whole sulfate-reducing bacteria cells [10]. Peroxidase mimetics can be also coupled in assays with native enzymes (e.g. glucose oxidase [13], amino-acid oxidase [19], xanthine oxidase [20]) to enable indirect determination of co-substrates of enzymatic reactions involving H_2O_2. The susceptibility of noble metal nanozymes to inactivation in presence of surface-active agents (e.g. Hg^{2+} ion) was also used in sensors based on analyte-induced switching off enzyme-like activity, as reported by Tseng et al. [14].

Among various modes of detection of peroxidation products, including fluorometry [14,20], chemiluminometry [21] and electroanalysis [22], spectrophotometry has gained the highest applicability [18,23]. Widespread use of common chromogenic substrates such as 3,3′,5,5′-tetramethylbenzidine, *o*-phenylenediamine and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) for HRP-mediated peroxidation was dictated by their high stability, sufficient sensitivity and compatibility in terms of optimum pH for HRP activity (slightly lower than 7.0) and efficiency of colored product generation (acidic media of pH between 4.0 and 5.5) [24]. In vast majority of literature reports characterization of NPs catalytic properties is performed with the use of conventional peroxidase substrates, whose applicability is greatly limited by the pH of the reaction medium. However, some essential differences in structure and mechanism of catalysis in comparison to HRP are reflected in particular features of noble metal-based catalytic nanoparticles. NPs resistance to inactivation in case of high concentrations of hydrogen peroxide and enhanced stability in a wide pH range allow them to be used in media of different composition, significantly beyond working optima of peroxidases [6,7].

Catechol derivatives: pyrogallol (PG), bromopyrogallol red (BPR) were known as peroxidase substrates to use at near – physiological pHs, but nowadays they are of relatively little importance because of widespread use of substrates working in acidic media [25–27]. BPR has been utilized in analytics as complexometric agent and acid-base indicator [28]. In turn PG has been applied for the determination of oxygen and spectrophotometric determination of metal cations [29].

Common limitation of catechol derivatives in HRP or HRP-like activity assays is their instability in alkaline media. Catechol derivatives have been known to autoxidize in the presence of oxygen to form superoxide radical anions ($O_2^{\bullet-}$) [30–32]. It should be pointed out that suppression of oxygen-dependent oxidation may lead to improvement of reliability and stability of catechol-based methods for HRP-like activity evaluation. Several strategies of inhibition of *o*-hydroquinones autoxidation have been already described. For example the removal of oxygen from sample solutions is indeed effective, but cumbersome, and it makes the analytical procedure more complex. Few studies documented noticeable undesired catalytic effect of metal cations (e.g. Fe^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}) on catechol derivatives, and upon their occurrence the application of chelating agents was recommended to retard the oxidation [33,34]. One of reported strategies of oxidation suppression involves complexation of catechol moieties by borate [30,31,35]. However, to date, no attempts towards inhibition of autoxidation by application of borate for peroxidase-like activity studies have been described. Therefore, best to our knowledge, *o*-diphenolic compounds have not yet been examined as substrates for metal-based catalytic nanoparticles as peroxidase mimetics in alkaline media.

It should be pointed out that only few substrates for peroxidase mimetics, capable of operation at basic media were reported to date [25,36,37]. However, in case of phenol [20], additional coupling agent – 4-aminoantipyrine is necessary. In addition, the stability of the product in pH > 9.0 is limited. In turn, products of 3-(4-dihydroxyphenyl)propionic acid or homovanillic acid peroxidation require spectrofluorometric detection [37]. Therefore, there is a need to develop chromogenic substrates, which can be utilized in alkaline media, and their peroxidated products are stable. It should be underlined that in our opinion peroxidase-like activity of nanoparticles in alkaline media is often overlooked, due to utilization of inappropriate substrates, designed for analysis in acidic pHs, when analyzing NPs.

In this work the applicability of selected catechol derivatives as chromogenic substrates for peroxidase-like activity assay of hyperbranched polyglycidol-stabilized gold nanoparticles (HBPG@AuNPs) is evaluated. The developed substrate systems based on PG and BPR, in contrast to the currently available hydrogen donors, can find application for characterization of catalytic properties of miscellaneous peroxidase mimetics in neutral and alkaline media. Therefore, this work should be especially useful for those, who work on characterization of new nanomaterials for applications as peroxidase mimetics. Moreover, proposed substrate systems can be utilized for signal generation in affinity biosensors and bioassays with optical signal detection, capable of operating at physiological pH.

2. Experimental

2.1. Reagents

Gold(III) chloride trihydrate, sodium borohydride, pyrogallol (PG), bromopyrogallol red (BPR), copper(II) nitrate, cobalt(II) sulfate, EDTA, diethylenetriaminepentaacetic acid (DTPA), Tris and boric acid were all purchased from Sigma Aldrich. Iron(II) chloride was purchased from Merck. Phosphoric acid (85%) was purchased from Chempur (Poland). Hydrogen peroxide (30%), and ethanol (96%) were purchased from POCh (Poland). Hyperbranched polyglycidol (HBPG) was obtained by ring-opening multibranching polymerization of glycidol (M_W ~ 3.2 kDa) in Chair of Polymer Chemistry and Technology, Faculty of Chemistry, Warsaw University of Technology [38]. All reagents were used without further purification.

Glassware used for AuNPs preparation was previously rinsed with *aqua regia*. Borate/phosphate (0.1 M/0.1 M) and Tris/phosphate (0.1 M/0.1 M) buffers were prepared by dissolution of solids: boric acid or Tris and concentrated phosphoric acid (85%) in Milli-Q water (resistivity above 18 MΩ cm), respectively. pH was adjusted to desired values using concentrated solution of NaOH. Hydrogen peroxide and PG stock solutions (5 M and 0.5 M, respectively) were freshly prepared daily and stored in absence of light. Stock solution of BPR (1 mM) was prepared by dissolving solid in 10% aqueous