Analytica Chimica Acta 856 (2015) 90–95

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A sensitive fluorescent assay for thiamine based on metal-organic frameworks with intrinsic peroxidase-like activity

Hongliang Tan *, Qian Li, Zhengchen Zhou, Chanjiao Ma, Yonghai Song, Fugang Xu, Li Wang *

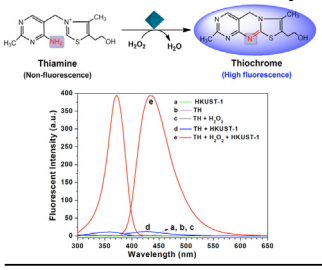
Key Laboratory of Functional Small Organic Molecule, Ministry of Education, Key Laboratory of Chemical Biology, Jiangxi Province, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang 330022, PR China

HIGHLIGHTS

- It has been found that copper-based MOFs possess peroxidase-like activity.
- Thiamine was used as fluorescent substrate for copper-based MOFs as peroxidase mimic.
- A simple and sensitive fluorescent method for thiamine detection was developed.

GRAPHICAL ABSTRACT

HKUST-1 as a peroxidase mimic can catalyze the conversion of non-fluorescent thiamine to high fluorescent thiochrome in the presence of H_2O_2.



ARTICLE INFO

Article history:
Received 2 August 2014
Received in revised form 14 October 2014
Accepted 17 November 2014
Available online 20 November 2014

Keywords:
Metal-organic frameworks
Peroxidase-like activity
Thiamine
Fluorescence

ABSTRACT

Metal-organic frameworks (MOFs) with tunable structures and properties have recently been emerged as very interesting functional materials. However, the catalytic properties of MOFs as enzymatic mimics remain to be further investigated. In this work, we for the first time demonstrated the peroxidase-like activity of copper-based MOFs (HKUST-1) by employing thiamine (TH) as a peroxidase substrate. In the presence of H_2O_2, HKUST-1 can catalyze efficiently the conversion of non-fluorescent TH to strong fluorescent thiochrome. The catalytic activity of HKUST-1 is highly dependent on the temperature, pH and H_2O_2 concentrations. As a peroxidase mimic, HKUST-1 not only has the features of low cost, high stability and easy preparation, but also follows Michaelis–Menten behaviors and shows stronger affinity to TH than horseradish peroxidase (HRP). Based on the peroxidase-like activity of HKUST-1, a simple and sensitive fluorescent method for TH detection has been developed. As low as 1 μM TH can be detected with a linear range from 4 to 700 μM. The detection limit for TH is about 50 fold lower than that of HRP-based fluorescent assay. The proposed method was successfully applied to detect TH in tablets and urine samples and showed a satisfactory result. We believed that the present work could improve the understanding of catalytic behaviors of MOFs as enzymatic mimics and find out a wider application in bioanalysis.

1. Introduction

Natural horseradish peroxidase (HRP) is an important heme-containing enzyme that has been studied for more than a century. It continues to attract considerable attention of researchers from a variety of disciplines because of its practical and commercial applications. However, natural HRP is a protein, which bears some

* Corresponding author. Tel.: +86 791 88120861; fax: +86 791 88120861.
E-mail addresses: hltan@jxnu.edu.cn (H. Tan), lwanggroup@aliyun.com (L. Wang).

http://dx.doi.org/10.1016/j.aca.2014.11.026
0003-2670/

serious disadvantages, such as instability and easy loss of catalytic activity in harsh chemical environment and high cost and time-consumption in preparation and purification procedures [1]. Thus, it is highly desirable to develop artificial enzymes with peroxidise-like activity. Since the first peroxidase mimic based on Fe_3O_4 nanoparticles was reported by Gao et al. [2], a variety of inorganic materials, such as platinum nanoparticles [3], Co_3O_4 [4], carbon dots [5], CuO [6], and single-walled carbon nanotubes [7], have been demonstrated to possess peroxidase-like activity. Compared with HRP, these peroxidase mimics are inexpensive and stable and have tunable catalytic activity [8]. Nevertheless, the species of peroxidase mimics are limited and laborious procedures are often required for the preparation of peroxidase mimics. Therefore, the development of new peroxidase mimics with simple preparation procedures, excellent stability and high catalytic activity is still a hot topic.

As an inorganic–organic hybrid material assembled by metal ions and organic linkers via coordination bonds, metal-organic frameworks (MOFs) have recently been emerged as very interesting functional materials due to their tunable structures and properties [9]. The limitless choices of metal ions and organic linkers afford an essentially infinite number of MOFs with different architectures and physicochemical properties for diverse applications. To date, the potential applications of MOFs have been examined in the areas including gas storage and separation [10], heterogeneous catalysis [11], chemical sensing [12], biomedical imaging [13], and drug delivery [14]. Among these applications of MOFs, chemical catalysis is particularly interesting owing to their properties of tunable pore sizes, high specific surface areas, and exposed active sites [15]. However, most studies of MOFs-based catalytic reactions focus on the model organic reactions [16], less work concerns with the application of MOFs as catalysts in biological assays. Very recently, it has been demonstrated that Fe^{3+}-based MOFs possess similar peroxidase-like activity with natural HRP. Based on the peroxidise-like activity of Fe^{3+}-based MOFs, several colorimetric methods for the detection of glucose and ascorbic acid have also been developed [17–19]. In spite of this, it is still highly desirable to develop new enzyme-like MOFs-based analytical methods due to the intrinsic low sensitivity of colorimetric method and the instability of chromogenic substrate in aqueous solutions [20,21]. Moreover, to the best of our knowledge, there have been no reports on the application of other metal ions-based MOFs as peroxidase mimics in biological assays except Fe^{3+}-based MOFs.

In this work, we attempt to explore the intrinsic peroxidase-like activity of Cu^{2+}-based MOFs by employing $Cu_3(BTC)_2$ (BTC = 1,3,5-benzene tricarboxylate), also known as HKUST-1, as a model MOF and thiamine (TH) as a peroxidase substrate, respectively. TH, known as vitamin B_1, is a natural nutrient that exists in many foods. In biological systems, TH is an important growth factor to maintain the normal functions of the nervous and cardiovascular systems [22,23]. Severer deficiency of TH can result in beriberi or the Wernicke–Korsakoff syndrome [24]. From a chemical perspective, however, TH is a very attractive fluorescent substrate for sensitive measurements of peroxides due to its low cost and easy accessibility [21,25,26]. TH itself is a non-fluorescent compound, but it can be readily converted to intensely fluorescent thiochrome (TC) in basic solution by appropriate oxidants. In fact, this principle has been widely applied in the fluorescent detection of TH [27–30]. On the other hand, it has been demonstrated that Cu^{2+}-based compounds and nanostructures possess intrinsic peroxidase-like activities and can catalyze the oxidation of some chromogenic and fluorogenic substrates in the presence of H_2O_2 [31–34]. So, the peroxidase-like activity of HKUST-1 is expected. Meanwhile, the conversion of non-fluorescent TH to strong fluorescent TC may also occur in the presence of H_2O_2 based on the peroxidise-like activity of HKUST-1 (Scheme 1). Consequently, a fluorescent method for the determination of TH would be developed.

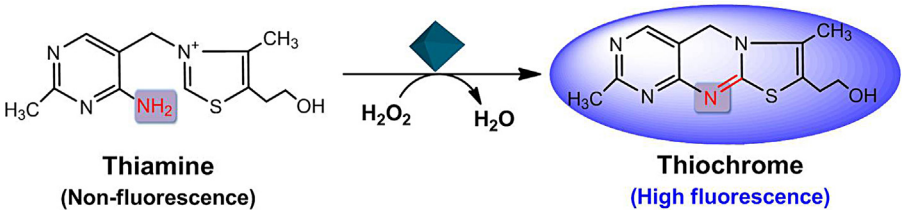
2. Experimental

2.1. Chemicals

All chemicals are obtained from commercial source and used without further purification. 1,3,5-Benzenetricarboxylic acid (BTC), thiamine, tryptophan (Trp), aspartic acid (Asp), alanine (Ala), lysine (Lys), leucine (Leu), terephthalic acid (TA), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenate (vitamin B_5), pyridoxine (vitamin B_6), folate (vitamin B_9), and cobalamin (vitamin B_{12}) were obtained from Aladdin (Shanghai, China); metal salts (Cu $(NO_3)_2$, NaCl, KCl, $NaNO_3$, Na_2SO_4), urea, uric acid, starch, glucose, lactose, and fructose were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water (18 MΩ cm) was used for the preparation of all aqueous solutions. Unless otherwise stated, all chemicals are of analytical reagent grade.

2.2. Instruments

The morphology of HKUST-1 was measured by S-3400 scanning electron microscopy (SEM, Hitachi, Japan) equipped with an energy dispersive spectra (EDS) detector. Fluorescence spectra were recorded on F-7000 fluorescence spectrophotometer (Hitachi, Japan). A 370 nm excitation wavelength was used to record the emission spectra, whereas the excitation spectra were recorded by setting the emission intensity at 435 nm. The UV–vis absorption spectra were measured by using UV-3900H spectrophotometer (Hitachi, Japan). Avatar 360 FTIR spectrometer (Nicolet, USA) was used to record the Fourier transform infrared (FTIR) spectra with the KBr pellet technique. The analysis of powder X-ray diffraction (XRD) spectrum was performed on D8 Advance X-ray diffractometer (Bruker, Germany). Thermogravimetric analysis (TGA) was conducted under a N_2 flow with a heating rate of 5 °C min^{-1}, using an SDT 2960 instrument. An ASAP 2020 instrument (Micromeritics) was used to obtain N_2 adsorption/desorption isotherm at −196 °C. The surface area, the micropore volume, the mesopore volume, and the total pore volume were calculated from the isotherms.



Scheme 1. HKUST-1 as a peroxidase mimic catalyzes the conversion of non-fluorescent TH to high fluorescent TC in the presence of H_2O_2.