Talanta 113 (2013) 142–147

Contents lists available at SciVerse ScienceDirect

Talanta

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

Activity of catalase adsorbed to carbon nanotubes: Effects of carbon nanotube surface properties

Chengdong Zhang [a,b,c,*], Shuiming Luo [a,b,c], Wei Chen [a,b,c]

[a] College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China
[b] Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Nankai University, Tianjin 300071, China
[c] Tianjin Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 27 November 2012
Received in revised form
7 March 2013
Accepted 10 March 2013
Available online 16 March 2013

Keywords:
Catalase
Carbon nanotubes
Adsorption
Surface properties

ABSTRACT

Nanomaterials have been studied widely as the supporting materials for enzyme immobilization. However, the interactions between enzymes and carbon nanotubes (CNT) with different morphologies and surface functionalities may vary, hence influencing activities of the immobilized enzyme. To date how the adsorption mechanisms affect the activities of immobilized enzyme is not well understood. In this study the adsorption of catalase (CAT) on pristine single-walled carbon nanotubes (SWNT), oxidized single-walled carbon nanotubes (O-SWNT), and multi-walled carbon nanotubes (MWNT) was investigated. The adsorbed enzyme activities decreased in the order of O-SWNT > SWNT > MWNT. Fourier transforms infrared spectroscopy (FTIR) and circular dichrois (CD) analyses reveal more significant loss of α-helix and β-sheet of MWNT-adsorbed than SWNT-adsorbed CAT. The difference in enzyme activities between MWNT-adsorbed and SWNT-adsorbed CAT indicates that the curvature of surface plays an important role in the activity of immobilized enzyme. Interestingly, an increase of β-sheet content was observed for CAT adsorbed to O-SWNT. This is likely because as opposed to SWNT and MWNT, O-SWNT binds CAT largely via hydrogen bonding and such interaction allows the CAT molecule to maintain the rigidity of enzyme structure and thus the biological function.

1. Introduction

Nanobiocatalysis, which refers to the application of enzymes immobilized on nanomaterials [1], is a rapidly growing research field. Various nanomaterials, such as nano-SiO_2, nano-TiO_2, nano-gold, carbon nanotubes (CNT), and graphene, have been explored as supporting matrices [2]. Among them CNT have attracted considerable interest because of their stability, high adsorption capacity, better retention of catalytic activity, and biocompatibility [3]. Particularly, CNT can act simultaneously as the immobilization matrix and as the electrochemical transducer, rendering wide-ranged applications in nanoelectronics, biosensor, and high-resolution imaging [4]. Moreover, CNT have an additional benefit, in that they can be surface-modified with solubilizing chains or targeting molecules, resulting in the improvement of performance and/or loading of the immobilized enzyme and enhancing their application in the biomedical field.

Both single-walled carbon nanotubes (SWNT) and multi-walled carbon nanotubes (MWNT) have been used to immobilize enzyme. SWNT are attractive for their higher surface area for protein interaction, while MWNT have the advantages of better dispersibility and lower cost. So far, most studies have focused on the development of effective immobilization methods using either SWNT or MWNT, but a few studies have attempted to understand the structural effects of CNT on enzyme loading, activity and stability [5–8], and the results indicate that the performance of enzyme–CNT complexes can be affected significantly by the structural properties of CNT. Pedrosa et al. [5] found that organophosphate hydrolase conjugated to oxidized SWNT (O-SWNT) exhibited better catalytic activity compared to that conjugated to oxidized MWNT, and they argued that this was attributable to the more uniform deposition of the enzyme on O-SWNT surface. Wang et al. [6] compared the activity and stability of covalently bonded NADH oxidase on N_α,N_α-bis(carboxymethyl)-L-lysine hydrate functionalized MWNT and SWNT. They observed that even though the two enzyme–CNT conjugates exhibited similar catalytic activities, higher enzyme loading and better stability were observed for SWNT conjugate than for MWNT conjugate. They argued that the larger surface area and higher surface curvature of SWNT allow less lateral interactions between the adjacent enzyme molecules on SWNT.

It has also been observed that the surface functionalities of CNT [7–9], in particular surface O-functionalities, can affect the

* Corresponding author at: College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China. Tel./fax: +86 22 6622 9517.
E-mail address: zhangchengdong@nankai.edu.cn (C. Zhang).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.03.027

activities of adsorbed enzymes. For example, Mu et al. [8] found that the enzyme bound to O-MWNT exhibited weaker activity than the enzyme bound to MWNT. They proposed that MWNT bound enzymes interacted through mostly π–π stacking interactions, whereas O-MWNT interacted with enzymes mainly through negatively charged O-MWNT surface with neutral or positively charged surface residues on the protein. The stronger nanotube-binding between carbonic anhydrase and O-MWNT induced more enzyme conformational changes, thus decreasing activity. In another study, Dong et al. [9] observed that soybean peroxidase exhibited higher enzymatic activity on highly oxidized SWNT than on less oxidized SWNT. They suggested that this phenomenon may be attributed to the stronger hydrophobic interaction between the enzyme molecules and less oxidized SWNT so that more of the secondary structure was changed consequently.

To date, how the activities of enzyme–CNT conjugates are affected by the nature of adsorptive interactions between enzyme and CNT are not fully understood. To further understand the underlying mechanism, in particular the effect of surface O-functionalization on enzyme activity, we examined the adsorption properties and enzyme activities of catalase (CAT), a heme-containing metallo-enzyme, adsorbed to three different CNT. CAT was used as a model enzyme because it is one of the most common enzymes in plant and animal tissues [10], and because immobilized CAT has wide application in many industrial fields [11]. SWNT, MWNT, and O-SWNT were used as the model CNT. The adsorption affinities and mechanisms of CAT to the three CNT were examined and the controlling mechanisms were analyzed. The conformational changes of enzymes upon immobilization were studied using Fourier transform infrared (FTIR) spectroscopy and circular dichroisom (CD) spectroscopy. The correlations between CAT–CNT adsorptive interactions and enzyme activities of adsorbed CAT are discussed.

2. Materials and methods

2.1. Materials

SWNT and MWNT were purchased from Nanotech Port Co. (Shenzhen, Guangdong Province, China). A portion of the SWNT was treated with HNO_3 and H_2SO_4 mixtures (1:3 by volume) to obtain carboxylic functionalized SWNT using a previously developed method [12]. Graphite was purchased from Sigma-Aldrich. Elemental analysis, surface area and pore size distribution, and ζ potential were determined using previously reported methods [13]. Selected physical–chemical properties of the CNT are listed in Table 1.

Table 1
Elemental analysis, surface area measurements and pore size distribution of various CNT.

Adsorbent	Elemental composition[a]			Surface area[b] (m^2/g)	Pore size distribution (%)[c]		
	C%	H%	O%		Micropore (< 2 nm)	Mesopore (2–38 nm)	Macropore (> 38 nm)
SWNT	91.7	2.0	4.2	417	1	78	21
O-SWNT	82.6	1.1	8.7	310	59	41	0
MWNT	96.1	1.3	2.8	372	8	69	23

[a] Analyzed with an elemental analyzer.
[b] Surface area determined by N_2 adsorption using the Brunauer–Emmett–Teller (BET) method.
[c] Pore size distribution was calculated by the Horvath–Kawazoe function.

CAT (EC 1.11.1.6, from bovine liver) was purchased from Sigma-Aldrich. All other chemicals and solvents used were of analytical grade or higher.

2.2. Adsorption experiments

The adsorption experiments were performed using a method similar to that of Cang-Yong and Pastorin [14]. CNT (1 mg) were added to 1 ml of 0.05 M phosphate buffer at pH 7.0 and the suspension was ultrasonicated (150 W, 40 kHz) for 1 h to enhance the dispersion. The sonicated sample was dispensed into an eppendorf microcentrifuge tube, and then exposed to a freshly prepared solution of enzyme in the same buffer. The mixture was shaken on a platform shaker for 4 h at 200 rpm under room temperature. After incubation the CNT were centrifuged at 8000 rpm using a microcentrifuge and the supernatant was removed. Typically six washes were performed, and fresh buffer was added each time to remove unbound enzyme. All supernatants were analyzed for protein content using the bicinchoninic acid method (BCA) or the μBCA assay. The amount of enzyme loaded onto the CNT was determined by measuring the difference in the concentration of enzyme in solution before and after exposing it to the dispersion of CNT in buffer. In a separate test, adsorption of CAT on graphite was done using the same approach.

Effect of pH on CAT adsorption was examined by using 50 mM acetate buffer at pH 4.5, 5.0, 5.5 and 50 mM phosphate buffer at pH 6.0 6.5, 7.0, 7.5, 8.0. To study the effect of ionic strength on adsorption, the ionic strength of the solution was adjusted using NaCl to 50, 100, 150, and 200 mM at pH 7. The adsorption experiments were performed using the procedure described above. CNT concentration was maintained the same as 1 mg/mL in all experiments and the initial enzyme concentration was 0.9 mg/mL for the experiment of pH effect and 0.5 mg/mL for the experiment of ionic strength effect, respectively.

2.3. Enzyme activity assays

The activity of CAT was measured according to the method of Alptekin et al. [15], using H_2O_2 as the substrate. Briefly, the stock solution of 20 mM H_2O_2 was prepared in 50 mM phosphate buffer at pH 7.0. Then, 10 μL of free or absorbed CAT solution was added to 990 μL of 20 mM H_2O_2 in quartz cuvettes and immediately mixed by inversion. H_2O_2 depletion was examined at 240 nm using a UV–vis spectrometer every 10 s for over a period of 90 s. One unit is defined as the amount of CAT required to decompose 1 μmol of H_2O_2 per min at 25 °C at pH 7.0. The stock solution of adsorbed enzyme was prepared by resuspending CAT–CNT conjugate in 1 ml of 50 mM phosphate buffer at pH 7.0. The presence of 10 μg/mL of CNT in the solution will not interfere with activity measurement [16].

Kinetic parameters of the free CAT and CAT adsorbed to CNT were investigated at various concentrations (2–25 mM) of H_2O_2 (in 50 mM phosphate buffer at pH 7.0) as the substrate. The enzyme loading was maintained approximately the same for all immobilized enzyme at 240 μg/mg. The Michaelis–Menten kinetic is defined as

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

where v is the reaction rate (mmol/min), $[S]$ is the concentration of substrate (mmol/L), V_{max} (mmol/min) is the maximum rate achieved by the system at maximum (saturating) substrate concentrations, and K_m (Michaelis–Menten constant, mmol/L) is the substrate concentration at which the reaction rate is half of V_{max}. K_m and V_{max} were determined from the Lineweaver–Burk double