Analytical Biochemistry 409 (2011) 74–80

Contents lists available at ScienceDirect

Analytical Biochemistry

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

An electrocatalytic transducer for L-cysteine detection based on cobalt hexacyanoferrate nanoparticles with a core–shell structure

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ARTICLE INFO

Article history:
Received 1 August 2010
Received in revised form 13 September 2010
Accepted 18 September 2010
Available online 24 September 2010

Keywords:
L-Cysteine
Core@shell nanoparticle
Cobalt hexacyanoferrate
Electrocatalysis
Electrocatalytic transducer

ABSTRACT

The electrocatalytic oxidation of L-cysteine (CySH) was studied on cobalt hexacyanoferrate nanoparticles with a core–shell structure (iron(III) oxide core–cobalt hexacyanoferrate shell) using cyclic voltammetry and chronoamperometry. Voltammetric studies represented two quasi-reversible redox transitions for the nanoparticles in phosphate buffer solution (pH 7.4). In the presence of CySH, the anodic peak current of the Fe(II)/Fe(III) transition was increased, followed by a decrease in the corresponding cathodic peak current, whereas the peak currents related to the Co(II)/Co(III) transition almost remained unchanged. The results indicated that the nanoparticles oxidized CySH via a surface mediation electrocatalytic mechanism. The catalytic rate constant, the electron transfer coefficient, and the diffusion coefficient involved in the electrooxidation process of CySH are reported here. Ultrasensitive and time-saving determination procedures were developed for the analysis of the CySH, and the corresponding analytical parameters are reported. According to the proposed methods, CySH was determined with detection limits of 40 and 20 nm in batch and flow systems, respectively. The proposed amperometric method was also applied to the analysis of CySH in human urine and serum blood samples.

There has been tremendous interest in the design, characterization, and applications of amperometric biosensors. The use of electrochemical biosensors based on metal hexacyanoferrates (MHCFs)[1] has been studied extensively [1,2]. The fast charge transfer rate and the insolubility of both the oxidized and reduced states of MHCFs make them good candidates for construction of electrochemical biosensors. In this regard, different MHCFs have been applied [1,2]. Among them, cobalt hexacyanoferrate with an open and zeolite-like structure is the nearest analogue of the well-known compound Prussian blue. It exhibits well-defined and reproducible electrochemical responses because both the oxidized and reduced structures of cobalt hexacyanoferrate are fairly open and permit transport of alkali metal counter cation providing charge balance during redox transitions.

Nanomaterials are a broad class of materials that can be made to exhibit novel and significantly improved physical, chemical, and biological properties because of their small dimension. These materials show increased reactivity compared with their bulk counterparts. This is due to the increased surface area and/or nanosize effect [3–11]. As the dimension is reduced, the overall reactivity increases. Some nanoscale materials also show surprising electrical conductivities and improved catalytic activity [12].

Recently, preparation and design of composite particles consisting of a core covered by the shell of different chemical compositions have attracted much attention due to their unique properties, different from those of single-component materials. The morphology and size of these core–shell materials can be easily tailored by changing the core materials' shape or size or the shell's thickness. Interesting properties of core–shell materials that are different from those of single-component materials (such as mechanical, optical, electrical, magnetic, and catalytic properties) are due to structure, size, morphology, and composition of their shells and cores [13].

Thiol compounds are of special significance in biochemistry and environmental chemistry, and studies on these compounds provide critical insight into the proper physiological function and diagnosis of disease states [14–17]. L-Cysteine (CySH [see Supplementary material S1]) is a sulfur-containing α-amino acid that plays an important role in biological systems because it binds in

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[1] *Abbreviations used:* MHCF, metal hexacyanoferrate; CySH, L-cysteine; n-Fe_2O_3@-NaCo[$Fe(CN)_6$], nanoparticles of Fe_2O_3 core–NaCo[$Fe(CN)_6$] shell; m-Fe_2O_3@NaCo[-$Fe(CN)_6$], microparticles of Fe_2O_3 core–NaCo[$Fe(CN)_6$] shell; n-NaCo[$Fe(CN)_6$], uniform NaCo[$Fe(CN)_6$] nanoparticles; EDTA, ethylenediaminetetraacetic acid; SEM, scanning electron microscopy; TEM, transmission electron microscopy; UCPE, unmodified carbon paste electrode; ECT, electrocatalytic transducer; LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation.

0003-2697/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2010.09.032

a special way and maintains the structure of proteins in the body. In addition, it may play an important role in the communication between the immune system cells [18]. CySH is also the sulfide donor for the biosynthesis of iron–sulfur clusters and the molybdopterin cofactor. CySH deficiency is accompanied by a number of clinical situations such as premature arteriosclerosis, leukemia, cervical cancer, diabetes, sepsis, cataracts, liver disease, skin lesions, slowed growth, Alzheimer's disease, Parkinson's disease, immediate aftermath of stroke, and AIDS (acquired immune deficiency syndrome) [19–21]. It is also used in the medicine and food industries [22]. It also serves as a model for the thiol group of proteins in a variety of biological media [23]. Therefore, sensitive quantification of this species is an important task within the biomedical community, and the determination of the protocols of CySH has been developed for both clinical and commercial purposes.

Numerous research efforts have been performed for the determination of CySH [3,24,25]. Compared with the fluorimetric and spectroscopic detection methods, the electrochemical techniques have the inherent advantages of ease of miniaturization, high sensitivity, and relatively low cost as well as being less sensitive to matrix effects than other analytical techniques. In addition, these techniques can be easily electronically interfaced to a computer and, in most instances, do not require the derivatization process. However, electrochemical detection of CySH still remains challenging. CySH electrooxidation at the conventional solid electrodes, such as noble metals and carbon-based electrodes, is usually plagued by sluggish electron transfer kinetics. It needs a large overpotential for the electrooxidation process to occur at a desirable rate to attain reasonably good sensitivity [26]. In addition, the high overpotential needed significantly reduces the detection selectivity, especially for biological samples. On the other hand, electrooxidation of CySH on these electrodes at highly positive potentials causes surface oxide formation as well as the fouling effect. To overcome these problems, some strategies have been developed and include application of pulse electrochemical detection [27,28], the use of mercury and diamond electrodes [24,27,28] and enzyme-based biosensors [29,30], and the design and applications of a variety of modified electrodes [24]. In the course of modified electrodes, the immobilized modifier on the electrode surface generally involves redox species that flip-flop between two redox states. CySH is then oxidized through an electrocatalytic conversion. Analysis of CySH with a physiological concentration of less than 300 μM has been improved greatly; however, the development of new modified electrodes for its determination is needed.

In the current study, an efficient electrocatalytic transducer based on cobalt hexacyanoferrate nanoparticles with a core–shell structure was employed for the electrooxidation and ultrasensitive detection of CySH.

Materials and methods

Materials

All of the chemicals used were of analytical grade from Merck (Germany) and were used without further purification.

Synthesis of n-Fe_2O_3@NaCo[Fe(CN)$_6$]

Nanoparticles of Fe_2O_3 core–NaCo[Fe(CN)$_6$] shell (n-Fe_2O_3@NaCo[Fe(CN)$_6$]) were synthesized using a solution precipitation procedure similar to that described in detail previously [4]. Briefly, nanoparticles of Fe_2O_3 were synthesized by mixing solutions of 0.10 M Fe^{2+} and 0.20 M Fe^{3+} and 2.0 M NaOH solution under stirring at 80 °C. Then the obtained Fe_2O_3 nanoparticles were suspended in a solution containing 0.10 M Na_3[Fe(CN)$_6$] + 10 mM HCl and stirred. Then a solution containing 0.10 M $CoCl_3$ + 10 mM HCl was added. The n-Fe_2O_3@NaCo[Fe(CN)$_6$] was then collected and washed.

Synthesis of m-Fe_2O_3@NaCo[Fe(CN)$_6$]

Microparticles of Fe_2O_3 core–NaCo[Fe(CN)$_6$] shell (m-Fe_2O_3@NaCo[Fe(CN)$_6$]) were synthesized similar to the procedure indicated for n-Fe_2O_3@NaCo[Fe(CN)$_6$]; however, 20 times higher concentrations of the reagents (Na_3[Fe(CN)$_6$] and $CoCl_3$) were used, and the reaction was performed at room temperature.

Synthesis of n-NaCo[Fe(CN)$_6$]

Uniform NaCo[Fe(CN)$_6$] nanoparticles (n-NaCo[Fe(CN)$_6$]) were synthesized based on a modified method that was published elsewhere [31]. Briefly, a 10-ml solution containing 0.01 M $CoCl_2$ + 0.01 M ethylenediaminetetraacetic acid (EDTA) was added dropwise to a 10-ml solution containing 0.05 M Na_3[Fe(CN)$_6$] + 0.05 M NaCl under vigorous stirring in a beaker. After complete addition, the mixture was further stirred for 5 min. Then a sufficient amount of acetone was added to precipitate a slurry product, and it was removed from the supernatant by a centrifuge. The final precipitate was dried overnight at room temperature under vacuum.

Apparatus

Electrochemical measurements were carried out in a conventional three-electrode cell containing 100 mM phosphate buffer solution (pH 7.4, which was employed as the running electrolyte throughout the work) powered by a Potentiostat/Galvanostat, an Autolab, and PGSTAT 302N (Eco Chemie, Netherlands). Ag/AgCl/3 M KCl and platinum gauze were used as the reference and counter electrodes, respectively. The system was run by a PC through NOVA software.

Scanning electron microscopy (SEM) was carried out using an X-30 Philips scanning electron microscope (USA). Transmission electron microscopy (TEM) was performed using a CEM 902A Zeiss transmission electron microscope (Germany) with an accelerating voltage of 80 kV.

Delivering the supporting electrolyte solution as the carrier stream and injecting CySH solutions were performed by a homemade peristaltic pump and a homemade injection valve with an interconnecting Teflon tube equipped with a 100-μl sample loop. A flow rate of 1.0 ml min^{-1} was employed. A glass cell mounted in a wall-jet configuration used a conventional three-electrode setup.

Working electrode preparation

Unmodified carbon paste electrode (UCPE) was prepared by hand-mixing carbon powder and mineral oil (Nujol) at a ratio of 80:20 (w/w). The paste was carefully mixed and homogenized in an agate mortar for 20 min. The resulting paste was kept at room temperature in a desiccator before use. The paste was packed into a cavity (3.6 mm diameter and 2 mm depth) at the end of a Teflon tube. Electrical contact was established by a copper wire connected to the paste in the inner hole of the tube. The electrode surface was gently smoothed by rubbing on a piece of weighing paper just prior to use. This procedure was also used to regenerate the surface of the carbon paste electrode.

Fe_2O_3 nanoparticle-modified carbon paste electrode (Fe_2O_3–CPE), n-NaCo[Fe(CN)$_6$]-modified carbon paste electrode (n-NaCo[Fe(CN)$_6$]–CPE), and m-Fe_2O_3@NaCo[Fe(CN)$_6$]-modified carbon paste electrode (m-Fe_2O_3@NaCo[Fe(CN)$_6$]–CPE) were prepared by