Materials Science and Engineering C 29 (2009) 1781–1789

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

A new zwitterionic imprinted polymer sensor using ethylenediamine tetraacetic acid and chloranil precursors for the trace analysis of L-histidine

B.B. Prasad [*], S. Srivastava, K. Tiwari, P.S. Sharma

Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India

A R T I C L E I N F O

Article history:
Received 6 November 2008
Received in revised form 30 December 2008
Accepted 5 February 2009
Available online 12 February 2009

Keywords:
L-histidine
Molecularly imprinted polymer
Charge-transfer complexations
Differential pulse
Cathodic stripping voltammetry

A B S T R A C T

A new zwitterionic ('gemini' like) L-histidine imprinted polymer, based on poly (ethylenediamine tetraacetic acid-co-chloranil), was synthesized for the modification of hanging mercury drop electrode. The imprinted polymeric film at the electrode surface facilitated L-histidine to be reduced electrochemically, as a consequence of the catalytic action of charge-transfer (chloranil-L-histidine complexation) reaction, to respond the corresponding differential pulse, cathodic stripping voltammetric signal without any cross-reactivity with interferents. The limit of detection (3σ) was found to be 0.128 ng mL^{-1} (RSD 0.2%) and results were found to be precise and accurate, without any false-positive contributions from human blood serum samples.

1. Introduction

L-histidine, (2-amino-3-(4-imidazolyl propanoic acid) (LH), a neurotransmitter, is an essential bioactive amino-acid component of many proteins. The other isomer, D-histidine is not bioactive. The chiral recognition and quantitative assay of LH isomer are associated with the diagnosis of LH metabolism disorders, particularly 'histedinemia' at elevated levels [1] in physiological fluids. (Normal level: 0.31 to 26.35 µg mL^{-1}; elevated levels: 29.5 µg mL^{-1} [2]). On the other hand, a persistent LH deficiency causes mild but chronic hyperammonemia called 'Friedreich ataxia' [3]. Other diseases like epilepsy, Parkinson's disease, and the failure of normal erythropoiesis develop, when LH reduces below the maintenance requirement (0.27 µg mL^{-1}) [4].

In general, most amino acids are non-electroactive at conventional carbon electrodes [5], but are reportedly active toward electrochemical oxidation at metallic (Au, Pt) electrodes [6,7]. As far as chemically modified electrodes are concerned [7,8], the success in the electrochemical detection of amino acids lies upon the practicability of electrodes, involving proper incorporation of either a catalyst or a redox mediator.

Several sophisticated technologies including sensors/biosensors [9,10], potentiometry [11], fluorescence with capillary electrophoresis [12], voltammetry [9], mass-spectrometry [13], chromatography [14], and spectroscopy [15], have been used for the LH detection. However, use of large sample volumes, expensive instrumentation, lack of reproducibility and selectivity, and eco-unfriendly solvents were serious concerns associated with these techniques.

Materials with recognition properties have wide applications, particularly in sensor development. The recognition properties of a material are related to its ability to form interactions with the analyte. These interactions might be established both as a consequence of the presence of particular chemical groups on the material (e.g. presence of hydroxyl, carboxyl, amino group etc.), and a complementarity between the analyte and the three-dimensional structure of the material at the nanometer scale-level, which can be defined as 'shape recognition'. A prominent technology for the synthesis of material with entailed recognition properties is molecular imprinting. The process involves a template-directed assembling of functional building blocks in solution, which is governed by thermodynamic forces. Preformed complex is further fixed by polymerization, forming a material or a surface with spatially disposed functional groups complementary to the template.

Molecular "receptors" using molecularly imprinted techniques were potentially used for the separation of histidine enantiomers [16–21]. However, these have some drawbacks such as tedious synthesis procedures [16,18], longer incubation time [17,19], longer response time [20], and template leaching [19]. Further, their sensitivities were limited to millimolar levels [16,18,20], and applications to real samples were also found limited. There is an obvious need for the sensor-surface modification, particularly with zwitterionic polymers, which can significantly resist the non-specific protein sorptions from complex biological media [22]. Furthermore, keeping in view of the notorious passivation of solid electrodes in ordinary aqueous solution of LH, a cationic surfactant micellar medium was found useful as a remedial approach for the voltammetric determination of LH at glassy carbon electrode (GCE) [5]. In this context, a zwitterionic 'gemini' surfactant modified GCE was reported to execute a direct-electron-transfer [23].

---

[*] Corresponding author. Tel.: +91 9451954449; fax: +91 5422368174.
E-mail address: prof.bbpd@yahoo.com (B.B. Prasad).

0928-4931/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.msec.2009.02.001

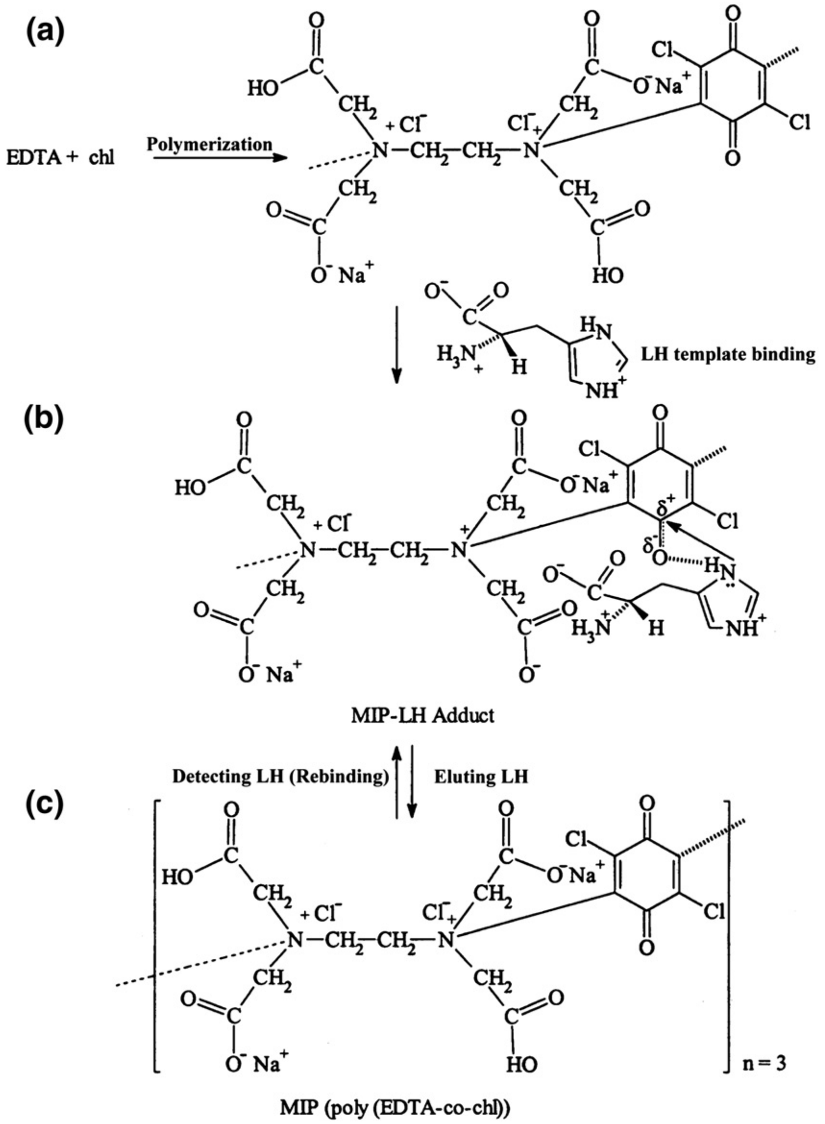

Fig. 1. (a) EDTA–chl polymerization, (b) binding mechanism of LH with MIP (charge-transfer complex formation), (c) imprinted polymer with shape and functional memory.

In the present work, adapting complexation between ethylenediamine tetraacetic acid disodium salt (EDTA) and chloranil (chl) [24], an innovative new molecularly imprinted polymer, poly (EDTA-co–chl), henceforthwith referred to as MIP, was prepared, to modify hanging mercury drop electrode (HMDE) and to attain the limit of detection as low as ng mL^{-1} level. Molecular imprinting of LH was made following Takagishi's system approach [25], where the template was incorporated at a later stage after pre-polymerization of the mixture of precursors solution. This obviated the need for the protection of N-terminal histidine residues in MIP preparation. The resultant macroporous MIP not only recognizes imprinted amino acid but also may be able to recognize the protein molecule as well.

Insofar as LH detection is concerned, electrochemical oxidation of LH at preanodized higher positive potentials (+1.5 V vs. Ag/AgCl) with chemically-modified electrodes often leads to the oxidation current largely inhibited by the strong adsorption of LH-oxidized product on the electrode surface [9]. Alternatively, in the present work, the direct electron-transfer from cathodic HMDE to the LH redox centres in zwitterion ('gemini'-like) [26] MIP cavities, without using an electron-transfer mediator is followed. The selection of chl moieties, in analogy with a surfactant for the direct electron-transfer of myoglobin within a zwitterionic gemini film [23], is apparently advantageous to create an electroconductive film onto the electrode surface. Here chl being an activated electron-acceptor residue at +0.1 V might inject positive holes in the LH (protein residue) as p-type semiconduction, and conductivity may be enhanced many fold in the presence of water [27]. Furthermore, the charge-transfer interactions (partly hydrogen bonding), between planar imidazole ring of the LH (–NH) and chl (–C=O) (Fig. 1), may constitute a typical donor–acceptor charge-transfer complex (LH–chl) behaving like an 'organic-metal' and influencing electronic effects in terms of redox activation [28].

2. Experimental

2.1. Materials and reagents

EDTA, chl, LH and other chemicals/interferents were purchased from GlaxoSmithKline Pharmaceuticals, India; Loba Chemie, India; Sisco Research Laboratories, India, and SD Fine, India. All chemicals were of analytical grade. The solvent dimethyl sulphoxide (DMSO) was of HPLC quality. Human blood serum samples were procured from a local patholab. The stock standard solutions (500 μg mL^{-1}) of LH were freshly prepared by dissolving 0.0125 g of LH in 25 mL deionized, triple distilled water. The working (test) solutions of LH were prepared from stock by appropriate dilutions with water, and their