Analytica Chimica Acta 853 (2015) 271–284

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Imprinted ZnO nanostructure-based electrochemical sensing of calcitonin: A clinical marker for medullary thyroid carcinoma

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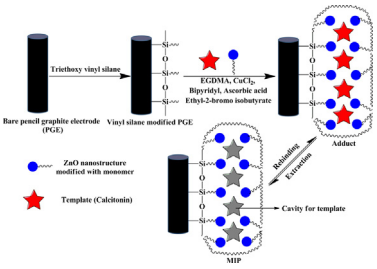
HIGHLIGHTS

- Molecular imprinting-based sensor for medullary thyroid carcinoma marker was developed.
- ZnO nanostructure was used as a platform for synthesis of imprinted polymer.
- Imprinted polymer was prepared by ARGET–ATRP method.
- A novel and biocompatible tyrosine amino acid derivative was used as monomer.
- Linear working range is found from 9.99 ng L^{-1} to 7.919 mg L^{-1} with LOD 3.09 ng L^{-1}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 17 October 2014
Accepted 24 October 2014
Available online 27 October 2014

Keywords:
Calcitonin
Zinc oxide nanostructure
Surface imprinting
Activator regenerated by electron transfer–atom transfer radical polymerization (ARGET–ATRP)
Biomarker-sensor

ABSTRACT

The present work describes an exciting method for the selective and sensitive determination of calcitonin in human blood serum samples. Adopting the surface molecular imprinting technique, a calcitonin-imprinted polymer was prepared on the surface of the zinc oxide nanostructure. Firstly, a biocompatible tyrosine derivative as a monomer was grafted onto the surface of zinc oxide nanostructure followed by their polymerization on vinyl functionalized electrode surface by activator regenerated by electron transfer–atom transfer radical polymerization (ARGET–ATRP) technique. Such sensor can predict the small change in the concentration of calcitonin in the human body and it may also consider to be as cost-effective, renewable, disposable, and reliable for clinical studies having no such cross-reactivity and matrix effect from real samples. The morphologies and properties of the proposed sensor were characterized by scanning electron microscopy, cyclic voltammetry, difference pulse voltammetry and chronocoulometry. The linear working range was found to be 9.99 ng L^{-1} to 7.919 mg L^{-1} and the detection limit as low as 3.09 ± 0.01 ng L^{-1} (standard deviation for three replicate measurements) (S/N = 3).

1. Introduction

In the recent years, cancer becomes the most feared global disease, based on the rate of death all over the world [1]. According to the International Agency for Research on Cancer, there were 12.7 million new cancer cases in 2008, among them approximately 7.6 million people died [2]. Based on the cancer registry data in

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http://dx.doi.org/10.1016/j.aca.2014.10.030
0003-2670/

India, it seems that 800,000 new cancer cases are reported every year [3]. According to world health organization (WHO), if the toll rate will not be changed, then the universal cancer cases will become two fold by the year 2030 [4]. The fast growth of cancer is not just because of the failure of the medicine or the treatment process; this is due to the lack of early stage detection of the cancer. The early diagnosis of cancer is crucial for patient survival and successful prognosis of the disease and for this reason sensitive and specific methods are required for early cancer diagnosis. The popular conventional methods for the detection of cancer are based on imaging and/or screening methods. Cancer imaging encompasses with the radiology, which is harmful for the human body to some extent. On the other hand, in the screening methods, a blood test and urine test are performed for the detection of cancer markers. Cancer markers are the most efficient tools for the early stage diagnosis of cancer disease. They are classified as an indicator of cancer and by detecting them the existence of the specific cancer can be verified. Cancer biomarkers are critical not only for the diagnosis of cancer, but also for monitoring its response to treatment or recurrence and, more recently, for the assessment of cancer risk. This means the necessary tools have to be extremely sensitive as well as selective to overcome the effects of interfering compounds present in real samples.

Since the discovery of hypocalcemia, hypophosphatemic polypeptide by Copp et al. [5] in 1961, calcitonin is known to be an excellent marker for medullary thyroid carcinoma (MTC) [6]. Calcitonin is a linear polypeptide containing 32 amino acids, secreted mainly by the parafollicular cells (also called C-cell) of the thyroid gland [7]. The amount of calcitonin in human serum is 0–100 ng L^{-1}. Human calcitonin has an important role in the calcium metabolism by reducing the blood calcium level [8]. The biological activity shown by calcitonin molecule is due to the presence of disulphide bridge between residues 1 and 7, eight specific amino acid residues at the N-terminus, and a proline amide moiety at the C-terminus [9]. The amino acid sequences of human calcitonin are given below:

Human calcitonin: COOH–Cys–Gly–Asn–Leu–Ser–Thr–Cys–Met–Leu–Gly–Thr–Tyr–Thr–Gln–Asp–Phe–Asn–Lys–Phe–His–Thr–Phe–Pro–Gln–Thr–Ala–Ile–Gly–Val–Gly–Ala–Pro–NH_2.

Detection of calcitonin can be used for the biomarking of malignant tumor, osteoporosis and medullary thyroid carcinoma (MTC) [10]. According to the table provided by Costante et al. in January 2009, the normal range of calcitonin in body is less than 10 ng L^{-1} but if the concentration reaches to a level in between 100 and 500 ng L^{-1}, then there is a probability of C-cell hyperplasia and when crossed 500 ng L^{-1}, medullary thyroid carcinoma (MTC) may occur. If the level is above 1000 ng L^{-1}, MTC is already formed in the body [10]. Various methods for the detection of calcitonin have been employed till date, like radioimmunoassay [11], enzyme-linked immunosorbent assay [12], high performance liquid chromatography (HPLC) [13], time-resolved fluoroimmunoassay [14], two-site immunofluorometric assay [15], room temperature phosphorescence immunoassay [16]. But these methods have certain disadvantages, like, the radioactive contamination in radioimmunoassay is harmful toward the human health. Similarly, other immunoassay techniques require either very sophisticated handling or large analysis time or both. So, a sensitive and simple technique for the clinical detection of calcitonin is still called for, which will be rather cost-effective too.

In modern days, electrochemical techniques draw our attention toward the sensitive determination of cancer markers in biological fluids [17] due to their simplicity, high sensitivity, low cost and relatively short analysis time than other techniques. But these existing electrochemical techniques suffer major drawback of selectivity [18]. The lack of selectivity issue can be well resolved by applying the molecular recognition elements in electrochemical techniques. According to the literatures, molecularly imprinted polymers (MIPs) are one of the most popular molecular recognition elements in electrochemical techniques [19–24]. MIPs are the polymer network formed by the monomer molecules in the presence of a cross-linker around a target molecule and after the extraction of target molecule; it leaves a cavity, which is specific to the size and shape of that template molecule. MIP for small molecules is widely known and easy to synthesize. But imprinting of large molecules like proteins and other biomolecules is still challenging due to their size, structure complexity and solubility factor [25]. Most of the proteins are water-soluble, which is a major problem for the MIP preparation in the organic solvent, resulting poor site accessibility and low binding capacity in the 3D polymer matrix [26]. To overcome these issues, various approaches have been proposed for the successful imprinting of proteins, such as surface imprinting [27], an epitope approach [28], covalent imprinting [29], electropolymerization [30,31], and the use of low cross-linking density hydrogels [32]. In general, surface imprinting allow the imprinted sites to be situated at the surface or in the proximity of the material's surface. So that entrapment of protein template in cross-linked polymers could be curtailed. Along with this, the presence of cavities at the surface ensures the complete removal of templates, low mass-transfer resistance and easy access to the target molecules [33]. Surface imprinting over nanosized sphere support materials with large specific surface area and is very appropriate for a template of the bulky structure like protein. Many particles, such as silica nanoparticles [34], magnetic nanoparticles [35], and quantum dots [36,37] have been successfully used as a platform for surface imprinting process. Nanostructure materials not only offer a high surface to volume ratio, but they also provide very unique and different electrical, optical, mechanical and magnetic properties compared to their micro sized particles [38].

Herein, for the first time, we have used ZnO nanostructures as a platform for the synthesis of calcitonin imprinted polymer. Indeed, ZnO nanostructures, due to their excellent electron transfer rate, can evoke the hidden electrochemical ability of biomolecules, and facilitate their direct electrochemistry [39–42]. The synthesized ZnO nanostructures have unique advantages, including high surface to volume ratio, non-toxic, low cost, chemical stability, eco-friendly and high electron communication features than their bulk counterpart.

Besides this, herein, a new technique for living radical polymerization is opted [43] to get well-defined chain architecture as well as low molecular weight distribution. Among controlled or living radical polymerization methods, atom transfer radical polymerization (ATRP) is the most successful one. But in ATRP, a problem arises due to the trapping of propagating radicals by oxygen. For this another constant deoxygenation step is always associated with ATRP. The deoxygenation step can be eliminated by activator regenerated by electron transfer (ARGET) ATRP process [44]. It is a modern technique which uses reducing agent such as ascorbic acid or tin(II) 2-ethylhexanoate for the formation of Cu(I) catalyst in situ from Cu(II) [44]. Here, the copper catalyst concentration is also reduced down to ppm level and for this it becomes an industrially important technique due to lesser catalyst requirement process.

Herein, a biocompatible tyrosine derivative as a monomer [2-acryloylamino-3-(4-hydroxy-phenyl)-propionic acid] was grafted covalently on the synthesized ZnO nanostructure. The monomer modified ZnO nanostructure along with template, initiator, catalyst, reducing agent and cross-linker was coated onto the vinyl group modified pencil graphite electrode (PGE), as shown in Scheme 1. Herein, ascorbic acid is used as a reducing