Analytica Chimica Acta 838 (2014) 31–36

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A new method to assay hypoxia-inducible factor-1 based on small molecule binding DNA

Nana Yang [a], Hao Li [b], Tingting Yin [a], Yue Huang [b], Lizhou Sun [a,**], Genxi Li [b,c,*]

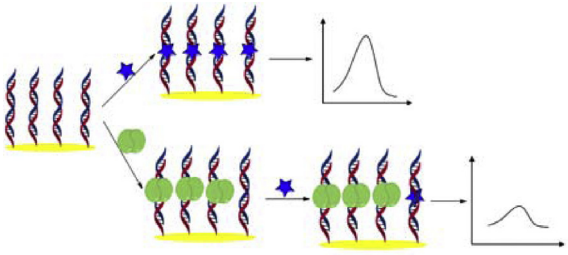
[a] Department of Obstetrics, The First Affiliated Hospital with Nanjing Medical University, Nanjing 210036, PR China
[b] Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China
[c] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, PR China

HIGHLIGHTS

- A novel electrochemical method has been established for hypoxia-inducible factor-1.
- The sensitivity of the method surpasses that of current immunoassays.
- The assay of placenta sample can be used to evaluate the severity of preeclampsia.

GRAPHICAL ABSTRACT

A novel method for HIF-1 detection is proposed by using electrochemical techniques based on small molecule binding DNA. The proposed method can be directly used to assay HIF-1 in placenta tissue, and the assay results can reliably reflect the severity of preeclampsia, a very dangerous condition during pregnancy.



ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 25 April 2014
Accepted 22 May 2014
Available online 29 May 2014

Keywords:
Hypoxia-inducible factor-1
Echinomycin
Biosensor
Electroanalysis
Preeclampsia

ABSTRACT

Hypoxia-inducible factor-1 (HIF-1) is among the most important indicators of hypoxia in evaluating severity of many diseases. In this work, a novel method for HIF-1 detection is proposed by using electrochemical techniques based on small molecule binding DNA. In this method, since the designed DNA sequence can specifically bind with either an electroactive small molecule or HIF-1, the signal readout is inversely proportional to HIF-1 concentration, thus a simple and easily-operated method for HIF-1 detection can be developed. With the proposed method, HIF-1 can be determined in a linear range from 5 to 25 nM with a detection limit of 2.8 nM. Furthermore, the proposed method can be directly used to assay HIF-1 in placenta tissue, and the assay results can reliably reflect the severity of preeclampsia, a very dangerous condition during pregnancy. The proposed method also shows desirable sensitivity, high selectivity and excellent reproducibility, so this method can have potential applications in clinical practice.

1. Introduction

Hypoxia is a common condition found in a wide range of physiological and pathological states. Hypoxia promotes various biologically significant processes, such as cell glycolysis, angiogenesis, cell invasion, metastasis, by inducing hypoxia-inducible factor-1 (HIF-1) expression [1]. So, HIF-1 is the most important indicator identified to date of the cellular response to hypoxia [2,3]. Elevated HIF-1 level is frequently correlated with metastasis and

* Corresponding author at: Department of Biochemistry, Nanjing University, Nanjing 210093, PR China. Tel.: +86 25 83593596; fax: +86 25 83592510/86663616.
** Corresponding author. Fax: +86 25 86663616.
E-mail addresses: lizhou_sun121@hotmail.com (L. Sun), genxili@nju.edu.cn (G. Li).

http://dx.doi.org/10.1016/j.aca.2014.05.045
0003-2670/

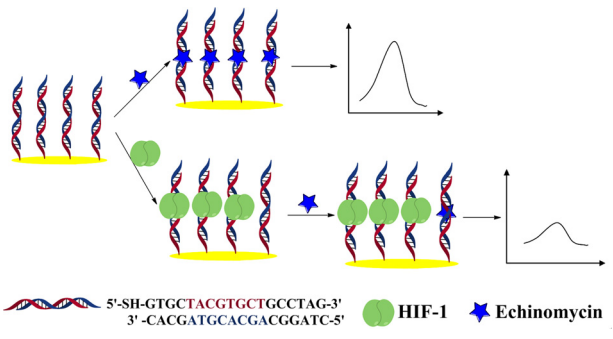
poor clinical outcomes in cancers of various origins [4,5]. HIF-1 overexpression can also imply the severity of hypoxia in other diseases such as preeclampsia. As a consequence, the assay of HIF-1 protein is critical for clinical prognosis and the monitoring of the curative effect of drug intervention [6].

In the present, only western blotting and ELISA have been proposed to assay HIF-1. Therefore, more methods should be developed on the one hand; on the other, the need of antibodies in the assays of western blotting and ELISA have posed limitations in their applications, because the highly complicated chemical structure of antibodies may greatly obstruct the use of novel sensing strategies.

In recent years, some low molecular weight equivalents to antibodies, such as structured DNA, peptides and small molecule drugs have been reported [7,8]. These molecules have far more simplified chemical structures, so they can be equipped with other function modules and can be orderly immobilized in the sensing layer without the loss of protein-binding affinity. Therefore, the development of chemically uncomplicated HIF-1 sensing reagent that enables highly sensitive, specific, and cost-efficient assays has aroused great interest in analytical chemistry as well as biomedical science.

In this work, sequence specific DNA is designed for both HIF-1 recognition and signal readout, so a simple and easily operated method with high sensitivity and selectivity is developed for HIF-1 assays. It has been known that some DNA sequences may have the characteristics of particular protein-binding motifs, such as the naturally occurring binding sites of transcriptional factors, and the artificially fabricated protein-binding surfaces on aptamers [9,10]. It has also been known that some DNA sequences can specifically bind with small molecules, such as organic metal complexes and various drug compounds via special sequence and conformation in the major groove. So, we propose that if the small molecules can be exploited as signal reporter, i.e., if a DNA sequence is designed to be the binding sites of both the target protein and a small molecule, then the readout signal from the small molecule will be inversely proportional to the concentration of the target protein, protein detection will be more easily and favorably performed. In this context, a DNA sequence featuring the hypoxia response element (HRE) is designed for the assay of HIF-1, based on the design that HRE will bind with either HIF-1 [11,12], or an electroactive small molecule, named Echinomycin (Echi) [13–16]. So, a new method for HIF-1 assay is established via the electrochemical signal readout of Echi inversely proportional to the amount of HIF-1. Meanwhile, since no bio-conjugation is required for the attachment of the electrochemical reporter Echi, performance of the HIF-1 assay is greatly simplified.

To demonstrate the feasibility of the proposed method, HIF-1 expression in placenta has also been determined to evaluate hypoxia during preeclampsia (PE). PE is a gestational idiopathic disease that complicates 5–8% of all pregnancies, resulting in



Scheme 1. Principle of the new method for HIF-1 assay. Not drawn to scale.

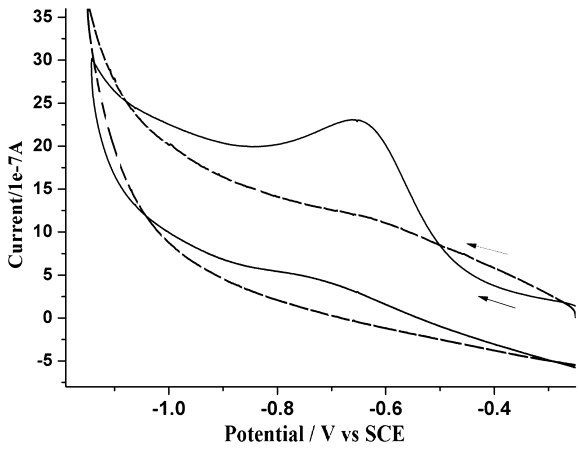


Fig. 1. Cyclic voltammograms obtained at the probe DNA (solid line) and the scrambled sequence (dotted line) modified electrode after incubation with 100 nM Echi at 37 °C for 1.5 h. Arrows mark the scan direction. Scan rate: 0.1 V/s.

significant prenatal morbidity and mortality [17]. Placental hypoxia is the principal clinical features of PE [18]. Our method enables efficient analysis of HIF-1 expression as a reliable indicator of the severity of PE.

2. Experimental

2.1. Chemicals and materials

Recombinant mammalian HIF-1α and HIF-1β were obtained from Abnova (America). Echi was ordered from Alexis (Switzerland). 6-mercapto-1-hexanol (MCH), tris(2-carboxyethy) phosphine hydrochloride (TCEP), bovine serum albumin (BSA), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB) from human plasma were purchased from Sigma–Aldrich. Nuclear extract kit was purchased from Active Motif. Ammonium formate was purchased from JK Scientific Ltd. (Beijing, China). Thiol-modified probe DNA (5′-GTGCTACGTGCTGCCTAG-SH-3′) and its complementary sequence (5′-CTAGGCAGCACGTAGCAC-3′), thiol-modified DNA with scrambled sequence (5′-GTGCTATC-TATCGCCTAG-SH-3′) and its complementary sequence (5′-CTAGGCGATAGATAGCAC-3′) (PAGE-purified) were synthesized from Takara Biotechnology Co., Ltd. (Dalian, China). The underlined sequence is the binding site of HIF-1 and Echi. The probe DNA and

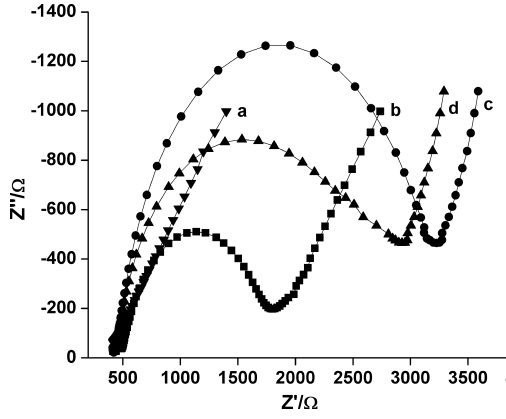


Fig. 2. Nyquist diagrams corresponding to (a) the bare gold electrode, (b) the probe DNA modified electrode, (c) the probe DNA modified electrode after being treated with 5 nM HIF-1, and (d) the probe DNA modified electrode after being treated firstly with 5 nM HIF-1, and then with 100 nM Echi. Electrochemical species: 5 mM $[Fe(CN)_6]^{3-/4-}$. Biasing potential: 0.224 V. Amplitude: 5 mV. Frequency range: 0.1 Hz–10 kHz.