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Electrochemical biosensor for epidermal growth factor receptor detection with peptide ligand

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ABSTRACT

Epidermal growth factor receptor (EGFR) that has been over-expressed in many different types of cancers has become logical targets for both diagnosis and therapy. Herein, we report an electrochemical peptide-based biosensor for the detection of EGFR, in which ferrocene moiety modified peptide ligands that are specific to EGFR were immobilized on gold electrode through Au–S interaction. Upon the binding of peptide ligands with EGFR, complex formation changes occurred on the electrode surface, which resulted in current increase. The amplitude of the current increase displayed a linear relationship with logarithm of the EGFR concentrations in the range of 1.0×10^{-6}–1.0×10^{-10} g L^{-1}, with a detection limit of 3.7×10^{-11} g L^{-1}. The proposed method has been applied for determination of EGFR in diluted human serum samples with satisfied results.

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1. Introduction

Epidermal growth factor receptor (EGFR) plays important roles in cell proliferation, survival, adhesion, migration and differentiation [1]. EGFR is also an important tumor-specific target, since in cancer cells, probably under genetic mutations, the EGFR-mediated downstream pathways are activated, leading to uncontrolled tumor proliferation and apoptosis [2]. Thus, increased EGFR expression is likely to be a strong prognostic and predictive feature in multiple tumor types and determination of EGFR may produce substantial diagnosis benefits.

The traditional techniques for EGFR detection include immunohistochemistry (IHC) on tissues, western blotting (WB) on membranes and enzyme-linked immunosorbant assay (ELISA) [3]. ELISA method is the gold standard for detection of protein concentration with high sensitivity and selectivity, but the procedures are complicated and also time consuming. IHC and WB methods need strong personnel skills to perform the detection. Recently, several other methods have been developed to detect EGFR. For examples, Weigum et al. used a microfluidic biochip to detect EGFR concentration through capture of cells by a porous membrane [4]. Vasudev et al. presented an electrochemical immunosensor for label free EGFR detection with high sensitivity [5]. Takahashi et al. demonstrated a noninvasive electrochemical detection of EGFR by scanning electrochemical microscopy [6,7]. The methods based on quartz crystal microbalance were applied to detect EGFR also [8,9]. Besides of the above mentioned methods, some other techniques, such as surface plasma resonance [10] and thin film transistor-based immunosensor [11], have been also applied to detect EGFR. However, though these sensors demonstrated high selectivity and sensitivity, in most cases, expensive and unstable antibodies were required, which limited their practical applications.

Peptide ligands are initially developed as research tools to dissect protein function within complex molecular regulatory networks [12–14]. They can be bond to a given target protein with high specificity and strong affinity *in vitro* and *in vivo*. Since peptide ligands are smaller than antibody, they show better cell membrane penetration and almost no immunogenic. Moreover, peptide ligands possess the benefits of low manufacturing costs, high activity and stability, reduced immunogenicity, efficient organ penetration and less complicated patenting issues [15]. All of these merits make peptide ligands powerful alternatives to traditional approaches for the analysis of target proteins. For examples, Choi et al. identified a peptide ligand for *Edwardsiella tarda* from a phage peptide library and tested two approaches for sensitive detection of the bacteria with the peptide labeled with fluorescein or biotin [16]. Wang et al. prepared a kind of beta-amyloid peptide (Abeta1-16) conjugated gold nanoparticles and used them as colorimetric indicator for studying the interaction of beta-amyloid peptide with metallic ions [17]. Gerasimov and Lai developed an electrochemical peptide-based biosensing platform for HIV detection [18].

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Electrochemistry detection possesses the characters of simple equipment and high sensitivity. In this study, we combine the advantages of electrochemistry and peptide ligand to develop an EGFR biosensor. The sensor is simple, rapid, ultrasensitive and high affinity with no immunogenicity. The proposed approach combines binding-induced changes in the dynamics of the peptide ligand (*i.e.*, the rate at which the redox label collides with the electrode surface) with the well-established electrochemical biosensing platform. A bi-functionalized peptide ligand with one terminal modified by electroactive ferrocene (Fc) group as the reporter and the other terminal thiol functioned as the anchor on the gold electrode surface is employed [19]. The immobilized Fc-labeled peptide ligand showed a voltammetric signal due to the one-step redox reaction of the ferrocenyl moiety. With the recognition of EGFR, an enhanced electrochemical signal is detected, which is used as the basis for quantitative analysis of EGFR. The proposed method has been applied to detect EGFR in human serum samples with satisfied results. We believe that this class of sensing platforms has a great potential and can be adapted to the detection of a wide range of disease-relevant targets, which would undoubtedly expand its practical applications.

2. Experimental

2.1. Chemicals

The peptide ligand employed in this study was chosen and designed according to the prior report [20] and synthesized by GL Biochem Ltd. (shanghai). Its amino acid sequence was YHWYGYTPQNVI (designated as GE11) [21,22]. A 9-mercapto-1-nonanol was added to the N-terminus of this peptide ligand to enhance the flexibility for better recognition and binding.

The target protein, epidermal growth factor receptor (EGFR), was purchased from ProSpec-Tany TechnoGene Ltd. (Israel). The ferrocene carboxylic acid (97%, FcCOOH), 6-mercapto-hexano (MCH), tris(2-carboxyethyl) phosphinehydrochloride (TCEP), sodium monohydrogen phosphate, sodium dihydrogen phosphate, potassium ferricyanide, potassium ferrocyanide were purchased from Sigma (St. Louis, MO). 1-Ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC), N-hydroxysuccinimide (NHS) and albumin from human serum (HSA) were purchased from Shanghai Medpep Co. Ltd (Shanghai). All chemicals were of analytical grade and used as received. The human serum samples donated by three volunteers were collected by the First Affiliated Hospital of Fujian Medical University. The study was conducted with written approval of the participants.

Standard EGFR samples were dissolved by 100 mM Tris–HCl buffer (pH 7.4, containing 200 mM NaCl) to get various concentrations of EGFR. Peptide ligand solution was prepared by dissolving it with 0.05 mol/L (pH 7.4) phosphate buffer solutions (PBS). The physiological buffer for sensor interrogation contains 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and pH 7.4 [18]. Deionized water (18.4 MΩ) purified by a milli-QTM system (Millipore) was used throughout the experiment.

2.2. Synthesis of ferrocene-labeled peptide ligand

Ferrocene was modified on peptide through the EDC-NHS method [23]. Briefly, 1 mM ferrocene carboxylic acid (FcCOOH) and 0.1 M 1-ethyl-3-[(3-dimethylamino) propyl]-carbodiimide hydrochloride (EDC) were first incubated for about 10 min. Then, 0.1 mM peptide chain and 0.1 M N-hydroxysuccinimide (NHS) were added to the above solution with the volume ratio of 1:1:1:1 and incubated together at room temperature for 4 h [24,25]. Excessive FcCOOH was dialyzed in TAE buffer solution for 24 h and following that the mixture was stored at 4 °C.

2.3. Sensor preparation

The gold electrodes (3 mm in diameter) were polished firstly with aqueous slurries containing 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powders on a polishing microcloth, followed by an ultrasonic treatment to get rid of the invisible alumina particles adsorbed on the surface of the electrodes. The electrodes were then washed with Milli-Q water for 5 min, dried by nitrogen stream to obtain gold electrodes with clean surface. After that, they were further cleaned and activated in a fresh 0.5 M H_2SO_4 solution through cyclic voltammetry (CV) scanning in the range of 0–+1.65 V until ideal CV curves were obtained. Finally, the solution containing 25 μM peptide ligand probes was dropped on the electrode surface and covered by a closed container for 2 h at room temperature to form a peptide-immobilized electrode. Next, the above modified electrode was passivated with 1.0 mM MCH for 60 min to obtain a well-aligned monolayer. The modified electrodes were incubated with different concentrations of EGFR at room temperature for 1.5 h for following assays. After each modification and binding step, the electrodes were washed again with Milli-Q water for 5 min and dried in a nitrogen stream to remove the excess materials that were not immobilized over the electrode surface.

2.4. Electrochemical measurements

All electrochemical measurements were carried out on a CHI660A electrochemical analyzer (Chenhua Instruments, Shanghai, China). Electrochemical impedance spectroscopy (EIS) studies were performed in 10 mM $[Fe(CN)_6]^{3-}/Fe[(CN)_6]^{4-}$ solution containing 0.1 M KCl with a three-electrode system, in which a gold electrode was used as the working electrode after pre-immobilization of peptide ligand, an Ag/AgCl electrode and a Pt wire were used as reference electrode and counter electrode, respectively. Impedance measurements were recorded between 0.1 MHz and 1 Hz at a sinusoidal voltage perturbation of 5 mV amplitude. A Randles equivalent circuit was used to fit the obtained impedance spectra. During the current detection, differential pulse voltammetry (DPV) was chosen as the scanning mode for detection, which had a potential interval of −0.2–+0.4 V with the amplitude of 50 mV and the sensitivity of 1×10^{-6} A/V.

3. Results and discussion

3.1. The principle of the biosensor

The mechanism of the electrochemical peptide biosensor is described in Fig. 1. 9-Mercapto-1-nonanol moiety is modified to the N-terminus of the peptide ligand, so that the ligand can be well immobilized on the gold electrode surface through Au–S covalent bonds and still owns high flexibility to bind its target protein. MCH is then modified onto the electrode to passivate the unmodified area of the electrode and a well-aligned monolayer is obtained. At this stage, the Fc-moiety is relatively far away from the surface of gold electrode, a lower current is detected. After the above modified electrode is incubated in the solution that contains the target (EGFR) for some time, EGFR is captured by the peptide, which causes the change of the conformation of the peptide, and subsequently significantly reduces the electron-transfer tunneling distance between the electrode and the redoxable label. As a result, the detected current is enhanced. As the increased current has a linear relationship with EGFR concentration, therefore, the concentration of EGFR can be detected indirectly.

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