Journal of Pharmaceutical and Biomedical Analysis 150 (2018) 1–8

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

SPR imaging biosensor for the quantitation of fibronectin concentration in blood samples

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ARTICLE INFO

Article history:
Received 28 June 2017
Received in revised form
28 November 2017
Accepted 28 November 2017
Available online 29 November 2017

Keywords:
Fibronectin
Surface plasmon resonance imaging
Biosensor

ABSTRACT

The purpose of this study was presentation of a new biosensor capable of determination of fibronectin. This biosensor was based on the specific interaction of anti-fibronectin antibody produced in rabbit with fibronectin. The surface plasmon resonance imaging (SPRI) technique was used as a detecting method. Optimization and characterization properties of the biosensor were studied. The determination of fibronectin concentration in natural samples was done. The results were compared with a reference method (Enzyme-Linked Immunosorbent Assay-ELISA). The analytically useful dynamic response range of biosensor is between 5 and 400 ng mL^{-1}. The detection limit is 1.5 ng mL^{-1} and limit quantification is 5 ng mL^{-1}. The proposed SPRI biosensor showed good selectivity for potential interferences. It was applied to determine fibronectin concentrations in plasma of healthy donors and of patients after thermal injury. Good correlations between results obtained using the SPRI biosensor and ELISA test (correlation coefficients for healthy donors 0.996, for patients 0.984) were obtained. The average fibronectin concentration of healthy donors was 140.5 ± 24.6 μg mL^{-1} and the average fibronectin concentration of patients was 601.5 ± 72.1 μg mL^{-1}, which was in agreement with results obtained by other investigators. The obtained results indicate that the developed biosensor may be a candidate for monitoring fibronectin concentration in blood samples.

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1. Introduction

Surface plasmon resonance imaging (SPRI) was first demonstrated in the late 1980s [1,2]. Since then, SPRI has found rapid increasing research interests. Extensive research work on SPR imaging has been conducted by Corn et al. [3]. In the literature we can find various SPRI systems, for example: Multi-parametric Surface Plasmon Resonance (MP-SPR) [4], Localized Surface Plasmon Resonance (LSPR) [5] and spectral surface plasmon resonance (λSPR) [6].

This technique measures the refractive index changes in the vicinity of thin metal layers in response to biomolecular interactions. The refractive index is proportional to the amount of immobilized molecules on the metal surface. The label-free nature of SPRI makes it an attractive alternative technique to others, in which labels have to be used. The labeling process can cause the loss of functional properties of biomolecules (functional proteomics).

SPRI biosensor allows the real-time biomolecules detection. SPRI experiment may be exploited to monitor interaction between molecules and determining the kinetics of interaction [7–9].

The combination of SPRI technique with the biosensor is a sensitive, quantitative and rapid measurement method with abundant applications in the bioanalytical field [10–15].

The analytically active layer (receptor) of biosensor is formed with biological material being immobilized on a suitable support. Receptor type affects the sensitivity and selectivity with respect to the test substance. The antibody or inhibitor may be used as recognition elements in SPRI biosensor. The numerous examples of successful use of SPRI biosensors for the determination of diagnostically significant biomolecules have been reported [6–8]. The SPRI biosensor may be one of the competitive techniques in

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https://doi.org/10.1016/j.jpba.2017.11.070
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comparison to the ELISA assay in the determination of diagnostically significant biologically active species [16,17].

Fibronectin (FN) is one of the biologically active species which may be important as a potential biomarker. It is a glycoprotein that is an essential component of the extracellular matrix (ECM). FN is present in plasma and in various types of tissue and plays important role in cell adhesion, growth, migration, differentiation, wound healing and embryonic development [18,19].

The basic structural unit of FN is a dimer composed of two similar but not always identical polypeptide chains of similar molecular weight of about 250 kDa. Each chain of fibronectin is composed with repeated motifs of the amino acid sequence designated as type I, II and III. There are 12 modules of the type I, two of type II and 15–17 of type III in one subunit. The modules are distributed irregularly and form a mosaic structure of the protein [18,19].

Fibronectin in mammals coexists in the multiple molecular forms. It exists in a soluble form – plasma (PFN), and two insoluble forms: bound to the cell membrane and present in the extracellular matrix as cross-linker. According to Hynes [20], PFN is a globular protein consisting of independently folded globular domains and with a relatively compact conformation. Insoluble FN is in extended conformation and may be assembled into fibronectin fibrils [20].

FN soluble form occurs not only in plasma, but in other biological fluids: amniotic fluid [21], cerebrospinal fluid [22], saliva [23,24]. The soluble fibronectins are synthesized by hepatocytes while the insoluble forms are produced by fibroblasts, macrophages, endothelial cells and epithelial cells [18].

Numerous studies have shown that the fibronectin deposition into the extracellular matrix and the turnover of FN is a dynamic process. The steady state exists between assembly of ECM, its remodeling and degradation. FN fibrils provides the matrix with stability and rigidity [19].

The change in expression of fibronectin, organization, degradation and its concentration in body fluids has been reported in various disease conditions. The levels of fibronectin increase in plasma of patients with gastric cancer [25,26], colorectal cancer [27], breast cancer [28], bladder cancer [29], rheumatic disease [30], ischemic heart disease [31], collagen vascular diseases, diabetes mellitus [32]. Fibronectin detected in cerebrospinal fluid and serum may be a potential epilepsy biomarker [22].

FN concentration in plasma has low values in septic patients compared to healthy subjects and patients with different non-infectious pathologies and FN appears to act as a marker of sepsis [33].

The most common methods used for determination of fibronectin and its degradation products are immunohistochemistry [34,35] and enzyme-linked immunosorbent assays (ELISA) [22,25,29,31,32] respectively.

The fibronectin concentration in plasma was also determined by laser nephelometry [33,36] or immunoturbimetric method [30,37]. A competitive radioimmunoassay (CRIA) for quantitating determination of human plasma fibronectin levels has been described [38]. The presence of cellular FN was determined in the plasma using a time-resolved fluorescence immunoassay (TRFIA) [39,40].

The classic version of SPR technique was used for determination of fetal fibronectin [41]. The interaction of fibronectin and the cells was studied using SPRI technique. This technique makes it possible to monitor cellular modifications of FN in relation to the extracellular matrix forms in real-time [42].

The aim of this work was to develop SPRI method for the determination of fibronectin concentration in biological fluids. The biosensor on the basis of fibronectin interaction with specific antibody was constructed. Next, the analytical parameters of SPRI biosensor were optimized and the biosensor's ability to determine fibronectin in biological samples was tested.

2. Experimental materials and methods

2.1. Reagents

Fibronectin from human plasma (lyophilized powder) as standard, anti-fibronectin antibody produced in rabbit, collagen type IV, glycoprotein GPIIb/IIIa, human albumin, bovine serum albumin (BSA), cysteamine hydrochloride, *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodimide (EDC) (Sigma, Steinheim, Germany, http://www.sigmaaldrich.com/safc/facilities/steinheim-germany.html), N-hydroxysuccimide (NHS), (Aldrich, Munich,Germany,http://www.sigmaaldrich.com/germany.html), photopolimer ELPEMER SD 2054 and hydrophobic protective paint SD 2368UV SG-DG (Peters, Kempen, Germany, http://www.peters.de) were used, as well as absolute ethanol, acetic acid, hydrochloric acid, sodium hydroxide, sodium chloride, sodium carbonate, sodium acetate, (POCh, Gliwice, Poland, http://www.poch.com.pl/). HBS-ES buffer pH = 7.4 (0.01 M HEPES, 0.15 M sodium chloride, 0.005% Tween 20, 3 mM EDTA), Phosphate Buffered Saline (PBS) pH = 7.4, carbonate buffer pH = 8.5 (BIOMED, Lublin, Poland, http://www.biomed.lublin.pl) were used as received. Aqueous solutions were prepared with milliQ water (Simplicity® Millipore). Argon N 5.0 with a content Ar ≥ 99,999% was used (AIR LIQUIDE Polska Sp.z o.o., Poland). ELISA kit for fibronectin determination (Abcam, Cambridge, United Kingdom, http://www.abcam.com) was used.

2.2. Biological samples

All blood samples were taken from children with thermal injuries during the hospital admission to the L. Zamenhof Children's Clinical Hospital of the Medical University of Bialystok, Pediatric Surgery Department (Bialystok, Poland). Patients were aged 9 months to 14 years old. The blood samples were collected from the median cubital vein using EDTA as an anticoagulant. Also, the blood samples of the control group were taken from healthy people (age 18–35 years old), who are Honorary Blood Donors of the Regional Blood Donor Centre of Bialystok, Poland.

Two milliliters of blood was centrifuged ($1000 \times g$) for over 15 min and filtered 3-times for the separation of blood plasma from the cells. The blood plasma samples were frozen and stored at −70 °C until their further use. The samples were diluted 1000–5000 times with PBS buffer directly prior to measurement.

The study had the approval of the Ethics Committee of the Medical University in Bialystok and all patients gave informed consent.

2.3. Procedures

2.3.1. Chip preparation

Basis of the biosensor for fibronectin determination was prepared as described in the previous paper [37]. The glass chips were covered with gold (50 nm on a 1 nm thick chromium layer). Next, gold surface was covered with photopolymer and hydrophobic paint. An array of 9 × 12 free gold surfaces was made – Fig. 3. The nine different solutions can be simultaneously applied on chip. Twelve independent measurements can be made for each solution, so twelve individual SPRI signals for each sample can be obtained.

2.3.2. Antibody immobilization

The chip surface was rinsed with absolute ethanol and water, dried in a stream of argon and was immersed in 20 mM cysteamine ethanolic solutions for a minimum of 18 h. The chips with immobilized linker were rinsed and dried as described above. In order to immobilize antibody,50 μL of antibody solution (4 μg mL^{-1}, designated from the experiments for optimization of antibody concentration) was mixed with 250 μL of

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