BIOLOGIJA. 2004. Nr. 1. P. 33–35

Piezoelectric affinity biosensor for diagnosing bovine leukemia

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A piezoelectric affinity sensor based on immobilised bovine leukemia virus (BLV) envelope protein *gp*51 is described. A quartz crystal microbalance (QCM) has been used to register interaction of *gp*51 with specific antibodies from the blood serum of BLV-infected cattle. Protein *gp*51 was cross-linked with glutaraldehyde on the platinized surface of quartz crystals to prevent fast desorption. The behaviour of coating containing the *gp*51 in a pure glycine buffer pH 7.3 as well as in a glycine buffer pH 7.3 mixed with blood serum from BLV-infected and not infected cattle was studied. The possibility to exploit the QCM-based bioanalytical system for fast diagnosis of BLV is discussed.

Key words: QCM, immunosensor, leukemia, retrovirus

INTRODUCTION

Life depends on the interaction of proteins, because virtually many cellular processes are regulated by protein–protein interactions. It is the reason why detection and investigation of protein–protein interaction processes is a very important problem of biochemistry in the postgenomic era. Investigation of interactions among proteins is important in the fields of immunology, proteomics, drug-discovery and bioanalytical chemistry. Experimental methods available to detect protein–protein interactions vary in their level of resolution and according to [1] can be classified into four major categories. In the first level of observation, multiprotein complexes can be detected using methods such as immunoprecipitation or mass-spectral analysis. The second category comprises measurements at the cellular level, where an 'activity-bioassay' is used to observe the interaction. The third comprises an 'atomic observation' in which the protein interaction is detected using, for example, X-ray crystallography. These experiments can yield specific information on the atoms or residues involved in the interaction. Hovever, neither of the three methods unveil the kinetic details of protein–protein interaction. It is the reason why for detection of protein interactions in real time the most interesting is the fourth group of methods, which gives a possibility to detect a binding event directly in the real time without any additional labels. The protein–protein binding event can be directly converted into a measurable signal mainly by optical, for example, surface plasmon resonance (SPR), electrochemical or piezoelectric, like QCM, transducers. QCM has already found application as a tool for studying electrochemical processes like control of mass transport to/from the electrode [2] and development of selective chemical sensors for complex matrixes of medical, environmental and industrial interest [3]. An effective combination of immunochemical reagents and QCM in an analytical device could provide the basis for direct gravimetrical detection of a wide range of analytes with a high sensitivity and specificity. In this method, changes of mass or densities of an immobilised biolayer are used for transduction and do not need any auxiliary reaction. To work label-free is very attractive, especially for the performing real-time kinetic measurements in the real time. On the other hand it will be very useful in designing immunosensors for measurements in hardly transparent solutions such as like blood. Until now, several QCM-based affinity biosensors have been created, for example, a model biosensor based on biotinylated polypyrrole modi-

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fied QCM for a fast and reagentless determination of avidin concentration [4], and for detection of genetically modified organisms based on immobilised DNA primers [5].

The major indispensable condition in designing QCM based affinity sensors is that one of the able-to-bind reagents is immobilised and at least one must be found as analyte [6]. It means that in affinity sensors the biologically active compound must form a unified system with an oscillator. One of the simplest and still very popular immobilisation technologies in creating different kinds of biosensors is cross-linking by glutaraldehyde. If a biologically active compound is extremely sensitive to glutaraldehyde, a more delicate immobilisation method based on application of glutaraldehyde vapour can be applied [7].

The aim of the present work was to apply QCM modified by *gp*51 for detection of a binding event between *gp*51 and specific (anti-*gp*51) antibodies in the real time.

MATERIALS AND METHODS

Materials. Bovine leukemia virus envelope forming protein *gp*51 was purchased from Biok (Kursk, Russia). The blood serum of healthy and BLV-infected cattle was obtained from National Laboratory of Veterinary (Vilnius, Lithuania). Analytical grade chemicals and triply distilled water were used to prepare the solutions.

Apparatus. Microgravimetric measurements were performed by using 6-MHz fundamental frequency AT-cut quartz crystals from Intelemetrics Ltd. (Oxford, U. K.) with circular sputtered platinum electrodes (geometric area 0.636 cm^2) pressed to the contact wires in-between too silicon rubber O-rings, which were placed between the holder and the glass tube, forming the bottom of a cell with a working volume of *ca*. 2 ml. The quartz crystal was connected to a home-made oscillator. QCM measurements were carried out using a 43–64 precision frequency counter connected to a PC through the IEEEE 488 interface. The measured frequency (counted to an accuracy of ± 0.01 Hz) was transferred to the PC every 1.3 s. The obtained data were amended by the Sigma Plot 4 program.

Immobilisation of biolayer: (*i*). 5 μl of 5, 1, 0.1, 0.01 mg/ml *gp*51 solution was evenly distributed on the platinized quartz crystal surface cleaned with 96% ethanol, (*ii*) then the crystal was deposited over 25% solution of glutaraldehyde for 10 min, (*iii*) a *gp*51-modified crystal was deposited in QCM cell, and measurements of resonance frequency were started in 0.1 M glycine buffer, pH 7.3.

RESULTS AND DISCUSSION

Optimisation of immobilisation procedure. Protein *gp*51 was immobilised according to the experimental protocol. To inactivate all free carbonyl groups of glutaraldehyde, to remove all not cross-linked proteins, *gp*51 modified crystals were treated with 0.1 M glycine buffer, pH 7.3. Since QCM is an oscillating quartz crystal, its frequency response (Δf) is related to a change of mass (Δm) [8]. Δf was registered as a function of Δm (Δm was not calculated since the viscoelastic effects of the blood serum containing solutions were not estimated in the present study). Two main parameters were estimated during the optimisation procedure: 1) the rate of mass decrease of the *gp*51-modified quartz resonator as a function of Δf/Δt in the 2nd phase in which not covalently bound particles like ions and unbound proteins were released; it is dominating during 5–30 min after incubation of the *gp*51-modified quartz resonator in phosphate buffer, pH 7.3 (Fig. A); 2) an increase of quartz resonator mass after a 3–15 min of exposure to a buffer solution / (blood serum of BLV-infected cattle) = 5 : 1. It was found that an optimal coating could be formed if 5 μl of 0.1 mg/ml *gp*51 solution was evenly distributed on the surface of a platinized quartz resonator. To prevent non-specific sorption, 0.5 M KCl solution in phosphate buffer, pH 7.3, and 0.1 M glycine buffer, pH 7.3 were tested. 0.1 M glycine buffer, pH 7.3, was found to by most useful for preventing non-specific sorption of blood serum proteins on the surface of platinized quartz crystals as well as non-specific interaction of proteins with immobilised *gp*51.

Investigation of immobilised *gp*51 layer. Figure A shows the development of a gravimetric signal after filling the QCM with glycine buffer, pH 7.3. One can see that during the first 5 min the resonance frequency registered with QCM is decreasing very rapidly, and after that it is constantly increasing. The first process dominate for the first 5 min and is related to an increase of QCM mass during the solvatation and swelling processes. Then the destruction of the biopolymeric film starts to dominate and is evenly occurring during a period of at least 30 min (the duration of this test was 30 min). The rate of this process can be described by a linear regression $y = -2210 + 36.8*X$ with the correlation coefficient $r = 0.99$. This process can be explained as a decrease of mass and/or weakening of connections between the layers of *gp*51 and the platinized quartz resonator.

Application of QCM for studying the interaction of *gp*51 with anti-*gp*51 antibodies. Since the previously described mass decrease proceeded constantly over a sufficient period of time and no fluc-

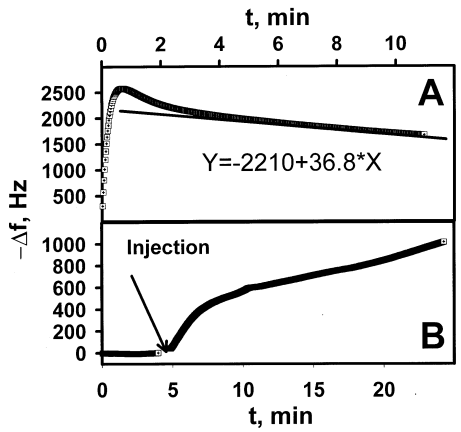

Figure. A. Frequency changes of *gp*51-modified quartz resonators in 0.1 M glycine buffer, pH 7.3. B. Frequency changes of *gp*51-modified quartz resonators in 0.1 M glycine buffer, pH 7.3, upon addition of blood serum of BLV-infected cattle (5:1)

tuations were detected, it seems reasonable to employ this system for the detection of *gp*51- and anti-*gp*51-binding events. As one can see from figure B that after addition of BLV-infected blood serum into a QCM cell the mass starts rapidly increasing continues and for 3–5 min. It means that the interaction between immobilised *gp*51 and high-affinity anti-*gp*51 antibodies is most effective during the first 3–5 min. It is in agreement with data on the interaction of a biotinylated polypyrrole modified quartz resonator with avidin, where the first interaction phase also lasts 2–5 min and depends on the concentration of avidin [4]. If the blood serum of BLV-free cattle was investigated, Δf changes were at least 15 times less than for BLV-infected cattle.

Conclusions and future trends. The results show that the presence of anti-*gp*51 in blood serum generates a significant Δf; it can be used as an analytical signal. These results allow us to conclude that QCM can be successfully applied for detection of anti-*gp*51 antibodies in blood serum. Real-time measurements in the future can be used for calculation of interaction constants between *gp*51 and anti-*gp*51 antibodies. Our attempts to regenerate the biosensor by high ionic strength or extreme pH solutions like 3 M KCl or 0.5 M glycine-HCl buffer, pH 2, were not effective enough or initiated the destruction of the biologically active layer. It means that in the future it is desirable to improve the procedure of biosensor regeneration.

ACKNOWLEDGEMENT

This work was financially supported in part by the Lithuanian State Science and Studies Foundation project number C 03047 and COST Program project number 853.

Received 4 April 2002
Accepted 12 November 2003

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PJEZOELEKTRINIS AFINAVIMO BIOJUTIKIS, SKIRTAS GALVIJØ LEUKOZEI NUSTATYTI

S a n t r a u k a

Ant platinos sluoksniu padengto kvarcinio osciliatoriaus kristalo buvo imobilizuotas galvijø leukozës viruso (GLV) baltymas *gp*51; tam buvo panaudoti glutaro aldehido garai. Mikrogravimetriniai galvijø leukozës virusu infekuotø ir sveikø galvijø kraujo serumø tyrimai buvo atliekami 0,1 M glicino buferiniame (pH 7,3) tirpale. Tokia terpë sumaþino nespecifinæ kitø baltymø sorbcijà ant *gp*51 modifikuotø kvarco kristalø. Parodyta tokiø pjezoelektriniø afinavimo biojutikliø taikymo galimybë, siekiant greitai nustatyti GLV.