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Aptamer-based biosensors for the detection of HIV-1 Tat protein

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Abstract

Two biosensors have been constructed using an RNA aptamer as biorecognition element. The aptamer, specific for HIV-1 Tat protein, has been immobilised on the gold surface of piezoelectric quartz crystals or surface plasmon resonance (SPR) chips to develop a quartz crystal microbalance (QCM)-based and an SPR-based biosensor, respectively. Both the biosensors were modified with the same immobilisation chemistry based on the binding of a biotinylated aptamer on a layer of streptavidin.

The binding between the immobilised aptamer and its specific protein has been evaluated with the two biosensors in terms of sensitivity, reproducibility and selectivity. A protein very similar to Tat, Rev protein, has been used as negative control.

The two biosensors both were very reproducible in the immobilisation and the binding steps. The selectivity was high in both cases.
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1. Introduction

The analytical power of biosensors is determined by the features of the molecular recognition element that is linked to the transducer. The recent understandings of nucleic acid functions and, specifically, the power of RNA as both informational and ligand binding molecule, have opened new scenarios in the development of new biosensors.

Moreover, the development of in vitro selection and amplification techniques [1,2] has allowed the discovery among random sequence populations of specific nucleic acid molecules (aptamers) that bind to the target molecule with high affinity and specificity. Aptamers have been selected to bind a wide range of different molecules such as organic dyes, amino acids, biological cofactors, antibiotics, peptides, proteins and whole cells [3]. Aptamers are suitable for applications based on molecular recognition as analytical, diagnostic and therapeutic tools. Recently, they have been used as biocomponents in order to develop biosensors (aptasensors) and allosteric ribozymes (aptazymes) [4] and their advantages over antibodies have been evidenced [5].

Development of aptasensors relies on the use of optical, acoustic and fluorescent methods to analyse in real-time the biological phenomena in solution or by immobilising the aptamer onto a solid support.

To explore the potential of RNA aptamers as small-molecule discriminating tools, the surface plasmon resonance (SPR) technology has been applied to study the selection of new aptamers against specific targets. In this regard, some studies have been published on S-adenosylhomocysteine (SAM) [6], the interaction TAR–Tat [7], tubulin [8], and monocyte chemoattractant protein-1 (MCP-1) [9].

A very interesting analytical study by using the same optical technique was used to detect the iron regulatory protein (IRP1), an indirect marker of oxidative stress, through the specific binding to an mRNA loop (iron responsive element, IRE) [10].

A second interesting technology used to generate aptasensors is the quartz crystal microbalance (QCM). This technique was used to study the interactions of HIV-1 TAR RNA with Tat protein, responsible for HIV-1 RNA virus infection [11]. A second system directly compared the performances obtained using a DNA aptamer and the

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antibody specific for human IgE chosen as target analyte [12]. Both receptors selectively detected 0.5 nM of IgE. The linear detection range was 10-fold higher when aptamers were used.

In this work, an RNA aptamer has been used as biorecognition element to develop aptasensors for the detection of HIV-1 Tat (Trans-Activator of Transcription) protein, using SPR and QCM transduction.

Tat is an HIV-1 RNA-binding protein that exhibits an inherent affinity against the TAR, Trans-Activating Response Element of the virus. Tat (Fig. 1A) is 101 amino acids long and it is encoded by two exons. It can be divided into 5 core domains among which the most important is the fourth one regulating its function and allowing its binding to its corresponding RNA element TAR. This region is positively charged and is rich in arginines [13,14].

A Tat-specific RNA aptamer has already been reported in literature [15,16]. This aptamer featured a similar structure to TAR and exhibited a 133-fold increased affinity for Tat, compared to TAR (Fig. 1B). The aptamer has been compared to an anti-Tat monoclonal antibody through a QCM-based biosensor [17].

In this work the analytical performances of an SPR- and a QCM-based biosensor realised using this aptamer as biorecognition element, have been examined and compared. The analytical performances of the two biosensors have been evaluated in terms of sensitivity, reproducibility and specificity.

2. Materials and methods

2.1. Reagents

N-hydroxysuccinimide (NHS), 1-Ethyl-3-(Dimethylaminopropyl) carbodiimide (EDAC), diethyl pyrocarbonate (DEPC), RnaseZAP™ and streptavidin were all purchased from Sigma Aldrich (Milan, Italy).

Recombinant Tat HIV-1 IIIB was purchased from ImmunoDiagnostics, (Woburn, MA, USA). Tat protein was stored as received (500 ppm in 0.2 M KCl, 5 mM glutathione) at −80 °C.

The biotinylated Tat-aptamer (IBA Göttingen, Germany) used in this study, has the following sequence: ACGAAGCUUGAUCCCGUUUGCCGGUCGAUCGCUUCGA [15]. All the solutions used in the experiments were prepared in DEPC-treated water, filtered (0.22 μm pore size filter (Nalgene, Milan, Italy)) and degassed daily prior to use. DEPC-treated H_2O was prepared adding 1 ml of DEPC to 1 l of MilliQ water and stirred overnight. The solution was then autoclaved at 1 atm for 20 min.

The buffer used for the experiments has the following composition: 0.01 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.15 M NaCl, 3 mM EDTA and 0.005% polyoxyethylene sorbitan monolaurate (Tween 20), pH 7.4. Bovine serum albumine (BSA) 0.1% was added to this buffer to prepare Tat protein solutions.

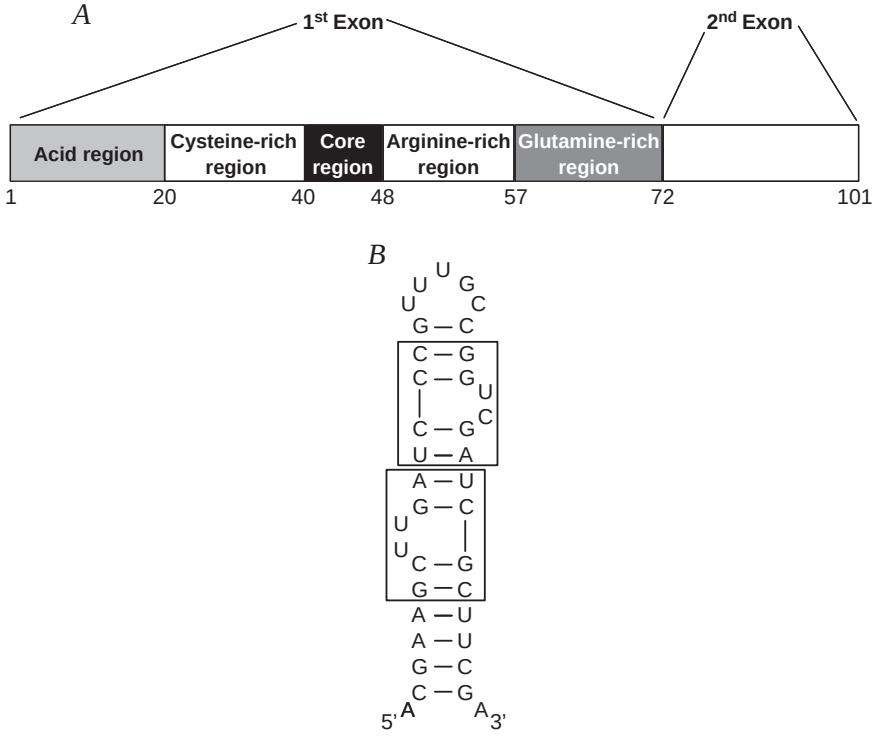

Fig. 1. (A) Domain classification of HIV-1 Tat protein with the functional domain involved in RNA binding. The first exon includes amino acids 1–72, while the second exon includes amino acids 73–101. (B) Secondary structure of the aptamer specific for Tat protein. The boxed sequence is the important region for Tat binding.

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