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Sensitive detection of interferon-gamma with engineered proteins and surface plasmon resonance biosensor

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Abstract

We present a novel surface plasmon resonance (SPR) biosensor for detection of human interferon gamma (hIFNγ), an important diagnostic marker for several infectious diseases. The biosensor employs engineered proteins derived from albumin binding domain (ABD) and selected with ribosome display to provide high affinity towards hIFNγ. We demonstrate that the biosensor provides rapid, sensitive, and cost-effective platform for the detection of hIFNγ and allows its detection at nanomolar levels both in buffer and diluted human plasma.

Keywords: Surface plasmon resonance; Biosensor; Scaffold; Engineered proteins; Interferon gamma

1. Introduction

Human interferon-gamma (hIFNγ) is an important inflammatory cytokine [1] and its levels provide diagnostic information on various infectious diseases, including AIDS and tuberculosis. Secreted cytokines, such as hIFNγ, are commonly detected using antibody-based sandwich immunoassays. These conventional strategies, such as ELISA, can detect very low concentrations of hIFNγ (~10 pg/mL), but they are time-consuming, require multiple steps, and involve enzymes and antibodies which are sometimes difficult to produce. As an alternative to natural antibodies [2], aptamers have been also used [3].

Protein engineering has become a powerful tool for generating proteins with desired properties for various applications, such as medicine and biotechnology (for review, see [4]). The engineered proteins stem from native proteins of small size, robust structure (scaffolds [5]) and natural recognition ability towards a specific analyte. Upon site-directed mutagenesis of several amino-acid residues, the binding site of the scaffold protein is

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transformed to acquire affinity towards analyte of choice while losing the affinity towards the original binding partner at the same time. A large library of possible mutations can be screened using cell-dependent display systems, or cell-free display, such as ribosome display, or non-display systems [4], in order to select mutants exhibiting strong affinity towards the target molecule. Due to their small size and rigid structure, the selected scaffold proteins can be then easily synthesized *de novo*, reducing the cost and complexity of the bioreceptor production.
In this work, we present an SPR biosensor for the detection of hIFNγ using novel high-affinity engineered proteins derived from the albumin binding domain of protein G [6] and a competition assay format. It is demonstrated that the developed biosensor is able to detect hIFNγ at concentrations down to nM both in buffer and diluted human plasma.

2. Materials and methods

2.1. Preparation of hIFNγ

The hIFNγ-AviTag was prepared using a synthetic gene coding for a 143 residue-long variant of hIFNγ with a C-terminal AviTag consensus sequence (GLNDIFEAQKIEWHE). This was produced from the pET-28b vector as a C-terminally biotinylated hIFN-γ-AviTag protein in *E.coli* BL21 (DE3)BirA cells, upon induction with 1 mM IPTG in the presence 50 μM d-biotin (Sigma-Aldrich). hIFN-γ-AviTag was extracted from inclusion bodies with 8 M urea in 50 mM Tris buffer (pH7.4) and purified by a combination of chromatographies on SP Sepharose and Phenyl Sepharose CL-4B (GE Healthcare) at pH 7.4. The eluted hIFNγ-AviTag protein was dialyzed into 50 mM ammonium acetate solution (pH 5.0).

2.2. Construction and production of ABD20-AviTag

The ABD20-AviTag proteins were constructed from the ABD20-TolA variant [6], in which the 306 residue-long TolA moiety was replaced by the AviTag sequence. The resulting 8.5 kD protein was produced in *E.coli* BL21 (DE3)BirA cells as above, extracted from inclusion bodies with 8 M urea, 150 mM NaCl in 50 mM Tris buffer (pH 8), purified by size exclusion chromatography and refolded by >100-fold dilution into the SPR running buffer.

2.3. SPR instrument:

In this work, we used a four-channel SPR system developed at the Institute of Photonics and Electronics AS CR, v.v.i., Prague, Czech Republic. The sensor is based on the attenuated total reflection method and wavelength spectroscopy of surface plasmons [7]. In this sensor, white light is used to excite surface plasmons on a thin gold film in four areas (sensing channels) which are interfaced with four separate chambers of a four-channel flow-cell.

2.4. Immobilization of hIFNγ-AviTag:

The hIFNγ-AviTag was immobilized to the gold sensor surface using covalent attachment to the self-assembled ω carboxyalkylthiol-self-assembled monolayer (SAM). The procedure of SAM formation is described in detail in Ref [7]. After the activation of carboxylic groups, the hIFNγ was flowed in the concentration of 5 μg/mL in SA buffer (10 mM sodium acetate, pH 5 at 25°C) until the saturation of the surface occurred. After short injection of buffer, the 500 μg/mL solution of BSA was introduced. The remaining carboxylic groups were deactivated with 5-minute injection of 1M ethanolamine–hydrochloride (pH 8.5).

2.5. Affinity analysis and competition assay:

To characterize the binding of biotinylated ABD20-AviTag [6] to the immobilized hIFNγ-AviTag, several different concentrations were injected to both measuring and reference channels for 10 minutes. Then, the running buffer was injected again and the dissociation phase of the interaction was monitored for another 10 minutes. The obtained kinetic curves were fitted using the 1:1 Langmuir model in BiaEvaluation software.

The detection of hIFNγ-AviTag was performed in running buffer RB (10 mM HEPES, 150 mM NaCl, 50 µM EDTA, 0.005% Surfactant P20, pH 7.4, 25°C) at a temperature of 25°C and a flow rate of 20 µl/min. The solutions of ABD20-AviTag variant at 100 nM concentration was spiked with hIFNγ-AviTag at concentrations 0 – 200 nM and injected into the measuring channels. After 10 minutes, the sample was replaced with running buffer and the dissociation phase was monitored. The absolute sensor response after 3 minutes of dissociation was used as the sensor response to the given concentration. Each concentration was measured using at least 3 different SPR chips.

3. Experimental

In this work, we used an ABD20 variant as binder of hIFNγ. An ABD20-AviTag protein of small size (8.5 kDa) was constructed, which had a C-terminal AviTag consensus sequence required for *in vivo* biotinylation and an N-terminal Trp leader sequence enhancing production and stability of the ABD scaffold. We investigated the interaction between the ABD20-AviTag protein in solution and hIFNγ-AviTag immobilized on the surface of the SPR sensor and found the affinity constant to be 1.1 ± 0.5 nM. This value is very close to K_d of 2.7 ± 0.3 nM of the ABD20-TolA protein carrying a longer C-terminal alpha-helical TolA moiety (306 residues), which was studied in our previous work under the same experimental conditions [6].

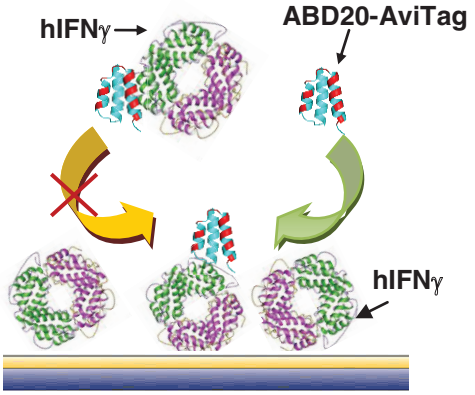

Figure 1: Scheme of the competition assay for detection of hIFNγ. The free hIFNγ captures the ABD20-AviTag and prevents its binding to hIFNγ immobilized on the sensor surface.

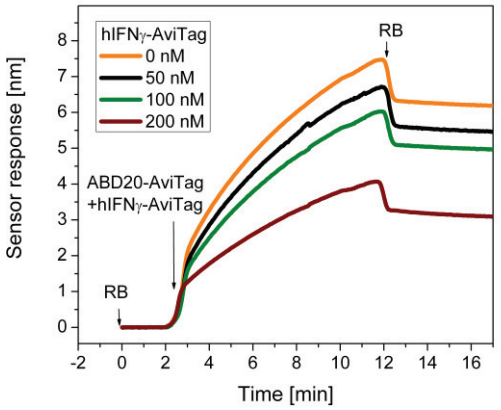

Figure 2: Sensor response to samples with different concentrations of hIFNγ and with the addition of 100 nM ABD20-AviTag. Higher concentration of hIFNγ resulted in lower sensor response. Arrows indicate injection of solutions

The ABD20-AviTag was used in a competition assay, in which ABD20-AviTag at a fixed concentration (100 nM) was spiked with various concentrations of hIFNγ-AviTag and flowed over the SPR chip surface with covalently immobilized hIFNγ (Figure 1). The hIFNγ present in the solution captures the free ABD20-AviTag and causes a decrease in sensor response compared to the channel with pure ABD20-AviTag. The sensor response to hIFNγ in buffer is shown in Figure 2. As follows from Fig.2, the sensor response to samples containing hIFNγ is lower than that obtained for the (blank) sample exposed to ABD20-AviTag in running buffer with no hIFNγ present and the decrease is proportional to the concentration of hIFNγ.

Figure 3 displays the absolute sensor response registered 3 minutes after the end of the sample injection normalized to the sensor response of the blank sample for different concentrations of hIFNγ. The calibration curve is shown in red and was obtained by the least square fitting. The limit of detection (LOD), which is defined as the analyte concentration that results in the sensor response equal to 3 times the standard deviation of noise, was determined to be below 1 nM concentration. This LOD is lower than the LODs achieved using biosensors and antibodies or aptamers as receptors [2, 3].

Figure 4 demonstrates the sensor response to 22% human plasma in running buffer, which was spiked with 100 nM ABD-AviTag and various concentrations of hIFNγ. As can be observed in Figure 4, shortly after the injection, the sensor response steeply increases, which is mainly due to a large refractive index change. At the end of the injection, the sensor response decreases again, but stays above the initial baseline, suggesting that there is significant

adsorption of plasma proteins to the surface of the sensor. The sensor response was registered 3 minutes after the end of the sample injection (inset of Figure 4). Despite the non-specific adsorption of plasma proteins, differences in absolute sensor response can be observed, which correlated well with the concentrations of hIFNγ. Even the lowest used concentration (10 nM) produced sensor response which was clearly different from the blank sample with no hIFNγ added.

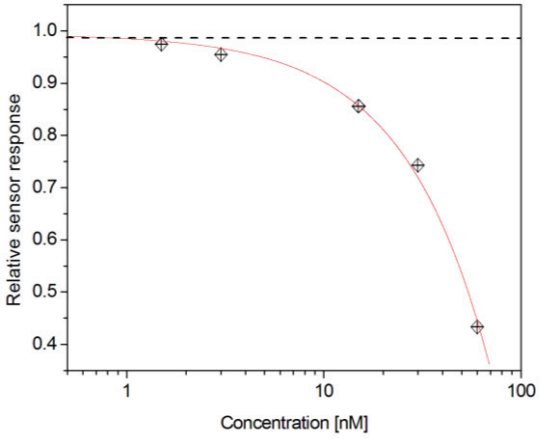

Figure 3: Calibration curve for IFNγ. Dashed line indicates the level of baseline noise, which determines the limit of detection.

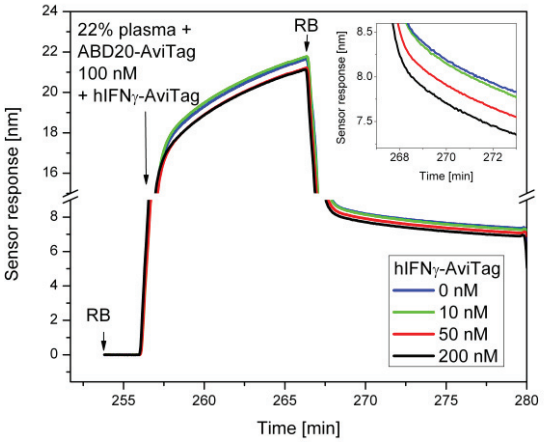

Figure 4: Temporal sensor response to hIFNγ in 22% plasma. Inset shows details of the dissociation phase.

4. Conclusions

We have developed a new biosensor based on surface plasmon resonance and scaffold of albumin binding domain (ABD) modified to bind human interferon gamma (hIFNγ). Using the competition detection scheme, the biosensor was demonstrated to detect hIFNγ at concentrations down to 1 nM in buffer and down to 10 nM in 22% plasma.

Acknowledgement

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