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Aptamer-Based QCM-Sensor for Rapid Detection of PRRS Virus †

Chakpetch Kuitio [1,2,3], Kiattawee Choowongkomon [1] and Peter A. Lieberzeit [3,*]

[1] Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; bacillus07@hotmail.com (C.K.); kiattawee.c@ku.th (K.C.)
[2] Genetic Engineering, Faculty of Graduate School, Kasetsart University, Bangkok 10900, Thailand
[3] Department of Physical Chemistry, Faculty for Chemistry, University of Vienna, Vienna 1090, Austria
* Correspondence: Peter.Lieberzeit@univie.ac.at; Tel.: +43-1-427-752-341
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Abstract: Porcine reproductive and respiratory syndrome (PRRS) is caused by an RNA virus and has substantial economic impact on swine industry. Screening pigs for infection is the best way to prevent spreading the disease. For that purpose, we developed biosensors based on aptamers, i.e., short ss-DNA that can bind to porcine reproductive and respiratory syndrome virus (PRRSV). The present study, demonstrates selectivity and sensitivity of PRRSV aptamer (7R) by the means of quartz crystal microbalance (QCM) measurements. The respective results show that 7R aptamer indeed binds to samples containing around 10^{10} PRRSV virus particles, but not to Pseudorabies virus (PRV) and Classical swine fever virus (CSFV).

Keywords: aptamer; quartz crystal microbalance (QCM), porcine reproductive and respiratory syndrome (PRRS)

1. Introduction

Porcine reproductive and respiratory syndrome is an important disease in swine industry. The disease has become endemic in many countries throughout the world following an epidemic phase. It is caused by infection with the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), a small single-stranded, non-segmented RNA virus [1]. The virus is enveloped, spherical and ranges in size from 45 to 80 nm in diameter. PRRSV is differentiated into two genetically distinct genotypes: Type 1, or European genotype, predominant in Europe, and Type 2, or North American genotype, that is mostly isolated on the American continent (North and South), as well as in Asia. Disease signs are similar to many other viral or bacterial swine diseases and can be blurred by co-infection with other pathogens. Therefore, diagnosis of PRRS currently is based on laboratory tests. PRRS virus can be identified by PCR, RT-PCR, ELISA and immunohistochemistry [1].

However some of these methods require long time for obtaining results. Hence it is important to find screening techniques for it. Aptamers are a good alternative to antibodies when developing sensors for PRRSV. They comprise of small oligonucleotides, such as single-strand deoxyribonucleic acid (ssDNA), ribonucleic acid (RNA), or small peptides. Aptamers usually bind their target species with high selectivity and affinity. They have been designed for a variety of analytes ranging from small molecules and ions, over drugs and toxins, to large molecules, such as peptides, proteins, and even whole cells, bacteria, and viruses. The advantages of aptamers include high affinity and specificity, production by chemical synthesis, long-term storability, and ease of modification. They also provide an economic and superior alternative to antibodies. In addition, aptamers show minimal or no immune response in vivo [2]. In this study we used the 7R aptamer, which specifically interacts with PRRSV. It was selected by capillary electrophoresis and

colorimetric methods during previous experiments and coated onto quartz crystal microbalances (QCM), i.e., high-resolution mass sensitive devices. Among others, this is based on the fact that QCM is the technique of choice for detecting interactions between nucleotides and their targets [3]. Therefore QCM can used to characterize selectivity and sensitivity of 7R aptamer.

2. Materials and Methods

2.1. DNA Aptamer

7R aptamer consisted of 40 nucleobases modified at the 5′ end with a thiol group (-SH). Its sequence is 5′-(ThiC6)GCTGTACCGTCTGCTAGGACACCATAACTTCTAGCAAACGC. Both selective and non-specific aptamer were synthesized by SIGMA-ALDRICH.

2.2. Virus Purification

MARC-145 cells infected by PRRSV were lysed by three consecutive freeze-thaw cycles following by collecting the supernatant of cell lysate by centrifugation. The virus was purified by ultra-centrifuge at 110,000 g for 24 h in 40–60% sucrose gradient.

2.3. Quartz Crystal Microbalance (QCM)

10 MHz dual electrode QCMs were coated with 7R aptamer diluted with cysteine (1:1000) on working site of gold-electrode. The reference side contained non-specific 40-mer diluted with cysteine at the same concentration to ensure similar chemical compositions of the two surfaces. After drop-coating, solutions were incubated at 4 °C overnight and washed with distilled water one hour at room temperature. During QCM measurements we used 1 mM TNE (Tris/NaCl/EDTA) buffer at pH 7.4 for obtaining the baseline signal. QCM were exposed to PRRSV test solutions containing around 10^{10}–10^{11} particles/mL and washed the target come out form working site by mixed 10% aqueous solution of acetic acid and deionized water. Selectivity tests with CSFV and PRV solutions were based on the same virus concentrations.

3. Result and Discussion

3.1. Sequence Analysis

7R specific aptamer of PRRSV was developed during previous studies and identified/quantified by capillary electrophoresis and UV-Vis detection. Figure 1 shows its secondary structure (calculated using the tool of the University of Rochester at https://rna.urmc.rochester.edu). As can be seen, the 7R aptamer comprises of a 40-mer of nucleotide and reveals one loop in its secondary structure. This loop is important for interaction with a virus particle, because it facilitates formation of electrostatic interactions or hydrogen bonds between the aptamer and its target, as well as stacking interactions between aromatic compounds and the nucleobases.

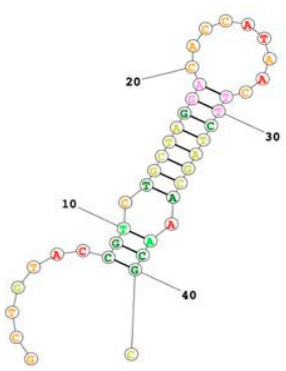

Figure 1. The secondary structure of 7R aptamer analysis by https://rna.urmc.rochester.edu.

3.2. 7R Aptamer as Sensitive Layer on QCM

During these measurements, PRRSV was diluted with 1 mM TNE buffer for sensitivity test. Figure 2 shows the QCM responses for solutions containing PRRSV 1.87 * 10^{10} particles/mL. The corresponding frequency shift from baseline on the working electrode is Δf = 150 Hz. Flushing the system with 10% acetic acid and water removes the virus particles: the frequency increases to baseline revealing fully reversibility. The frequency shift increases when adding higher concentrations corresponding to mass loading. Frequency shifts on the reference side reach only 20–25% of those values. So the QCM results clearly indicate that 7R aptamer can bind PRRSV. Furthermore, virus particles can be removed by acetic acid solution, because the conformation of the DNA aptamer changes and thus releases virus into the solution.

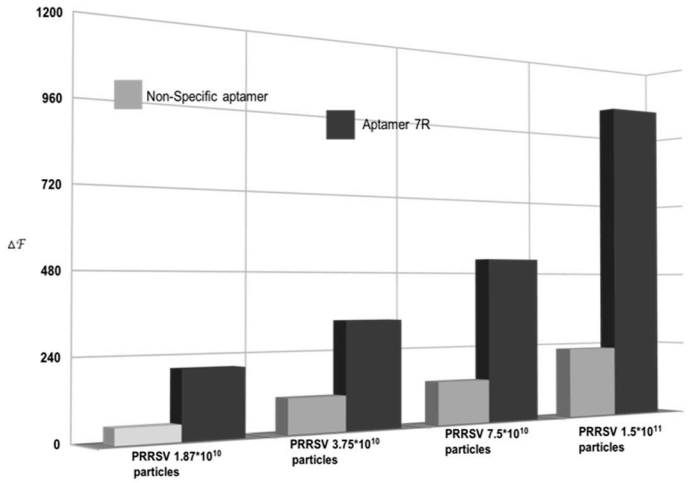

Figure 2. QCM frequency shifts PRRSV 1.87 * 10^{10} particles/Ml–1.5 * 10^{11} particles/mL.

3.3. Selectivity of 7R Aptamer

As can be seen from Figure 3, CSFV and PRV at concentrations of around 1.5 * 10^{11} particles/mL lead to much lower frequency responses than PRRSV: they are at −95 Hz for CSFV and −110 Hz for PRV, respectively. In contrast to this, PRRSV– the target of the 7R aptamer—results in frequency shift by-1320 Hz. Therefore, selectivity factors are obviously at least six, which strongly supports the claim that the 7R aptamer is suitable for detecting the PRRSV in situ on the surface of quartz crystal microbalances (QCM).

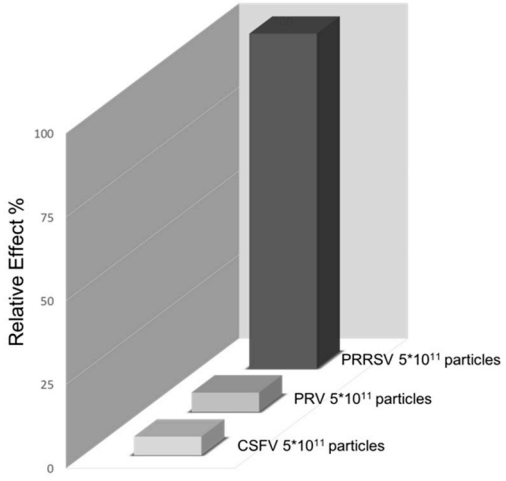

Figure 3. Selectivity of 7R-aptamer immobilized on QCM against CSFV, PRV, PRRSV, respectively, at 1.87 * 10^{10} particles/mL—1.5 * 10^{11} particles/mL. The PRRSV signal is used as the reference standard.

4. Conclusions

Although concentrations are on the high side, all results indicate successful implementation of 7R aptamer/QCM sensors. The resulting systems lead to appreciable selectivity and acceptable sensitivity, although especially the latter needs further improvement. The main recognition behavior is based aptamer-virus binding which mimics antibody/antigen interactions. The results show that QCMs are in principle suitable for screening aptamers and for testing their binding properties on the way to develop biosensors toward a specified target analyte. In further steps, this may lead to reversible (or quasi-reversible) biomimicking sensors for that target analyte.

Author Contributions: C.K. carried out the experiments and prepared the first version of the present manuscript. K.C. developed the aptamer concept and the corresponding experimental strategy and edited the respective parts in the manuscript. P.A.L. designed QCM experiments and prepared the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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