Detection of cancer marker ebna-1 by aptamer based biosensors

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The Epstein-Barr virus (EBV) is a human herpes virus that infects the majority of World´s adult population. Following primary infection, EBV immortalized a portion of hosts B-lymphocytes and establishes a latent infection that persists in the patient for life. EVB is the causative agent of infectious mononucleosis and has been associated with several malignancies including Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease, and lymphoproliferative disorders in immunodeficient individuals. All EVB associated disorders shows a distinct viral gene expression patter, but in all of them the nuclear antigen 1 (EBNA-1) is constitutively expressed [1].

EBNA-1 is a DNA binding protein located in the nucleus that is necessary for the maintenance and replication of the viral genome. In EVB associated tumors and latently infected B-lymphocytes, viral genome persist as a multicopy episome. For viral genome maintenance and replication, EBNA-1 forms homodimers that recognize specific sequences in the EVB genome. In addition to its role in viral episome maintenance, it was suggested that EBNA-1 can contribute to the oncogenic process by up-regulating the apoptosis suppressor protein in EVB-associated B-lymphomas [2]. Furthermore, it was reported the direct binding of EBNA-1 to cellular promoters and the correlation between EBNA-1 bound promoters and changes in gene expression [3].

In the present work, we develop an aptamer based system to detect EBNA-1 using Quartz Crystal Microbalance (QCM). The EBNA-1 binding aptamer was obtained by annealing 30-mer consensus oligonucleotides, and 16 T bases and a thiol group were added at the 5´end to facilitate its immobilization on the gold electrode. QCM experiments were performed using a Q-Sense E4 unit (Q-Sense AB, Sweden) and the shifts in frequency (Δf) was monitored online.

Scheme 1A show the label free detection of EBNA-1. In order to get a calibration curve, different standard solutions of EBNA-1, ranging from 20 nM to 0.5 nM, were applied to the biosensors. As shown in Fig. 1A, there is a linear relationship between the shift in frequency and the concentration of EBNA-1 in the range of 0.5 and 10 nM, with a correlation coefficient of 0.991 and a detection limit of 0.5 nM.

To detect EBNA-1 concentrations below 0.5 nM, we used a signal amplification system based on the alkaline phosphatase (AP) catalyzed oxidative hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate [4], showed in scheme 1B. The oxidative hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate yields the accumulation of the insoluble indigo derivative on the surface of the electrode that provides an amplification route for the detection of EBNA-1. The precipitate generates an increase of mass on the QCM sensor, resulting in a decrease in the resonance frequency of the electrode.

As shows the in Fig.1B, the frequency shift shows a linear relationship with the logarithm of the EBNA-1 concentration over a range of 5-100 pM, with a correlation coefficient of 0.994 and a detection limit of 5 pM. This means that using the signal amplification system the sensitivity was improved 100 times in comparison with that of the label free detection of EBNA-1 (0.5 nM).

References

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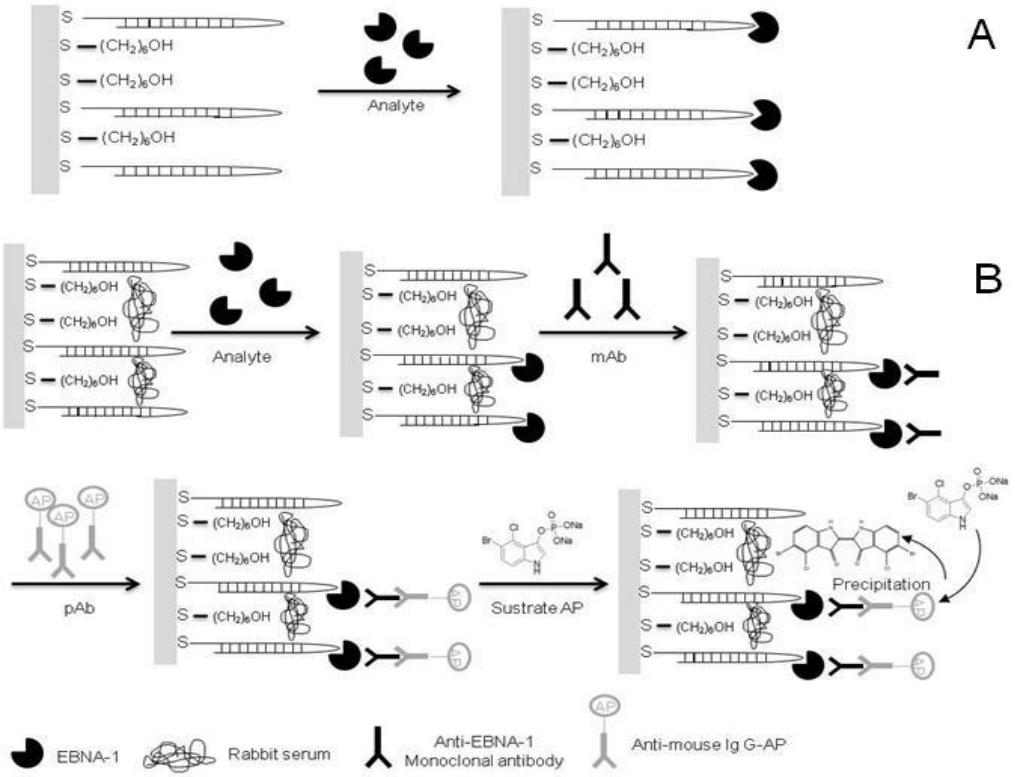
[4] Pavlov V., Willner I., Dishon A., Kotler M.,Biosensors and Bioelectronics, **20** (2004) 1011-1021.

Figures



***Scheme 1**. Schematic diagram of A) the label free detection of EBNA-1 and B) detection of EBNA-1 by the 5-bromo-4-chloro-3-indolyl phosphate based amplification system.*

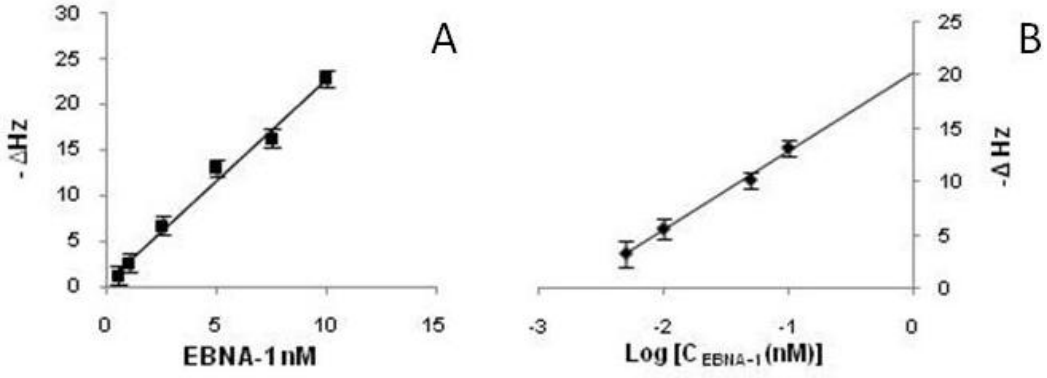


***Figure 1**. Calibration curve A) linear relationship between EBNA-1 concentration and frequency shift in the range of 0.5-10 nM. Calibration curve B) linear relationship between the amplified frequency shift and the logarithm of EBNA-1 concentrations. The error bars represent the standard deviation of three measurements.*