

Electrochemical Aptasensors Based on Carbon Nanotubes and Conducting Polymers

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DNA and RNA aptamers are single stranded oligonucleotides with high affinity to proteins or other ligands, comparable to those of antibodies. The aptamers are selected in vitro by the SELEX method [1]. In solution, the aptamers maintain a unique configuration that contains a specific binding site to the ligand. Aptamers can be easily modified by biotin, SH or amino- groups, leading to a variety of immobilization strategies on solid supports. Using simple molecular engineering based on DNA hybridization it is possible to create aptamer dimers with two binding sites like that in antibodies [2]. These aptamer dimers (aptabodies) are characterized by improved sensitivity to the analyte, for example to thrombin. We have shown that typical guanine quadruplexes that form a binding site for thrombin are stable in aptamer dimers [3]. Currently there is increased interest in the development of aptamer based biosensors for the detection of proteins and other molecules using various methods of detection, such as optical, acoustical and electrochemical [4,5]. These biosensors could be used in fast and low cost medical diagnostics. The sensitivity of detection depends not only on the selectivity of the binding site, but also on the supporting part of the aptamer that serves for immobilization onto a solid support. Using multiwalled carbon nanotubes (MWCNTs) as an immobilization matrix we developed a high sensitive biosensor for the detection of human thrombin [2] and cellular prions (PrP^C) [6] in biological liquids. In this work we analyzed in detail the properties of DNA aptasensors sensitive to thrombin, immobilized either on a gold support covered by neutravidin or on a surface of MWCNTs. We have shown that the immobilization of aptamers and aptamer dimers at MWCNTs improved the sensitivity of the sensor for thrombin and allowed detection in a complex matrix such as blood plasma. Using single molecule force spectroscopy (SMFS) we studied in detail the forces between enhanced single stranded aptamers (BFA) and aptamer dimers (BFF) immobilized on an AFM tip and the thrombin immobilized on a mica surface. By varying the pulling velocity in force distance cycles the formed thrombin – aptamer complexes were ruptured at different force loadings allowing determination of the energy landscape. It turned out that the BFA aptamer shows a higher binding force at the investigated loading rates and a significant lower dissociation rate constant, k_{off} , compared to BFF. The lower binding strength of BFF in comparison with those of BFA may be due to certain steric hindrance between two G-quadruplexes of this aptamer dimer. However, the potential of the aptabody BFF to form a more stable double bound complex could clearly be shown.

Using thickness shear mode acoustic method (TSM) we analyzed in detail the interaction of thrombin with DNA aptamers of various configurations immobilized at the neutravidin layer chemisorbed on TSM quartz crystal transducer and showed enhanced sensitivity for the detection of thrombin by aptamer heterodimer (BFH) [8]. The obtained results agree well with those of SMFS studies.

By means of electrochemical quartz crystal microbalance method (EQCM) we performed comparative analysis of the sensitivity of DNA aptamers and antibodies specific to human cellular prions (PrP^{C}) immobilised on a surface of MWCNTs (Fig. 1). We found that the detection limit (LOD) for both aptamers (50 pM) and antibodies (20 pM) was rather low indicating high, but comparable sensitivity (Fig. 2). The LOD was also much better in comparison with immobilisation of aptamers on a surface of conducting co-polymers and using QCM and surface plasmon resonance (SPR) as detecting methods. Higher stability of aptamers in comparison with antibodies and possibility to easy regenerate aptasensors make them rather promising candidates for practical applications.

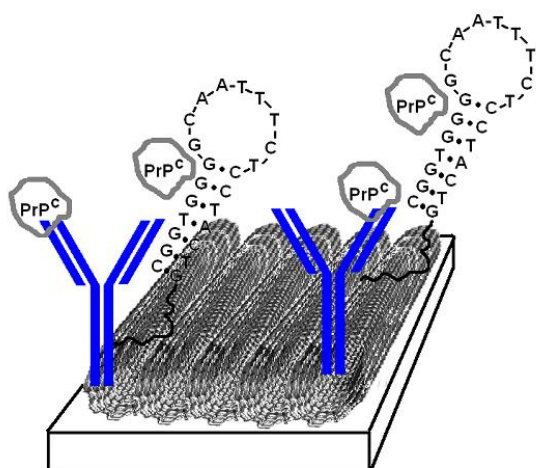


Fig. 1. Schematic representation of the sensor surface composed of MWCNTs and immobilised DNA aptamers and antibodies with bounded PrP^{C} .

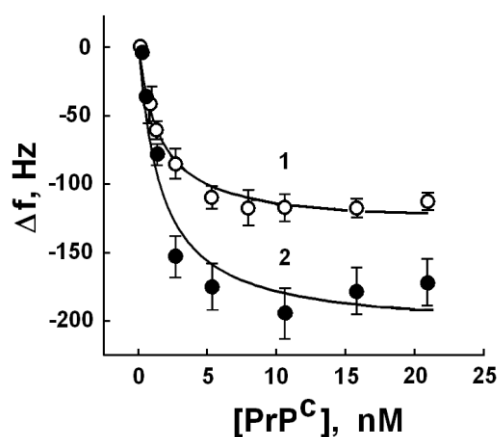


Fig. 2. The plot of the frequency changes as a function of PrP^{C} concentration for biosensor based on: 1 – single-stranded aptamer and 2 – BAR 223 antibody immobilized on the surface of MWCNTs. The points are experimental results and the lines are fit according to Langmuir isotherm (error bars: SD, $N = 3$) [7].

Acknowledgements

Financial support of Agency for Promotion Research and Development under the projects No. LPP-0341-09 and SK-FR-0025-09, Slovak Academy of Sciences under the project mnt-era.net (proposal No. 2009-50), VEGA 1/0794/10 and COST TD1003 is gratefully acknowledged.

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