DNA aptamers are single stranded oligonucleotides (<100 bases) with high affinity to proteins or other ligands, comparable to those of antibodies. The aptamers are selected in vitro by the SELEX method [1]. Once the sequence is selected, aptamers can be multiplied with high precision and purity. In solution, the selected sequence maintains an unique 3D configuration that contains 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. Thus, aptamers can serve as simple and highly sensitive artificial receptors. Currently there is increased interest in development of aptamer based biosensors for detection proteins and other molecules using various method of detection, such us optical, acoustical and electrochemical [2,3]. These biosensors could be used in medical diagnostics as a fast and low cost method of detection the dissease indicators, such are increased concentration of thrombin or prions. The sensitivity of detection depends not only on the selectivity of binding site, but also on supporting part of the aptamer that serve for immobilisation onto a solid support. Aptamers are rather flexible and using simple molecular engineering it is possible to increase their sensitivity. This has been demonstrated simultaneously by Hasegawa et al. [4] that used thymine linker for connection of two DNA aptamers sensitive to fibrinogen and heparin-binding exosites of thrombin and by us for thrombin aptamer dimers (so called aptabodies) that were prepared by hybridization of aptamer supporting parts [5,6]. Using multiwalled carbon nanotubes (MWCNT) as an immobilization matrix we developed high sensitive biosensor for detection human thrombin [5] and cellular prions (PrP C) [7] in biological liquids.

In this work we extended our study to comparative analysis of efficiency of DNA aptamers and antibodies for detection human cellular prions using electrochemical quartz crystal microbalance (EQCM), quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) methods. We also analyzed possibility of amplification the detection using gold nanoparticles conjugated to aptamers or antibodies. The DNA aptamer specific for PrP C was designed according to Takemura et al. [8] and purchased from Thermo Fisher Scientific (Germany). This aptamer was extended by dT 15 spacer (5'-CGG TGG GGC AAT TTC TCC TAC TGT dT 15-3') and has been modified at 3' end either by SH group or biotin. Gold nanoparticles (diameter 10 nm, Sigma Aldrich, USA) were used for modification of aptamers or antibodies. Monoclonal antibodies BAR 233 and PRI 308 which recognizes the PrP C sequence within amino acids 141-152 and 106-126, respectively, were from Spibio (Montigny France), AG4 and AH6 antibodies recommended for detection of N-terminal amino acid residues 31-51 and C-terminal amino acid residues 90-230, respectively of PrP C were from TSE Resource (UK). The receptors
Contribution (Oral)

(apartmers or antibodies) were immobilized either on MWCNT, polyamidoamine dendrimers of four generation (G4) conjugated with protein A, or on a surface of conducting co-polymer.

The highest sensitivity of detection of PrP\(^C\) indicated by limit of detection (LOD) has been obtained by EQCM method and using aptamers or BAR 223 antibodies immobilized on MWCNT (LOD 50 and 20 pM, respectively). Less sensitivity was obtained when antibodies were immobilised on a surface of QCM transducer modified by G4 dendrimers conjugated with protein A. In this case the detection considerably depends on the type of antibody used as it is demonstrated on (Fig. 1) where the plot of the frequency changes as a function of PrP\(^C\) is shown. Best LOD has been obtained for PRI 308 (0.8 nM), however amplification of the QCM detection by means of DNA aptamers (Fig. 2) or antibodies AH6 conjugated with gold nanoparticles improved LOD substantially (110 pM). The sensitivity of detection PrP\(^C\) by QCM using antibodies and SPR using DNA aptamers was comparable. However, the advantage of aptamers-based SPR detection, consisted in possibility of sensor regeneration by means of washing the surface with 0.1 M NaOH.

**Fig. 1.** The plot of the changes of the resonant frequency as a function of PrP\(^C\) concentration for QCM biosensors based on various antibodies against PrP\(^C\).

**Fig. 2.** The kinetics of the frequency changes following addition of PrP\(^C\) and amplification of the sensor response by the injection DNA aptamers modified by gold nanoparticles (Apta-gold). PBS is phosphate buffer used for removing non specifically adsorbed molecules.

Acknowledgements

Financial support of Agency for Promotion Research and Development under the projects No. APVV-0362-07 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.

References