INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Ankana Kakoti
Roll Number	:	10610610
Programme of Study	:	Ph.D.

Thesis Title: DEVELOPMENT AND CHARACTERIZATION OF DNA APTAMER AND MICROFLUIDIC PAPER BASED PLATFORM FOR DETECTION OF HEART TYPE FATTY ACID BINDING PROTEIN

Name of Thesis Supervisor(s)	:	Prof. Pranab Goswami
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SHORT ABSTRACT

The current investigation is centered on the development of specific aptamers against human heart type fatty acid binding protein (FABP3), a novel early marker for detection of acute myocardial infarction (AMI). It also encompasses the detection of FABP3 using the developed aptamers on a specially designed paper based microfluidic device (μPAD). For generating specific aptamers for FABP3, recombinant FABP3 was selected as the target while recombinant FABP1, FABP4 and FABP7 were chosen as controls. The coding sequences of all the four proteins were separately sub cloned into the pGEX-4T2 expression vector and transformed into BL21 (DE3) cells. Recombinant FABP3, FABP1 and FABP4 expression was induced at an IPTG concentration of 100 μM for 12 hrs at 37 ^{o}C, while expression of FABP7 was accomplished at an IPTG concentration of 50 μM for 12 hrs at 30 ^{o}C. The recombinant proteins were purified using glutathione agarose affinity chromatography and confirmed by SDS-PAGE and Western blots. All the recombinant proteins retained their correct secondary structures suggested by their CD spectra which were dominated by β-sheets. Systematic Evolution of Ligands by Exponential Enrichment (SELEX) was employed to generate aptamers against FABP3. A total of 20 SELEX cycles were performed, out of which 8 were counter SELEX cycles incorporating the control proteins. The enriched pool was cloned into TA vectors and the positive clones were selected on the basis of blue-white screening and restriction enzyme digestion. Sequencing and alignment studies of 50 positive clones revealed seven sequences were enriched at levels more than the others. EMSA and CD studies in the presence of FABP3 and the control proteins were conducted to study the specificity of the enriched sequences. Out of the seven aptamers, two aptamers (N13 and N53) were found to bind FABP3 specifically with dissociation constants (K_d) 0.0743 ± 0.0142 μM and 0.3337 ± 0.1485 μM, respectively. Both the aptamers shared a stem loop structure as predicted by Mfold which was confirmed through CD studies. Aptamer stability studies were performed to evaluate its behavior at different pH, temperature and ionic strength. Both the aptamers were stable at high salt concentrations in the settings of the binding buffer. Additionally at neutral and slightly alkaline pH, the aptamers were stable, however at acidic and highly alkaline conditions, the CD spectra of the

aptamers deviated from the normal. Both the aptamers were thermally stable with melting temperatures of 60.7 ± 0.9 ^{o}C and 53 ± 8.0 ^{o}C for N13 and N53, respectively. Furthermore, singular value decomposition (SVD) analysis of 3D melting curves indicated that both the aptamers exist in a simple two state form without the formation of higher ordered structures. The evolved aptamers though showed comparable specificity towards the target, a significant sequence variation between them and their different binding affinity to the target prompted to explore the distribution pattern of diverse chemical forces involved in these interactions to corroborate the bindings. Considering the large sizes of the aptamers, limited proteolysis of the aptamer-protein complex was performed to map the amino acids involved in binding, which was then used to screen docked structures. The N13 aptamer led interaction with stronger affinity, involving more salt bridges (three) and fewer hydrogen bonds (two), whereas N53 had less number of salt bridges (two) with higher number of hydrogen (eight) and hydrophobic interactions. It is suggested that the greater footprint of N53 incited synergistic conformational changes in N53 and FABP3 leading to decrease in binding affinity during the recognition. To utilize the aptamers so developed as a bio recognition element on a µPAD for FABP3 detection, we designed a microfluidic platform using a modified negative photolithography technique. The modified technique allowed the partial polymerization of photoresist on the rear of the device, which acts a leak proof layer. The presence of the partial photoresist layer was confirmed through AFM, FESEM and normal imaging under a microscope. This modification reduces the cost of laminating both sides of the device thereby reducing the overall device cost to $ 0.048 (as on Dec. 2014). The partial layer of photoresist on the device channel limits sample volume to 7 ± 0.2 µl as compared to devices without the partial photoresist layer which requires a larger sample volume of 10 ± 0.1 µl. The partially laminated device was then coupled with an externally fabricated test zone that allowed controlled loading of reagents as well as modification steps for incorporating reagents covalently without any spillage to the other zones of the device. In the coupled device the wicking speed was reduced to 1.8 ± 0.9 mm/min compared to the completely laminated device with an inbuilt test zone (3.3 ± 1.2 mm/min), thus extending the reaction time between the analyte and the reagents in the test zone. The efficacy of the prepared device was studied with colorimetric assays for the non-specific detection of protein by tetrabromophenol blue, acid/base with phenolphthalein indicator, and specific detection of proteins using the HRP-DAB chemistry. Finally, for the detection of FABP3 on the developed µPAD, using the aptamers generated, the principle of salt induced aggregation of gold nanoparticles was exploited. The detection served as a Yes/No format for FABP3 presence with a minimum detection limit of 54 ng/ml.