INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Development of DNA Aptamers against selected Celiac disease epitopes in Glutenin

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SHORT ABSTRACT

Celiac disease, an autoimmune enteropathy of small intestine is caused by the indigestible immunostimulatory peptide sequences present in gluten protein. Gluten is present in the cereals like wheat, rye and barley. Consumption of gluten free diet is the only option available for celiac disease patient. The immunostimulatory peptide sequences are present in both the gliadin and glutenin fractions of gluten. Prior research were emphasized on detection of gliadin protein mostly, by different methods including antibodies and aptamers. This thesis focuses on the *in-vitro* selection and characterization of aptamers against two 14 mer peptide sequences, one from the high molecular weight glutenin of wheat (*Triticum aestivum*), GQGQQGYYPTSPQQ (GQ-14), which contains a celiac disease epitope QGYYPTSPQ and the other from low molecular weight glutenin, SQQQPPFSQQQQPV (SV-14), which contains epitope PFSQQQQPV. The aptamer sequences apt_J91P (56 bp) and apt_M09P (76 bp) were selected through conventional SELEX method against peptide targets GQ-14 and SV-14 respectively. The binding characterization between apt_J91P and GQ-14 by ITC reveals the dissociation constant of 2.26 µM and 4.385 mM for the primary and the secondary site of binding respectively. The dissociation constants are 17.6 µM and 8.33 mM for the primary and the secondary site of binding between apt_M09P and SV-14 respectively. The binding was also characterized by circular dichroism spectroscopy. The limit of detections of the aptamers evaluated by direct-ELAA method are found to be 16.0875 µM for apt_J91P and 20.00 µM for apt_M09P against its respective targets. The preliminary investigations carried out to check the applicability of aptamers apt_J91P and apt_M09P in development of bioassays, gold nanoparticle based aptamer assay and aptamer mediated magnetic bead based extraction assay demonstrated target binding and specificity. The future scope include improvement the binding affinity of the aptamers for detection of the target peptides in real wheat samples.