DEVELOPMENT OF PROGRAMMABLE BIOSENSOR USING SOLID PHASE PEPTIDE SYNTHESIS ON MICRO CHIP

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ABSTRACT

A miniaturized solid phase peptide synthesis (SPPS) system aiming novel programmable biosensor is developed. This SPPS system can synthesize various oligopeptide probes. Oligopeptides synthesized on single bead trapped at the center of microchannel using vacuum pump. After probe synthesis, sample solution containing fluorescence labeled bio-molecule added to the beads and interacts with synthesized probe molecules. Some bio-molecules bound with these probes and confirm their attachment through fluorescence. Different probe sequences have different binding affinity towards biomolecules specifically. Hence, this system is expected to realize a bio-sensing chip which can flexibly change target molecule on-demand and on-site by changing oligopeptide sequence.

KEYWORDS

Programmable system, oligopeptide probe, SPPS, single bead.

INTRODUCTION

There has been a tremendous demand of modern technique that has great potential for on-site diagnosis of various analysts. In this context, biosensors have the potential benefits of miniaturization, automation, integration, simplicity, high sensitivity and potential ability for real time and ability to make analysis possible in cases where time and place cannot be selected i.e., on-site analysis.[1,2] But, most of conventional biosensor like immunosensor can't change target molecule on-site and on-demand because probes were prepared for specific target molecules.[3] So it has become necessary attempt to develop further flexible and highly versatile microchip based system to deal with unexpected hazards and other malfunctions. Here, we proposed a concept of 'Programmable biosensor', which can flexibly change the target molecule by changing the sequence of biologically active peptide probe molecule on chip. Figure 1 shows the schematic of proposed programmable bio-sensing system.

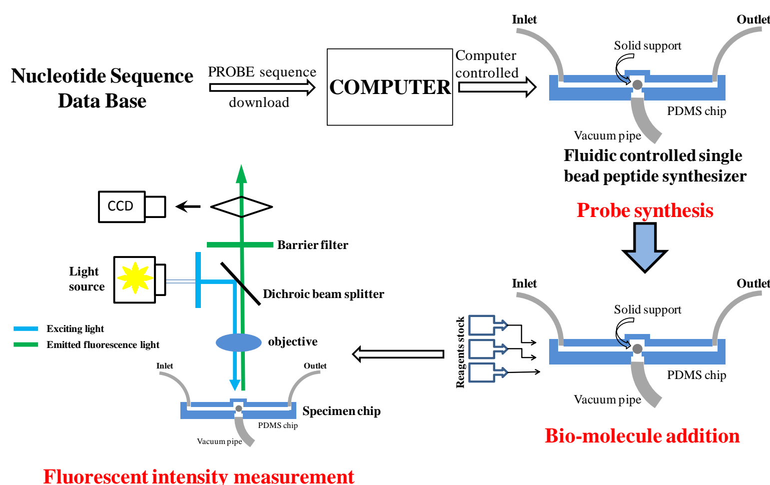

Figure 1. Demonstration of programmable biosensor.

This miniaturized system consists of fluidic controlled single bead peptide synthesizer, fluorescence detecting microscope and computer. In case of unexpected hazards, users can download on-demand sequencing of peptide probe for the hazard from database via network. Miniaturized peptide synthesizer system customizes the sensor module to be ready for diagnosis of the hazard on-site. Oligopeptide probe was synthesized on a single bead trapped at the center of micro channel. The purpose of using chip with a single bead control was designed to synthesize probe with high purity, easy handling and stable immobilization of bead on to the micro-scale solid phase holder.

In this work, three different tetrapeptide sequences (I) Arg-His-Lys-Ser (AHLS), (II) Asp-Glu-Asp-Glu (AGAG) containing hydrophilic amino acids and (III) Leu-Ala-Gly-Val (LAGV) containing hydrophobic amino acids respectively, were synthesized on single resin bead using SPPS. Probe synthesized on bead was analyzed by protein sequencer based on Edman degradation method. Then, synthesized probes were demonstrated against fluorescence labeled target analyte, a sample solution containing YOYO 1 labeled T4 DNA molecule will flow through the channel and target analyte will bind with synthesized probe. The probe: target binding confirms through the fluorescence microscope. The function that probe molecule carried out is dependent on the type of amino acids involved in the sequence. Hence, as a small step in the area of universal diagnostic devices, we demonstrated the specific binding of oligopeptide probes with the Target T4 DNA molecule. In this system, we can flexible and successfully change the target molecule by changing the oligopeptide probe sequence on chip.

EXPERIMENT

Fabrication of PDMS chip was done by soft prototyping method.[4] For small chain peptide (oligopeptide probe) synthesis, an F-moc protecting strategy was applied to on-chip SPPS.[5] Peptide chain elongation was carried out as circulatory reaction which comprised deprotection, activation, coupling and washing in each cycle (figure 2).

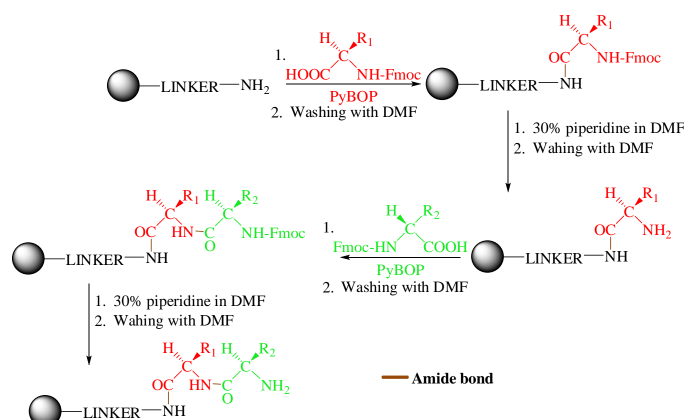

Figure 2. Scheme of SPPS using F-moc protecting amino acids.

It started with swelling resin bead by suspending in DMF for 3 min in a small eppendorf tube. Load a single bead and cover the reaction chamber by a piece of PDMS for regular flow through the channel and also prevention of evaporation of solutions.

Bead was first rinsed by DMF. An F-moc protected amine group of the resin was deprotected by 20% piperidine. After deprotection, residual reagent was removed by DMF washing. The first F-moc protected amino acid (Fmoc-Val-OH) and PyBOP pre-dissolved in 20 µL coupling reagent 1 (0.1M HOBt) and 15 µL coupling reagent 2 (0.5M NMM) were injected into the chip. After the coupling reaction, washing step was repeated. Then another deprotection procedure was carried out to initiate a new cycle. When the peptide elongation was completed, final deprotection step was carried out by deprotection reagent followed by washing with the DMF.

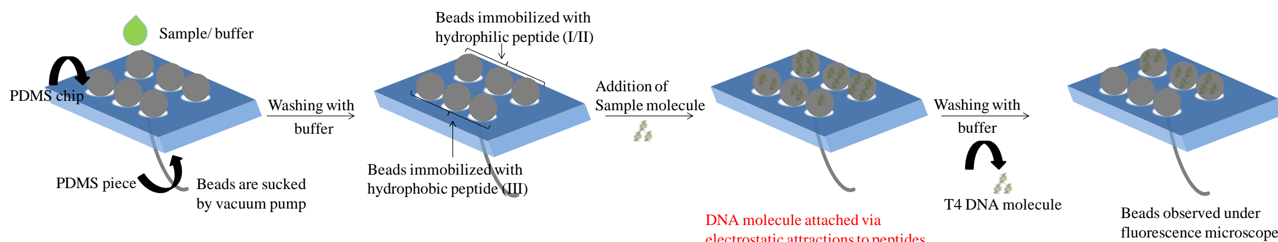

Figure 3. Experimental set up for peptide: DNA interaction.

Using fabricated miniaturized SPPS system as Biosensor, on-chip oligopeptide-DNA interaction was performed. Three divergent oligopeptide sequences (I), (II) and (III) were used for binding. Beads immobilized with oligopeptides were fixed on PDMS chip as shown in figure 3 by vacuum pump and washed by 0.5×TBE buffer solution to remove any contaminations attached. Then 20 µl of YOYO1/T4 DNA solutions was added and again washed with buffer solution.[6] After washing, the resulting beads were observed under a fluorescence microscope. We repeat this cycle again for more interaction with the DNA molecule.

RESULTS AND DISCUSSION

Synthesized tetrapeptide sequence (Leu-Ala-Gly-Val) was analyzed on bead by protein sequencer and successfully obtained chromatograms with 90% yield (figure 4).

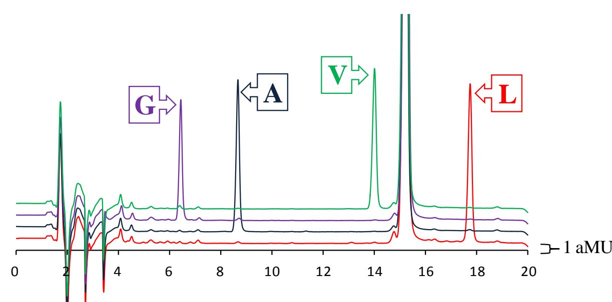

Figure 4. Chromatogram of Leu-Ala-Gly-Val (LAGV) with 90% yield.

After probe synthesis, on-bead analyte:target binding experiment was performed to evaluate the affinity of synthesized peptide probe towards DNA. Microscopy technique has been employed to detect the fluorescence image of beads.

Figure 5 shows typical fluorescence images of microchip containing beads after peptide interaction with T4 DNA solution.

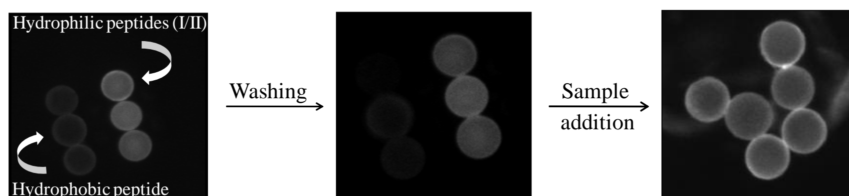

Figure 5. Fluorescence images of beads immobilized with the peptide.

Tetrapeptide probes were used to check the affinity, based on the charge possessed by side chain of amino acids. Hydrophilic sequence I contain amino acids with positive charged side chain, II contain amino acids with negative charged side chain and hydrophobic sequence III contains amino acids with electrically neutral side chain.

Fluorescent intensity graph (figure 6) shows the different affinity of probes toward target molecule. Linear increase in fluorescent intensity in case of peptide sequence I and II clearly shows the strong adsorption due to electrostatic interaction between T4 DNA molecule and peptide containing amino acids with (+/-) charged side chain respectively. While there is a strong fluctuation in fluorescent intensity in case of peptide sequence III, due to weak binding between T4 DNA and hydrophobic peptide. Hence hydrophilic peptide sequences I/II and YOYO1 labeled T4 DNA molecule interactions shows the specific binding of the specific sequence with the target molecule.

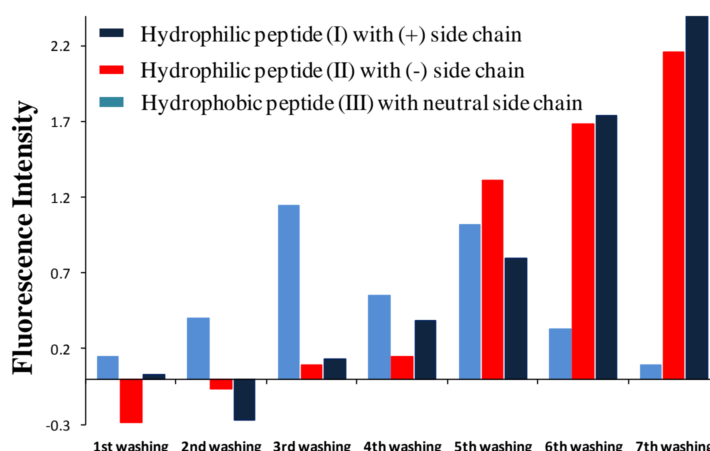

Figure 6. Fluorescent intensity graph from peptide:T4 DNA binding.

CONCLUSION

A novel concept involving μ-TAS incorporating miniaturized SPPS system which can synthesize different peptide sequence on chip was developed. The analysis system performed two processes on a microchip: solid phase peptide probe synthesis and demonstrate binding with the target analyte. These processes were integrated onto the microchip successfully. With slight modification of the probe molecule, the same chip design can be used for multi-target detection and can provide a simple, cost-effective and integrated microchip solution for target detecting applications. Hence, we developed a programmable biosensor which can change the target molecule by changing the sequence of probes on to the chip.

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