World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:01, 2016

A Novel Concept of Optical Immunosensor Based on High-Affinity Recombinant Protein Binders for Tailored Target-Specific Detection

Authors : Alena Semeradtova, Marcel Stofik, Lucie Mareckova, Petr Maly, Ondrej Stanek, Jan Maly

Abstract : Recently, novel strategies based on so-called molecular evolution were shown to be effective for the production of various peptide ligand libraries with high affinities to molecular targets of interest comparable or even better than monoclonal antibodies. The major advantage of these peptide scaffolds is mainly their prevailing low molecular weight and simple structure. This study describes a new high-affinity binding molecules based immunesensor using a simple optical system for human serum albumin (HSA) detection as a model molecule. We present a comparison of two variants of recombinant binders based on albumin binding domain of the protein G (ABD) performed on micropatterned glass chip. Binding domains may be tailored to any specific target of interest by molecular evolution. Micropatterened glass chips were prepared using UV-photolithography on chromium sputtered glasses. Glass surface was modified by (3-aminopropyl)trietoxysilane and biotin-PEG-acid using EDC/NHS chemistry. Two variants of high-affinity binding molecules were used to detect target molecule. Firstly, a variant is based on ABD domain fused with TolA chain. This molecule is in vivo biotinylated and each molecule contains one molecule of biotin and one ABD domain. Secondly, the variant is ABD domain based on streptavidin molecule and contains four gaps for biotin and four ABD domains. These high-affinity molecules were immobilized to the chip surface via biotin-streptavidin chemistry. To eliminate nonspecific binding 1% bovine serum albumin (BSA) or 6% fetal bovine serum (FBS) were used in every step. For both variants range of measured concentrations of fluorescently labelled HSA was 0 – 30 µg/ml. As a control, we performed a simultaneous assay without high-affinity binding molecules. Fluorescent signal was measured using inverse fluorescent microscope Olympus IX 70 with COOL LED pE 4000 as a light source, related filters, and camera Retiga 2000R as a detector. The fluorescent signal from non-modified areas was substracted from the signal of the fluorescent areas. Results were presented in graphs showing the dependence of measured grayscale value on the log-scale of HSA concentration. For the TolA variant the limit of detection (LOD) of the optical immunosensor proposed in this study is calculated to be 0,20 µg/ml for HSA detection in 1% BSA and 0,24 µg/ml in 6% FBS. In the case of streptavidin-based molecule, it was 0,04 µg/ml and 0,07 µg/ml respectively. The dynamical range of the immunosensor was possible to estimate just in the case of TolA variant and it was calculated to be 0,49 – 3,75 µg/ml and 0,73-1,88 µg/ml respectively. In the case of the streptavidin-based the variant we didn´t reach the surface saturation even with the 480 ug/ml concentration and the upper value of dynamical range was not estimated. Lower value was calculated to be 0,14 µg/ml and 0,17 µg/ml respectively. Based on the obtained results, it´s clear that both variants are useful for creating the bio-recognizing layer on immunosensors. For this particular system, it is obvious that the variant based on streptavidin molecule is more useful for biosensing on glass planar surfaces. Immunosensors based on this variant would exhibit better limit of detection and wide dynamical range.

Keywords : high affinity binding molecules, human serum albumin, optical immunosensor, protein G, UV-photolitography
Conference Title : ICBST 2016 : International Conference on BioSensing Technology
Conference Location : Zurich, Switzerland
Conference Dates : January 12-13, 2016