氏　　名	タラ　バハドゥル
学位の種類	博士（理学）
学位記番号	理工博　第 280 号
学位授与の日付	平成 30 年 11 月 1 日
課程・論文の別	学位規則第 4 条第 1 項該当
学位論文題名	Development of Sensing Probes of Peptide Aptamers by Incorporation of Non-Natural Amino Acids 非天然アミノ酸を導入したペプチドアプタマー・センシングプローブの開発（英文）
論文審査委員	主査　教授　相垣　敏郎 委員　教授　川原　裕之 委員　連携大学院教授　伊藤　嘉浩

【論文の内容の要旨】

Aptamers are experimentally-selected oligonucleotides (RNA, ssDNA) or peptide ligands that bind to specific target molecules. Although the primary function of aptamers is specific binding to the targets, their functions can be advanced by incorporation of functional compounds for various applications. In this thesis, I tried to prepare two types of sensing probes of peptide aptamers by incorporation of functional groups: a fluorogenic probe by modification of selected aptamer with environmental sensitive dye conjugated amino acid and an electrochemically sensing probe by selecting such aptamers from a peptide library containing an electrochemically active amino acid.

In the first part, aiming to prepare fluorogenic probe, I modified a reported peptide aptamer for a tumor maker, epithelial cell adhesion molecule (EpCAM), with an environment sensitive dye, 7-nitro-2,1,3-benzoxadiazole (NBD). I replaced the less important amino acids of EpCAM-binding aptamer (1) from “alanine scanning” with NBD-coupled aminophenylalanine one-by-one. One of the NBD-incorporated peptides exhibited fluorescence only on the cell surfaces expressing EpCAM. However, the binding affinity was reduced by the incorporation of NBD. Therefore, it was considered to be necessary to directly select aptamers from random sequence libraries incorporated with the functional group to construct aptamer probes without reducing affinity.

In the second part, I aimed to construct an electrochemical detection system for influenza virus by harnessing electro-polymerization properties of 3,4-ethylenedioxythiophene (EDOT). Here peptide aptamers were directly selected from random sequence of peptide library containing EDOT-coupled aminophenylalanine using ribosomal display method. After six round of selection, sequence were analyzed by next generation sequencing and high frequency three sequences were prepared by solid phase peptide synthesis method with EDOT-coupled aminophenylalanine. One of the selected peptide aptamers bound to the virus with a moderate affinity (EC50 = 9.6±2.3 μM) and effective selectivity. As I expected, the aptamer in the absence of virus was electrochemically polymerized via EDOT, although the polymerization was suppressed in the presence of virus presumably due to hindrance by the virus. As a result the polymerized material deposited on the working electrode reduces the electric current with the decrease of virus concentration. The detection limit of this system was 12.5 μg mL^{-1} ($P < 0.05$) of virus, which is comparable to the sensitivity of immune-chromatography. This work represents the first example of the *in vitro* selection of an electrosensitive peptide aptamer for the development of electrochemical biosensors.

(1) Shiba K.,Kokubun K., Suga K., PCT/ JP 2013/074655.