DEVELOPMENT OF BIOASSAYS FOR THE DETECTION OF BENZYLISOQUINOLINE ALKALOIDS,HIGENAMINE AND BERBERINE

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論文題名　：DEVELOPMENT OF BIOASSAYS FOR THE DETECTION OF BENZYLISOQUINOLINE ALKALOIDS,HIGENAMINE, AND BERBERINE（ベンジルイソキノリンアルカロイド、ヒゲナミンおよびベルベリン検出のためのバイオアッセイの開発）

区　　分　：甲

論　文　内　容　の　要　旨

Benzylisoquinoline alkaloids (BIA) are one classes of the natural compounds which are well known as the pharmacologically active substances. Thus far, there are various kinds of medicines and dietary supplements which are derived from the plant containing BIA in the market. The specific and reliable analytical methods for determination of the individual BIA content in plant samples and plant-containing samples are required for quality control of these products.

Higenamine (HM) is the BIA which are well-known as β-adrenoreceptor agonist. HM is recognized as the prohibited compounds in sport games according to World Anti-Doping Agency (WADA). Here, the immunological approaches for (*S*)-HM determination in plant samples were developed (Figure 1). The antibody against HM was detected after immunization of mouse using HM conjugated with bovine serum albumin (BSA) as an antigen. The monoclonal antibody against (*S*)-HM (MAb E8) was produced from the systematically screened hybridoma (colony E8) obtained by fusing the mouse splenocytes with myeloma (SP2/0). The MAb E8 was characterized and utilized for indirect competitive enzyme-linked immunosorbent assay (icELISA). The detectable range of the developed icELISA was 7.81–125 ng/mL with the limit of detection (LOD) of 4.41 ng/mL. Various validation methods were conducted to ensure the precision and accuracy of the developed icELISA. The plant samples analysis revealed that the (*S*)-HM content detected from icELISA were correlated to those obtained from HPLC-UV. The results confirmed that this developed icELISA can be a useful tool for screening of (*S*)-HM-containing plants to prevent the unintentional used of the HM in athletes [1].

Apart from that, the usefulness of MAb E8 was expanded as it was used for development of lateral flow immunoassay (LFA) for (*S*)-HM detection. The detection sensor of the system was colloidal gold nanoparticle conjugated with MAb E8. The paper-made device comprised of rabbit anti-mouse IgG antibodies and HM-γ-globulin conjugates as control and test zones, respectively. The bounded HM on the strip and free (*S*)-HM in the samples were competitively reacted with the detection sensor. The results of these microenvironment phenomena were represented as the pink spot on the test zone, where the intensity of the spot inversely relate to the content of free (*S*)-HM in the samples. The LOD of the developed LFA was 156 ng/mL. The sensitivity, selectivity, repeatability, and accuracy of the assay were systemically assessed to ensure the reliability of the LFA. As a result, the fast and reliable HM determination assay, LFA, was successfully developed [2]. The developed system could expand the versatility of detection to be possible in more scenarios.

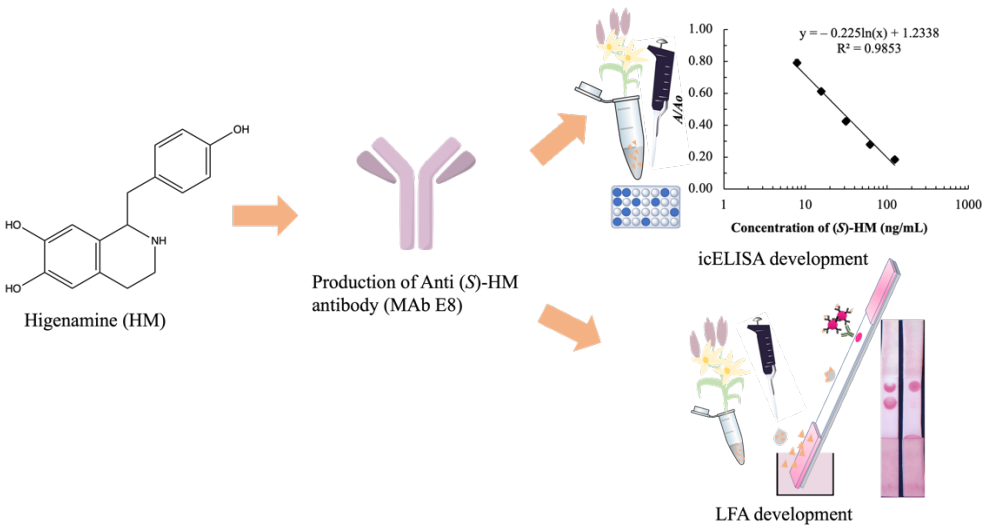

Figure 1 schematic diagram of determination methods development of (*S*)-HM.

Berberine (BBR) was also the BIA of interest in this study. BBR was contained in over 10 Kampo medicine preparations. Here, the aptamer-based fluorometric assay for BBR detection in Kampo medicine preparations was developed (Figure 2). The assay based on the fluorescent enhancement of BBR when it binds with aptamer. The specific aptamer against BBR was selected using colloidal gold nanoparticle-based systematic evolution of ligands by exponential enrichment (GOLD-SELEX). The aptamer candidate with highest fluorescent intensity was used as the template for structure evolution of the aptamer. By truncation, mutation, and polymerization of the candidate aptamer, the 80-mer aptamer was successfully obtained. The aptamer-based fluorometric assay was developed with determination range of 0.780–25.0 µg/mL with LOD of 0.431 µg/mL. The validation processes were implemented to confirm the reliability of the assay. Moreover, the BBR content in Kampo medicine preparations determined by developed aptamer-based fluorometric assay and those determined by HPLC-UV were correlated. This can be the useful tool for BBR determination in Kampo medicine samples.

In conclusion, this study successfully developed the assay for BIA detection based on the binding affinity of the macromolecule and ligand. These immunological assays and aptamer-based assay could be applied for BIA detection in plant samples as an alternative method or use alongside with conventional detection methods.

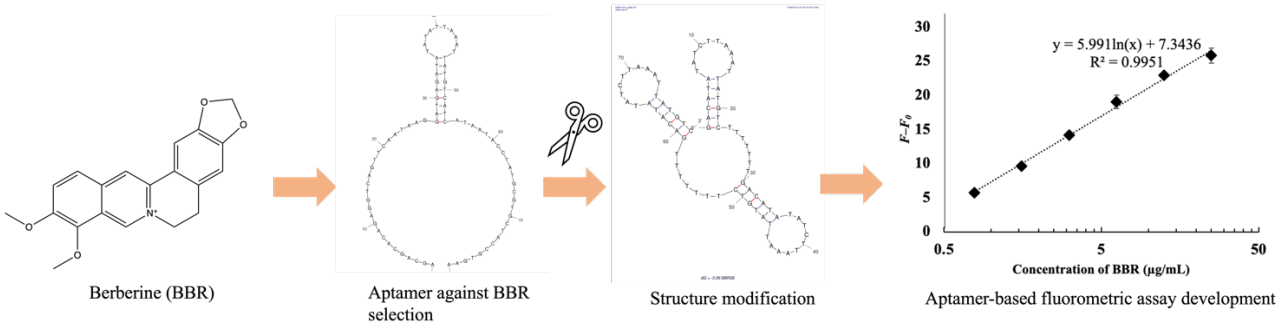

Figure 2 schematic diagram of determination methods development of BBR.

[1] Nuntawong P. Tanaka H. Sakamoto S. Morimoto S. Planta Med., **86** (11) 760-766, 2020.

[2] Nuntawong P. Ochi A. Chaingam J. Tanaka H. Sakamoto S. Morimoto S. Drug Test Anal., **13** (4) 762-769, 2021.