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Immunocapture couples with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for rapid detection of type 1 dengue virus

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ABSTRACT

A facile method for accurate detection of type 1 dengue virus (DV1) infection from complex biological mixtures, using type specific immunocapture coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), was developed. The biological mixtures were treated with magnetic particles coated with a monoclonal antibody directly against type 1 dengue virus. After immunocapture purification, the DV1 was eluted with 30% acetic acid, directly spotted with seed-layer method, and analyzed by MALDI-TOF MS for DV1 capsid protein. The detection limit of the assay was ~10^5 pfu/mL by MALDI-TOF MS. The immunocapture could unambiguously differentiate the DV1 from other serotypes of the dengue viruses and Japanese encephalitis virus, and could be used as a specific probe to detect DV1 from complex biological mixtures.

1. Introduction

Dengue infection is the most important mosquito-borne viral infection worldwide, especially in the tropics and subtropics [1]. It is estimated that more than 2.5 billion people living in over 100 countries are at high risk for endemic dengue transmission. Furthermore, dengue infections are responsible for as many as 100 million cases of dengue fever (DF) annually. There are four serotypes of dengue virus (DV-1, 2, 3, 4), which are antigenically related but distinct from each other. A primary infection with any of the four serotypes of DV leads to life-long immunity to this serotype but only partial or temporary immunity to the others. Secondary infection with different serotypes, due to antibody-dependent enhancement phenomena, may lead to more severe diseases, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2,3]. Annually, there are more than 200,000–500,000 cases of DHF, causing 22,000 deaths mainly among children under the age of 15 [4,5]. The case fatality rates of DHF and DSS are as high as 10–15% if no proper treatment is given in early stage [3,6,7].

Up-to-date, neither effective drugs/protective vaccines for DF/DHF nor timely and accurate diagnosis of specific DV strains are available. The development of serotype-specific assay for DV detection is of importance both to the treatment of patients and to epidemiological surveillance. Several protocols for dengue detection have been described. Traditional virus isolation and the viral infectivity titer assay is the "gold standard" method used to detect and type the dengue infection. However, long cell-cultured periods of a week or longer for virus growth is time-consuming and not suitable for high-throughput detection [8]. Alternatively, serological diagnosis of dengue infection includes capture of immunoglobulin M (IgM) antibodies, which need to be produced at least 5 days after the onset of fever. Moreover, the test is complicated by the cross-reactive antigenic determinants shared by the four dengue virus serotypes and other flaviviruses. Previously, based on genomic information, molecular techniques such as polymerase chain reaction (PCR), reverse transcription-PCR, and real-time PCR have been shown to be faster assays than cell culture and IgM antibodies assays [9–12]. Possible false-positive reactions, due to cross-contamination of PCR products, and time-consuming of stained agarose gel electrophoresis, however, have limited the high-throughput screening. Recently, biosensor-based diagnostic methods including piezoelectric [13], optical [14–16], and electrochemical [17,18] have been developed. Faster techniques are needed for the early diagnosis of dengue infection.

In the last several years, mass spectrometries including electrospray ionization mass spectrometry (ESI MS) and matrix-assisted

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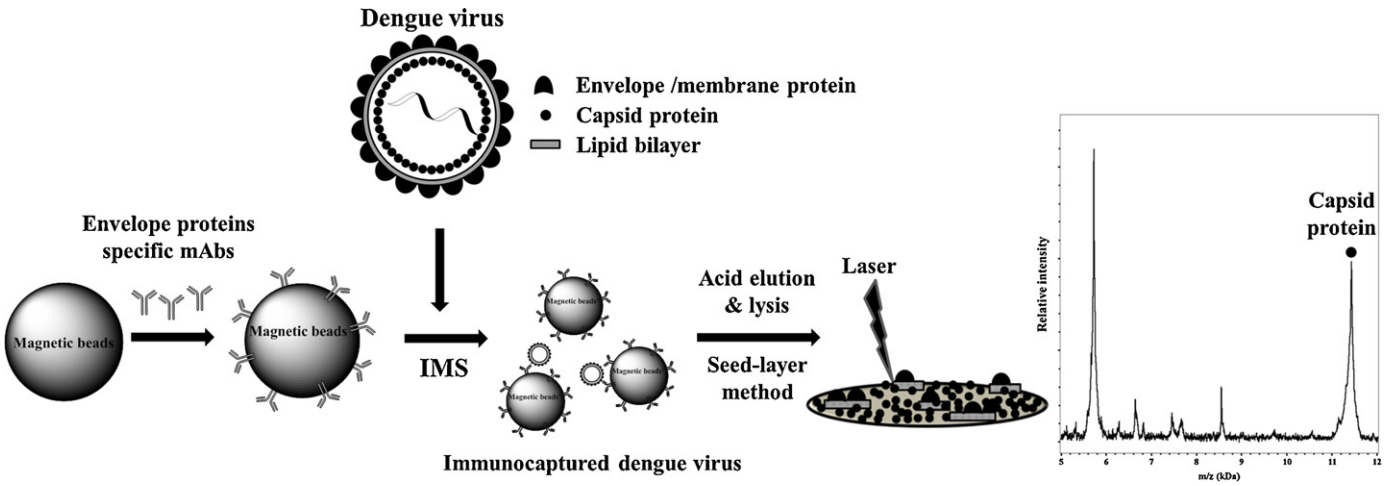

Fig. 1. Experimental strategy for detection of dengue virus by immunocapture and MALDI-TOF MS.

laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have emerged as rapid and sensitive tools to characterize microorganisms [19], including bacteria [20,21], fungi [22], spores [23], and viruses [24–26]. In the electrospray ionization, the analyte(s) of interest is dispersed by electrospray and ionized by mixing water with volatile organic compounds. However, clogging of samples on devices, low tolerance to salt concentration, and difficulty on deconvolution of multiply charged proteins, are usually experienced [19]. Alternatively, because of its large mass range, higher tolerance to contaminants including salts, detergents, and buffer components, and robustness in instrumentation, MALDI-TOF MS has been applied in the identification of unique protein biomarkers for individual microorganisms. Furthermore, in order to reduce the analysis time and ease of operation, other approaches such as immunomagnetic separation (IMS) have also been coupled with MALDI-TOF MS for virus detection [27–30]. Despite the utilization of the methodologies in various fields, the application of immunocapture coupled with MALDI-TOF MS for detection of DVs remains unexplored. In this study, we developed a simple method for DV1 detection using magnetic bead conjugated type 1 specific monoclonal antibody coupled with MALDI-TOF MS (Fig. 1).

2. Experimental

2.1. Chemicals and materials

The MALDI matrices: α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), protein standard I, and peptide calibration standard were purchased from Bruker Daltonics. ZipTip C18 was purchased from Millipore. Hybond-P PVDF transfer membrane, nProtein A sepharose and Ni sepharose were purchased from GE healthcare life science. *Escherichia coli* host strain BL21(DE3) and plasmid pET28a were purchased from Novagen. All other materials were from Sigma, unless specified differently.

2.2. Virus preparation

The used prototype of DV strains: DV-1 (Hawaii), DV-2 (*PL046*), DV-3 (*H84*), DV-4 (*H241*), and JEV(*T1P1*) were propagated in C6/36 cells. The virus titers were determined by plaque assay in BHK21 cell [31]. The DV viruses were prepared in BSL-2 (Biosafety level-2) environment and inactivated by exposure to UV light for 30 min after experiments [15].

2.3. Cloning, expression and purification of DV1 E395 proteins

A cDNA encoding DV1 E395 (residues 1–395) was amplified from viral RNA by M-MLV Reverse Transcriptase according to the manufacturer's instructions (invitrogen). For gene subcloning, the sense strand oligonucleotide primer sequence was 5′-gAATTCATgCgATgCgTgggAAT (*EcoR* I site underlined) and the antisense strand oligonucleotide primer was 5′-CTCgAgTTATCCTTTCTTgAACCAgC (*Xho* I site under line). PCR conditions were as follow: denaturation 94 °C 5 min, followed by 25 cycles of (i) denaturation (94 °C 30 s), (ii) annealing (55 °C 30 s), and (iii) elongation (72 °C 90 s), followed by a final 10 min elongation period at 72 °C. The PCR product was ligated into pET28a plasmid using *EcoR* I and *Xho* I restriction sites, sequenced and transformed into *E. coli* strain BL21(DE3) to express and purify E395 protein following the previous published procedure [32]. The emergence of protein in the fractions was collected in ~1.5 mL/tube. Fractions containing protein were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.4. Generation of mAbs against DV1 E protein

Six-week-old female Balb/c mice were intraperitoneally immunized at 2-week intervals with inactivated DV1 (~10^5 particles) mixed with complete Freund's adjuvant twice and then given three boosts of recombinant DV1 E395 protein (100 μg) mixed with incomplete Freund's adjuvant. Hybridomas were generated according to the previous published procedures [33]. Hybridoma supernatants were screened for the presence of antibodies against DV1 with indirect ELISA coated inactive DV1. Positive hybridoma cells were cloned by limiting dilutions and the serotype-specific mAbs were further identified with dot blot using C6/36 cells infected with four different serotypes DV. The mAb was purified with nProtein A sepharose.

2.5. Identification of serotype specificity of anti-E mAb with Dot blot assays

Different dengue virus solutions and negative control: JEV solution, C6/36 cell cultured medium, and PBS-diluted human serum as the target were used to identify the serotype specificity of anti-E mAbs. To perform the dot blot assay, 50 μL of different targets were spotted on a methanol-activated Hybond-P PVDF membrane with Dot Blotter (Major science) and detected