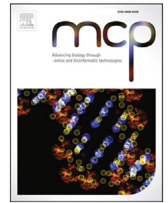
Molecular and Cellular Probes 29 (2015) 158–166

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr



Nanoparticle-based lateral flow biosensor for visual detection of fish nervous necrosis virus amplification products

Dimitra K. Toubanaki*, Maritsa Margaroni, Evdokia Karagouni*

Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, 127 Vas. Sofias Ave., 11521 Athens, Greece

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form
5 March 2015
Accepted 12 March 2015
Available online 20 March 2015

Keywords:
Lateral flow strip biosensor
Gold nanoparticles
Nervous necrosis virus
Nodavirus
RT-PCR
Dicentrarchus labrax

ABSTRACT

Lateral flow paper biosensors are an attractive analytical platform for detection of human and veterinary disease pathogens because they are optimal for accurate, rapid and sensitive analysis in research laboratory setups, as well as field analysis. Since diseases of viral etiology have been wreaking havoc in aquaculture industry, as well as the environment, the present study aims at the development of a gold nanoparticle-based biosensor for fish nervous necrosis virus (Nodavirus) nucleic acids detection. Total viral RNA, isolated from fish samples was subjected to reverse transcription PCR amplification. The PCR products were mixed with a specific oligonucleotide probe and applied next to oligonucleotide conjugated Au NPs. A red test line was formed when nodavirus product was present. The visual detection of the RT-PCR product was completed within 20 min. Following optimization, the biosensor was able to visually detect 270 pg of nodavirus initial total RNA. The present study describes a simple, accurate, robust and low cost method for nodavirus detection in biological samples. Apart contribution on basic research, the proposed biosensor offers great potential for commercial kit development for use on the site of fish culture by fish farmers. This fact will have great impact on environmental safety and disease monitoring without time consuming and costly procedures.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Viral nervous necrosis (VNN), also named vacuolating encephalopathy and retinopathy (VER) or encephalomyelitis, is a disease that causes high mortalities (80–100%) in several marine fish species in Europe, America, Japan, Southeast Asia, and Australia [1]. In recent years, VNN has attracted much attention because of its ecological and economic impacts on the aquaculture industry. Especially in the Mediterranean area, VNN has emerged as a major problem for aquaculture facilities, as well as wild marine species, since it has no effective vaccine or treatment [2,3]. The causal agent of the disease is the nervous necrosis virus (NNV), also known as nodavirus, which has been reported worldwide in at least 30 different fish species, including groupers, sea bass and Atlantic halibut, indicating a wide host range [1,4–7].

Fish nodavirus (genus: *Betanodavirus*, family: *Nodaviridae*) is an icosahedral, non-enveloped virus with diameter of ~25 nm [1]. Its genome is composed of two, single-stranded, positive-sense RNA molecules of 3.1 kb (RNA 1) and 1.4 kb (RNA 2). RNA 1 directs the synthesis of the RNA-dependent RNA polymerase (100 kDa) [8] and RNA 2 encodes the synthesis of the coat protein of the virus (42 kDa) [9]. RNA 3 is a subgenomic transcript of the RNA1 segment which contains an open reading frame for the B2 protein [10]. Nodavirus has been classified in four genotypes: striped jack NNV (SJNNV), red-spotted grouper NNV (RGNNV), tiger puffer NNV (TPNNV) and barfin flounder NNV (BFNNV). Fish nodaviruses are quite different from insect nodaviruses (*alphanodaviruses*) [11]. A nodavirus found only in shrimps and prawns, *Macrobrachium rosenbergii* nodavirus has been identified. Its sequence could not be assigned to either of the existing genera and it has been proposed to be classified as *Gammanodavirus* [12].

Fish nodavirus has been detected and analyzed with several methods including light- and electron-microscopy [1,13], immunofluorescence antibody test (IFAT) [14], enzyme-linked immunoassay (ELISA) [15] and *in situ* hybridization [16]. However, polymerase chain reaction (PCR) [9] and reverse transcriptase PCR (RT-PCR) [17] have become the main diagnostic methods for fish nodaviruses, as they can detect tiny quantities of viral RNA in any tissue. Analysis of PCR products (amplicons) is, usually, carried out by agarose gel electrophoresis, followed by ethidium bromide

* Corresponding authors. Tel.: +30 210 647 8825; fax: +30 210 647 8828.
E-mail addresses: dtouban@pasteur.gr (D.K. Toubanaki), mmargaroni@pasteur.gr (M. Margaroni), ekaragouni@pasteur.gr (E. Karagouni).

http://dx.doi.org/10.1016/j.mcp.2015.03.005
0890-8508/© 2015 Elsevier Ltd. All rights reserved.

staining. Several methodologies for rapid and accurate nodavirus detection have been developed recently, including: real-time quantitative PCR [18]; loop-mediated-isothermal-amplification (LAMP) [19] and real-time LAMP [20] assays; an immunomagnetic reduction (IMR) assay which employs magnetic nanoparticles coated with dextran and antibodies [21]; a microfluidic LAMP system, needing slab-electrophoresis for products detection [22]; a RT-PCR microfluidic chip with laser-induced fluorescence technology on the capillary electrophoresis module of the chip [23]; and an integrated microfluidic chip with molecular beacons and nucleic acid sequence-specific captured probes conjugated to magnetic beads [24]. Even though each mentioned methodology has several advantages, they require expensive and specialized instrumentation, highly-trained personnel or they are time-consuming and cumbersome. Especially the methods that utilize gel electrophoresis for products detection are based on products size detection, which might result in false positives, and are using the highly mutagenic ethidium bromide.

Paper-based sensing has emerged as an attractive analytical platform. Lateral flow paper biosensors (LFB) are optimal for accurate, rapid and sensitive analysis in research laboratory setups, but they have the potential to be used for field analysis. Especially the use of functionalized nanoparticles, as recognition labels has accelerated nucleic acids/proteins sensing by LFBs, by increasing their sensitivity and specificity. In general, lateral flow biosensors are prefabricated strips of materials containing dry reagents that are activated by applying the fluid sample. They are designed for disposable single use and for applications where an on/off signal is sufficient [25]. LFBs are considered one of the most promising technologies owing to their simplicity, rapid analysis, low cost, high sensitivity and specificity. Several lateral flow biosensors have been developed to detect many analytes such as DNA, mRNA, miRNA, proteins, biological agents and chemical contaminants [26].

In that aspect, the present study aims at the development of a gold nanoparticle-based LFB for nodavirus nucleic acids detection. Specifically, in order to increase the detection accuracy, simplify and speed up the total time for PCR-based analysis, we propose a paper lateral flow biosensor which has been evaluated in applications such as hepatitis C virus detection [27]; genetically modified organisms (GMO) detection [28]; bacterial infections detection [29] and single nucleotide polymorphisms (SNPs) genotyping [30,31]. In brief, total viral RNA, isolated from fish samples was subjected to reverse transcription PCR amplification. The PCR products were mixed with specific oligonucleotide probe and applied next to oligonucleotide conjugated gold nanoparticles (Au NPs). A red test line was formed when nodavirus product was present. The visual detection of the RT-PCR product was completed within 20 min. Detection parameters were optimized and the proposed LFB discriminate NNV infected from non-infected samples successfully.

2. Experimental section

2.1. Oligonucleotides

All oligonucleotides used in this study were synthesized by VBC-Biotech (Vienna, Austria). A 5′-thiolated $(dT)_{30}$ oligonucleotide (SH-dT_{30}: 5′–SH–TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′) was used for conjugation on gold nanoparticles. A $(dA)_{20}$ oligonucleotide (dA_{20}: 5′- AAAAAAAAAAAAAAAAAAAA-3′) was used for construction of the control zone of the biosensor. The 20-mer 5′-TAAGAAATTGGCAAAACCCG-3′ was labeled at the 5′-end with biotin and used as an upstream primer (UpNdv_B) for PCR of nodavirus coat protein cDNA. The 20-mer 5′-TATCCGTCTGTTCCTGTCCC-3′ was used as downstream primer (DpNdv). The 20-mer 5′-CCTTAGACACAGGTGCGTCA-3′ was used as the nodavirus-specific probe (Probe_Ndv). The oligonucleotide primers and probe were designed based on the published sequence for nodavirus coat protein gene (GenBank accession number: Y08700.1). Finally, B-dA_{20} (5′-B-AAAAAAAAAAAAAAAAAAAA-3′) reference oligonucleotide was used as control for biosensor construction.

2.2. Tailing of oligonucleotide probes with dTTP or dATP

The 5′-SH modified $(dT)_{30}$ oligonucleotide was tailed enzymatically with dTTP, whereas the $(dA)_{20}$ probe, the biotinylated control oligonucleotide B-dA_{20}, and the nodavirus-specific probe (Probe_Ndv) were tailed with dATP. The reactions were performed in a total volume of 20 μL, containing 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate (pH 7.9), 0.25 mM $CoCl_2$, 3.5 mM dTTP or dATP (2 mM of dATP for probe Ndv), 10 units of terminal deoxynucleotidyl transferase (TdT; New England Biolabs, Ipswich, MA, USA), and 700 pmol of oligonucleotide (400 pmol for probe_Ndv). The reactions were carried out at 37 °C for 60 min and terminated by heating to 70 °C for 10 min. After completion of the tailing reaction the poly(dA)-tailed Ndv probe was mixed with 1.5 μL of 40 mM N-methylmaleimide (Sigma–Aldrich, Steinhem, Germany) solution in dimethyl-sulfoxide (DMSO, 2.7 mM final concentration). The tailed probes were stored at −20 °C.

2.3. Preparation of oligonucleotide conjugated gold nanoparticles

Gold nanoparticles (40 nm, 7.2×10^{10} particles/mL; Sigma–Aldrich, Steinhem, Germany) were modified with oligonucleotide addition, through thiol group conjugation [27]. The reactions were carried out by adding 75 pmol of tailed $(dT)_{30}$ oligonucleotide and 0.8 μL of absolute pyridine to 1 mL of the gold nanoparticle solution (~0.12 pmol). The mixture was incubated at 4 °C, for 24 h. Subsequently, the solution was subjected to “aging” by the addition of NaCl up to a concentration of 90 mM. The solution was allowed to stand for another 24 h at 4 °C, and the excess of reagents was removed by centrifugation at 1300 × g for 30 min. The supernatant was discarded, and the pellet was redispersed in 100 μL of an aqueous solution containing 30% sucrose, 0.25% Tween-20, 0.25% sodium dodecyl sulfate (SDS), and 45 mM NaCl, by vortexing and brief sonication.

2.4. Preparation of the lateral flow biosensors

The dry reagent lateral flow biosensors (4 × 60 mm) consisted of a cellulose immersion pad, a glass-fiber conjugate pad, a nitrocellulose diagnostic membrane, and a cellulose absorbent pad. The parts were assembled on a plastic adhesive backing as follows: The diagnostic membrane (HF240MC100, 25 mm in length; Millipore, Billerica, MA, USA) was placed on center of a laminated card by the manufacturer. The conjugate pad (GFCP000800, 8 mm; Millipore, Billerica, MA, USA) was then placed below the membrane, the immersion pad (CFSPOO1700, 17 mm; Millipore, Billerica, MA, USA) was placed below the conjugate pad, and the absorbent pad (same as immersion pad) was positioned above the membrane. Each part overlapped 2 mm to ensure that the solution could migrate through the biosensor. The TLC applicator, Linomat 5 and WinCats software (Camag, Muttenz, Switzerland), were employed to construct the test zone and the control zone by loading streptavidin (SA) from *Streptomyces avidinii*, and poly(dA), respectively, on the membrane. In details, a solution containing 4 g/L SA (Sigma–Aldrich, Steinhem, Germany), 150 mL/L methanol, and 20 g/L sucrose was loaded at a density of 1.6 μg/4 mm membrane. A solution containing 4 μM poly(dA)-tailed probe, 500 mL/L methanol, and 20 g/L sucrose was loaded at a density of 2.4 pmol/4 mm membrane. The membrane

Download English Version:

https://daneshyari.com/en/article/2199674

Download Persian Version:

https://daneshyari.com/article/2199674

Daneshyari.com