Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biosensors and Bioelectronics 20 (2005) 1690–1695
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Short communication

Polyaniline synthesis and its biosensor application

Zarini Muhammad Tahir[a,1], Evangelyn C. Alocilja[a,*], Daniel L. Grooms[b]

[a] *Biosystems Engineering, Michigan State University, East Lansing, MI 48824, USA*
[b] *Large Animal and Clinical Sciences, Michigan State University, East Lansing, MI 48824, USA*

Received 21 May 2004; received in revised form 28 July 2004; accepted 4 August 2004
Available online 15 September 2004

Abstract

In this study, five polyaniline compounds were synthesized using different protonic acids and incorporated into a conductometric biosensor used for bovine viral diarrhea virus detection. The biosensor was developed and evaluated by the authors for bacterial pathogen detection in previous studies. The biosensor consisted of two parts: the immunosensor and the electronic data collection system. Liquid sample moved through the immunosensor surface by capillary action. The specificity of the biosensor was based on the unique binding characteristics of the polyclonal and monoclonal antibodies immobilized on the immunosensor. Polyaniline was used in the biosensor architecture as the transducer due to its electronic and bio-molecular properties. Results showed that the biosensor was sensitive at a concentration of 10^3 cell culture infective dose per milliliter (CCID/ml) of BVDV antigens. The promising results on the BVDV detection demonstrated that the conductometric biosensor was interchangeable for different target molecules of detection. Further modification could be implemented to evaluate the biosensor as a rapid diagnostic device to detect other infectious disease outbreaks in livestock population.

Keywords: Polyaniline; Bovine viral diarrhea virus; Conductometric; Immunosensor; Conducting polymer; Biosensor

1. Introduction

Bovine viral diarrhea virus (BVDV) is one of the most insidious and economically devastating viral pathogens in cattle (Baker, 1995). This virus is a single-stranded, enveloped RNA virus that can cause acute diarrhea, fetal infection, and mucosal disease. Mucosal disease results in high fever, epithelial lesions, anorexia, and death (O'Rourke, 2002). Infections in adult cattle have been reported in the Great Lakes region with mortality approaching 50% (Carmen and Dreumal, 1994). The estimated loses at the national level range between 10 and 40 million dollars per million calvings (Houe, 2003). Therefore, a rapid diagnostic device for BVDV detection is valuable, as part of preventive measures in controlling BVDV infections.

Conducting polymers have considerable flexibilities in modifying their chemical structures. By chemical modeling and synthesis, it is possible to modulate their electrical and mechanical properties (Gerard et al., 2002). Moreover, the polymer itself can be modified to bind with protein molecules (Situmorang et al., 2000). Conducting polymers are also known for their ability to be compatible with biological molecules in neutral aqueous solutions (Gerard et al., 2002). Additionally, conducting polymers have the ability to efficiently transfer the electric charges produced by biochemical reactions to electronic circuits (De Taxis du Poet et al., 1990).

Polyaniline is one of the most important conducting polymers (Trivedi, 1997). It is the first conducting polymer to be commercialized and now has applications ranging from batteries (Osama et al., 1992) to biosensors (Kim et al., 2000). Most polyaniline syntheses are carried out using aniline hydrochloride solution or a mixture of an aniline monomer and a dilute hydrochloride acid (Stejskal and Gelberg, 2002). In order to obtain a conducting form of polyaniline, three steps are required: (1) polymerization of aniline with hydrochloride

* Corresponding author. Tel.: +1 517 355 0083; fax: +1 517 431 2892.
E-mail addresses: muhamm20@msu.edu (Z.M. Tahir), alocilja@egr.msu.edu (E.C. Alocilja).
[1] Tel.: +1 517 3553011; fax: +1 517 431 2892.

0956-5663/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2004.08.008

acid, (2) treatment of the obtained product with base to produce non-conducting polyaniline, and (3) successive protonation with an appropriate acid to form a conducting polyaniline salt (Laska and Widlarz, 2003a). Each step is a separate process and requires purification and filtration processes.

Most of the recent studies on polyaniline are concentrated on controlling the macromolecule properties of the polymer, such as its conductivity, molecular weight, and solubility in water (Salaneck and Lundstrom, 1987; Stejskal et al., 1996, 1998; Laska and Widlarz, 2003a). One way to modify the polyaniline properties is by polymerization in various acids. Inorganic acids such as sulfuric acid (Neoh et al., 1990), phosphoric acid (Yoon et al., 1996), and water soluble organic acids, such phosphonic and sulphonic acids (Laska and Widlarz, 2003a,b), have been investigated with the expectation of increasing conductivity and solubility in water. It has also been reported that the temperature at which the polymerization is conducted controls the molecular structure of the synthesized polyaniline. When polymerization of aniline is carried out at a sub-zero temperature, a marked increase in both molecular weight and crystalinity have been observed (Stejskal et al., 1998).

A conductometric biosensor for bacterial pathogen detection has been developed by the authors in previous studies (Muhammad-Tahir and Alocilja, 2003a,b). The architecture of the biosensor utilizes a lateral flow format that allows the liquid sample to move from one membrane to another. The biosensor uses antibodies as the biological sensing element and polyaniline as its transducer. Polyaniline acts as an enzyme amplifier to provide signal amplification in the recognition process (Sergeeva et al., 1996; Kim et al., 2000; Grennan et al., 2003; Morrin et al., 2003). The use of polyaniline as an enzyme switch, which yields “on” and “off” responses, has also been demonstrated by Iribe and Suzuki (Iribe and Suzuki, 2002).

This paper will describe the characteristics of polyaniline synthesized in various acids and how the polyaniline-based biosensor performs for BVDV detection. The objectives of this study were (1) to synthesis and characterize polyaniline compounds protonated with phenylphosphonic acid, 4-hydroxybenzenesulfonic acid, sulfobenzoic acid, hydrochloride acid, and percholoric acid and (2) to evaluate the performance of the polyaniline-based biosensor for BVDV detection in pure culture.

2. Materials and methods

2.1. Reagents

Aniline, glutaraldehyde, *N*,*N*-dimethylformamide, Polysorbate 20 (Tween-20), phenylphosphonic acid (PPA), 4-hydroxybenzenesulphonic acid (HBSA), sulfobenzoic acid (SBA), hydrochloride acid (HCl), percholoric acid (PA), ammonium persulphate, Tris buffer, peptone water, and phosphate buffer were purchased from Sigma–Aldrich (St. Louis, Missouri). The nitrocellulose membrane, cellulose membrane, and fiberglass membrane were purchased from Millipore (Bedford, Massachusetts). A benchtop dispenser (Integrated dispensing solution, Inc., Agoura Hills, CA) with silver paste was used to make the electrodes on the capture membrane. Affinity purified swine anti-BVDV polyclonal antibody (USDA: NADL, Ames, IA) and purified mouse anti-BVDV 15c5 (gp48) monoclonal antibody (Ed DuBovi, Cornell University, Ithaca, NY) were used as the biological receptors.

2.2. Preparation and characterization of polyaniline

In this study, polyaniline compounds were polymerized separately using PPA, HBSA, SBA, HCl, and PA based on a standard procedure of oxidative polymerization of aniline monomer in the presence of ammonium persulfate (Seegeeva et al., 1996; Laska and Widlarz, 2003a,b). Each resulting polyaniline compound was characterized as to pH and conductivity in solid and liquid forms. The pH level of all polyaniline compounds was determined by using a pH meter by Orion (Model 290, Baton Rouge, LA). The conductivity meter by Oakton (Vernon Hills, IL) was used to determine the conductivity of the polymer samples in water. A mixture of each polyaniline compound and potassium bromide was compressed into pellets and was used to determine the conductivity of the solid polymer by using a four-point-probe (Signatone model S-301, Gilroy, CA). Transmission electron microscope (TEM) (JEOL 2100FEF 200 kV field emission, Center for Advance Microscopy, Michigan State University) was used to inspect the physical attributes of each polyaniline compound.

2.3. Biosensor construction

As indicated earlier, the conductometric biosensor consisted of two parts: an immunosensor and an electronic data collection system. The immunosensor was comprised of four, one-time-use, disposable membranes: sample application, conjugate, capture, and absorption membranes (Fig. 1). Silver electrodes were fabricated on the capture membrane to electrically connect the immunosensor to the electronic data acquisition system consisting of a copper wafer and an ohmmeter linked to a computer. The detailed fabrication of the biosensor is described in previous publications (Muhammad-Tahir and Alocilja, 2003a,b).

2.4. Conjugate membrane preparation

A mixture of the monoclonal antibody (150 μg/ml) and each of the synthesized polyaniline compound (0.1 g/ml) was left to react for 1.5 h at 21 °C. To inactivate the non-reacted aldehyde group, 0.1 M Tris buffer with 0.1% Tween-20 (ph 9) was added to the mixture and left to react at 21 °C for 0.5 h. The conjugate was then precipitated by centrifugation