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Review

Recent advances in peptide probe-based biosensors for detection of infectious agents ☆

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ABSTRACT

Recent biological terrorism threats and outbreaks of microbial pathogens clearly emphasize the need for biosensors that can quickly and accurately identify infectious agents. The majority of rapid biosensors generate detectable signals when a molecular probe in the detector interacts with an analyte of interest. Analytes may be whole bacterial or fungal cells, virus particles, or specific molecules, such as chemicals or protein toxins, produced by the infectious agent. Peptides and nucleic acids are most commonly used as probes in biosensors because of their versatility in forming various tertiary structures. The interaction between the probe and the analyte can be detected by various sensor platforms, including quartz crystal microbalances, surface acoustical waves, surface plasmon resonance, amperometrics, and magnetoelastics. The field of biosensors is constantly evolving to develop devices that have higher sensitivity and specificity, and are smaller, portable, and cost-effective. This mini review discusses recent advances in peptide-dependent rapid biosensors and their applications as well as relative advantages and disadvantages of each technology.

Contents

1. Introduction

Throughout history, pathogenic microorganisms have significantly impacted human activities, whether by causing disease or by being used deliberately in biological warfare (Crawford, 2007; Lim et al., 2005). The anthrax attacks that occurred post-9/11 in the United

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States highlight the potentially deadly threat posed by the intentional use of biological threat agents (BTA) against both civilians and the military (Fennelly et al., 2004). In addition, recent outbreaks of *Escherichia* and *Salmonella* in the United States make clear the danger of microbial pathogens disseminated through contaminated food (http://www.cdc.gov/; http://www.fda.gov/oc/opacom/hottopics/Salmonellatyph.html; (Goldschmidt, 2006)). Infectious agents also have an indirect effect on agricultural and other related commodities. For example, the recent discoveries in the United States of Huanglongbing disease (citrus greening) in citrus crops and Pierce's disease in grapes threaten to have a severe economic impact on the specialty crop industry at the state, national and international levels (Bové, 2006; Hopkins and Purcell, 2002). To minimize the effects of natural outbreaks or deliberate attacks, near real-time detection of infectious agents is an essential first step in mounting an appropriate response.

Traditionally, infectious agents were detected and identified using standard microbiological and biochemical assays that were accurate but time-consuming. Traditional methods required isolation and/or culturing of large quantities of the infectious agents, and therefore needed several days to complete the analysis. More recently, molecular approaches to identify infectious agents have supplanted traditional microbiological methods because they are more sensitive and take less time. Molecular approaches such as the polymerase chain reaction (PCR) amplification and analyses of unique DNA sequences and/or 16S rDNA are highly accurate and sensitive (Deisingh and Thompson, 2002). However, these assays require specialized instruments and still take several hours to perform. In addition, DNA-based molecular techniques are limited to the detection of whole organisms and cannot detect toxins and other extracellular products of infectious agents. New techniques are needed that combine the accuracy and breadth of traditional microbiological approaches with the enhanced accuracy and sensitivity of molecular approaches.

Biosensor technology is one such technique that brings together the accuracy and sensitivity of standard approaches with improvement in rapidity of detection. Biosensors also offer the possibility of continuous and real-time monitoring of the environment for the presence of infectious agents to allow timely implementation of preventive and protective measures. The majority of biosensors take advantage of the affinity between a probe molecule and an analyte. Hence, specificity of the probe:analyte interaction is critical for designing an effective biosensor. The sensor platform that detects the probe:analyte interaction and generates a measurable signal needs to be sensitive enough to discern infectious agents even at low concentrations.

An ideal field-ready biosensor should differentiate between pathogenic and non-pathogenic organisms with high sensitivity and accuracy (Ivnitski et al., 2003). Although great technological improvements have been made in continuous collection of environmental samples and increased sensitivity in detection of infectious agents (Chase et al., 2005; Christensen et al., 2006; Jones et al., 2005; Keer and Birch, 2003; Makino and Cheun, 2003), field-ready biosensors continue to be plagued by background interference during collection, duration of detection time and portability (Petrenko and Sorokulova, 2004). Furthermore, an ideal early warning biosensor system should be designed as an array that can simultaneously detect a multitude of infectious agents while minimizing the probability of false alarms. In this mini review, we discuss recent advances in near real-time peptide-based biosensors for the capture and detection of various infectious agents with an emphasis on label-free detectors suitable for field deployment. Due to the time-consuming nature of the polymerase chain reaction (PCR), we have intentionally minimized discussions of biosensor platforms that contain a PCR step.

2. Label-free biosensors

A label-free biosensor consists of a sensing element or probe/receptor molecule tethered to a stable sensing surface. A sensing transducer detects the probe:analyte interaction and provides a measurable signal for the binding reaction (Goldschmidt, 2006; Petrenko and Sorokulova, 2004). Ideal biosensor characteristics have been described by Ivnitski et al. (1999) and these are summarized in Table 1. Specificity is achieved by using a probe that interacts only with the target analyte. This probe is absolutely critical in the overall design and success of a biosensor because it reduces the incidence of false positives. In addition, an effective probe must have high affinity and avidity for the target analyte in order for the sensor to detect the analyte in a complex sample. Without a strong and highly specific probe:analyte interaction, the biosensor loses its efficacy and advantages over the traditional microbiological and molecular biological detection methods. Other desirable features include a long shelf life, reproducibility, capability for continuous monitoring, and portability.

The sensitivity of biosensors is determined in part by the ability of the sensor platforms to generate detectable signals even with a low concentration or frequency of probe:analyte interactions. The majority of sensor platforms detect a perturbance that is created when the unbound probe binds the target analyte, and then translate that interaction into a measurable signal. Label-free biosensors do not require secondary or tertiary reactions to generate measurable signals (i.e., ELISA or DNA sequencing), and are thus ideal for continuous and near real-time monitoring of infectious agents. Sensor platforms with low detection limits can detect the presence of even a minute quantity of infectious agents. (Specifics of each sensor platform are described below in Section 4).

Biosensors are classified as single-use sensors, intermittent-use sensors, and continuous-use sensors (Kissinger, 2005). Intermittent-use sensors, typically found in laboratory settings, are accurate and have the capability for data storage. In comparison, single-use and continuous-use sensors currently have relatively poor accuracy and sensitivity (Kissinger, 2005). Further research and development are needed to produce continuous real-time monitoring biosensors that are small, affordable, accurate and sensitive.

3. Peptide-based receptor molecules (probes)

Peptides are remarkable in their ability to form various tertiary structures that interact with numerous molecules. This ability is clearly demonstrated in antibodies, which contain highly specific complementarity determining regions (CDR) for recognition of various antigens. As a result, peptides have been explored as ideal probe molecules for biosensors. In this section, relative advantages, disadvantages, and experimental application of various peptide probes in biosensors will be discussed.

3.1. Antibodies, antibody fragments, llamabodies

Due to their specificity and affinity for diverse analytes, antibodies have been a natural choice for molecular receptors and probes in

Table 1
Ideal characteristics of a biosensor (adapted from (Ivnitski et al., 1999)).

Low limit of detection	Detection of a single bacterial cell in a small volume (<100 ml)
Species specific	Ability to differentiate an individual bacterial species in a mixed population
Strain specific	Ability to differentiate an individual bacterial strain from other strains of the same species
Assay time	Short, 5–10 min/assay
Precision	1%
Assay protocol	Simple, no addition of reagents to facilitate detection
Measurement	Direct, no pre-enrichment or secondary amplification
Format	Highly automated design
Operator	No special skill required to operate assay
Viable cell count	Able to classify live versus dead cells
Size	Small, portable, hand-held, hardy

Necessary aspects of a field-deployable biosensor.