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Optical Lectin Based Biosensor as Tool for Bacteria Identification

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Abstract

Biosensor techniques are based on biospecific interaction between the biological parts of biosensor with the analyte. In biosensor construction, antibodies are usually used for the detection of analytes such as microorganism, because of very strong and highly specific interaction. The disadvantages of this assay are a long time needed for antibody isolation and purification as well as difficult regeneration of biosensor chip. The use of lectins instead of antibodies could solve these problems because a several hundred lectins are commercially available and their stability in standard buffers is better compared to monoclonal antibodies. While antibody can only be used to detect that antigen it was designed for, lectin as low affinity molecule may bind several different pathogens. Using the discriminative effect of an artificial neural network the application of a lectin array will compensate for the lower specificity. Microbial surfaces bear many of the sugar residues capable of interacting with lectins. The ability of lectins to react with microbial glycoconjugates means that it is possible to employ them as probes and sorbents for whole cells, mutants and numerous cellular constituents and metabolites, and it makes them useful tools for identification or typing of bacteria. Lectins are attractive reagents for the clinical diagnostic laboratory because of their diverse specificity, commercial availability, a wide range of molecular weights, and their stability in standard buffers. The construction of lectin biosensor could be an advantage method for detection of pathogenic bacteria.

Key words: biosensors, lectins application, bacteria identification

Introduction

Foodborne illness has increased dramatically throughout the world. It is estimated that there are a few hundred million cases of foodborne illness each year resulting in several thousands of deaths. Pathogenic bacteria in foods are the cause of 90% of this reported illness. Council of Agricultural Science and Technology in 1998 released eighteen recommendations for producer organizations, governmental employees, scientists, and public interest groups. One of the important tasks for research is to develop rapid, accurate detection methods for foodborne pathogens and toxins.

Conventional methods for microorganism identification are sensitive, but they require too long time for detection. Typically, a small sample of the analyzed food is homogenized, incubated and pathogens are identified after at least one to three days by stains, biochemical tests and/or serological reaction (De Boer and Beumer, 1999; Artault *et al.*, 2001). Some new methods for detection of microorganisms have been developed recently including chromatography, spectroscopy, immunomagnetic-electrochemiluminiscence, immunomagnetic separation with flow cytometry, impedance monitoring, nucleic acid hybridization and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. PCR-based methods are also much used for sensitive detection (Toze, 1999), but require careful sample preparation and is more time-consuming than the proposed technique. Beside these methods, biosensor techniques play an important role since they are based on biospecific interaction between the biological parts of the biosensor with the analyte (microorganisms). In biosensor construction, antibodies are usually used for the detection of microorganisms because of very strong and highly specific interaction. The disadvantage of this assay format is the demand for antibodies against the antigen, which takes more than 3 months to obtain (including immunization, purification and shipment). Even if purified antibodies against desired antigen are obtained, cross-reactivity may occur, resulting in lower specificity. For regeneration of the biosensor chip very strong agents are needed because of strong antibody-antigen binding.

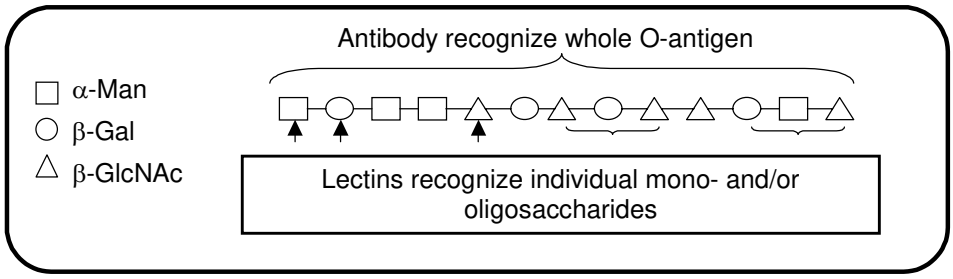

Fig. 1. Comparison of the binding specificity between O-antigen and lectin or antibody

The use of lectins instead of antibodies could solve these problems, since several hundred lectins with different saccharide specificities are commercially available. Lectins compared to monoclonal antibodies have a better stability, but on the other hand in some cases lower specificity. At first sight lower sensitivity is a disadvantage, but a high affinity antibody binds only the antigen it was designed for and can only be used to detect that antigen, while a low affinity molecule (lectin) may bind several different ligands (pathogens). Using the discriminative effect of an artificial neural network the application of a lectin array will compensate for the lower specificity. Figure 1 shows difference between lectin and antibody recognizing of O-antigen structure.

Lectins

Lectins are sugar-binding proteins of non-immune origin that agglutinate cells and/or precipitate complex carbohydrates. They contain at least two sugar-binding sites and they are capable of binding glycoconjugates even in presence of various detergents. The specificity of lectins is usually defined by the mono- or oligo-saccharides that are best at inhibiting the agglutination or precipitation the lectin causes. The precise physiological role of lectins in nature is still unknown but they have proved to be very valuable in a wide variety of applications *in vitro*. They have been isolated from various sources: bacteria, plant, animal, virus, and fungi and they are present in cells and biological fluids (Duverger *et al.*, 2003). They are involved in a wide variety of biological functions including bacterial growth, recognition, adhesion, cancer metastasis, bacterial and viral infections (Lis and Sharon, 1998; Varki, 1993; Ashraf and Khan, 2003)

Bacterial lectins play an important role in the initial stages of infection by mediating the interaction of pathogens with host cell surface glycoconjugates. By virtue of their ability to mediate adhesion to host tissues, the lectins can be considered as bacterial virulence factors (*Listeria* references+ *Streptomyces*). Thus, lectin-deficient mutants of the pathogens are often unable to initiate infection (Sharon and Ofek, 2001). Plant lectins are extensively used in purification, detection and structural characterization of glycoconjugates, investigation of cell-surface architecture, blood typing and identification and differentiation of various procaryotic and eucaryotic cells and as epidemiologic as well as taxonomic markers (Lis and Sharon, 1998). The main drawbacks of employed approaches were in the need for fluorescent-labelled or enzyme-labelled lectin preparations (Graham *et al.*, 1984; Singh and Doyle, 1993) and in many cases the use of non-immobilized lectins.

Direct aggregation of suspended microorganisms is one of the important applications of lectins in microbiology. Almost all microorganisms express surface-exposed carbohydrates which are potential lectin-reactive site. The lectin receptors of microorganisms are listed in Table I (Calderon *et al.*, 1998).

Table I
Lectin receptors of microorganisms (Calderon *et al.*, 1998)

Fungi	Arabinans, capsules, cell wall glucan, chitin, galactans, mannans, secreted proteins
Gram-negative bacteria	Capsules, cytoplasmic membranes, lipopolysaccharides, lipooligosaccharides, outer membranes, peptidoglycan, surface array glycoproteins
Gram-positive bacteria	Capsules, group-specific polysaccharides, lipotechoic acids, peptidoglycans, surface arrays, teichoic acids, teichuronic acids
Protozoa	Galactomannans, glycoproteins, glycolipids, lipophosphoglycans, phosphoglycans

Free, non-immobilized lectins were applied in most tests for identification of bacteria mainly in seventies and eighties years of last century (Reeder and Estedt, 1971; Hamada *et al.*, 1977; DeLucca, 1984; Davidson *et al.*, 1982; McSweegan and Pistole, 1982) but also in recent years (Hynes *et al.*, 1999; Aabenhus *et al.*, 2002; Hynes *et al.*, 2002; Annuk *et al.*, 2001). While the earlier works were focused on qualitative demonstration of bacteria agglutination by individual lectin or panel of lectins, the recent works deal about differentiation and phenotyping of bacterial isolates based on agglutination assays.

Immobilized lectins were successfully used for isolation of bacteria (*Listeria monocytogenes*, *Salmonella* sp., *Staphylococcus aureus*, *E. coli*) from milk and ground beef. Lectin-derivatized surfaces were also used to capture and concentrate microorganisms and even enveloped viruses with subsequent detection by MALDI mass spectrometry.

Biosensors

Biosensors use a combination of biological receptor compounds (antibody, enzyme, nucleic acid, lectin, *etc.*) and the physical or physico-chemical transducer directing, in most cases, real-time observation of a specific biological events (*e.g.* antibody-antigen, lectin-carbohydrate interaction) (Leonard *et al.*, 2003). Biosensors may be divided into four basic groups, depending on the method of signal transduction: optical, mass, electrochemical, and thermal biosensors (Goepel, 1991; Seyhi, 1994).

Optical biosensors are very attractive because they allow direct label-free and real-time detection of bacteria. They are frequently used for medical purposes and they are very promising for assessment of many diseases enhancing quality of people's life. Surface plasmon resonance (SPR) is a powerful optical biosensing technique for non-label bioaffinity interaction analysis (antigen-antibody, lectin-saccharide, and receptor-hormone). It is phenomenon that occurs during optical illumination of a metal surface and it can be harnessed for biomolecular interaction analysis (Liedberg *et al.*, 1995). SPR allows to monitor the process of interaction in real-time and in continuous mode providing a highly automated device enabling routine analysis without need for trained personnel. Six companies currently manufacture a variety of biosensor hardware based on SPR technique: Biacore AB (Uppsala, Sweden), Affinity Sensors (Franklin, MA), Windsor Scientific Limited (Berks, UK), BioTul AG (Munich, Germany), Nippon Laser and Electronics Lab (Hokaido, Japan), and Texas Instrument (Dallas, TX). Biacore AB released the first commercial instrument in 1990 and approximately 90% of commercial biosensor publication cites the Biacore instrument (Rich and Myszka, 2000). Biacore 3000 instrument has a few advantages compared with previous models:

- It allows measure interaction between analyte and four different ligands at the same time;
- Lower sample consumption due to smaller flow cell volume;
- Online data subtraction;
- Micro-sampler recovery.

Although lectins have been commonly used for characterization and determination of tumor-associated markers, synthetic carbohydrates, synthetic glycopeptides, and microbial carbohydrates using SPR technique (Mecklenburg *et al.*, 2002, Mislovicova *et al.*, 2002, Holmskov *et al.*, 1996; Okazaki *et al.*, 1995), only one report has been published in which lectin (Con A) immobilized on SPR chip was used to capture cells (erythrocytes) (Quinn and O'Kennedy, 2001). SPR has not yet been reported for monitoring of the interaction between lectin and microorganism for diagnostic purposes.

In our work we probed possibility to utilize lectins for bacteria identification. Eight lectins with various specificities (Table II) were covalently attached on Biacore™ sensor chips CM5 *via* their amine groups and analyte containing only endotoxin or whole bacteria cells was injected onto the surface.

The lectins listed in Table II were used for differentiation of bacteria based on lectin-endotoxin (lipopolysacharide; LPS) interaction. Only endotoxins (*Salmonella toucra* O48, *Salmonella typhimurium*, *Escherichia coli* O104, *Escherichia coli* C600, *Klebsiella oxytocyta* 666, and *Citrobacter youngae* O2) as well as whole bacteria (*Citrobacter freundi*, *Salmonella typhimurium*, *Escherichia coli* O157, and non-pathogenic *Escherichia coli*) were applied. Biacore 3000 was employed for determination of lectin-LPS interaction.

A contribution of non-specific interaction between lipid part of endotoxin and lectins was occurred and it was reduced by addition of detergent. The obtained lectin binding patterns were well correlated with theoretical patterns derived from carbohydrate structure of O-antigens. The binding pattern of bacteria *Salmonella typhimurium* corresponded with one obtained by lectin-lipopolysaccharide interaction (Masarova *et al.*, 2004a). The experiment with whole cells showed the possibility to distinguish bacteria

Table II
Lectin panel used in biosensor preparation

Lectin	Abbrev.	Carbohydrate specificity	Mr
Canavalia ensiformis	Con A	α-Man, α-Glc	104 000
Maackia amurensis	MAA	α-SA	140 000
Ulex europeaus I.	UEA I.	α-Fuc	63 000
Erythrina cristagalli	ECA	α/β-GalNAc α/β-Gal	54 000
Sambucus nigra	SNA	α-SA-α-Gal	150 000
Dolichos biflorus	DBA	α-GalNAc	120 000
Triticum vulgaris	WGA	β-GlcNAc	36 000
Galanthus nivalis	GNA	α-Man	52 000

with cross-reactivity in immunoassays (*Citrobacter freundi*, *Escherichia coli* O157:H7) by using of lectin panel (Masarova *et al.*, 2004b).

Such method allows creating a "bacteria library" based on their interaction with lectins. The obtained results signified the construction of lectin biosensor would be an advantage method for detection of pathogenic bacteria. In the case of gram-negative pathogen even cell free lipopolysaccharide can be monitored as indicator of pathogenic infection.

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