Biosensor

16

A biosensor is an analytical device incorporating a biorecognition element intimately associated with or integrated within a transducer that converts the biological response into an electrical signal. It is a sensor that integrates a biological element with a physiochemical transducer to produce an electronic signal proportional to a single analyte which is then conveyed to a detector. The biological response could be anything from enzyme activity or antibody/receptor binding to cell responses. The transduction to an electrical signal could also be diverse. In simple words, it is a device made by fusion of a biocomponent and an electronic gadget used to detect the presence of the substance to be monitored (Chaplin 2009).

Biosensor technologies include transduction platforms based on electrochemical (potentiometric, amperometric, impedance), piezoelectric, thermal or optical methods (reflectrometric interference spectroscopy, interferometry, optical waveguide lightmode spectroscopy, total internal reflection fluorescence, surface plasmon resonance). These techniques have been adapted to detect analytes of interest based on the interaction with or functionality modification of a biological target, which could be nucleic acids, enzymes, antibodies, receptors, cell organelles, or whole cells. The specificity of the detection is determined by the biological component of the method.

16.1 Immobilization of Biological Material

Immobilization of the biological component is the key step while constructing biosensor which can be carried out in many of the following ways as listed out by Palit and Mukherjee (2007),

- Adsorption—the biological material is contacted with a surface-active material such as graphite, alumina, clay, and glass.

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- Covalent attachment—chemical groups on the biological material that are not essential for activity such as non-essential amino acid residues of enzymes are attached to chemically activated supports like synthetic polymers, cellulose, and glass.
- Cross-linking—intermolecular covalent linkages can be formed between macromolecules with the aid of bifunctional cross-linking agents such as di-isothiocyanates, alkylating agents, and dialdehydes such as glutaraldehyde.
- Physical entrapment—gels or polymers such as polyacrylamides, silica gel, starch are cross-linked in the presence of enzyme or other biological material which is thereby trapped or captured.
- Microencapsulation—materials are trapped by the membranes of various polymers, usually in capsules with mean diameters ranging from 5 to 300 μm.

16.2 Components of a Biosensor

- Bio-element.
- Sensor element.
- Processor circuit.

16.3 Construction of Biosensor

Biosensor consists of biological and physical components. Biological components include enzyme, nucleic acid, antibody, etc. while physical components include transducer, amplifier, etc. The biological and physical analyte interact to produce some physical charge detectable by the transducer. As a first step, the biological component is immobilized on the transducer. Then the analyte must be transported from the solution to the biological component through simple diffusion for reaction. Then the biological component interacts specifically to the analyte which produces a physical change near the surface of the transducer. Depending on the physical change produced, the type of biosensor varies. The transducer then detects and measures this change and converts it into an electric signal. This signal is very small and is amplified by an amplifier before it is fed into the microprocessor. The signal is then processed, interpreted, and displayed in suitable units (Fig. 16.1) (Palit and Mukherjee 2007).

16.3.1 Pre-requisites for Developing Biosensor

- Selection of suitable bioreceptor molecule which requires knowledge on bio-chemistry and biology.
- Selection of suitable immobilization method which requires knowledge on chemistry.

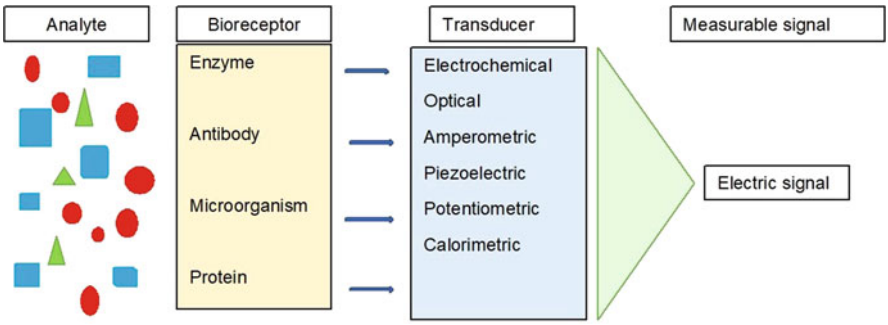

Fig. 16.1 Biosensor and its components

- Selection of suitable transducer for which knowledge on electrochemistry and physics is necessary.
- Designing a biosensor considering measurement ranger, linearity, and minimization of interference which requires knowledge on kinetics and mass transfer.
- After designing of the biosensor, it must be miniaturized and mass produced. Modern fabrication technology and micromachining technology are used in fabrication of biosensors.

16.3.2 Characteristics of Good Biosensor

- It should be highly specific for the analyte.
- The reaction used should be independent of factors such as pH, temperature, etc.
- The response should be linear over a range of analyte concentrations.
- Device should be small, durable, easy to use, and cheap.

16.4 Types of Biosensors

16.4.1 Calorimetric Biosensors

Most of the enzyme catalyzed reactions produce heat. Calorimetric biosensors measure the change in temperature of the solution containing the analyte following the enzyme action and interpret it in terms of the analyte concentration in the solution.

16.4.2 Potentiometric Biosensors

They use ion-selective electrodes that convert the biological reaction into electric signal. The electrodes employed are most commonly, glass pH electrodes for

cations, glass electrodes coated with a gas selective membrane, or solid state electrodes. E.g., pH meter.

16.4.3 Amperometric Biosensors

They function by the production of current when potential is applied between two electrodes, the magnitude of current being proportional to the substrate concentration. These biosensors are used to measure redox reactions, an example being determination of glucose using glucose oxidase.

16.4.4 Optical Biosensors

These biosensors measure both catalytic and affinity reactions. They measure a change in the fluorescence or in absorbance caused by the products of catalytic reaction. E.g., biosensor using luciferase enzyme for detection of bacteria in food samples.

16.4.5 Acoustic Wave Biosensors

These are also called piezoelectric devices. Their surface is usually coated with antibodies which bind to the complimentary antigen present in the sample solution. This leads to increased mass which reduces their vibrational frequency. This change is used to determine the amount of antigen present in the sample.

16.5 Applications

- Glucose monitoring in diabetes patients.
- Environmental applications (the detection of pesticides and river water contaminants).
- Detection of pathogens.
- Determining levels of toxic substances before and after bioremediation.
- Detection and determining of organophosphate.
- Routine analytical measurement of folic acid, biotin, vitamin B12, and pantothenic acid as an alternative to microbiological assay.
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey.
- Drug discovery and evaluation of biological activity of new compounds.
- Detection of toxic metabolites such as mycotoxins.

16.6 Applications in Fisheries

16.6.1 Biosensors in Food Analysis

Biosensors in food industry uses optic coated with antibodies to detect pathogens and food toxins. The light system in these biosensors has fluorescence. A range of immune-and ligand-binding assays for the detection and measurement of small molecules such as water-soluble vitamins and chemical contaminations (drug residues) such as sulfonamides and beta-agonists has been developed.

16.6.2 Biosensors in Aquatic Environment Assays

Due to water pollution, fish has shown reproductive dysfunction with males displaying feminization. The biochemical responses of organisms to organic and metal compounds in the water can be measured and used as a biomarker to study the level of pollution. Most commonly, Cytochrome P4501A is used as it is responsive to a number of organic chemicals including aromatic hydrocarbons and dioxins. The induction of this gene by these contaminants is measured by changes in protein expression or mRNA levels. Alternatively, metallothionein is utilized, which are induced specifically by metals.

Similarly, gene encoding green fluorescent protein (GFP) is fused to a number of promoters which will respond to water pollutants. These include the promoters from some inducible genes such as those encoding heat shock proteins or metallothioneins which are induced by general stress, heavy metals, or chemical toxins, those contain estrogen response elements being induced by estrogens or xenoestrogens. The availability of the GFP as a reporter gene has enabled the use of transgenic organisms as biosensors for water contamination providing rapid and visible results while eliminating the need for enzymatic or specific protein assays.

16.6.3 Aequorin

Aequorin is a photoprotein isolated from the bioluminescent jellyfish (*Aequorea victoria*). Upon addition of calcium ions (Ca^{++}) and coelenterazine, a reaction occurs whose end result is the generation of blue light in the 460–470 nm range. Aequorin has been incorporated into human B cell lines for the detection of pathogenic bacteria and viruses in the CANARY assay (Cellular Analysis and Notification of Antigen Risks and Yields). The B cells are genetically engineered to produce aequorin. Upon exposure to antigens of different pathogens, the recombinant B cells emit light as a result of activation of an intracellular signaling cascade that releases Ca^{++} inside the cell.

16.6.4 Green Fluorescent Protein (GFP)

Green fluorescent protein (GFP) is also a photo protein isolated and cloned from the jellyfish *A. victoria*. GFP, like aequorin, produces a blue fluorescent signal, but without the required addition of an exogenous substrate. All that is required is an UV source to activate the fluorescent properties of the photoprotein. This ability to autofluorescence makes GFP highly desirable in biosensing assays since it can be used on line and in real time to monitor intact, living cells. Additionally, the ability to alter GFP to produce light emissions besides blue (i.e., cyan, red, and yellow) allows it to be used as a multianalyte detector. Consequently, GFP has been used extensively in bioreporter constructs within bacterial, yeast, nematode, plant, and mammalian hosts.

16.6.5 Biosensors in the Detection of Fish Toxins

Biosensors technologies can detect different toxins in seafood. Several biosensor techniques have been developed that allow the detection of tetrodotoxin. One of the approaches uses an anti-tetrodotoxin specific antibody as the biological reporter and amperometric detection with a screen-printed electrode. The format of the assay is an indirect competition assay where the amount of current generated by *p*-aminophenol, the product of the enzymatic activity of the alkaline phosphatase label of the specific antibody, is inhibited by the presence of tetrodotoxin in a sample that competes with the electrode immobilized tetrodotoxin.

Another tetrodotoxin biosensor based on inhibition of cell function has been designed using murine spinal cord neuronal networks cultured on microelectrode arrays. The biological response is monitored as extracellular potentials. In this system, tetrodotoxin quantification was based on spike rate inhibition.

16.6.6 Lab-on-a-Fish from PNNL

Pacific Northwest National Laboratory has developed a biosensor named "Lab-on-a-fish" that can collect data about a fish, including its location, heartbeat, tail movement, and burned calories, as well as the temperature, pressure, and magnetic field of its surrounding environment. It also uses electrocardiogram and electromyogram. This will help to understand the impact of climate change and infrastructure development on ecosystem health and develop suitable management and conservation strategies. This particular device was developed in order to create a better fish passage at hydraulic structures.

16.6.7 BIOLAN for Sulfite Testing

BIOLAN has developed a portable biosensor for sulfite monitoring in shrimp farming. It requires minimal sample and is highly suitable for field applications to ensure the right amount of metabisulfite in water. The optimized biosensor provides accurate sulfite quantification from 8 to 110 g/L of metabisulfite, a repetitivity expressed as an imprecision lower than 7%, and as an irreproducibility lower than 10%.

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