Current Trends in Biotechnology and Pharmacy
Vol. 12 (1) 108-117 January 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Overview of enzyme based biosensors and their applications

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Abstract

Enzymes are biocatalysts that govern life processes. Each biochemical reaction of cell metabolism is catalyzed by one specific enzyme. Enzymes can catalyze reactions in different states but most commercial enzymes, being water soluble are difficult to recover at the end of the catalytic process. This restricts the use of soluble enzymes to essentially batch-operations followed by disposal of these expensive enzymes containing solution. A possible approach to resolve this problem is to attach enzymes onto a solid/ semi solid support material. Such an attachment restricts the free movements of the enzyme molecules and renders them insoluble in aqueous media. The process of attachment of enzymes on an insoluble solid/semisolid material by physical or chemical bonding known as immobilization. In the present review we have discussed in brief about the enzyme-based biosensors, their history of development, classification and their applications in various filed with special emphasis on medicine and analytical assays.

Keywords: Enzymes, Biosensors, Biocatalyst, Immobilization

Introduction

Enzymes are powerful biological catalysts which serve to accelerate the chemical reactions of living cells. Without enzymes, most of the biochemical reactions would be too slow to even carry out life processes. Enzymes display great substrate selectivity and perform best at optimum pH and temperature. Besides their role as biocatalysts, enzymes are being increasingly used in industries, medicine and analysis of biomolecules (1-5).

Enzymes are not changed during the reactions, as they are soluble in reactants or products, so it is difficult to separate them. Therefore, if an enzyme can be attached (immobilized) to an insoluble support material by any means (physically or chemically), it can be used repeatedly after the products have been removed. Immobilization thus offers an effective way to enhanced stability and also the recovery of enzyme at the end of catalytic process, which improve activity and purity of products (6-11).

Every immobilization technique is not suitable for all enzymes. Hence, it is important to understand the physical and chemical changes in enzyme when it undergoes immobilization. Numerous factors can affect the rate of the enzyme's catalytic activity; changes have been observed in the stability and kinetic properties of enzyme due to the microenvironment and product's characteristics (12-13).

It is most important to choose a method of attachment which prevent loss of enzyme activity as well as it should not allow change in chemical nature and binding site of the enzyme. It is desired to avoid reaction with the essential binding site group of the enzyme. Alternatively, an active site can be protected during attachment as long as the protective groups can be removed later on without loss of enzyme activity (14-15).

The use of immobilized enzymes in industries, pharmaceutical companies and in biochemical analysis has increased tremendously

in recent years (16-19). In this review, we discuss the basic principles of enzyme based biosensor, their history of development, classification and their applications in various filed with special emphasis on medicine and analytical assays.

Historical development of enzyme-based biosensors : The science and technology of immobilized enzymes has experienced a phenomenal growth in recent years (20-23). The very first report on the immobilization of proteins via adsorption of invertase on activated charcoal was reported by Nelson and Griffin (20), way back in 1916. The first enzyme electrode, an amperometric system, was described by L.C. Clark and C. Lyons (21) and S. J. Updike and G.P. Hicks (22), using glucose oxidase entrapped onto a polarographic oxygen electrode, for the measurement of glucose in biological solutions and tissues. In such amperometric or voltammetric probes, the current, produced upon application of a constant applied voltage, is measured. The first potentiometric enzyme electrode was described by G. Guilbault and J. Montalvo (23). The historical data of biosensor development are shown in Table1.

Methods of preparation of enzyme electrode based biosensors : Biosensor can be prepared in one of the four possible ways viz., membrane entrapment, physical adsorption, matrix entrapment and covalent bonding (36-37). In the membrane entrapment, a semi permeable membrane separates the analyzer and the enzyme, where the sensor is attached to the enzyme. The physical adsorption is dependent on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic

Table 1 Historical data of biosensor development

Year	Event	Reference
1916	First report on the immobilization of proteins: adsorption of invertase on activated charcoal	(20)
1922	First glass pH electrode	(24)
1956	Invention of the oxygen electrode	(25)
1962	First description of a biosensor: an amperometric enzyme electrode for glucose	(21)
1969	First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea	(26)
1970	Invention of the ion-selective field-effect transistor (ISFET)	(27)
1972-75	First commercial biosensor: Yellow Springs Instruments glucose biosensor	-
1975	Invention of the pO_2/ pCO_2	(28)
1976	First bedside artificial pancreas	(29)
1980	First fiber optic pH sensor for in vivo blood gases	(30)
1982	First fiber optic-based biosensor for glucose	(31)
1983	First surface plasmon resonance (SPR) immunosensor	(32)
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for the detection of glucose	(33)
1987	Launch of the MediSense ExacTech blood glucose biosensor	-
1990	Launch of the Pharmacia BIACore SPR-based biosensor system	(34)
1998	Launch of Life Scan FastTake blood glucose biosensor	
1998	Merger of Roche and Boehringer Mannheim to form Roche Diagnostics	(35)

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forces to attach the enzyme to the surface of the sensor (36). The porous entrapment is based on forming a porous encapsulation matrix around the enzyme that helps in binding it to the sensor. In the case of the covalent bonding the sensor surface is treated as a reactive group to which the enzyme can bind.

To construct an enzyme electrode, the enzyme must react most selectively with the substance to be determined. It is important to check the purity of enzyme, availability and also the selectivity. The better the immobilization procedure and the more stable the enzyme; longer it can be used analytically. Generally the physically entrapped enzyme is stable for about 3 weeks or 100 assays and the chemically bound enzyme for over a year and up to 1000 assays (37).

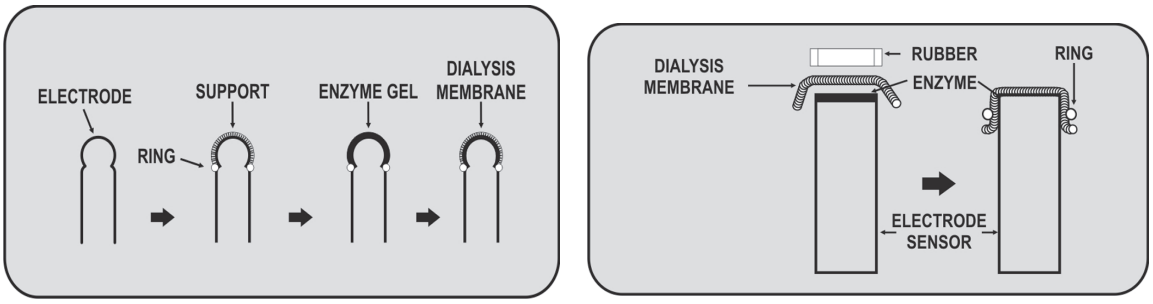
The electrode probe may be assembled as described by the diagram in (Figure-1) using configuration-A, for the physically entrapped enzymes and configuration-B for the chemically bound or soluble entrapped enzymes. The base sensor used is chosen according to the enzyme reaction to be studied. It must respond either to one of the products or to one of the reactants of the enzyme system.

Working of biosensor and its classification :

Biosensor is a device which is associated with the electronic signal processors that are primarily responsible for the display of the results in a user-friendly way. Biosensor obtains quantitative and semi-quantitative information using biological recognition element (biochemical receptor), which is in a space contact with a transducer (21). The readers are usually custom-designed and manufactured to suit the different working principles of biosensors (38). Enzyme based biosensor has large protein molecule that acts as a catalyst in chemical reactions, but remains unchanged at the end of reaction. An enzyme upon reaction with a substrate forms a complex molecule which under appropriate conditions forms the desirable product molecule releasing the enzyme at the end.

Biosensors may be classified according to a biological recognition material, a way of transformation of a physical–chemical signal into electrical one, by a transducer, a registered reaction or an analyzed substance.

Enzyme reactors can operate batch-wise or continuously. Batch processes with the enzymes usually hydrolyses in an aqueous reaction medium. Despite its numerous applications have several drawbacks, as enzymes are poorly stable and hard to recover in such systems, leading to



(A) (B)

Fig. 1. Different methods for preparation of enzyme electrodes based biosensors (configuration-A by Physical entrapment and configuration-B by Chemically attached enzyme)

low productivity. Poor stability is usually the limiting factor in any enzyme process so that enzyme stabilization during reactor operation is a major concern (39, 40). Immobilized enzymes can be used in batch processes but in this case the enzyme is recovered to be used in subsequent batches until the accumulated inactivation makes necessary to replace the spent biocatalyst. As a consequence, specific productivity (mass of product/mass of biocatalyst time of operation) is increases and bioreactor design becomes flexible to suit the needs of a given process (41).

Types of biosensors

Resonant Biosensors : A resonant biosensor uses wireless modules for data transmission, communication and alarm. Photonic sensor technologies are advantageous as they are immune to outside electromagnetic interference, permit compact format and some types enable effective light input and output (42). The functioning of resonant biosensors based on fact that, the molecule (or antigen) gets attached to the membrane and the mass of the membrane changes. The resulting change in the mass subsequently changes the resonant frequency of the transducer (43).

Optical-detection Biosensors : The optical-detection biosensors device is based on detecting changes in absorption of a gold layer. In this device the changes in absorbance or fluorescence of an appropriate indicator compound and do not need a total internal reflection geometry. One of such example is, a fully operational prototype device detecting casein in milk has been fabricated. Another widely used biosensor is based on the micro-array analysis which could be proved to be a vital tool in research (44).

The biosensor also can be made based on optical diffraction or electrochemiluminescence, where transduced signal measures light. In optical diffraction based devices, a silicon wafer is coated with a protein via covalent bonds. The wafer is exposed to UV light through a photo-mask and the antibodies become inactive in the exposed regions. When the diced wafer chips are incubated in analytes, antigen-antibody bindings are formed in the active regions, thus creating a diffraction grating (45). This grating produces a diffraction signal when illuminated with a light source such as laser. The resulting signal can be measured or can be further amplified before measuring for improved sensitivity.

Thermal-detection Biosensors : These class of biosensors are coupled with temperature detectors. They are constructed by combining immobilized enzyme molecules with temperature sensors (46). When the analyte comes in contact with the enzyme, the heat of reaction of the enzyme is measured and is calibrated against the analyte concentration. The total heat produced or absorbed;is proportional to the molar enthalpy and the total number of molecules in the reaction. The measurement of the temperature is typically accomplished via a thermistor, and such devices are known as enzyme thermistors. Their high sensitivity to thermal changes makes thermistors ideal for such applications (46).

Ion-Sensitive Biosensors : Ion-sensitive sensors work on the principle that the interaction of ions with a semiconductor changes the electric potential of the semiconductor surface (47). The potential changes can then be measured to evaluate the desired parameter. Sensors with ion Sensitive Field Effect Transistor (ISFET) can be constructed by covering the sensor electrode with a polymer layer. This polymer layer is selectively permeable to 4 analyte ions. The ions diffuse through the polymer layer and in turn cause a change in the FET surface potential. This type of biosensor is also called an ENFET (Enzyme Field Effect Transistor) and is primarily used for pH detection (47).

Electrochemical Biosensors : Electrochemical Biosensors involves the generation of ions by various chemical events that change the electrical properties of the analyte solution (48). The concentration of the analyzer is then measured with respect to this change. Such biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc(49,

50). A comparative discussion of different types of electrochemical biosensors is given in Table 2 (49-53).

Electrochemical Biosensors further can be classified based on the measuring electrical parameters such as: (1) conductimetric, (2) amperometric and (3) potentiometric.

Conductimetric : Conductometric biosensors are based on principle that when electrochemical reactions produce ions or electrons, the overall

Table 2. Different types of transducers used in biosensor construction

Transducer	Examples
Electrochemical	Clark electrode; mediated electrodes; ion-selective electrodes (ISEs); field-effect transistor (FET)-based devices; light addressable potentiometric sensors (LAPS)
Optical	Photodiodes; waveguide systems; integrated optical devices
Piezoelectric	Quartz crystals; surface acoustic wave (SAW) devices
Calorimetric	Thermistor; thermopile
Magnetic	Bead-based devices

Table. 3. Analytical application of enzyme based biosensors

Sr. No.	Analyte	Enzymes	Detection principle	Reference
1	Alanine aminotransferase	Pyruvate oxidase	Colorimetric	(55)
2	á-Amylase activity	Glucan 1,4-á-glucosidase + GOD	H_2O_2 or O_2	(56)
3	Urea	Urease	Cation electrode	(23)
4	Lactic acid	LDH + Cataiase	$Fe(CN)_6$ redox electrode	(57)
5	Galactose	Galactose oxidase	H_2O_2	(58)
6	Glucose	GOD	O_2	(22)
7	Phenol	Tyrosinase	O_2	(59)
8	Glutamine	Glutaminase	pH	(60)
9	Lactate/glucose	LOD/GOD	O_2	(61)
10	Cholesterol esters	Cholesterol esterase/ oxidase	H_2O_2	(62)
11	Ascorbic acid	Ascorbic acid oxidase	Thermistors	(63)
12	Sucrose	Invertase	Thermistors	(64)
13	Sucrose	Invertase, Mutarotase, GOD	O_2	(65)
14	Maltose	Amyloglucosidase + GOD	H_2O_2	(66)
15	Sucrose	Invertase, Mutarotase, GOD	O_2	(67)
16	Amino acids	Amino acids oxidase	O_2	(68)
17	Phosphate	Acid phosphatase + GOD	O_2	(69)
18	Carboxylic acid	Alcohol oxidase	O_2	(70)
19	Penicillin	Penicillinase	H^+	(71)
20	Glucose	GOD	Optic fiber	(72)

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conductivity or resistivity of the solution changes. This change is measured and calibrated to a proper scale. Conductance measurements have relatively low sensitivity. The electric field is generated using a sinusoidal voltage (AC) which helps in minimizing undesirable effects such as Faradaic processes, double layer charging and concentration polarization.

Amperometric : Amperometric transducers measure the flux of the electroactive species they are set up to detect. They have been used most widely to determine the decrease in oxygen tension or the increase of product (H_2O_2) in enzymatic oxidation-reduction reactions (51, 52).

Potentiometric: Potentiometric biosensors are generally chosen so as to detect the product of the enzyme reaction. In particular, pH electrodes and ammonium ion selective electrodes have been used for hydrolytic enzyme based electrodes (53,54).

Applications of biosensors in various fields

The commercial value of enzymes is linked to their applications as process catalyst and various medical fields (i.e. medical diagnostics, in-vitro and in-vivo diagnostics etc.) as described below (22, 56-72).

Biosensors for medical diagnostics:- Biosensors have wide range of application, the most important application is in the field of medical diagnostics for both In vivo and in vitro .

In vitro diagnostics:- In vitro medical diagnostics has a worldwide good market income but this required strict pre-market approval for these diagnostic tests. These diagnostic tests fall in following categories.

Centralized tests in hospitals:- These tests conducted in hospitals include tests for glucose, lactate, uric acid, viruses, and a variety of pathogenic microbes.

Tests in doctors clinics:- Analysers (in the form of portable biosensors) to be used in the nursing homes or in private clinics of practicing doctors, are also being developed for testing glucose, lactate, creatinine and urea.

Analytical application of biosensors:- The analytical application of enzyme based biosensors are receiving increasing attention as it has a direct role in various fields and majorly in medical field (22, 56-72). For the determination of a specific analyte, choice of enzymes and detection principle are compiled in Table 3, which give easier access to the various procedures and the choice of method to serve as a starting point of detection of analytical assays for a given analyte.

Conclusion

Immobilization of enzymes improves stability of enzymes. Recent use of immobilized enzymes in analytical applications is enjoying tremendous popularity. Today's biosensor market is dominated by glucose biosensors and pregnancy kits. Here in the present review we have tried to look into the historical background, classification, growth and development of the enzyme biosensor field from a strong commercial viewpoint. The current status of the technology is evaluated and future trends in this dynamic and fast-moving field are also anticipated.

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