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Recent Trends of Biosensors in Japan

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Abstract

Remarkable progress has been made in the development of bioelectrochemical sensors, and various kinds of biosensors have been developed and applied in analytical chemistry. Many of these enzyme, microbial, and immunosensors have been recently developed in Japan and in this paper, recent trends in Japanese biosensor development are described.

Index Entries: Bioelectrical sensors; biosensors; sensors, bioelectrical; immobilized biocatalysts; biocatalysts, immobilized; electrochemical sensors; enzyme sensors; immunosensors; microbial sensors; organelle sensors

Introduction

Recently the applications in analytical chemistry of enzymes and other biologically active substances have been gaining popularity and indeed, one of the most important areas in enzyme engineering is the analytical application of immobilized biologically active substances. Biosensors consist of immobilized biocatalysts and physicochemical devices. The chemical information of the substrates is transformed to physical information such as an electric signal, by the biosensor. Various kinds of biosensors using transducers, such as electrodes, thermistors, photon counters, and so forth, have been developed for the determination of biorelated compounds. Electrochemical monitoring of these compounds has definite advantages in food, fermentation, clinical, and environmental analyses and proc-

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ess control. For example, (1) wide concentration ranges are measurable without dilution; (2) the sample for assay does not need to be optically clear; (3) the measurement values of the sensor are obtained directly as electric signals, and so on. Extensive investigations on the development of electrochemical sensors have been made by author's groups.

Electrochemical Sensors

The principle of the sensor is based on selective detection by electrochemical devices of substances involved in the biochemical reaction system. In potentiometric methods, the concentrations of the ions involved in the biochemical reaction is determined by an ion-selective electrode. The Ammonia electrode, the pH electrode, and the carbon dioxide electrode are each used as such devices. On the other hand, in the amperometric method, it is determined from the current values resulting from electrode reaction of electroactive substances involved in the biochemical reaction, and the oxygen electrode, the fuel cell-type electrode, and a polarographic device are used. Table 1 shows various kinds of electrochemical sensors classified by the biocatalysts.

Enzyme Sensors

The enzyme sensor consists of immobilized enzymes and electrochemical devices. This sensor has an excellent selectivity for biological substrates because it employs enzymes as catalysts. The sensor can directly determine a single compound in a complicated mixture without the need for a prior separation step. Since the sensor's characteristics, such as a high sensitivity, long-term stability, and rapid response, markedly depend on nature of immobilized enzymes, developmental investigations for the enzyme sensor were mainly devoted to improving the properties of immobilized enzymes. Recently, sensors for the assay of water-insoluble substances, such as lipids, have been developed and applied to the determination of lipids in sera (*8–10*). Potentiometric detection of enzyme inhibitors (aprotin) was studied using titanium electrodes chemically modified with enzymes (trypsin) (*40*). Table 2 summarizes the characteristics of various enzyme sensors.

TABLE 1
Electrochemical Sensors

Sensor	Catalyst	Substrate
Enzyme sensor	Enzyme	Amines, carboxylic acids, glucose, lipids, uric acid, and urea
Microbial sensor	Microorganism	Alcohols, amino acids, antibiotics, carboxylic acids, sugars, and vitamins
Immunosensor	Antigen or antibody	Hormone, human serum albumin, immunoglobulin G, α-fetoprotein, and syphilis
Organelle sensor	Organelle	NADH and succinate

TABLE 2
Characteristics of Enzyme Sensors

Sensor	Immobilized enzyme	Device	Range, mg/mL	Response time, min	Stability, days	Ref.
Glucose	Glucose oxidase	Oxygen electrode	0.5–360	0.17	100	*1*
Glucose	Glucose oxidase	Oxygen electrode	0–4000	0.25	60	*2*
Glucose	Glucose oxidase	Oxygen electrode	0–7000	6	88	*3*
Cholin	Cholin oxidase	Oxygen electrode	0.1–13	0.1	30	*4*
Lactate	Lactate oxidase	Oxygen electrode	0.05×10^{-4}–0.8×10^{-4}	1		*5*
Monoamine	Monoamine oxidase	Oxygen electrode	7–28	4	7	*6*
Pyruvate	Pyruvate oxidase	Oxygen electrode	8.8–70	2	10	*7*
Total cholesterol	Cholesterol esterase, cholesterol oxidase	Pt–Pt(H_2O_2)	10–5000	3	30	*8*
Phospholipid	Phospholipase D Cholin oxidase	Pt–Pt(H_2O_2)	750–5000	2	30	*9*
Neutral lipid	Lipoprotein lipase	pH electrode	4.4–44	1	14	*10*
Urea	Urease	Ammonia electrode	60–1200	3	14	*11*
Uric acid	Uricase	CO_2 electrode	17–420	5	10	*12*

Microbial Sensors

Enzymes are generally expensive and unstable. Since many compounds that inhibit enzyme reaction are sometimes present in culture media, enzyme sensors are not always applicable to fermentation processes. On the other hand, microorganisms themselves contain various enzymes, and as a result, immobilized living whole cells can be applied to electrochemical sensors. Recently, many techniques have been developed for immobilizing such living whole cells (*13*). Thus, microbial sensors using immobilized whole cells and electrochemical devices have been developed by the authors (*14–16*). Microbial sensors also have potential applications in the fermentation industries and in environmental analysis (*17*). Single enzymes, multi-enzyme systems, coenzymes, and even whole functions of living microorganisms can be utilized for the microbial sensors. Such microbial sensors are classified into two categories, as shown in Fig. 1.

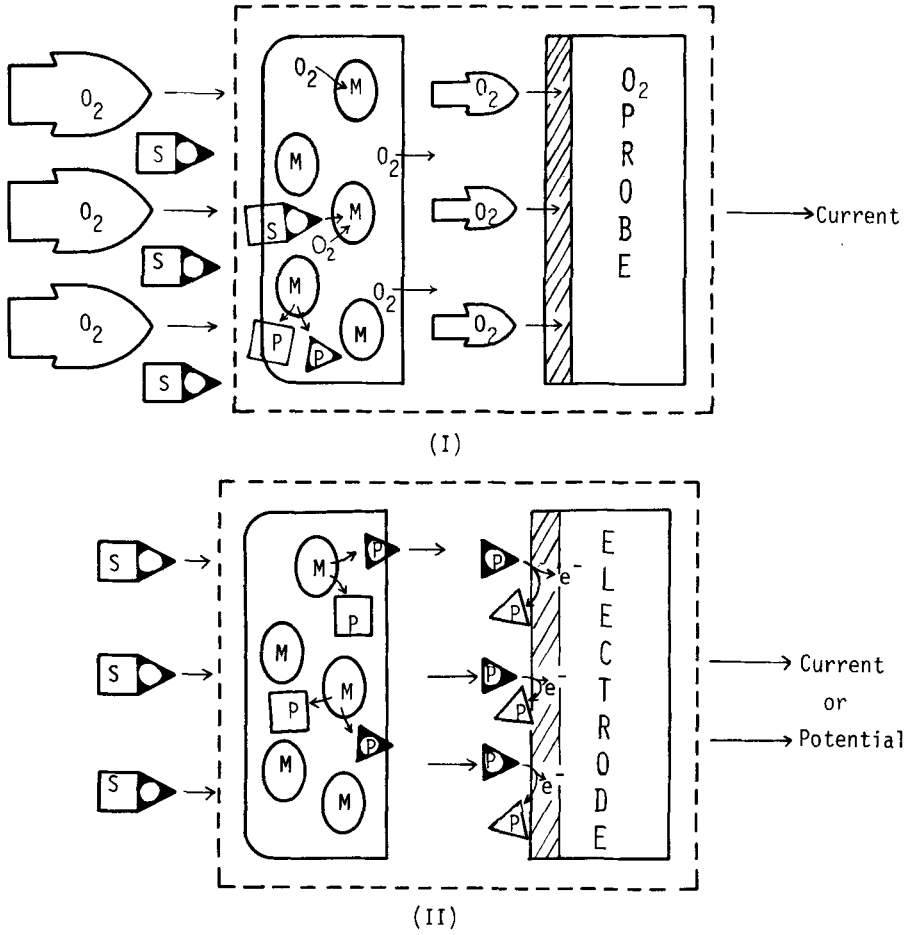

Fig. 1. Principle of the microbial sensor. (I) Amperometric determination of respiration activity. (II) Amperometic or potentiometric determination of metabolites (electroactive substances). S, substrate; P, products (electrochemically inactive); P product (electrochemically active); M, immobilized microorganism.

The principle of the amperometric microbial sensors is as follows. Assimilation of organic compounds by microorganisms can be determined from the respiration activity of microorganisms. The respiration activity can be directly monitored by an oxygen electrode. Therefore, it is possible to construct a microbial sensor for organic compounds using immobilized microorganisms and an oxygen electrode. Various microbial sensors that have been developed are summarized in Table 3. Several microbial sensors have been already applied to on-line measurements of organic compounds that exist in fermentation process.

Immunosensors

Immunosensors originated from investigations of antigen-bound membranes, which are known to generate a transmembrane potential in assoication with the immunochemical reaction with free antibody in a solution (*33–34*). This sensor is characterized by electrochemical determination of the antigen–antibody complex formed at the membrane–solution surface. The immunosensor can be applied to serology tests for syphilis (*35*), blood typing (*36*), and serum albumin determination (*37*). Titanium electrodes chemically modified with antigen or antibody were also developed by Tsubomura et al. (*38*). The shift of potential of the electrodes because of their reactions with their counterparts have been measured and applied to detect human chorionic gonadotropin (HCG) and antibody of HCG (*38–40*). On the other hand, a new approach to the construction of a biosensor was investigated by Fujiwara et al. (*41*). Thin-layer potentiometric measurements of lipid antigen–antibodies were performed using tetrapentyl ammonium (TPA^+) ion loaded liposomes, TPA^+ ion-sensitive electrodes, and a Ag/AgCl plate-shaped reference electrode. These sensors were applied to evaluate the level of ganglioside, dinitrophenylated antibody–hapten, and cardiolipin antibody hapten (*42, 43*).

Enzyme immunoassays utilizing an enzyme as a labeling agent have been d eveloped for clinical analysis. Recent enzyme immunosensors coupling an enzyme immunoassay system and an electrochemical device have been developed for sensitive and rapid microassay (limiting concentration: 10^{-9}–10^{-2} g/mL, of immunoglobulin G (Ig G), HCG, and α-fetoprotein. Table 4 shows the characteristics of several enzyme immunosensors. Catalase was used as the labeling agent in each sensor.

Organelle Sensors

Organelles in cells show high biochemical activity. An organelle sensor consisting of immobilized electron transfer particles from mitochondria and an oxygen electrode has been applied to the determination of NADH (the reduced form of nicotinamide adenine dinucleotide) (*49*). An organelle sensor using immobilized rat liver microsome for SO_x is also under investigation in our laboratory.

New Trends in Electrochemical Sensors

New sensors hybridizing enzymes and microbial cells can be applied to the determination of an enzyme activity in biological fluids and, moreover,

TABLE 3
Characteristics of Microbial Sensors

Sensor	Immobilized enzyme	Device	Range, mg/mL	Response time, min	Stability, days	Ref.
Acetic acid	*T. brassicae*	Oxygen electrode	10–100	15	30	*18*
Ammonia	*N. europea*	Oxygen electrode	0–1.3	8	14	*19*
Assimilable sugars	*B. lactofermentum*	Oxygen electrode	20–200	10	20	*20*
BOD	*T. cutaneum*	Oxygen electrode	0–60	20	17	*21*
Ethyl alcohol	*T. brassicae*	Oxygen electrode	3–30	15	30	*22*
Glucose	*P. fluorescens*	Oxygen electrode	2–20	10	14	*23*
Methyl alcohol	*Unidentified bacterium*	Oxygen electrode	2–22.5	10	10	*24*
Nystatin	*S. cervisiae*	Oxygen electrode	0.5–80[a]	60	20	*25*
Cephalosporins	*C. freundii*	pH electrode	60–500	10	7	*26*
Nicotinic acid	*L. arabinosus*	pH electrode	0.05–5	60	30	*27*
Glutamic acid	*E. coli*	CO_2 electrode	8–800	5	15	*28*
Formic acid	*C. butyricum*	Fuel cell	10–1000	20	20	*29*
Vitamin B_1	*(L. fermenti)*[b]	Fuel cell	0.001–0.05	360	60	*30*
Cell population						
S.cerevisiae		Fuel cell	10^{10}–10^{11}[c]	15	60	*31*
L.fermentum		Fuel cell	10^{10}–10^{11}[c]	15	60	*31*
B.subtilis		Fuel cell	10^8–2×10^9[c]	5	17	*32*

[a]Units/mL. [b]Free cell. [c]Numbers/L.

TABLE 4
Characteristics of Enzyme Immunosensors

Sensor	Device	Range, g/mL	Response time, min	Ref.
Immunoglobulin G	Oxygen electrode	10^{-4}–2.0×10^{-3}	30	*44,45*
Insulin	Oxygen electrode	2.4×10^{-4}–6.0×10^{-4}		*46*
Human chorionic gonadotropin	Oxygen electrode	2.0×10^{-2}–10^{2a}	10	*47*
α-Fetoprotein	Oxygen electrode	10^{-11}–10^{-8}	0.5	*48*

[a]IU/mL.

electromicrobioassay for amino acids (*5, 50*), vitamins, and antibiotics. Furthermore, studies are now being directed toward developing biosensors using animal, plant, and cultured tissues.

In conclusion, the development of electrochemical sensors for various kinds of substrates appears quite promising, and is very attractive for possible use in the routine analysis of many kinds of substrates in analytical chemistry.

Other Biosensors

Biochemical measurements using thermistor (biothermal sensor), piezoelectric element (biosound-sensitive sensor), and photon counter (bioluminous) sensors are now under investigation in our laboratory.

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