PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Immunosensors [and Discussion]

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Immunosensors

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The current trends and future aspects of the research and development of immunosensors are overviewed. A non-labelled immunosensor, whose selectivity depends on immunochemical affinity of an antigen for its corresponding antibody, has been developed as the basis for the potentiometric determination of an antigen, with an antibody-bound membrane or electrode. Non-labelled immunosensors for syphilis antibody, blood typing, human chorionic gonadotropin (HCG), and human serum albumin have been investigated.

In contrast with non-labelled immunosensors, labelled immunosensors may be characterized by marked enhancement of sensitivity. Of these labelled immunosensors, enzyme immunosensors that use the chemical amplification of a labelling enzyme for sensitivity are promising. Enzyme immunosensors with an oxygen electrode have been developed to determine AFP, HCG, IgG and toxin. Bioaffinity sensors with a preformed metastable ligand–receptor complex, which are similar to the enzyme immunosensor have been found effective for the determination of thyroxine (T_4), biotin, and insulin.

1. Introduction

To date, various biosensors have been developed which exploit the fact that the use of biomolecules is effective in providing a sensor with high selectivity (Aizawa 1983; Suzuki 1984). The biosensor depends on matrix-bound biomolecules for molecular recognition and on an electronic device for signal transduction, as schematically illustrated in figure 1. Highly selective biosensors may be prepared by coupling enzymes with potentiometric or amperometric devices, and have found increasing use, particularly in biomedical analysis. Other new developments include a handful of examples of microbial sensors incorporating matrix-bound microbial cells that act on selected substrates.

In the previous decade there were many reports describing immunosensors, which couple immunochemical reactions to various electrodes. The selective reaction between an analyte (antigen) and the corresponding antibody ensures the specificity of the immunosensor in biological fluid analysis. Immunosensors fall into two general types: non-labelled and labelled.

Castillo *et al.* (1966) have shown that immune damage to biological membranes can be mimicked in bilayer lipid membranes (BLM). Related experiments have also been carried out on liposomes (Michaels *et al.* 1976; Rosenstreich & Blumenthal 1977). Janata (1975) coated a platinum electrode with a thin film of polyvinyl chloride containing Concanavalin A. The electrode responded in electrode potential to the corresponding saccharide. An immunoresponsive polymer membrane has been developed by Aizawa *et al.* (1977*a*). An antigen-containing membrane responded specifically (in transmembrane potential) to the corresponding antibody in solution. From these findings, it has been proposed that a non-labelled immunosensor may be produced.

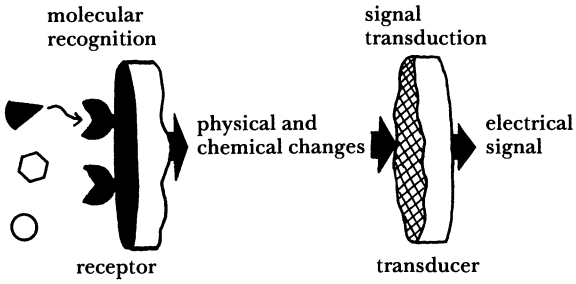

FIGURE 1. Schematic diagram of a generalized biosensor.

There are many types of label that can be used in immunoassay systems: (1) radioisotopes, (2) fluorophores, (3) enzymes, (4) particles, and (5) precipitins. Although sensitivities obtained by radioimmunoassay (RIA) are high, alternative techniques are being developed to overcome the need for radioactive labels. These include enzyme immunoassay (EIA) and fluoroimmunoassay (FIA). A novel immunosensor has been developed by Aizawa *et al.* (1976) on the principle of EIA, which is dependent on the immunochemical affinity for selectivity and on the chemical amplification of an enzyme for sensitivity. Chemical amplification is particularly effective in ultratrace analysis. Various chemical amplification systems are available to enhance the sensitivity of biosensors; these systems should prove of practical importance in designing biochemical sensing systems.

Several other electrode immunoassays are now available. Applications of ion-selective electrodes (ISE) have been extended to involve immunoassays. New electrodes selective for organic compounds have been developed, and new uses for conventional electrodes have been found for direct detection of the antigen (or antibody) or of an appropriate label. Interest has since increased in the coupling of immunochemical reactions to various electronic devices.

Like many important movements in science, progress in immunosensors draws inspiration from concurrent developments in other areas. This review describes the current trends and the future aspects of the research and development of immunosensors.

2. NON-LABELLED IMMUNOSENSORS

(*a*) *Potentiometric immunosensors with antigen-(or antibody-) coated membrane*

A potentiometric immunosensor, which derives its selectivity from the immunochemical affinity of an antigen for its corresponding antibody, has been developed for the determination of syphilis antibody in human sera (Aizawa *et al.* 1977*a–c*, 1979*a*). Several other potentiometric immunosensors have also been reported (Janata 1975; Yamamoto *et al.* 1978; Aizawa *et al.* 1980*a*). These sensors consist of a solid matrix (membrane and electrode)-bound antigen (or antibody); thus the antigen–antibody complex formation on the matrix is followed by measuring membrane (or electrode) potential, as schematically represented in figure 2.

If an antigen is attached to a membrane, the membrane reacts specifically with the corresponding antigen at the membrane surface, as shown in figure 3. Such an immunochemical reaction of the membrane-bound antigen with free antibody in solution may cause a change of charge density at the membrane–solution interface, generating a transmembrane potential change.

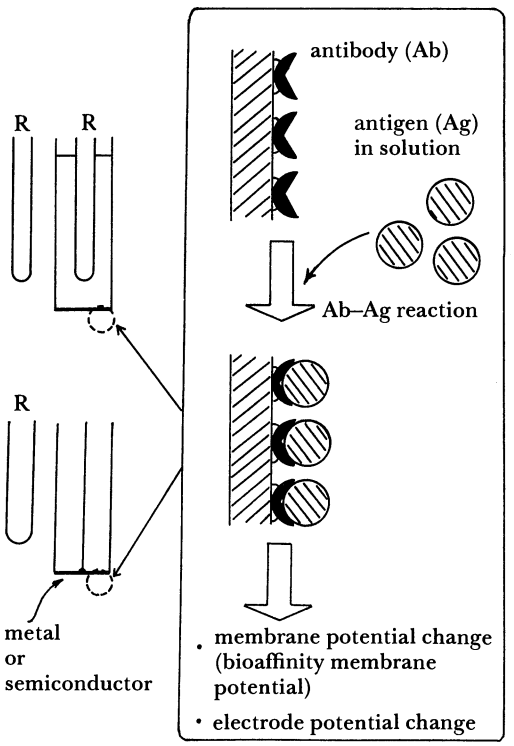

FIGURE 2. Schematic diagram of a non-labelled immunosensor; R, reference electrode.

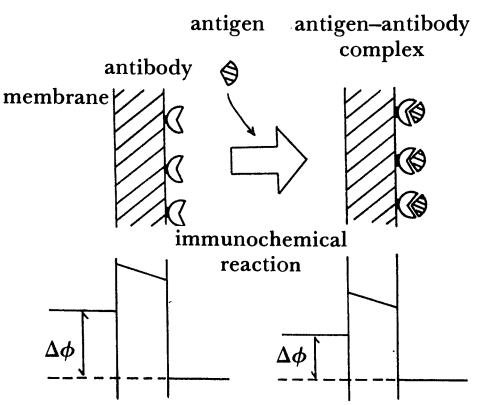

FIGURE 3. Potential profiles across an antibody-bound membrane before and after immunochemical reaction.

The membrane potential across a fixed charge membrane, $\Delta\phi$, is approximated by the following equation, when uni-univalent electrolytes are separated:

$$\Delta\phi = (RT/F)\{\ln(C_1/C_2) - \ln[-\theta + \sqrt{(\theta^2 + 4C_1^2)}]/[-\theta + \sqrt{(\theta^2 + 4C_2^2)}] + (1-2t)\ln[(1-2t)\theta + \sqrt{(\theta^2 + 4C_1^2)}]/[(1-2t)\theta + \sqrt{(\theta^2 + 4C_2^2)}]\}, \quad (1)$$

where C_1 and C_2 $(C_1 > C_2)$ are electrolyte concentrations, θ is the charge density of the membrane phase, and t is the ion transport number in the membrane phase (Kobatake 1965). The first and second terms result from the surface potential and the third term from diffusion

potential. In extremely high electrolyte concentrations ($C \gg \theta$), the membrane potential is approximated by diffusion potential. On the other hand, the surface potential may be predominant when electrolyte concentration is infinite.

The potential profiles across the antibody-bound membrane are presented in figure 3, along with the conceptual illustration of the immunochemical reaction on the membrane surface.

(*i*) *Immunosensors for syphilis*

An immunosensor for syphilis was constructed by using an Ogata antigen-binding membrane, as represented in figure 2. The membrane contained 37.5 μg Ogata antigen per square centimetre of membrane. Figure 4 presents the relation between the immunochemically induced potential change and the concentration of the antibody solution with which the

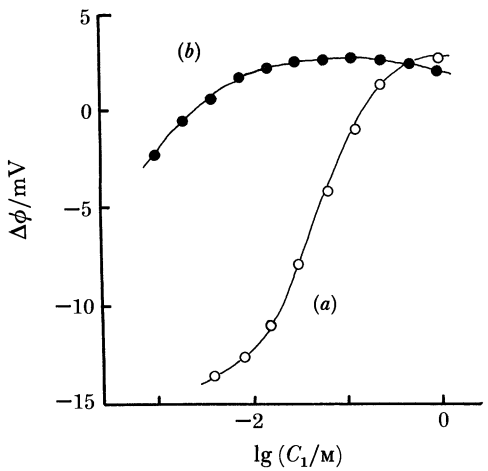

FIGURE 4. Immunochemically induced potential change across an Ogata antigen-bound membrane. (*a*) Before immunochemical reaction; (*b*) after immunochemical reaction.

immunosensor was contacted. The antibody solution was made up by the dilution of positive control serum, containing the Wasserman antibody, which was used for non-treponemal serology tests for syphilis (supplied from DADE Division, American Hospital Supply Co., Miami), with physiological saline. One arbitrary unit is equivalent to the antibody concentration of positive control serum, the reactivity of which was assayed at a dilution of 1:32 by rapid plasma reagin (RPR) and unheated serum reagin (USR) tests. The immunosensor was extensively washed after each immunochemical reaction.

An appreciable potential change was observed even at an antibody concentration of 1/800 (log[antibody] = −2.9), which was comparable to an 800-fold dilution of positive control serum. The immunochemically induced potential increased with an increase of the antibody concentration.

(*ii*) *Electrochemical typing of blood*

The erythrocyte membrane lipid contains the blood group determinant oligosaccharide (table 1). Blood group may be determined by immunochemical agglutination of the blood group substance with agglutinin (antibody) contained in serum. Electrochemical typing of blood was performed with control sera for blood group tests. A membrane containing type A

blood-group substance (EL(A) M) was mounted in an immunosensor (figure 2). The immunosensor contained $\frac{1}{512}$ M NaCl as an inner electrolyte solution. A potential of −7.2 mV was generated when the sensor was immersed in a $\frac{1}{64}$ M NaCl solution. A membrane containing type B blood-group substance (EL(B) M) developed a membrane potential of −12 mV under the same conditions.

TABLE 1. BLOOD-GROUP DETERMINANTS OF ERYTHROCYTE MEMBRANES

blood group	structure
O	Gal-GlcNAc – – – Fuc
A	GalNAc–Gal–GlcNAc – – – Fuc
B	Gal–Gal–GlcNAc – – – Fuc

Abbreviations: Gal, galactose; Fuc, fucose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine.

Type A serum was allowed to react with the EL(A) M and the EL(B) M of immunosensors at 25 °C for 30 min. After washing with a 0.9% (by mass) NaCl solution and water, the immunosensors were placed in a $\frac{1}{64}$ M NaCl solution. The transmembrane potentials across the EL(A) M and EL(B) M were −7.2 mV and −33.6 mV, respectively. Only the EL(B) M showed a negative shift in membrane potential; therefore the test serum contained anti-B antibody.

Similar experiments were performed with type B serum. An EL(A) M and EL(B) M showed membrane potentials of −8.2 mV and 12 mV, respectively, before the reaction. The membrane potentials changed to −34 mV and 7.2 mV, respectively, due to the reaction. Only the EL(A) M membrane potential changed after the reaction, because the serum contained anti-A antibody.

Table 2 summarizes the membrane potential changes resulting from the contact of each membrane with a test serum. The data are average values for repeated tests. Based on these results, the following electrochemical typing of blood is possible:

Type A: EL(A) M, none; EL(B) M, negative shift.
Type B: EL(A) M, negative shift; EL(B) M, none.
Type AB: EL(A) M, negative shift; EL(B) M, negative shift.
Type O: EL(A) M, none; EL(B) M, none.

Any negative shift in membrane potential is attributed to the agglutination of membrane-bound blood-group substance; therefore, blood can be typed by checking whether the test serum causes the EL(A) M and EL(B) M to shift their membrane potential.

TABLE 2. MEMBRANE-POTENTIAL CHANGE (mV) DUE TO AGGLUTINATION OF MEMBRANE-BOUND BLOOD GROUP SUBSTANCE

(EL(A)M and EL(B)M are membranes containing type A and type B blood-group substances, respectively. The membrane was allowed to react with each serum for 30 min. The membrane potential was measured before and after reaction, while maintaining C_1/C_2 at 8.)

	EL(A)M	EL(B)M
Type A serum	0±2	−28±2
Type B serum	−22±2	0±2

(*iii*) *Immunosensors for HSA*

An antibody-bound membrane was used to construct an immunosensor for HSA. The structure of the immunosensor is illustrated in figure 2. An electrolyte solution (4 mM KCl) was contained in the immunosensor.

The immunosensor was immersed in a phosphate buffer containing 1 mg ml^{-1} HSA at 37 °C for 1 h. After thorough washing, the immunosensor was placed in 32 mM KCl. The sensor output was reduced by 4.8 mV. The output change is caused by the immunochemical adsorption of HSA to the antibody-bound membrane of the sensor. The sensor output difference decreases with a decrease in HSA, reaching the minimum at a concentration of 10^{-6} g ml^{-1}.

(*b*) *Potentiometric immunosensors with antibody-coated electrode*

Yamamoto *et al.* (1978) covalently immobilized anti-human chorionic gonadotropin (HCG) antibody on the surface of a TiO_2 electrode. The antibody-bound electrode responded in electrode potential to HCG in solution; this result suggested a possible development of another potentiometric immunosensor.

3. Labelled immunosensors

(*a*) *Enzyme immunosensors with an oxygen electrode*

An enzyme immunosensor is an analytical device which is dependent on the immunochemical affinity for selectivity and on the chemical amplification of a labelling enzyme for sensitivity.

When catalase, which catalyses the evolution of oxygen from hydrogen peroxide,

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2, \qquad (2)$$

is the labelling enzyme for an antigen, the enzyme immunosensor is constructed by assembling an antibody-bound membrane and an oxygen-sensing electrode. In heterogeneous enzyme immunoassay, the labelling enzyme is measured by amperometry with the oxygen-sensing device. The enzyme immunosensor requires an extremely short time for measuring the labelling enzyme; consequently, rapid and highly sensitive enzyme immunoassay may be accomplished with the enzyme immunosensor (Aizawa *et al.* 1976, 1979*b*, 1980*b*).

The enzyme immunosensor is prepared by attaching the antibody-bound membrane to a Clark-type oxygen electrode (figure 5) which has an oxygen-permeable plastic (e.g. Teflon) membrane on the cathode surface and responds sensitively and rapidly to oxygen.

(*i*) *Enzyme immunosensor for AFP*

A sheet of anti-α-fetoprotein (AFP) antibody membrane, attached to the sensor was placed in contact with 260 'catalase units' of catalase-labelled AFP at pH 7 and 37 °C for 2 h. After thorough washing, the sensor was placed in phosphate buffer. When it gave a steady-state current from dissolved oxygen, 100 μl of 3% (by volume) hydrogen peroxide was injected. The sensor responded very rapidly to the generation of oxygen, (figure 6). A steady current was obtained within 30 s.

A calibration curve for competitive enzyme immunoassay for AFP with the sensor is presented in figure 7. It shows that AFP can be determined in the range 5×10^{-11}–5×10^{-8} g ml^{-1}

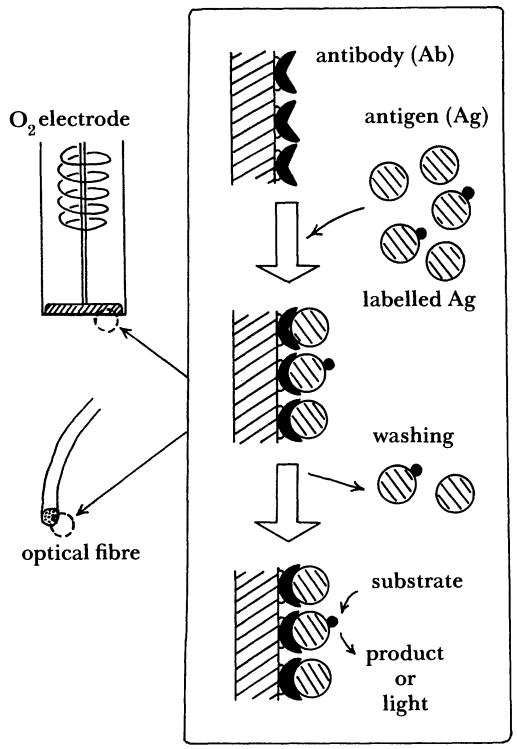

FIGURE 5. Schematic diagram of an enzyme immunosensor that incorporates an oxygen electrode.

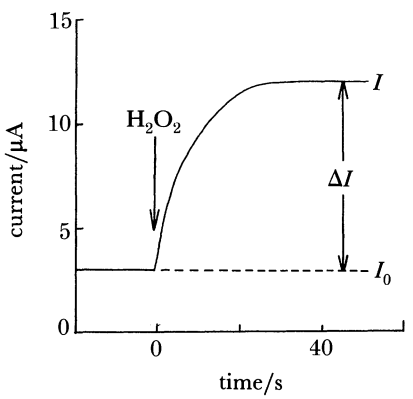

FIGURE 6. Response curve of an enzyme immunosensor for AFP; catalase was used as a label.

The standard deviation for 25 assays of 10^{-9} g AFP was 15%. All the assays were performed with different antibody membranes.

It is noted that the enzyme immunosensor provides very rapid and sensitive enzyme-linked immunosorbent assay (ELISA) compared with photometric ELISA.

(*ii*) *Enzyme immunosensor for toxin*

Ochratoxin A (OTA), a secondary metabolite of *Aspergillus ochraceus*, *Penicillium viridicatum* and strains of some other species of both genera, causes nephropathy in animals and probably also in man. Recently, carcinogenicity has also been demonstrated by feeding experiments. Its

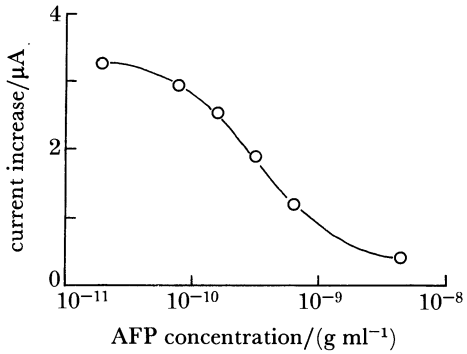

FIGURE 7. Calibration curve for competitive enzyme immunoassay for AFP with an enzyme immunosensor (catalase label).

occurrence, especially in grains, seems to be prevailing not only in European and North American countries but also in Asian countries. OTA has most commonly been determined by chemical analysis, which requires a tedious separation from a sample. Biosensors and radioimmunoassays have also been used, but there were problems in operation. A simplified and rapid analysis for OTA is urgently needed. An enzyme immunosensor for OTA has recently been developed (Hongyo *et al.* 1987).

The toxin sensor consists of an amperometric oxygen electrode and an OTA-bound membrane. Catalase-labelled OTA, which is added at a fixed amount to a sample solution, may react competitively with membrane-bound OTA and with the free OTA to be determined. The sensor is then assayed for amperometric determination of catalase activity. OTA has been selectively and sensitively determined by measuring the sensor output.

Competitive immunoassay of OTA was carried out by using the immunosensor for OTA. OTA was determined under a constant concentration of catalase-labelled antibody. The sensor response was obtained for each concentration of free OTA, where the experiment was carried out in the PBS buffer at 35 °C. The change of sensor output decreased with the addition of free OTA to be determined.

Standard curves under different concentrations of labelled antibody are presented in figure 8. The standard curve shifted to the lower concentration range with a decrease in antibody concentration. The minimum-detection limit was in the order of 10^{-10} g ml^{-1}, when the concentration of labelled antibody was fixed at 20 μg ml^{-1}. Table 3 shows the characteristics of both non-labelled and labelled immunosensors.

(*b*) *Bioaffinity sensors with a preformed metastable ligand–receptor complex*

A new biosensor has been designed on the basis of bioaffinity difference between two ligands, one a determinant and the other an analogue compound in a given binding reaction. In general, an analogue compound shows a lower affinity for the binding protein than a determinant does. Therefore, one can expect the following displacement reaction when a membrane-bound analogue compound, complexed with its binding protein, is exposed to a determinant molecule. The binding protein is displaced from the membrane-bound analogue molecule; it is supposed that the displacement depends on the bioaffinity difference of an analogue molecule and a determinant one, as well as on the determinant concentration. The determinant concentration may easily be measured by detecting the residual molecular complex that remains on the

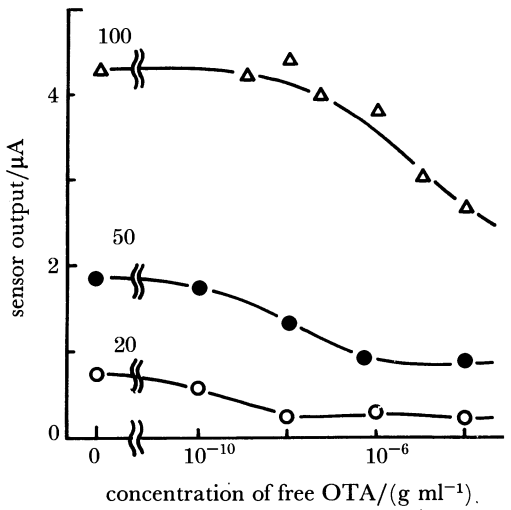

FIGURE 8. Standard curves for OTA determination at different concentrations of catalase-labelled antibody (given in micrograms per millilitre).

TABLE 3. CHARACTERISTICS OF NON-LABELLED AND LABELLED IMMUNOSENSORS

		biosensor assemblies	
determinant	biosensor	receptor	transducer
albumin	immunosensor	anti-albumin	Ag/AgCl electrode
	enzyme immunosensor	anti-albumin (catalase label)	O_2 electrode
IgG	enzyme immunosensor	anti-IgG (catalase label)	O_2 electrode
		(GOD label)	O_2 electrode
HCG	immunosensor	anti-HCG	TiO_2 electrode
	enzyme immunosensor	anti-HCG (catalase label)	O_2 electrode
AFP	enzyme immunosensor	anti-AFP (catalase label)	O_2 electrode
HBs antigen	enzyme immunosensor	anti-HBs (POD label)	I^-electrode
antibody:			
syphilis	immunosensor	cardiolipin	Ag/AgCl electrode
blood group	immunosensor	blood-group substance	Ag/AgCl electrode
antibodies	immunosensor	antigen-bound liposome (TPA^+ marker)	TPA^+ electrode

membrane surface. High sensitivity can be attained by chemical amplification, by using an enzyme catalyst as a label. A biosensor based on the above principle may be termed a 'bioaffinity sensor

(*i*) *Bioaffinity sensor for thyroxine* (T_4)

The working principle of a bioaffinity sensor for thyroxine (3,5,3′,5′-tetraiodothyronine, T_4) (Ikariyama & Aizawa 1982), a thyroid hormone, is diagrammatically shown in figure 9. The sensor is composed of membrane-bound T_4 and enzyme-labelled antibody, i.e. thyroxine is

chemically immobilized on a membrane and the membrane-bound T_4 undergoes immunochemical reaction with enzyme-labelled anti-T_4 antibody to form an immunocomplex. Membrane-bound T_4 has less affinity for the antibody than free T_4 in solution. Attachment of the membrane where antigen–antibody complex is formed on the surface of a galvanic-type oxygen electrode results in a bioaffinity sensor for T_4. When the sensor is immersed in a solution containing free T_4 (to be determined) the antigen–antibody complex will be dissociated on exposure to T_4. The dissociation may be enhanced with an increase in the analyte concentration. The released enzyme-labelled antibody will then form a stable complex with T_4. Consequently, T_4 can be determined by measuring the enzyme activity of enzyme-labelled antibody on the T_4 membrane.

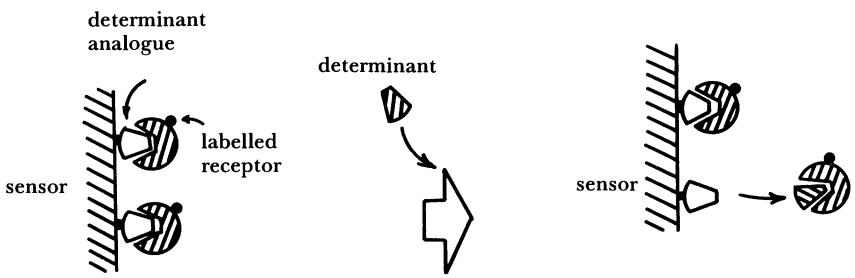

FIGURE 9. Schematic diagram of a bioaffinity sensor.

(ii) Bioaffinity sensor for biotin

Avidin, an egg-white protein, forms a very stable complex with biotin (vitamin H). The protein can also bind analogue compounds of biotin, such as 2-[(4-hydroxyphenyl) azo] benzoic acid (HABA) and lipoic acid, to form metastable complexes. These ligand–avidin complexes dissociate on exposure to biotin to form a very stable biotin–avidin complex. Figure 10 shows the association constants of a few biotin-related compounds in a ligands–avidin binding reaction.

The sensor (Ikariyama & Aizawa 1985) is fabricated from a membrane, on which a molecular complex between an analogue compound of biotin and an enzyme label is prepared, and a galvanic oxygen electrode. HABA and lipoic acid are employed as the analogue

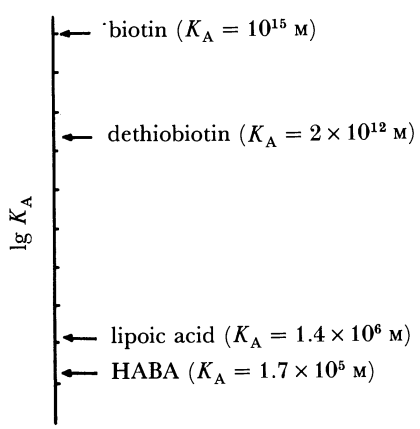

FIGURE 10. Association constants of biotin-related compounds in a ligand–avidin binding reaction.

compounds; biotin and dethiobiotin are the determinants. Sensitization is to be attained by an enzyme amplification technique:

$$[\text{avidin}] + 4[\text{HABA}] \rightarrow [\text{avidin}][\text{HABA}]_4 \xrightarrow{4[\text{biotin}]} [\text{avidin}][\text{biotin}]_4 + 4[\text{HABA}] \qquad (3)$$

Figure 11 illustrates the relation between the incubation time and the change in sensor output. The current change was caused by the molecular complex receptor that remained on the sensor. Biotin recognition with the receptor finished within 10 min after the start of

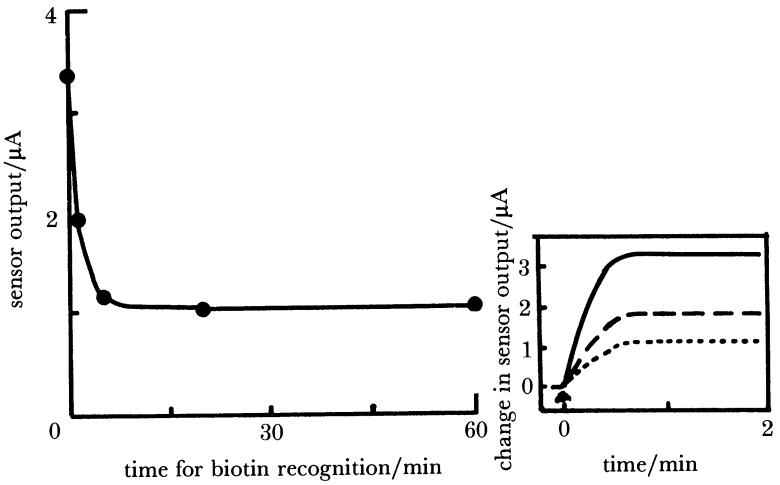

FIGURE 11. Time course of biotin sensor output.

biosensing. The measurement of the remaining receptor finished within 1 min as shown in figure 11. Similar results were observed with membrane-bound lipoate complexed with the labelled avidin. The catalase-labelled avidin that was adsorbed was not fully dissociated even in the presence of excess biotin. Approximately half of the receptor was undissociated. The HABA-immobilized membrane binds the labelled avidins by complex formation (specific binding) and adsorbs them on its nonspecific binding sites. The labelled avidin, specifically bound to membrane-immobilized HABA (or lipoic acid), is expected to dissociate easily in the presence of excess biotin, whereas the nonspecifically adsorbed avidin is not. The response time of the proposed bioaffinity sensor was no longer than 10 min.

(*ii*) *Bioaffinity sensor for insulin*

In a bioaffinity sensor for bovine insulin (Ikariyama & Aizawa 1983) porcine insulin is used as the analogue compound. A porcine insulin-bound plate undergoes immunoreaction with peroxidase-labelled anti-(bovine insulin)antibody to form an immunocomplex. When the plate, on the surface of which the immunocomplex is formed, is immersed on a solution containing free bovine insulin (to be determined), the complex may be dissociated. The dissociated enzyme-labelled antibody then forms a stable complex with bovine insulin in solution. Insulin is thus determined by measuring peroxidase retained on an immunoplate. The luminol–H_2O_2 system is employed to detect peroxidase activity. The emitted light is transferred to a photomultiplier through an optical fibre.

The characteristics of bioaffinity sensors for thyroxine, biotin, and insulin are summarized in table 4.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 4. Characteristics of bioaffinity sensors

ligand	determinant	binding protein	measurable range of concentration/ (g ml^{-1})	detection
HABA	biotin	avidin	10^{-9}–10^{-7}	electrochemical
	dethiobiotin	avidin	10^{-9}–10^{-7}	electrochemical
lipoic acid	biotin	avidin	5×10^{-10}– 10^{-8}	electrochemical
thyroxine (modified)	thyroxine	antibody	10^{-8}– 10^{-5}	electrochemical
insulin (porcine, modified)	bovine insulin	antibody[a]	10^{-7}– 10^{-4}	optoelectronic
insulin (bovine, modified)	bovine insulin	antibody[a]	10^{-8}– 10^{-6}	optoelectronic

[a] Anti-bovine-insulin antibody.

4. Concluding remarks

Immunosensors have been realized by a fascinating combination of immobilized antibody (or antigen) with an electronic device. High selectivity may be attained, by both non-labelled and labelled immunosensors, due to the specific affinity of antigen to the corresponding antibody. However, the immunochemical reaction of matrix-bound antibody (or antigen) is influenced by non-specific adsorption of coexisting substances onto the matrix. Several methods have been proposed to prevent the non-specific adsorption, although they are not satisfactory. It is stressed that further research should be concentrated on the development of improved materials for immunosensors.

Non-labelled immunosensors are characterized by high selectivity and simple operation. Most of these sensors are based on potentiometric measurement. Further investigation is required to seek for novel non-labelled immunosensors with high selectivity and sensitivity.

In contrast with non-labelled immunosensors, labelled immunosensors have succeeded in enhancing sensitivity while retaining high selectivity. Enzyme labels have successfully been incorporated into the immunosensor to work as effective chemical amplifiers. The lower limit of detection is of the order of 10^{-11} g ml^{-1}. Further improvement of sensitivity appears promising.

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Discussion

J. D. R. Thomas (*Applied Chemistry Department, UWIST, Cardiff, U.K.*).

(*a*) Professor Aizawa mentioned that the antigen determination using the catalase-antigen–antibody approach took only a very short time. How is the system regenerated for the next determination, as the antigen–antibody binding on the sensor is quite strong?

(*b*) How was the antibody immobilized on the electrode or associate membrane?

M. Aizawa. (*a*) The system is not regenerated; antigen–antibody complex fixed on the system may be dissociated by acids or protein denaturing reagents. It is, however, tedious to confirm the potentiality of the regenerated system. At present our effort is concentrated on developing a disposable type of immunosensor.

(*b*) The antibody was immobilized on the electrode or polymer membrane by the covalent method.

I. J. Higgins (*Biotechnology Centre, Cranfield Institute of Technology, and The Leicester Biocentre, University of Leicester, U.K.*). Professor Aizawa mentioned the development of species-selective immunosensors for identification and enumeration of particular microbial species. How sensitive and selective are these devices, and are they prone to interference? Have they yet been tested in materials where they might find application, e.g. biological fluids or foodstuffs?

M. Aizawa. Biosensors for living cells incorporate the antibody to microbial cells in the binding protein to the membrane surface markers of B lymphocytes. We have not tested the system in biological fluids or foodstuffs. The sensor responded specifically to B lymphocytes in a mixture of T and B lymphocytes.

W. J. Albery, F.R.S. (*Department of Chemistry, Imperial College of Science & Technology, South Kensington, U.K.*). In Professor Aizawa's toxicity sensor for OTA, a competition is run between

the OTA attached to the membrane and the free OTA in solution. For this procedure to be successful, the amount of OTA bound to the membrane must be very reproducible. How is the required reproducibility achieved?

M. Aizawa. As Professor Albery pointed out, the amount of OTA bound to the membrane should be very reproducible. We do not know whether the present procedures have been carried out under optimal conditions. The reproducibility falls within 10%.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES