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Chemical Amplification in Biosensors

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In the last decade biosubstances such as enzymes and antibodies have been used in conjunction with electrochemical sensing devices to form bioselective sensors (biosensors), which include enzyme sensors, microbial sensors, and immunosensors. An enzyme sensor is the union of an enzyme, that biological catalyst which acts sensitively and specifically with almost all organic and inorganic compounds in nature, with an electrochemical sensor. The result is a sensor which is useful for the assay of organic and inorganic compounds in a manner as simple as a pH measurement with a glass electrode. Since an enzyme is water-soluble, it is immobilized (insolubilized) on solid matrix at or near the sensing device surface to serve as biocatalyst for specific molecular recognition. Due to marked progress in enzyme immobilization techniques, many enzyme sensors have been developed for inorganic and low molecular weight compounds, and evaluated particularly in clinical fields.

For biological and biomedical sensing purposes, the possibilities for making sensitive and selective biosensors for a specific protein are an exciting prospect. An immunosensor, which depends its selectivity on immunochemical affinity of an antigen to the corresponding antibody, has been developed for the determination of syphilis antibody in human sera.<sup>1,2)</sup> The immunosensor provides ultimately high selectivity in the measurement of macromolecules such as proteins. One of the problems remained unsolved, however, is to enhance sensitivity of the immunosensor for the trace analysis of a specific serum component.

The use of labeling agents for the measurement of antigens and antibodies has stimulated the new and expanding field of immunoassay. Among them, enzyme immunoassay, involving the use of antigen or antibody labelled with an enzyme, is competing with radioimmunoassay (RIA) in sensitivity. This paper describes the development of a novel immunosensor based on the principle of enzyme immunoassay, which is dependent on the immunochemical affinity for selectivity and on the chemical amplification of an enzyme for sensitivity.

Catalase, which catalyzes the evolution of oxygen from hydrogen peroxide  $\text{H}_2\text{O}_2 \longrightarrow \text{H}_2\text{O} + 1/2 \text{O}_2$ , was used as an enzyme label for an antigen. A new immunosensor was constructed by assembling an antibody-bound membrane and an oxygen probe, consisting of a platinum cathode, a lead anode, an alkaline electrolyte, and an oxygen-permeable Teflon membrane. The antibody-bound

membrane was prepared from cellulose acetate, 1,8-diamino-4-aminomethyl octane (TA) and glutaraldehyde (GA). Cellulose acetate (250 mg) was dissolved in 5 ml of dichloromethane, and 200  $\mu$ l of 50% GA was added followed by 1 ml of TA. The solution was cast on a glass plate and allowed to stand at room temperature to complete intermolecular cross-linking of TA via GA. The membrane peeled off was placed in a 0.1% solution of GA (pH8.0) at 30°C for 2 h, and was in contact with a solution of antibody. The antibody-bound membrane was firmly fixed onto the Teflon membrane of the oxygen probe.

Several antigens, including alpha-fetoprotein (AFP)<sup>6)</sup>, human chorionic gonadotropin (HCG)<sup>5)</sup> and immunoglobulin G (IgG)<sup>3,4)</sup> were determined according to the following three steps.

Step 1: Nonlabelled antigen to be assayed is added to a solution containing a known amount of the catalase-labelled antigen. Nonlabelled and labelled antigens competitively react with the membrane-bound antibody, resulting in formation of an antigen-antibody complex on the membrane surface of the sensor.

Step 2: The sensor is rinsed with saline to remove nonspecifically adsorbed antigen from the membrane. The antigen-antibody complex remains stable on the membrane surface.

Step 3: The sensor is contact with a hydrogen peroxide solution for enzyme activity assay. The catalase complexed with the membrane-bound antibody decomposes hydrogen peroxide into oxygen and water. The enzymatically generated oxygen diffuses to the platinum cathode through the Teflon membrane and is electrochemically reduced there, with a resulting increase in cathodic current.

Instead of counting the enzyme label, the sensor quantitates the enzymatically and quantitatively generated oxygen; thus the enzyme appears to act as an amplifier (chemical amplification). The sensor responds so rapidly that the enzyme activity assay finishes within 30 s, although the conventional enzyme immunoassay requires several hours. AFP, for instance, was determined in the concentration range  $10^{-11}$  -  $10^{-8}$  g ml<sup>-1</sup>. The newly developed immunosensor with chemical amplification appears feasible in biomedical sensing systems.

#### References

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