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AN IMMUNO SENSOR FOR SPECIFIC PROTEIN

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An immuno sensor for determining specific protein has been developed. The measuring system is characterized by the potentiometric determination of potential difference across an antigen-binding membrane. A lipid antigen containing cardiolipin, phosphatidyl choline, and cholesterol was immobilized to an acetylcellulose membrane. The membrane-bound antigen retained the immunochemical reactivity to Wassermann antibody. The assymmetric membrane potential developed was dependent on the concentration of the antibody.

In the past several years, there has been almost explosive development of electrochemical sensors specific for ions and for organic substances. Some of these sensors depend upon enzymes for their specificity [1-6]. Such enzyme electrodes have been already evaluated in some clinical laboratories for use in measuring important body fluid constituents, such as glucose in blood and serum, to aid medical diagnosis.

Several functional membranes have been prepared in our laboratory by immobilizing an enzyme [7], photochromic compound [8,9], and antigen [10]. The membrane-bound antigen retained the immunochemical reactivity to bind specifically a corresponding antibody. Since a drastic membrane potential shift was found to be associated with the immunochemical reaction between the membrane-bound antigen and the free antibody in a solution, the membrane was called an immuno-responsive membrane [10]. Such an immuno-responsive membrane can be utilized to detect a

specific antibody.

In this report, we intend to describe the development of a new sensor (an immuno sensor) which depends upon the use of immunochemical reaction for its specificity and upon the potentiometric determination of transmembrane potential for its operation. To test this idea, a lipid antigen-binding membrane was applied to assemble a specific immuno sensor for the corresponding antibody employed (Wassermann antibody). The lipid antigen, for use in non-treponemal serology tests for syphilis, contained 0.01 % cardiolipin, 0.04 % phosphatidyl choline and 0.20 % cholesterol in ethanol. Positive Control Serum (DADE) and MONI-TROL IX Serum (DADE) were used as a Wassermann antibody-containing and an antibody-free sera, respectively.

Acetylcellulose (250 mg) was dissolved in 6 ml of acetone. One milliliter of the lipid antigen was homogeneously mixed with the acetone solution of acetylcellulose. The resulting solution was cast on a glass plate (18 x 10 cm^2). The antigen-binding membrane was allowed to stand at 25°C under reduced pressure to dry. An acetylcellulose membrane containing no antibody was prepared in the same manner. In order to confirm the immunochemical reactivity of the membrane-bound antigen, the antigen-binding membrane was reacted with the antibody and then with complement. The complement was fixed by the resulting membrane. This indicates that the membrane-bound antigen retained the immunochemical reactivity.

The immuno sensor was assembled as illustrated in Fig.1. An antigen-free and an antigen-binding membranes were fixed to the respective bottom of the compartment I and III. The compartment I and III were filled with physiological saline. The reference electrodes used were Ag/AgCl. The measurements were conducted at 37°C.

Physiological saline was added to the compartment II. Potential difference between the pair of electrodes remained zero. The saline contained in the compartment II was replaced by the Wassermann antibody-free serum solution which was made up by a 1000-fold dilution of MONI-TROL IX Serum with physiological saline. Since the solution contains no Wassermann antibody, the immunochemical reaction (antigen-antibody complex formation) cannot take place at the membrane-solution interface. However, a potential difference of -0.02 mV was developed as shown

in Fig.2 probably due to the non-specific adsorption of serum proteins.

In contrast, a marked potential difference was generated when a solution of the antibody-containing serum, which was prepared by diluting Positive Control Serum with physiological saline at a 1000-fold dilution, was added to the compartment II. The potential difference gradually increased with time, reaching a steady state value in several minutes as demonstrated in Fig.2. At the time directed in Fig.2, the antibody concentration was doubled by adding the antibody-containing serum to the solution in the compartment II. The potential difference increased again as presented in Fig.2. The extent of the induced potential difference depended sharply on the antibody concentration. These results indicate that the potential difference results from the specific adsorption of the antibody to the membrane by the immunochemical reaction.

Several membrane theories have shown that the asymmetric membrane potential may arise from the difference between two diffused surface potentials on two sides of the membrane which are produced by the fixed charge at the membrane surface and the surrounding electrolyte solution [11]. It has been shown by the previous work that the immunochemical reaction of the antigen-binding membrane resulted in no appreciable change in transport number of ion but associated with marked change in the charge density of membrane phase [10]. In the present sensoring system, the antibody may attach to only one side of the antigen-binding membrane. The attachment of antibody to the membrane was found to cause a decrease in negative charge as described in the previous paper. Since cardiolipin possesses negative charge of the phosphate groups, the antigen-binding membrane can get hold of negative charge. The negatively charged groups of the membrane-bound antigen might be hindered by the Wassermann antibody. Thus, the membrane surface faced to the compartment II might be charged less negatively compared with the other surface. Under the assumption that the concentrations of electrolyte containing in three compartments are equivalent, the potential diagram of the immuno sensor is postulated as depicted in Fig.3. The potential difference across the antigen-binding membrane is attributed to an asymmertic membrane potental.

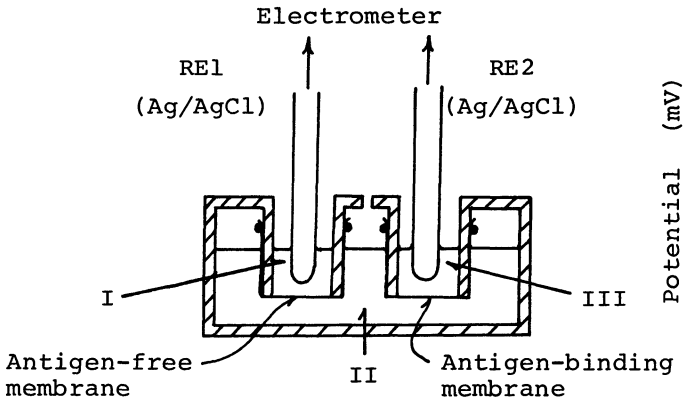

Fig.1 Schematic representation of the immuno sensor

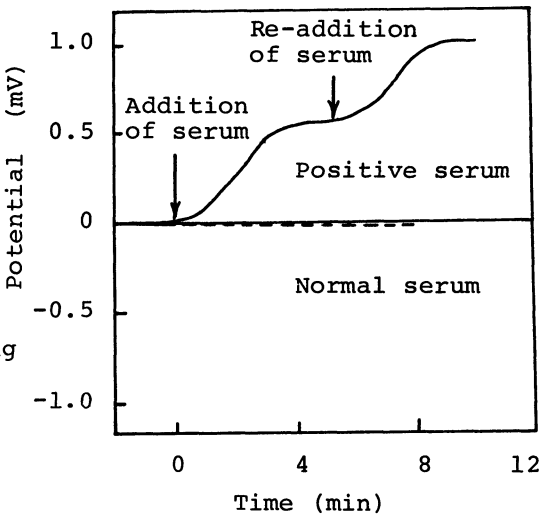

Fig.2 Response of the immuno sensor

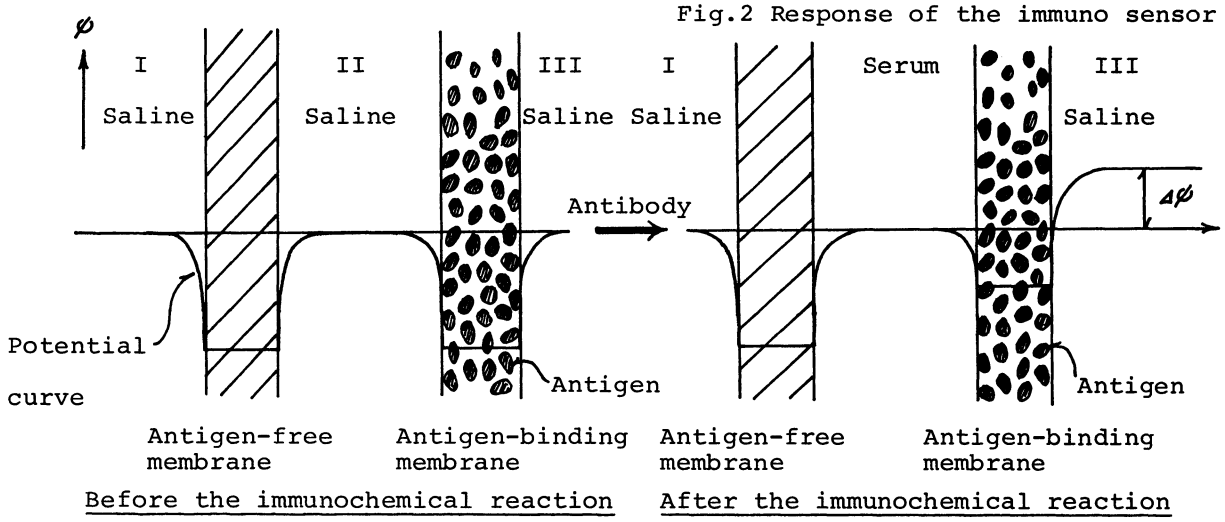

Fig.3 Postulated potential diagrams of the membranes

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