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LIPOSOME IMMUNOELECTRODE[1)]

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A new immunoelectrode system is reported, where thin-layer potentiometric measurement of complement and antibody levels in microliter serum was performed using tetrapentylammonium ion(TPA^+) loaded liposomes and TPA^+ ion selective electrode.

Recently, clinical and biochemical analysis has become extremely important for the diagnosis of patients and fundamental medical research. Particularly, the development of convenient and simple methods of immunoassay is highly desirable. Important contributions along this line have been made with some different view points[2)].

We report here a new method of immunoelectrodes, where thin-layer potentiometric measurement of complement and antibody levels in microliter serum is performed using tetrapentylammonium ion(TPA^+) loaded liposomes and TPA^+ ion selective electrode(ISE).

The principle of the present method is shown in Fig. 1. Kinsky[3)] first reported that amphipatic antigens solubilized in the liposome bilayer are effective in the production of immunologically sensitive liposome that release trapped markers in the presence of appropriate antibody and complement source. Several different markers have been utilized for immune reactions of liposomes, including glucose for spectrophotometric study[3)] and spin labels for ESR study[4)]. In the present study, TPA^+ was trapped in liposomes. Upon immune lysis reaction, TPA^+ ions are released from liposomes and are monitored by TPA^+ ISE.

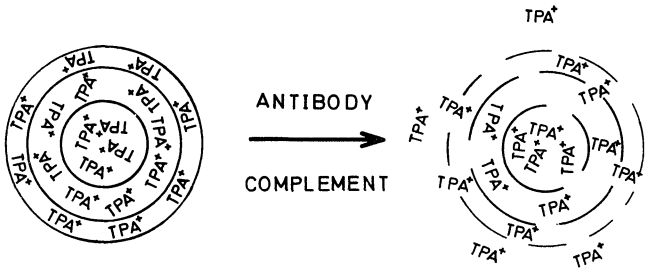

Fig. 1 Immune lysis of liposomes and resulting leakage of TPA^+ ion for analytical amplification.

The liposomes were prepared as before[5] using dipalmitoylphosphatidylcholine, cholesterol, dicetylphosphate and an appropriate antigen in molar ratio of 2:1.5:0.2:0.01. The dried lipids were swollen in 0.15 M TPA^+. The swollen liposomes were dialyzed against a modified veronal buffer saline(VBS)[6] for ca. 4 hours to remove untrapped marker. Since the molecular size of TPA^+ ion is sufficiently large, the background leakage of TPA^+ ion was found to be negligible during the course of the experiment.

The PVC type TPA^+ ISE was constructed following the Higuchi's method[7] from a fluoride ISE body of Denki Kagaku Keiki Co. with a 0.01 M KCl internal solution. The electrode thus made exhibited Nernst response from 0.1 M to 10^{-7} M of TPA^+.

One of the most difficult problems for electrochemical immunoassay is the amount of sample volume needed. We employed a special type of reference electrodes, a plate-shaped Ag/AgCl reference electrode, so that necessary sample volume became 10 microliters or less. The ISE body is placed on the plate reference electrode with an upright position. Containers such as a beaker is not needed, instead, a small thin layer space between the plate reference electrode and the flat bottom of the ISE sensor is conveniently used for holding a few microliters of sample solution. Schematic representation of the method is shown in Fig. 2. The details of this thin-layer potentiometric method was described elsewhere[8]. Plate Ag/AgCl reference electrodes were made by anodic oxidation of silver plate(4 x 7 cm, 2 mm thick) in 0.1 M KCl solution at +0.5 V vs. SCE for ca. 5 mins. The potential shift of the Ag/AgCl plate reference electrode against Cl^- ion interference from one serum sample to another was found negligible, because serum used in this study was of 2000 to 200 volume dilutions. All chemicals used were of analytical grade.

As an illustrative example of antigens, ganglioside[9], an intermediate of human brain metabolism, was chosen in the present experiment.

Fig. 3 shows the potentiometric calibration curve to ganglioside antibody

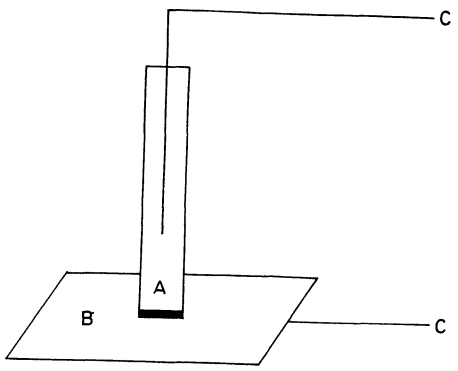

Fig. 2 Schematic diagram of thin-layer potentiometric assembly.

A. Tetrapentylammonium ion selective electrode.
B. Ag/AgCl plate reference electrode.
C. To mV meter.

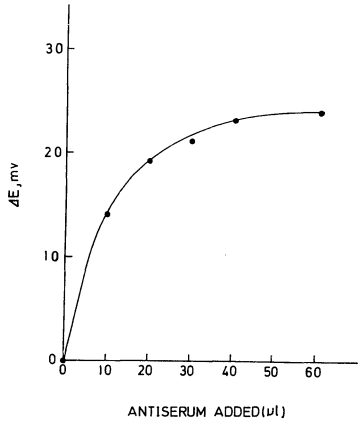

Fig. 3 Potential vs. relative concentration of ganglioside antiserum.

Antiserum was made in rabbits according to Ref. 9). Excess complement(from fresh guinea pig serum), and known amounts of sensitized liposomes and antiserum(56° C, 30 mins. heat) were mixed and incubated for 5 mins. at 37° C. Ten microliters added corresponds to 2000 volume dilution of stock antiserum. Potentials were recorded after 3 mins. for equibrium on a mV meter Model HM5BS at 21° C(accurate to ±0.5mV).

based on the complement mediated release of TPA^+ marker ion from liposomes: The observed potential change is ca. 24 mV upon changing the concentration of antiserum from infinite to 300 volume dilutions. Of course, it was confirmed that this potential change is only due to the corresponding immuno reaction rather than change in ionic strength, pH and so on. This result well claims the usefulness of this method for the accurate assay of antibodies. Also shown in Fig. 4 is the calibration curve for the potential vs. relative complement concentration from infinite to 250 volume dilutions. This result indicates that complement levels are also determined accurately with the present method. The antigen level can also be measured using the back titration technique which was employed by Hsia[10] in the case of tempocholine marker method.

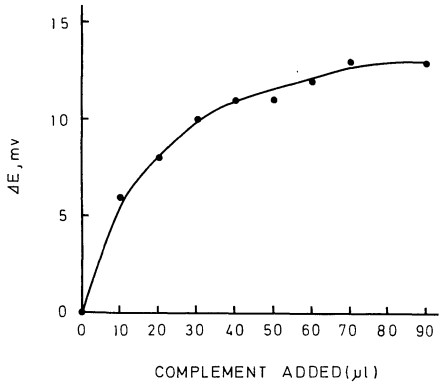

Fig. 4 Potential vs. relative concentration of complement.

Excess ganglioside antiserum, and known amounts of sensitized liposomes and fresh guinea pig serum(source of complement) were mixed and incubated for 5 mins. at 37° C. Ten microliters added corresponds to 2000 volume dilution of stock guinea pig serum.

The present method is advantageous over tempocholine marker(ESR)[4] and glucose marker(spectrophotometry)[3] methods in terms of simplicity of measurement and economy to fabricate, having comparable sensitivity. Another advantage of the liposome immunoelectrode system is the easiness of continuous monitoring the time course of immune lysis of liposomes which is one of current topics in membrane immunochemistry.

Further study is underway for evaluating the method using samples such as dinitrophenylated antibody hapten, and cardiolipin antibody hapten systems.

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