

The mouse antiphthalate immunoglobulin which now has a hydrophobic alkyl chain attached to it, tightly associates with the lipid vesicle membrane due to hydrophobic forces. Antibody that does not become associated is separated by gel filtration. The antibody-vesicle reagent can be used in an immunoassay as described in Example 2, supra.

EXAMPLE 11

THE USE OF SURFACTANTS AS LYTIC AGENTS IN THE IMMUNOASSAY

Lipid vesicles entrapping calcein and having mouse antiphthalate on their surface were prepared as described in Example 6, supra. The vesicles (19 nmoles of lipid in 1 ml) were incubated with 100 picomoles of phthalate for 30 minutes at a temperature of 37° C. At the end of this incubation, agarose beads containing aminophthalate attached to the bead surface by the method of Cuatrecasas and Parikh, *Biochemistry*, 11,2291-2299 (1972) were added to the assay mixture and the mixture allowed to incubate while being gently mixed for 30 minutes. The resulting mixture was allowed to stand for 30 minutes and the beads then separated from the solution by decantation. Vesicles which have reacted with phthalate in the first step do not bind to the beads and remain in solution while those that did not bind to phthalate in the first step bind to the phthalate exposed on the beads. A surfactant (Triton X-100, supra.; 0.05 ml) was added to the solution which contains the vesicles not attached to the beads, to lyse the vesicles, releasing entrapped calcein. The increase in fluorescence signal from the released calcein was proportional to the amount of phthalate in the original suspension. This forms the basis for an immunoassay for any substance to which an antibody can be made and does not require the use of complement to lyse the lipid vesicles.

EXAMPLE 12

A UNIVERSAL REAGENT FOR USE IN THE IMMUNOASSAY WHICH USES SURFACTANT TO LYSE THE LIPID VESICLE

Lipid vesicles entrapping calcein and having a rabbit antimouse antibody on their surface are prepared as described in Example 10, supra. These are a universal reagent which can be used with any mouse antibody to prepare an immunoassay kit for the antigen recognized by the particular antibody used. To assay for the levels of a particular antigen, in this case phthalate, mouse antiphthalate antibody is first incubated for 30 minutes with 1 nmole of phthalate in 1 ml of saline. The mixture is then added to a test tube containing 10 mg of agarose beads containing aminophthalate attached to the bead surface as described in Example 11, supra., and incubated for an additional 30 minutes with gentle shaking. A defined amount (50 nmoles) of vesicles having the rabbit antimouse antibody on their surface are added to the mixture and the incubation is continued for 30 minutes more. The beads are then allowed to settle for 10 minutes. The supernatant solution is decanted off of the beads and poured into a tube containing a detergent solution 0.5% Triton X-100 which lysis the vesicles that remain in the solution. The signal from the released calcein is directly related to the amount of phthalate in the original solution. In a similar fashion a wide variety of other analytes can be assayed for, limited only by one's ability to provide antibody to the analyte.

EXAMPLE 13

A UNIVERSAL REAGENT

In addition to antibodies that can recognize antibodies, e.g.; rabbit antimouse immunoglobulins, certain proteins bind to the constant region of antibody molecules. One such protein is produced by the bacterial *Staphylococcus aureus* called protein A. This protein can be attached to the lipid vesicle surface by the procedure given in Example 6, supra., and when used in the assay procedure described in Example 12 can be used in the immunoassay of the invention.

EXAMPLE 14

LYOPHILIZATION OF IMMUNOASSAY REAGENT

Lipid vesicles encapsulating alkaline phosphatase and with rabbit antimouse Fab' fragments on their surface are prepared as described in Example 7, supra., and are then freeze dried. After rehydration with distilled water and incubation for 30 minutes at a temperature of 37° C. the vesicles are mixed with mouse antiphthalate antibody to obtain a reagent of the invention which may be used in an immunoassay, carried out as described in Example 7, supra.

The invention also comprises diagnostic kits, containing reagents of the invention and which are useful for the determination of the presence or absence of ligands in biological fluids such as blood, blood serum, saliva, urine and the like. The kits are particularly useful for the detection and immunoassay of reagents, immunoglobulins and the like. The diagnostic kits of the invention comprise a container, housing in appropriate vessels (1) a reagent of the invention as described above, (2) a lytic agent and optionally (3) buffer solutions, (4) positive control solutions of known ligands in known concentrations and (5) amplification system components as desired to suspend the reagents of the invention in.

What is claimed:

1. An immunoreactive liposome reagent for use in the determination of a chemical compound capable of entering into an immunospecific reaction with a known antibody, said reagent comprising
 - a suspension of liposomes containing reporter molecules, and
 - a surface array of molecules of such antibody attached to the liposomes through glycolipid molecules anchored in the liposomes and linked to the antibody molecules by a diimidazole coupling agent.
2. The reagent of claim 1, wherein the glycolipid is a melibionamide and the diimidazole coupling agent is 1,1-carbonyldiimidazole.
3. The reagent of claim 2, wherein the melibionamide is tetradecylmelibionamide.
4. A method of producing an immunoreactive liposome reagent having an array of surface-bound antibody molecules, said method comprising
 - providing a glycolipid,
 - linking such antibody molecules covalently to molecules of the glycolipid, in an aqueous medium, with a diimidazole coupling agent, and
 - mixing the resultant antibody-glycolipid couple with liposomes in an aqueous medium to produce binding of the antibody to the liposome surface through the linked glycolipid.