

FIG. 2 is a negative stain electron microscopic photograph of DOPE-DNP-cap-PE (88:12) liposomes at a magnification of 1.12×10^6 ;

FIG. 3 illustrates the immunospecificity of DOPE liposome lysis by antibody attached to the glass slide;

FIG. 4 illustrates the immunospecificity of DOPC liposome lysis by antibody attached to a glass slide;

FIG. 5 illustrates the effect of the attached antibody concentration on DOPE liposome lysis. Anti-DNP IgG (c) or normal IgG (d) at indicated concentrations was used to coat a glass slide, and the DOPE liposome lysis was measured by calcein release;

FIG. 6 illustrates the inhibition of DOPE liposome lysis by free hapten. DNP-Gly (e) or Gly (f) of indicated concentration was added to attached anti-DNP IgG on a glass slide before liposome addition.

FIG. 7 illustrates the inhibition of DOPE liposome lysis by free antibody. Liposomes were preincubated with free anti-DNP IgG (g) or normal IgG (h) at indicated concentrations before adding to the attached antibody on a glass slide; and

FIG. 8 is a schematic representation of the solid-state immunoliposome assay of the present invention.

DETAILED DESCRIPTION

Liposomes are microscopic vesicles composed of closed lipid bilayers. See: Papahadjopoulos, *Ann. N.Y. Acad. Sci.*, 308 1 (1978). Due to their relatively simple composition and their flexibility for chemical, physical and immunological manipulations, liposomes are a favorite material for membrane lytic assays.

When compared to other immunoassay techniques, there are several advantages in using a membrane lytic immunoassay: (1) a single lytic event can lead to the release of many signal molecules and hence there is a high degree of signal amplification, (2) it is rarely necessary to separate the immune complex from the free antibody or antigen and hence it is generally a homogeneous assay; and (3) optical measurements such as colorimetric and fluorometric techniques can be used and hence it avoids any requirement for the use of radioisotopes. For these reasons, membrane lytic assays have received increasing attention in the recent development of immunoassays.

The immunoliposome assay of the present invention will be illustrated by referring to the assay for one particular entity, e.g. an antigen. The general principles and techniques described herein for assaying an antigen can then be applied to assay for other species such as, for instance antibodies, haptens, etc.

In order to aid in the understanding of the present invention, the following terms as used herein and in the claims have the following meanings:

Analyte—the compound or composition to be measured, which may be a ligand, such as an antigen, hapten or an antibody. For example, in a preferred embodiment, the analyte may be either an antigen or an antibody.

Ligand—any compound for which an immunological receptor naturally exists or can be made. When the ligand is an antibody, the immunological receptor can be an antigen or an anti-antibody.

Ligand-lipid complex—a covalently bonded species comprising the analyte of interest and a lipid composition compatible with the lipid or lipids used to form the liposomes for the assay herein. If the ligand of interest cannot be directly used to stabilize the lipid bilayer for the formation of vesicles, the ligand must first be cou-

pled to a suitable lipid using conventional coupling chemistry. Similar coupling chemistry is described herein below for coupling between the anti-ligand and the inert solid support. Lipids useful for such couplings include ($>C_{10}$) fatty acids, phospholipids, ($>C_{10}$) hydrocarbons, large cyclic hydrocarbons, polycyclic hydrocarbons and others readily selectable by those skilled in the art. The ligand-lipid complex is employed to stabilize an otherwise unstable liposome composition. For example, in one embodiment, an antigen covalently bound to a derivative of phosphatidylethanolamine (N-caproyl-) was employed to stabilize liposomes formed predominantly (88 mole percent) of phosphatidylethanolamine.

Marker compound—any compound capable of ready detection other than a radiotracer. Especially useful herein are markers such as enzymes, see Cole U.S. Pat. No. 4,342,825 (incorporated herein by reference) chemiluminescent species, colorogenic agents and fluorogenic agents. The most preferred marker compounds are self-quenching fluorescent dyes. These compounds include water soluble derivatives of fluorescein such as carboxyfluorescein and calcein. Another suitable marker is a combination of water soluble fluorophore, e.g., 8-amino-naphthalene-1,3,6-trisulfonic acid and a water soluble quencher, such as p-xylene bis(pyridinium)bromide. As the dye/quencher combination is released from the liposome at lysis, dilution allows for dequenching of and thus detection of the fluorophore. See for example, Ellens et al., *Biochem.*, 23 1532 (1984).

Receptor—any compound or composition capable of recognizing a particular spatial and polar organization of another molecule. Natural receptors include antibodies, enzymes, lectins, and the like. For any specific ligand, the receptor may be generally termed an anti-ligand. Depending upon the circumstances the terms may be interchangeable, i.e., receptors in one case can be ligands in another. For example, in a preferred embodiment, the receptor for an antigen is an antibody, while the receptor for an antibody is either an anti-antibody or, preferably, that antibody's cognate antigen.

System—a combination of analyte, ligand and/or receptor reagents, usually formulated with ancillary reagents such as buffers, salts, stabilizers and the like, and supplied in individual containers, generally in the form of an assay kit. For example, a system for detecting the presence and/or the concentration of an antigen would include appropriate containers with (1) antigen or a chemical derivative thereof in the membrane of liposomes and (2) a fluorescer or other suitable marker encapsulated in the liposomes of (1); (3) an antibody for the antigen attached to a solid support; and (4) antigen standards of known concentration for preparing a curve for comparison of known dye release with the unknown dye release or a predetermined comparison curve and one standard for a control. Alternatively, the kit can include merely: (1) suitable reagents for preparing the ligand-lipid complex; (2) other liposome forming components, as necessary; (3) a marker compound; (4) analyte standards; and (5) receptor standards. The user can readily formulate specific reagents depending upon the particular assay requirements at hand. A detection means can also be supplied as part of the system, but this is not typically required.

In one embodiment of this invention, an assay for an antigen involves the lateral phase separation of a liposome formed from an antigen-lipid complex resulting in