

LIPOSOMES WITH GLYCOLIPID-LINKED ANTIBODIES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to immunoassaying of organic compounds and more particularly relates to a novel class of reagents useful for such immunoassaying.

2. Brief Description of the Prior Art

Immunochemical reactions have served as a basis for the assay of a wide variety of organic compounds (particularly biologically active compounds) for many years.

The term "immunochemical reaction" is used herein to refer to that class of chemistry known as "immunochemistry". Immunochemistry is chemistry classically concerned with the physical interaction between "antigens" and "antibodies".

"Antigens" are high molecular weight compounds, usually protein or protein-polysaccharide complexes, which upon entry in the blood stream of a vertebrate stimulate the transformation of the small lymphocytes of the B-type into lymphoblasts. The lymphoblasts synthesize and secrete "antibodies" specific to the antigen stimulator. The antibodies are proteins possessing reactive sites specifically complimentary to a reactive feature or site on the stimulating antigen. Antibodies generally have the property of rendering the antigen harmless to the host organism, by occupying the immunologically active sites on the antigen molecules, and sometimes also by forcing precipitation or agglutination of the antigen, or by other protective mechanisms.

In some but not all applications it becomes difficult or meaningless to maintain the classical distinction between antigen and antibody, because in many regards the relation between antigen and antibody is reciprocal and each precipitates or agglutinates the other. The basis for the distinction resides in the history of the particular substance, and this can become irrelevant outside the original antibody-generating organism, for example in reagent applications. More specifically, and as an illustration, immunoglobulins such as IgA, IgG, IgM, IgD and IgE are by the above definitions antibodies (actually a class of antibodies) since they are produced by plasma cells of the lymphoid system in response to the presence of an antigen (usually a multiplicity of antigens). However, the immunoglobulins can also be antigenic in behavior and responsible for the production of the specific antibodies known as anti-IgA, anti-IgG, anti-IgM, anti-IgD and anti-IgE, respectively. For this reason the antigen-antibody relationship may be advantageously described in this reciprocal way: an antibody is the "immunological-homologue" of the antigen which produced it, and vice versa. An antibody and its corresponding antigen are thus homologues of each other. They may also be said to be homologous to each other.

In any event, the immunochemical antigen-antibody relationship forms the basis for immunoassay of either "homologue". Procedurally, the various known techniques of immunoassay for the immunoreactant homologues (antigen, antibody), i.e.; radioimmunoassay, fluorescent immunoassay and enzyme immunoassay are substantially identical. Each technique comprises, in general, the separation of bound and labeled immunoreactant from unbound, labeled immunoreactant. This may be done, for example by immobilizing one of

the immunoreactants, labeling one of the immunoreactants with a marker or tag to monitor its presence and reacting the immobilized immunoreactant with the free immunoreactant and measuring the degree of reaction through monitoring of the labeled immunoreactant. The main difference between the various techniques resides in utilization of different reagents as markers or tags for visualization and measurement of the immuno-reaction.

Radioimmunoassay is a popular and highly sensitive technique, particularly when the material being assayed for is in relatively small concentrations. It has found commercial acceptance. However, radioimmunoassay procedures are not entirely satisfactory for all purposes. The reagents employed are of limited stability and shelf-life. Their use is often subject to special handling and license. Personnel carrying out the procedure require special protection, special facilities and extraordinary training. The art has been searching for equally sensitive immunoassay procedures, employing non-radioactive reagents.

Among immunoassay procedures which obviate the need for radioactive reagents is the so-called spin membrane immunoassay technique (see for example U.S. Pat. No. 3,850,578). The technique is based on the fact that a lipid-linked antigen may be solubilized in a lipid bilayer membrane matrix to form antigen sensitized (bound) lipid vesicles. The sensitized vesicles, encapsulating spin resonance labels, will bind antibodies through the active, bound antigen. The antigen-antibody bound vesicles are disposed to lysis under certain conditions wherein those lipid vesicles not attached to antibody will not lyse. Release of the encapsulated spin labels is measurable to determine therefore the quantity of bound antibody. The method does not measure, at least directly, the presence of antigen, but only antibody.

The immunoassay of the present invention combines the sensitivity of a radioimmunoassay with the advantages of the spin membrane immunoassay, without the need for radioactive reagents. This sensitivity is greater than obtained in the spin membrane immunoassay. More specifically, in the immunoassay of the present invention, as the quantity of compound assayed for increases, the signal generated by the assay technique increases proportionately. In contradistinction, the signal generated by the spin membrane immunoassay technique decreases as the quantity of compound assayed for increases. Thus, sensitivity of the method of the invention is greater [this is due to the fact that the signal from totally lysed vesicle (no antibody present) and the lysis that occurs when a small quantity of antibody is present is the difference between two large numbers and thus the signal to noise ratio is low]. In the method of the invention, when no antigen is present, very little signal is given off. The assay is therefore simpler to interpret.

Another advantage of the immunoassay of the invention resides in the reagent, prepared by coupling antibody to a lipid vesicle surface. The same method of coupling may be used regardless of the antibody or vesicle composition used. In contradistinction, the prior art spin lable immunoassay requires different amphipathetic vesicle molecules for attaching different antigens.

Still another advantage of the immunoassay of the invention resides in the ability to use a wide variety of different reporter compositions. In the spin membrane