

BINDING ASSAYS IN AUTOMATED APPARATUS WITH LIPOSOME COMPATIBLE SURFACTANTS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the field of specific binding assays, particularly immunoassays, for determining substances of clinical interest. Specific binding assays are based on the specific interaction between a ligand, i.e., a bindable analyte under determination, and a binding partner therefor, i.e., receptor. Where one of the ligand and its binding partner is a hapten or antigen and the other is a corresponding antibody, the assay is known as an immunoassay.

2. Brief Description of the Prior Art

Many properties of natural cell membranes can be duplicated in simple lipid bilayer systems, referred to as liposomes. One of these properties is lysis. When a vesicular, e.g., liposome, membrane contains an externally accessible antigen it will react with corresponding antibody, causing agglutination. When the antigen-sensitized liposome reacts with corresponding antibody in the presence of complement the membrane is irreversibly damaged and can no longer function as the intact selective permeability barrier. This is immunolysis.

The extent of immunolysis has been monitored by using antigen-sensitized liposomes containing any of a wide variety of entrapped marker molecules, which are released upon immunolysis. See Hixby, et al., Proc. Nat. Acad. Sci., 64: 290-295 (1969); Kinsky, et al., Biochemistry, 8: 4149-4158 (1969); Kinsky, et al., Biochemistry, 9: 1048 (1970). See also Six, et al., Biochemistry, 13: 4050 (1974); Uemura, et al., J. Biochem, 87: 1221 (1980); and Uemura, et al., J. Immunol. Methods, 53: 221-232 (1982).

Specific binding assay systems have been proposed, using a multilayered lipid membrane vesicle which has been prepared or treated to have surface-bound ligand or ligand analog and a marker or reagent substance enclosed within the vesicle. The remaining reagents for the assay include: (1) a binding partner, e.g., antibody, for the ligand; and (2) complement to effect lysis of the vesicle upon binding of the binding partner to surface-bound ligand. Generally, see McConnell, U.S. Pat. No. 3,850,578 and McConnell, et al., U.S. Pat. No. 3,887,698 and Gregoriadis, et al., *Liposomes in Biological Systems*, John Wiley & Sons, N.Y. (1980), especially Chapter 12 entitled "Liposomes as Diagnostic Tools". Immunoassay systems have been disclosed in which the use of enzyme-encapsulating liposomes is suggested. Hsia, et al., U.S. Pat. No. 4,235,792 describes a competitive homogeneous immunoassay method which employs immunolysis of an antigen-sensitized liposome containing a marker. Enzymes are among the markers disclosed (col. 6, lines 24-28).

Numerous references in the literature have used various surfactants to achieve the chemical lysis of liposomes. This has been based on the recognized effect of such surfactant compounds on lipids and the integrity of lipid-containing membranes. Surfactants have been used to lyse liposomes, inter alia, in the development and characterization of immunoassays such as are described above. See, for example, Cole, U.S. Pat. No. 4,342,836 and the references cited therein. Another and substantially different type of liposome immunoassay is described in co-pending U.S. Ser. No. 528,496, which was filed on Sept. 1, 1983 and is assigned to the instant as-

signed. This also describes the use of surfactants to disrupt liposomes.

Many automated analyzers, including those of the continuous flow type, require the presence of surfactants in reagent compositions used therewith to provide appropriate hydrodynamic and optical properties to the liquids being analyzed. As such, the prior art has provided no way to accommodate these conflicting limitations of liposome specific binding assays and requirements relating to automated analysis systems.

SUMMARY OF THE INVENTION

Liposome specific binding assays offer a new approach to in vitro diagnosis. Adaptation of homogeneous immunoassays to automated clinical chemistry has become especially attractive. In particular, it is highly desirable to utilize homogeneous immunoassay methodologies on continuous flow-systems. In contrast to the prior art, this invention provides liposome-containing reagent compositions which can be utilized on automated analyzers including continuous flow systems. An especially critical component of these formulations is surfactants which are compatible with liposomes (i.e., do not lyse or otherwise modify them).

Accordingly, the present invention provides a specific binding assay composition for determining a ligand in a sample, which composition comprises (a) a binding partner for the ligand, (b) a selectively accessible vesicle having a surface incorporated ligand or ligand analog, (c) a substance which modifies vesicle accessibility in response to binding of surface-incorporated ligand or ligand analog and the binding partner, (d) a detection system which responds to modification of vesicle accessibility to produce a detectable response, and (e) at least one surfactant which does not modify vesicle accessibility. Several embodiments of such surfactants are disclosed, each characterized in having a polyoxyethylene component.

The invention further provides a specific binding assay method, including a unique continuous flow specific binding assay method. The method comprises reacting said sample with a composition comprising: a binding partner for said ligand; a detection system having a first and second component; a selectively accessible vesicle having a surface incorporated ligand or ligand analog and, within said vesicle, a first component of said detection system which is reactive with said second component to produce a detectable response; a substance which modifies vesicle accessibility in response to binding of surface-incorporated ligand or ligand analog and the binding partner; and at least one surfactant which does not modify vesicle accessibility; and observing any detectable response so-produced. Immunoassay determinations of a broad spectrum of analytes is made possible without operator intervention and without risk of sample-to-sample carryover.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Preferred embodiments of the present invention include a specific binding assay reagent composition and method of using the test composition. Specific terms in the following description which refer only to a particular embodiment are exemplary of all of the embodiments unless otherwise indicated.

Sample fluids on which tests are performed include biological, physiological, industrial, environmental, and