

in proportions such that the interaction of unbound analyte-cytolysin conjugate with said vesicles results in the release of said marker material; and

(B) measuring said marker material thereby released which is related to the amount of said analyte initially present in said liquid medium.

An antibody molecule is capable of functioning as an analyte in the present invention, but an antibody-cytolysin conjugate can have utility in a heterogeneous assay wherein a separation of reaction products occurs prior to the measurement step. Another aspect of this invention therefor involves a novel antibody-cytolysin conjugate.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a standard assay curve to be used for digoxin analysis which relates the amount of marker material released from lipid vesicles by unbound ouabain-melittin conjugate to the concentration of digoxin initially present in the liquid medium.

FIG. 2 is a standard assay curve to be used for analysis of biotin which relates the amount of marker material released from lipid vesicles by unbound biotin-melittin conjugate to the concentration of biotin initially present in the liquid medium.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a sensitive method and novel materials for detecting and/or determining the amount of a wide variety of organic materials present in clinical, environmental, and other test samples. In the context of this disclosure, the following terms shall be defined as follows: "analyte" is the substance, or group of substances, whose presence or amount in a liquid medium is to be determined, which additionally have the capability of being attached to a cytolysin substance to form an analyte-cytolysin conjugate; "cytolysin" is any substance or agent of molecular weight from about 100-100,000 daltons which can change the permeability of biomembranes; "analyte derivative" refers to both unmodified and chemically modified analyte molecules, and/or their chemical combinations with spacer arms, which can be chemically bound to cytolysin molecules by covalent, ionic, or other bonding techniques; "spacer arm" is a bifunctional molecule used to chemically bond an analyte derivative to a cytolysin molecule while maintaining some distance between the two molecular entities; "binding agent" is any substance, or group of substances, which has specific binding affinity for the analyte to the exclusion of other substances such that reaction occurs between a particular analyte and binding agent to form a bound complex; and "binding analog of the analyte" is any substance, or group of substances, which behaves essentially the same as the analyte with respect to binding specificity for the binding agent for the analyte; "vesicles" are either natural or synthetic sacs believed to consist of lipid bilayers which separate an internal compartment which can sequester marker material from the external media in which the vesicles are suspended; and "marker material" is any substance sequestered within a vesicle which is not detectable by instrumental methods until released from the vesicle, or until rendered detectable by another substance which as a result of an alteration in vesicle membrane permeability followed by diffusion into the vesicle and be chemical modified to a substance which is detectable by instrumental methods.

The present homogeneous method may be applied to the detection of any analyte for which a binding agent exists. The binding agent may consist of an antibody in the form of whole antiserum, an IgG fraction, as affinity-purified monospecific material, a monoclonal antibody, a monovalent antibody or of other specific binding proteins like lectins, hormone receptors, or serum transport proteins. The quantitative measurement aspect of the invention results from the fact that free analyte present in the test sample and the analyte-cytolysin conjugate are both capable of reacting in a competitive fashion with binding agent. In the absence of analyte, the concentration of the process components is adjusted such that there is no release of sequestered marker material from the vesicles. In the presence of analyte, excess analyte-cytolysin conjugate exists which alters the permeability of the vesicles resulting in a release of marker material at a rate and in an amount proportional to the amount of analyte initially present. An amplification is obtained in the measurement process due to the fact that a single interaction between an analyte-cytolysin conjugate and a vesicle can result in the release of a large number of detectable marker material molecules.

Various protocols can be employed in assaying for a wide variety of analytes. The determination of the amount of analyte initially present in the test sample can be carried out by correlation with either the amount of marker material released after a given contact time of reagents, or with the rate of release of such marker material under conditions which enable comparison with a standard curve produced for known amounts of the reagents. Contacting times of the reagents can vary from 10 seconds to one hour at temperatures in the range of from about 4° to 40° C. and at a pH in the range of about 5-10, usually 6-8. The measurement can be carried out manually, or with reagents packaged to utilize automated analyzers.

To illustrate the analyte-cytolysin conjugate and homogeneous assay of the instant invention, an aliquot containing an unknown amount of analyte is added to buffered incubation medium containing substrate for the enzyme which is used as marker material sequestered in the vesicles. A known amount of antibody specific for the analyte is added to the medium and briefly incubated prior to the addition of a known amount of analyte-cytolysin conjugate. After further brief incubation, a known amount of vesicle preparation is added and the amount of substrate converted by enzyme after diffusion into the vesicle is monitored as a function of time. Comparison with a standard curve relating analyte concentration with substrate conversion obtained under the same conditions of time intervals and reagent amounts enables the determination of the unknown amount of analyte.

The antibody-cytolysin conjugate of the present invention can be used in a heterogeneous assay for the detection of any analyte which has antigenic or haptenic properties, i.e., the ability to elicit the formation of anti-analyte antibody when injected into a host. To illustrate the use of an antibody-cytolysin conjugate in a heterogeneous assay, an aliquot containing an unknown amount of analyte is mixed with a buffered solution containing cytolysin labeled anti-analyte antibody (C-Ab). The C-Ab should be in molar excess over the highest concentration of analyte expected to be found in a clinical sample. The mixture is then incubated during which time a fraction of the C-Ab will immunochemi-