

SPECIFIC BINDING ASSAYS UTILIZING ANALYTE-CYTOLYSIN CONJUGATES

TECHNICAL FIELD

This invention relates to a highly sensitive and rapid method of analysis for the quantitative determination of the amount of a specific analyte in liquid medium, and to novel analyte-cytolysin conjugates which alter the membrane permeability of vesicles containing marker material.

BACKGROUND OF THE INVENTION

Clinical laboratory chemical diagnostic tests are an important component of health care delivery. The utilization of these tests by physicians to monitor drug levels where only a narrow therapeutic range exists, to guide decisions on treatment and surgical options, and to screen patients for the early detection of disease has rapidly increased the number of tests performed annually. With almost 6 billion tests performed in 1976 and 12.2 billion estimated to be performed in 1986 [Luning Prak Associates Survey, 1980], speed, accuracy, and cost control are important objectives. The desire to measure such analytes as drugs, hormones, and metabolites at micromolar (μM) to picomolar (pM) levels in complex body fluid matrices has led to the development of sophisticated test methodology which can be implemented by automated techniques at reasonable cost.

Broadly applicable, accurate screening assays are therefore needed to monitor the presence and quantity of biological materials. Various methods have been utilized in the past including liquid and gas chromatography, mass spectrometry, and numerous bioassay techniques. These methods are time consuming and not easily applied in large-scale, automated screening programs.

In recent years, a number of immunoassay techniques have been developed to take advantage of the specificity of antibody reactions while avoiding the complicating features of radiochemical labelling. The use of vesicles containing sequestered detectable marker material can provide a stable, sensitive, and flexible measurement system for quantifying such medically important materials as cardiac glycosides, antibiotics, therapeutic drugs, hormones, and vitamins. In addition, methods of analysis for toxins, food and packaging additives, and environmental pollutants at extremely low concentration are required.

Haga et al. [Biochem. Biophys. Res. Commun., Vol. 95, 187-192 (1980) and Anal. Biochem., Vol. 118, 286-293 (1981)] describe a liposome-based immunoassay in which horseradish peroxidase is sequestered within a lipid vesicle formed from a mixture of lecithins including phosphatidylethanolamine to which analyte has been covalently bonded. The lipid vesicle is therefore specific for the analyte of interest, and lysis is induced by complement (from guinea pig serum) in combination with the antibody specific for the analyte. Such systems require the preparation of lipid vesicles with specific analyte "tags" and also the use of the unstable, complex complement system to release the detectable marker material used to quantify the amount of analyte initially present. Long incubation periods are frequently required which increase analysis time.

Hsia et al. [New York Academy Sci., Vol. 308, 139-148 (1978) and U.S. Pat. No. 4,235,792] describe complement mediated immunoassay techniques

wherein the lysis of lipid vesicle with a synthetic sensitizer incorporated in the lipid bilayer leads to the release of marker material, in particular of spin labelled molecules quantified by electron spin resonance techniques.

The assay system requires the preparation of a specific lipid vesicle for each analyte of interest to mediate attack and lysis by the complement system in the presence of antibody.

U.K. Patent Application No. 2069133A and U.S. Pat. No. 4,342,826 describes a process for sequestering enzyme marker within lipid vesicles in a manner which enhances the so-called signal to noise ratio of the reagents. The lipid vesicles must be specifically labelled with either antigen or antibody to render them immunoreactive in the presence of complement.

Thelestam et al. [Biochem. Biophys. Acta, Vol. 557, 156-169 (1979)] describe a variety of microbial, plant, and animal cytolysins and efforts to classify cytolysins by measuring changes in the permeability of human fibroblasts. Melittin, the polypeptide lytic factor of bee venom, was among those agents tested, but no immunodiagnostic or analytical applications of the cytolytic agents are disclosed, and the effect on synthetic lipid vesicles was not considered.

Sessa et al. [J. Biol. Chem., Vol. 244, 3575-3582 (1969)] examine the mechanism by which melittin disrupts or lyses biomembranes using both erythrocytes and lipid vesicles as model systems. No disclosure of melittin-analyte conjugates or their relevance to immunodiagnostic analytic methods is made.

At this time there exists a clear need for membrane lytic immunoassay systems which do not require unstable complement or specific antigen or antibody tagged vesicles. A system in which specificity resides in a lytic agent would provide great flexibility since the same sequestered marker vesicle preparation would be utilized in all assays. Such a system could also incorporate a variety of detectable marker materials to take advantage of the instrumental methods available to the user.

Summary of the Invention

A sensitive, homogeneous assay to measure analytes in solution has been discovered which utilizes novel analyte-cytolysin conjugates and vesicles containing marker material wherein the vesicle membrane permeability changes resulting from interaction with such conjugates can be modulated by analyte-specific binding agent. The assay also utilizes a standard vesicle preparation applicable for analysis of a wide variety of analytes by many different instrumental methods. Specifically, one aspect of this invention involves the synthesis of an analyte-cytolysin conjugate wherein at least one analyte derivative molecule is attached per cytolysin molecule such that said conjugate is capable of both reacting with binding agent of the analyte and altering vesicle membrane permeability to release marker material. Another aspect of this invention is a method for determining the amount of an analyte in liquid medium comprising the steps of:

- (A) forming a reaction system by contacting said liquid medium with
 - (1) analyte specific binding agent;
 - (2) analyte-cytolysin conjugate; and
 - (3) vesicles containing marker material sequestered within the vesicle