

immunoassay one is restricted to the use of spin lable compositions and in a radioimmunoassay one is restricted to using radioactive reporter compositions. This is an important advantage since one can prepare reagent lipid vesicles with different reporter compositions encapsulated in different vesicles, to which different antibodies are attached. Specific antibodies, for example one to insulin and one for glucagon, may be attached to the two different sensitized vesicle preparations and the two compounds may be assayed for simultaneously in a single procedure.

The preferred reagents of the invention are prepared from very pure forms of antibodies, which account for their high degree of sensitivity to specific antigens. This high degree of specificity is a distinct advantage in use of the reagents for clinical (medical) diagnostic purposes.

Other advantages associated with the reagents and immunoassay of the invention will be described more fully hereinafter.

SUMMARY OF THE INVENTION

The invention comprises a reagent which is useful for the immunoassay of a chemical compound capable of entering into an immunochemical reaction with a known antibody, which comprises;

a fluid dispersion of a plurality of homogeneous immunoreactant particles, said particles comprising a water-soluble, non-radioactive reporter composition encapsulated within a lipid vesicle;

each of said vesicles having bound to its outer surface a specific highly purified polyclonal antibody or a monoclonal antibody possessive of an active epitopic site which is capable of immunochemical reaction with said compound and which will be lysed in the presence of a lysing agent when the epitopic site is occupied by an immunochemical reaction.

The reagents of the invention are useful to assay for chemical compounds capable of entering into an immunochemical reaction with a corresponding antibody, i.e.; a ligand, provided the ligand is not capable by itself of lysing the lipid vesicle portion of the reagent of the invention. The ligands assayed for by the method of the invention may be monoepitopic or polyepitopic and include for example polypeptides, proteins, polysaccharides, nucleic acids, combinations thereof and the like. Representative proteins assayable by the method of the invention are a wide variety of:

protamines,
histones,
albumins,
globulins,
scleroproteins,
phosphoproteins,
mucoproteins,
chromoproteins,
lipoproteins,
nucleoproteins,
glycoproteins and the like;

Representative of specific polypeptide and protein hormone ligands advantageously assayable for by the method of the invention are:

parathyroid hormone (parathromone),
thyrocalcitonin,
insulin,
glucagen,
relaxin,
erythroporetin,

melanotropin (melanocyte-stimulating),
somatotropin,
corticotropin (adrenocorticotropic hormone),
thyrotropin,
follicle-stimulating hormone,
luteinizing hormone (interstitial cell-stimulating hormone),
gonadotropin, prolactin, pepsin
and the like.

Other ligands include a wide variety of drugs, metabolites, virus derived antigens (such as hepatitis B surface antigen), bacterial antigens and derived antibodies (such as syphilis antibodies), parasite derived antigens, allergens and the like. Included among drugs of interest are alkaloids and their metabolites; barbiturates and their metabolites; aminoalkylbenzenes such as the amphetamines, catecholamines, their metabolites and derivatives; prostaglandins, which differ by degree and sites of hydroxylation and unsaturation; antibiotics, their metabolites and derivatives; nucleosides and nucleotides, and the like.

The invention also comprises the use of the reagents of the invention for the immunoassay of chemical compounds capable of immunochemical reaction with the antibody bound to the lipid vesicle.

The term "reporter composition" as used herein means a water-soluble, non-radioactive compound or composition containing a non-radioactive compound which is either directly or indirectly involved with the production of a detectable and measurable signal upon release from encapsulation. Representative of reporter compositions are water-soluble chromogens, e.g. fluorescers and chemiluminescers, catalysts, both enzymatic and non-enzymatic, molecules having an enzymatically labile bond which upon enzymatic cleavage provides a compound which can be detected, either directly or indirectly, and the like. More specific examples of reporter compositions will be provided hereinafter.

The term "non-radioactive" as used throughout the specification and claims means a chemical compound, isotope or composition (usually having an atomic weight over 207) which does not exhibit radioactivity, i.e.; spontaneous nuclear disintegration (unaffected by chemical or physical influences) of the compound, isotope or composition with emission of nucleons or of electromagnetic radiation.

The term "lipid vesicle" as used throughout the specification and claims means a man-made (synthetic) liposome.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Lipid vesicles (synthetic liposomes) have been known for a number of years as convenient carriers of encapsulated water soluble materials. Several methods are available to make lipid vesicles, encapsulating water-soluble materials; see for example Bangham et al. in *J. Mol. Biol.*, 13:238-252 (1965); D. Papahadjopoulos and N. Miller (*Biochim. Biophys. Acta*, 135:624-638[1967]); Batzri and Korn (*Biochim. Biophys. Acta*, 298:1015 [1973]); Deamer and Bangham in *Biochim. Biophys. Acta*, 443:629-634 (1976); Papahadjopoulos et al. in *Biochim. Biophys. Acta*, 394:483491 (1975); German Pat. No. 2,532,317; and U.S. Pat. Nos. 3,804,776; 4,016,100 and 4,235,871.

Lipid vesicle wall forming compounds are generally well known as are the methods of their preparation. For