

ASSAY UTILIZING ATP ENCAPSULATED WITHIN LIPOSOME PARTICLES

FIELD OF THE INVENTION

This invention relates to biological assays. Specifically, the invention employs sensitized liposomes having ATP encapsulated therein for determining the presence of biological analytes such as antigens and DNA probes.

BACKGROUND OF THE INVENTION

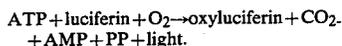
Through recent innovations in the areas of instrumentation, biological reagents, and high quality, inexpensive immunological reagents, it is now feasible to perform new types of immunoassays that were previously difficult or too costly. Additionally, recent advances in DNA probes and hybridization technologies have created a need for new amplification techniques which are nonradiative and sensitive enough to detect a DNA (deoxyribonucleic acid) hybridization event.

In the clinical treatment and diagnosis of disease, it is advantageous to conduct testing in the physician's office or at a clinical laboratory where fast results are desirable. Instrumentation and reagent development have generally proceeded together, as exemplified by scintillation and gamma counters for radioimmunoassays, Elisa readers and spectrophotometers for enzyme immunoassays and more recently fluorometers for fluorescent immunoassays. Inexpensive luminometers capable of reading light emissions from chemiluminescent and bioluminescent reactions, are now bringing these technologies toward commercial introduction for immunoassays. Accordingly, reagents which can be used with these inexpensive luminometers must also be developed.

Bioluminescent reactions such as the luciferin-luciferase reaction for the determination of adenosine triphosphate (ATP) make the detection of ATP one of the most sensitive luminescent reactions available. A significant number of publications exist in this area, illustrated by the following:

DeLuca, M. (1976) *Advance in Enzymology* Vol. 44:37-68 John Wiley & Sons, N.Y. McElroy, W. D., Seliger, H. H. & DeLuca (1974) *The Physiology of Insecta* Vol. 11:411-460, Academic Press, N.Y. These survey articles disclose that the reaction of luciferase, ATP and D-luciferin, produces free pyrophosphate and enzyme bound luciferyladenylate. The luciferyladenylate complex is subject to two processes which limit the speed of the initially emitted light, namely a conformational change and an abstraction of a proton from luciferyladenylate (DeLuca, M. & McElroy *Biochem* 13, 921-925, 1974). Luciferyladenylate is oxidized with oxygen under production of AMP and excited oxyluciferin, (which both remain enzyme bound), and carbon dioxide. Oxyluciferin is transformed to a ground state by emitting a photon. The energy for emitting the photon has been obtained from the oxidation of luciferin. Luciferin analogs make it possible to optimize the luciferin-luciferase ratio with respect to production cost of the reagent and increases stability of the light level.

U.S. Pat. No. 4,286,057 to Wulff discloses that the luciferase of the firefly (*Photinus pyralis* et al) catalyzes the following reaction;



The light that is produced in this reaction is emitted with a yield of virtually 1 Einstein per mole of ATP, and has a wavelength of 562 nm at the peak. The reaction is extremely sensitive, and permits the quantitative determination of ATP concentration down to 10^{-13} M per liter. This patent further teaches that the addition of AMP so modifies the properties of firefly luciferase, that the product inhibition by oxyluciferin, which usually occurs in the course of the reaction, is eliminated. The result is that, when a defined ATP concentration is measured, instead of the flash-like signal-time curve, a substantial constancy of signal over more than 15 minutes is achieved.

ATP determinations are based on a highly sensitive technique, resulting from the demonstration that luminescence in fireflies (*Photinus pyralis*) requires ATP and cannot use other sources of energy (Hastings J. W., *Ann Rev Biochem* 37:603, 1968). Light production with firefly lantern extract appears to be strictly proportional to the amount of ATP, and depends on the presence of luciferinluciferase, oxygen and magnesium ions. Under optimal conditions, each molecule of reacting ATP produces one photon of light. By means of present ultrasensitive photometers, this firefly bioluminescent reaction has now become the most sensitive method for ATP measurement.

In addition to ATP research, extensive work has been performed to stabilize and derivatize liposome particles as immunological reagents. The following publications are illustrative of this work:

U.S. Pat. No. 3,850,578 to McConnell discloses the use of sacs including erythrocyte ghosts, liposomes, or vesicles, which are lysable and capable of use in an immunoassay by containing an epitope on the surface of the sac which will be bound by its respective antibody. The antibody binding can occur through complement mediation resulting in lysis of the sac, or separation of agglutinated sacs followed by lysing of the sacs. The lysed sacs are loaded with a water soluble stable free radical compound, which can be detected when released into an environment external to the sac.

Kinsky et al, *Biochemistry* Vol. II, No. 22, 1972 pp. 4085-4093, describes the preparation and use of sensitized liposomes which are capable of being bound by specific antibodies and undergoing complement mediated immune lysis. Markers such as, for example, glucose entrapped in the liposomes are released and can be measured. The sensitizer is defined as the component which serves to render the liposome sensitive to lysis by a specific antiserum in the presence of complement. The sensitizer molecule comprises an amphiphilic body portion having a polar tail and a polar intermediate portion which is joined to an antigenic head.

Robinson, *Trans. Faraday Soc.* 56:1260-1264 (1960), and Papahadjopoulos et al *Biochim. Biophys. Acta* 135:639 (1967) describe a method of forming phospholipid dispersions from an ether-lipid aqueous two-phase system involving evaporation of the ether by bubbling nitrogen through the mixture.

Chowhan et al, *Biophys. Acta*, 266:320-342 (1972) describe a similar evaporation technique from a chloroform-aqueous two phase system using an excess aqueous phase and the slow removal of the chloroform phase in order to produce a uniform population of phospholipid vesicles.

Bangham et al *J Mol Biol.* 13:238-252 (1965) describes multilamellar lipid vesicles which could be char-