Background

Chemiluminescence describes the emission of light that occurs as a result of chemical reactions. These reactions frequently involve the release of large amounts of energy. This energy is dissipated in the form of photons when electronically excited molecules, produced as a result of the reaction, relax to a stable ground state. The unique ability of chemiluminecence to provide a high sensitivity detection system has been exploited in medical diagnostics through the use of aryl Acridinium Ester (AE) compounds.

The technology for linking AE compounds to binding reagents such as antibodies or oligonucleotide probes is well established. These labelled reagents can be designed to target specifically an infinite range of substances of biomedical interest which are then quantified by the intensity of the light generated in the chemiluminescent reaction. Such assays were initially developed to investigate human diseases by analysing changes in blood constituents such as hormones. Subsequently, they have been applied to the detection and monitoring of tumour products, infectious agents such as HIV, markers of coronary heart disease and the gene mutations that give rise to inherited diseases. Acridinium based assays have been developed in a wide range of assay formats including fully automated systems (eg Centaur, Siemens Medical Systems; Tigris, Gen-Probe Inc) and microtitre plate immunoassays.

These analytical methods are not confined to applications in the clinical laboratory. They can, for example, be used to assess the toxicological impact of environmental pollutants through monitoring changes in the expression of marker genes in organisms, tissues and cell lines. The sensitivity of detection, long-term stability and ease of use combine to make acridinium labels a logical and preferable alternative to radioactivity.

Comparison with Other Technologies

AE labels are stable compounds which can be oxidised under alkaline conditions to generate intense chemiluminescence. They can be detected in a simple luminometer with greater sensitivity than radioisotope labels such as ^{125}I. Light emission from acridinium esters is rapid, the reaction being completed typically in less than 1 second.

Since AE provides a direct labelling system, very few manipulations are required in comparison to assays based on enzyme labels, where it is necessary to convert substrates to detectable products following completion of the immunochemical reaction. With a measurement time of only 1 second, AE chemistry provides an extremely rapid and user-friendly detection system.

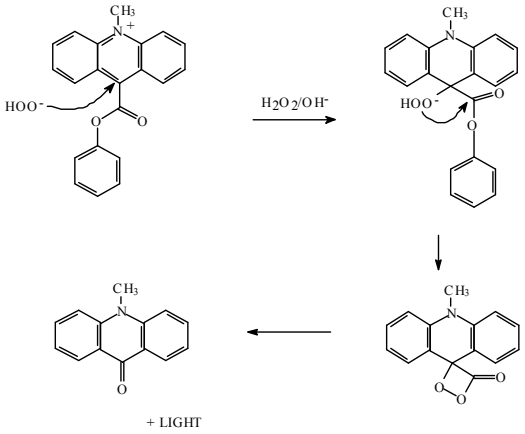
In ELISA systems, the working range of an assay is limited by the fact that light transmission decreases as an exponential rather than a direct function of product concentration, thus restricting the range over which measurements can be made. At low product concentrations, sensitivity is limited by the fact that transmission is maximal, making small changes in the signal difficult to differentiate. ELISA optimisation represents a trade-off between sensitivity and working range. In contrast, when measuring chemiluminescence, a standard photomultiplier tube can record light intensity in a linear fashion over at least 6 decades of product concentration against a background that is virtually zero. This means that assays based on AE can be optimised over an extremely wide working range without compromising analytical sensitivity.

In comparison with fluorescence, where light energy itself is used to produce electronically excited states, the low background generated by chemiluminescence offers a significant advantage. It is the high signal/noise ratio obtained with photon counting that makes chemiluminescence one of the most sensitive end-point detection systems.

AE Chemistry

The AE labels used in Invitron's chemiluminescence detection system are stable compounds which provide high quantum light yields with extremely accurate quantification. Light emission at a wavelength of 425 nm occurs when the AE is oxidised in the presence of alkali to yield N-methyl acridone via a dioxetanone intermediate, according to the scheme shown in figure 1. A wide range of instrumentation is now commercially available for the sensitive measurement of chemiluminescence in both tube and microtitre plate assay formats.

Figure 1: Chemical reaction of acridinium ester



In practice, the reaction is carried out by the use of hydrogen peroxide at alkaline pH. Since the peroxide is unstable at high pH, it is not practicable to prepare a single reagent to trigger the reaction. Two detection reagents are therefore used:

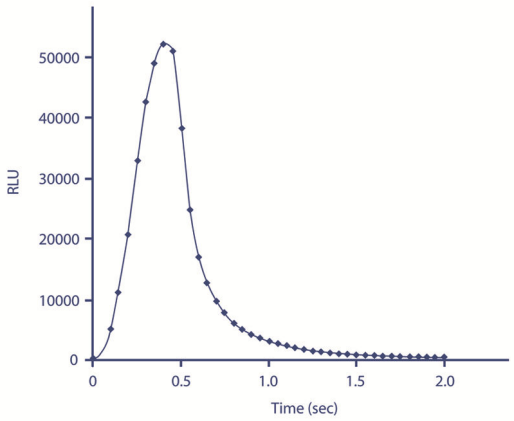
- Reagent 1 is a dilute solution of hydrogen peroxide in 0.1 M nitric acid.
- Reagent 2 is 0.25 M sodium hydroxide containing a surfactant.

In a typical microtitre plate assay, reagents are incubated in antibody-coated wells. After washing to remove any unbound reagent, the following protocol is used to trigger the AE light emission in a luminometer:

1. Add Reagent 1 (100µl)
2. Time delay (approx 2 seconds)
3. Add Reagent 2 (100µl)
4. Measure light output using a luminometer (approx 1 second)

This AE measurement procedure relies on the fact that mixing of the reagents takes place extremely rapidly and efficiently. A kinetic data plot showing the emission of light during a typical AE reaction can be seen in figure 2.

Figure 2: Kinetic data plot for an AE 'flash'



Bibliography

For more detailed information on AE chemiluminescence and its applications, further reading can be found in the following publications:

1. Weeks I *et al*. Acridinium esters as high specific activity labels in immunoassay. Clin Chem 29: 1474-1479 (1983).
2. Weeks I *et al*. Immunoassays using acridinium esters. Methods Enzymol 133: 366-387 (1986).
3. Sturgess ML *et al*. An immunochemiluminometric assay for serum free thyroxine. Clin Endocrinol (Oxf) 27: 383-393 (1987).
4. Woodhead JS, Weeks I. Immunochemiluminometric assays based on acridinium labels with a microtitre plate luminometer. Clin Chem 37: 472 (1991).
5. Turner G *et al*. Urinary growth hormone excretion as measured by a sensitive immunochemiluminometric assay. Ann Clin Biochem 30: 180-185 (1993).
6. Weeks I. Chemiluminescence Immunoassay, Elsevier, Amsterdam, 1992.