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Short Communication

Chemiluminescent and bioluminescent immunoassays*

L. J. KRICKA and T. P. WHITEHEAD

Department of Clinical Chemistry, Wolfson Research Laboratories, University of Birmingham, Birmingham B15 2TH, UK

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Introduction

Analytical techniques based on chemiluminescence and bioluminescence have proved to be very sensitive, and detection limits in the sub-attomolar range have been reported [1]. It is not therefore surprising that the application of these reactions in immunoassay should have been investigated. Two major types of application have emerged. Firstly, the use of a component of a chemiluminescent or bioluminescent reaction as a label, and second the use of such reactions to detect a conventional label [2].

Components of chemiluminescent and bioluminescent reactions as immunoassay labels

Most attention has focussed on luminol and isoluminol labels [2]. These react with an oxidant (e.g. peroxide) in the presence of a suitable catalyst (e.g. microperoxidase) to produce light according to the reaction scheme shown in Fig. 1. More recently acridinium

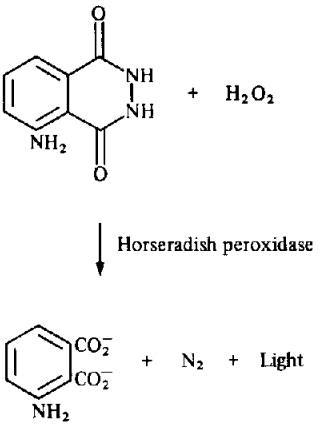

Figure 1
Luminol reaction for chemiluminescence.

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esters [1, 2] and phenanthridinium esters [4] (Fig. 2) have been used as labels, and reagents for immunoassays utilising these labels have become commercially available from Ciba-Corning and from Mallinckrodt, respectively.

Components of bioluminescent reactions have had limited success as immunoassay labels. Luciferases (enzymes which catalyse bioluminescent reactions) tend to lose activity when coupled to antigens and antibodies, whilest NAD (a component of the marine bacterial bioluminescent reaction) and ATP (a component of the firefly reaction) require complex chemical modification before they can be used as labels. Table 1 lists representative examples of immunoassays using (components of) chemiluminescent and bioluminescent reactions as labels.

Chemiluminescent and bioluminescent detection of immunoassay labels

All of the commonly used enzyme labels can now be detected using a light emitting reaction (Table 2). An advantage of this kind of immunoassay is that it combines the amplification effect of an enzyme label with the sensitivity of a luminescent reaction.

Table 1
Immunoassays which use components of chemiluminescent and bioluminescent reactions as labels

Label	Analyte	Detection limit	Reference
Luminol	IgG	5 ng/mL	9
Isoluminol	Progesterone	25 pg	10
Acridinium ester	Alpha-fetoprotein	1.3 KIU/L	11
Phenanthridinium ester			4
Firefly luciferase	Methotrexate	500 fmol	12
ATP	Myoglobin	50 ng/mL	13

Table 2
Chemiluminescent and bioluminescent assays for enzyme labels

Label	Detection system	Reference
Alkaline phosphatase	D-luciferin-*O*-phosphate, firefly luciferase, ATP	14
Beta-galactosidase	Galactose dehydrogenase, bacterial luciferase system	15
Glucose oxidase	bis (2,4,6-trichlorophenyl)oxalate-8-anilnonaphthalene-1-sulphonic acid	16
Pyruvate kinase	Firefly luciferase system	17
Glucose-6-phosphate dehydrogenase	Bacterial luciferase system	18
Horseradish peroxidase	Luminol-peroxide-p-iodophenol	5

Several luminescent assays for horseradish peroxidase labels have been developed and the most effective is the enhanced chemiluminescent assay technique [5]. This involves reacting peroxidase with a mixture of luminol, peroxide and an enhancer such as *p*-iodophenol. Peroxidase acts catalytically in the chemiluminescent oxidation of luminol by peroxide and the enhancer acts synergistically to increase the magnitude of light emission. Other cyclic diacyl hydrazide-oxidant combinations can be used, e.g. isoluminol-perborate, and several alternative enhancers are available, e.g. *p*-hydroxycinnamic acid, 2,4-dichlorophenol, firefly luciferin. Enhanced chemiluminescent endpoints have been incorporated into a wide range of enzyme immunoassays based on peroxidase labels and some examples are listed in Table 3.

Table 3
Enhanced chemiluminescent enzyme immunoassays

Analyte	Enhancer	Solid support	Reference
Alpha-fetoprotein	Firefly luciferin	Polystyrene bead	19
IgE	p-iodophenol	Plastic tube	20
Ferritin	Firefly luciferin	Mictotitre plate	21
Factor VIII related antigen	p-iodophenel	Microtitre plate	22

A characteristic of enhanced chemiluminescent reactions is that the light emission is intense and prolonged. This makes this type of end-point ideally suited to small batch analysis because the stable light emission eliminates the requirement for carefully timed initiation and, incubation periods. Microtitre plates and strips of microtitre wells provide an ideal solid phase for small batch analysis and several enhanced chemiluminescent enzyme immunoassays have been designed around this format. The microtitre wells must be constructed from opaque plastic in order to prevent the measurement of light from a well being influenced by light emission from adjacent wells ("cross-talk"). Several luminescence microtitre plate readers are now available (e.g. Dynatech Laboratories) which are capable of measuring the light emission from all 96 wells of an opaque microtitre plate in less than one minute. A complete system for enhanced chemiluminescent enzyme immunoassays which uses strips of microtitre wells and a luminescence microtitre plate reader has recently been marketed under the trade name of "Amerlite" by Amersham International [6].

Photographic Immunoassays

If a chemiluminescent or bioluminescent reaction is sufficiently intense then it is possible to detect the light using photographic film. Photographic assays for a range of analytes have been described including metal ions (e.g. cobalt, lead, manganese), cofactors (e.g. NADH, ATP), steroids (e.g. cholylglycine) [7].

This approach to analysis has now been extended to immunoassay, in particular enhanced chemiluminescent enzyme immunoassays. The combination of photographic film and enhanced chemiluminescence is ideal because (a) the intense nature of the light emission renders it readily detectable photographically and (b) the prolonged nature of the emission permits the reactions to be initiated prior to exposure to the film. A simple camera luminometer has been designed which uses instant photographic film (typically, Polaroid Type 612, ASA 20,000) to detect light emission from the wells of a polyvinyl chloride microtitre plate or from the surface of a membrane [8]. It consists of a light-tight box located on top of a film holder. A loose fitting labyrinth ensures a light-tight fit between the lid and the body of the instrument. A reaction vessel holder is located on top of a slidable shutter inside the light-tight box and when the shutter is opened the holder drops on to the film to produce a contact print. Results of photographic assays are usually assessed by visual comparison of exposures (exposed, partially exposed, unexposed) produced by samples and standards. Semi-quantitative results can be achieved by interposing neutral density filters between the film and the glowing reaction vessels. Some examples of photographic enzyme immunoassays are collected in Table 4.

The simplicity of a camera luminometer (small, light-weight, portable, no power requirement) recommends it for use in extra-laboratory analyses (e.g. field tests). Its

Table 4

Analyte	Solid support	Detection system	Reference
Cytomegalovirus specific IgG	PVC microtitre plate	Luminol-peroxide-6 hydroxybenzothiazole	24
Factor VIII related antigen	PVC microtitre plate	Luminol-peroxide p-iodophenol	25
Ferritin	PVC microtitre plate	Luminol-peroxide firefly luciferin	21
Malaria specific IgG	PVC microtitre plate	Luminol-peroxide-p-iodophenol	25
Rubella specific IgG	PVC microtitre plate	Luminol-peroxide-p-iodophenol	25

potential for this type of application has been demonstrated with enhanced luminescent enzyme immunoassays for specific antibodies in blood (e.g. antibodies to malarial parasites, antibodies to cytomegalo virus).

Conclusion

Interest in the fundamental and applied aspects of chemiluminescent and bioluminescent reactions continues to grow. In the case of luminescent immunoassay, development has progressed to the stage where reagents in the form of kits are now available for several analytes. The main advantages of chemiluminescent and bioluminescent reactions are sensitivity and simplicity. These advantageous characteristics have been effectively exploited in the development of ultra-sensitive immunoassays and in the development of simple and rapid photographic immunoassays. The latter application is expected to assume increasing importance in view of the mounting interest in simple analytical systems for use in field laboratories, clinics, the home or at the patients' bedside.

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