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CHEMILUMINESCENT ASSAYS BASED ON ACRIDINIUM LABELS

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The synthesis and study of the chemistry of acridinium compounds was reported during the last century. The synthesis of acridine-9-carboxylic acid, which is a useful precursor for the synthesis of chemiluminescent acridinium salts, was described by Lehmstedt and Wirth as early as 1928 (1). The chemiluminescence of bis(methylacridinium nitrate) was first described by Gleu and Petsch in 1935 (2) and has subsequently been better known as lucigenin. More detailed studies of the chemiluminescence of acridine compounds were carried out by Rauhut and co-workers (3) and by McCapra et al (4).

It is established that a wide range of organic molecules are potentially capable of being used as end-points for binding assays such as immunoassays and oligonucleotide probe hybridisation assays. It is possible to make use of these by coupling the chemiluminescent molecule itself into the binding reaction by attachment to one of the components thereof. Alternatively a label can be chosen which is itself not chemiluminescent but rather is one which can initiate the reaction of a chemiluminescent molecule. Examples of the latter include the use of horseradish peroxidase or xanthine oxidase labels to initiate luminol chemiluminescence and the use of alkaline phosphatase to cleave phosphorylated stable dioxetanes.

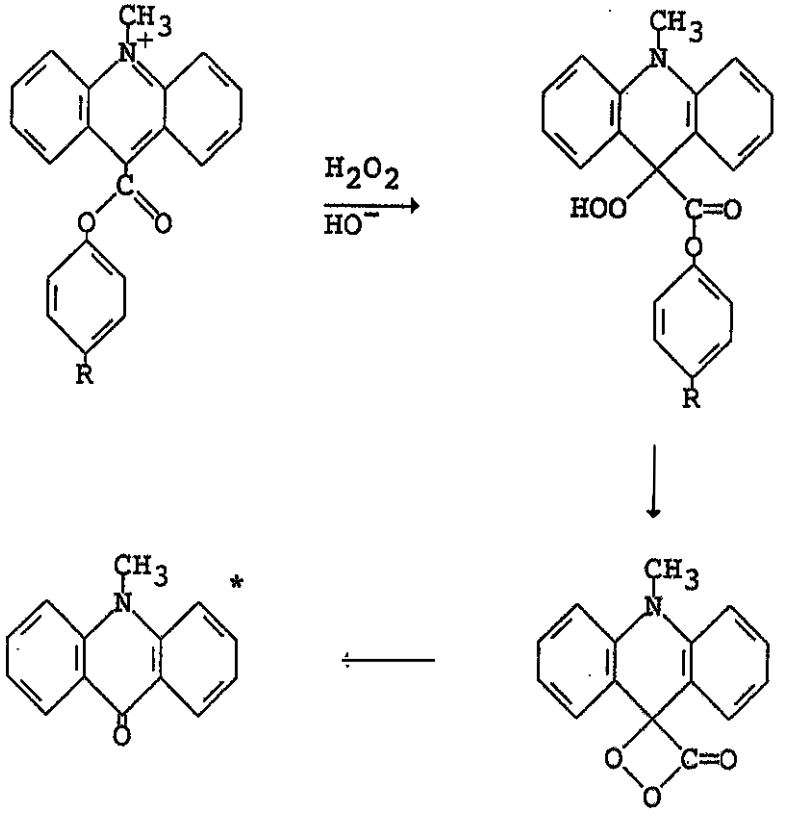
There are far fewer examples of different chemiluminescent molecules being used in the "direct" labelling mode. In the past, phthalhydrazide molecules have been most widely used for this purpose, particularly isoluminol derivatives such as aminobutylethylisoluminol (ABEI).

Such phthalhydrazides can be stimulated to emit chemiluminescence under a variety of conditions. Most commonly in the present application initiating reagents consist of alkali, hydrogen peroxide and a "catalyst". A wide variety of oxidising species can yield phthalhydrazide chemiluminescence and many chemical species are capable of "catalysing" or otherwise

modulating the reaction. Transition metal cations whether free, chelated or part of metalloproteins display a range of "catalytic" activities in this respect.

By contrast, certain acridinium salts exhibit chemiluminescence in the presence of dilute alkaline hydrogen peroxide without the need for a catalyst. Such an oxidation system is very simple and robust and also yields low chemiluminescence backgrounds. Most commonly, chemiluminescent acridinium salts are aryl esters, thioesters, active aliphatic esters and active amides. Derivatives of these can be synthesised to enable them to be coupled to a component of the required binding reaction to form the labelled reagent.

Phthalhydrazide molecules frequently exhibit loss of chemiluminescence activity when coupled to the relevant species, particularly proteins. Acridinium salts possess inherently high quantum yields which are maintained when the molecules are coupled in the appropriate manner. In part this is due to the fact that under such circumstances the emitting species, N-methylacridone, is dissociated from the rest of the labelled molecule and is thus less subject to microenvironmental quenching effects (Fig. 1).



The chemiluminescent salt most widely demonstrated for immunoassay purposes possesses an N-succinimidyl group. Here the N-succinimidyl ester moiety enables the molecule to be smoothly incorporated into species containing primary and secondary aliphatic amines, though other active groups can be incorporated into the synthesis of the acridinium salt if required.Many immunoassays have been described which are based on this label (Table 1). Of historical interest is an immunochemiluminometric assay for thyrotrophin (TSH) based on acridinium labelled antibodies (5). As early as 1984 this system demonstrated the high sensitivity achievable relative to conventional techniques. The use of high affinity monoclonal antibodies labelled to high specific activity with the chemiluminescent acridinium ester enabled a ten-fold improvement in sensitivity to be achieved over state-of-the-art methods.

Table 1. Some immunoassays based on acridinium salts

Labelled Antibody	Labelled Antigen
Alphafetoprotein	Progesterone
Ferritin	Calcitonin
Complement C9	Growth hormone releasing factor
Tamm-Horsfall glycoprotein	Albumin
Thyrotrophin	
Growth hormone	
Parathyroid hormone	
Thyroxine	
Fluphenazine	

Recently, acridinium labelled reagents have been adapted to full automation. Ciba Corning Diagnostics have developed an instrument (the ACS:180) capable of running their range of "Magic Lite" immunoassays in an automated, random access mode. Sample throughput is 180 samples per hour and time to first result is 15 minutes. The system has on-board reagent capacity to run 13 different analytes. A further development has been to make use of new instrumentation to configure simple, robust screening assays using acridinium labelling technology. Microtitre plates are particularly well-suited for this purpose but the constraints of existing instrumentation have not made it possible to exploit the advantages of of acridinium labels in this format. The introduction of microtitre plate luminometers with "on-board" reagent injection facilities now permits the high performance of acridinium based assays to be combined with an easy-to-use microtitre plate format. This system has been used in our laboratory to develop screening assays for thyrotrophin in 3mm neonatal blood spot discs and also for alphafetoprotein in a 50ul maternal serum sample. These

assays have a one hour incubation, following which the plate is washed and inserted into the luminometer. This end-point detection is more rapid and robust than enzyme-driven systems which involve addition of substrate followed by a delay period prior to quantitation. These assays are described in greater detail elsewhere in this volume.

The acridinium salt described earlier has also been successfully used as a label for oligonucleotide probe hybridisation assays marketed by Gen Probe Inc as the PACE System. In such applications, labelled probe (typically 20-30 bases) forms duplexes with target DNA or RNA if present. The duplexes are then selectively bound to magnetisable particles which permits separation and quantitation of chemiluminescence, the intensity of which is proportional to the amount of target nucleic acid present.

A novel approach to hybridisation assays also developed by Gen Probe involves the development of a homogeneous assay which does not require the use of a separation step prior to measurement (6). This is based on the chemical properties of the acridinium label which permits its hydrolysis under alkaline conditions, the hydrolysis products being non-chemiluminescent. However, when the labelled probe is involved in duplex formation with target nucleic acid, the acridinium ester is resistant to hydrolysis and thus retains its chemiluminescent activity. In this way, labelled probe can be added to the sample and, if target is present, duplexes will form. The mixture can then be exposed to alkaline conditions at elevated temperature and then chemiluminescence emission quantified in a luminometer. The intensity of chemiluminescence is thus proportional to the amount of target nucleic acid present.

This overview thus demonstrates the ability of acridinium salts to yield sensitive yet simple end-points for monitoring binding reactions.

REFERENCES

1. K. Lehmstedt and E. Wirth. Ber. Dtsch. Chem. Ges. 61, 2044 (1928).
2. K. Gleu and W Petsch. Angew. Chem. 58, 57 (1935)
3. M.M. Rauhut, D. Sheehan, R.A. Clarke and A.M. Semsel. J. Org. Chem. 30, 3587 (1965).
4. F. McCapra and D.G. Richardson. Tetrahedron Lett 43, 2167 (1964).
5. I. Weeks, M. Sturgess, K. Siddle, M.K. Jones and J.S. Woodhead. J.Endocrinol. 20, 489 (1984).
6. L.J. Arnold, P.W. Hammond, W.A. Wiese and N.C. Nelson. Clin. Chem. 35, 1588 (1989).