Thermochemiluminescence and its application in immunoassay

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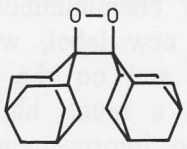
SUMMARY

Adamantylideneadamantane 1,2-dioxetane (1) is a 1,2-dioxetane with a half-life of about 16,000 years at room temperature. The half-life of 1 decreases exponentially at higher temperatures and is only 25 seconds at 240°C.

Blue light is emitted during the decomposition of the four-membered 1,2-dioxetane ring of 1. The photons, which are emitted during the chemical reaction can be counted by a sophisticated photon counter. This photon counter can distinguish an amount of $3*10^{-13}$ gram of 1 (10^{-15} mole) from the background luminescence.



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The decomposition of 1 is called thermochemiluminescence (TCL). The luminescence can be amplified by a variety of fluorescent molecules. Some derivatives of compound 1 in combination with a fluorescent label can be used as molecular labels. A molecule covalently bound to a derivative of 1 can give a light signal.

A thermochemiluminescence immunoassay has been developed in which unknown concentrations of a hormone, a tumor marker and proteins can be detected at very low concentrations. This thesis describes the properties of 1 and its derivatives and the development of a thermochemiluminescence immunoassay.

In Chapter 1, an introduction is given to the immunoassay technology and to the participation of bioluminescence and chemiluminescence in this technique.

Chapter 2 deals with the properties of 1 and its derivatives. Different decomposition mechanisms of 1,2-dioxetanes are discussed, the covalent coupling of derivatives of 1 to antibodies and other proteins, the quenching of 1,2-dioxetane chemiluminescence by other compounds, reproducibility of the TCL measurements and the handling of TCL data are described.

Chapter 3 shows the screening of a series of materials, which are appropriate being used at 240-260°C (the detection temperature). It has been shown that a polyimide -Kapton 500H- demonstrates excellent behaviour as a solid phase in immunoassay.

Chapter 4 describes the interaction of 1 and its derivatives with cyclodextrins. It is demonstrated that complexes of 1,2-dioxetanes and cyclodextrins can be measured in a reproducible way and a perfect linear dose response curve over 6 orders of magnitude can be obtained. Also it is shown that quenching of 1,2-dioxetane chemiluminescence can be prevented in some cases by complexing of 1,2-dioxetanes with cyclodextrins.

Chapter 5 shows the development of a thermochemiluminescence immunoassay for HCG (human chorionic gonadotrophin, a hormone which indicates pregnancy). A concentration of 10 ng HCG/ml serum can be detected in a quantitative manner. Experiments are described in which these results are further improved. A calculation is given of the potential of the thermochemiluminescence immunoassay sensitivity.

Chapter 6 describes the automation of the measurement protocol, allowing 240 samples to be counted successively without manual interference.

Finally, in the concluding remarks the advantages and the disadvantages of thermochemiluminescence are discussed.