World Academy of Science, Engineering and Technology
International Journal of Mathematical and Computational Sciences
Vol:14, No:12, 2020

# Caged Compounds as Light-Dependent Initiators for Enzyme Catalysis Reactions

**Authors :** Emma Castiglioni, Nigel Scrutton, Derren Heyes, Alistair Fielding

**Abstract :** By using light as trigger, it is possible to study many biological processes, such as the activity of genes, proteins, and other molecules, with precise spatiotemporal control. Caged compounds, where biologically active molecules are generated from an inert precursor upon laser photolysis, offer the potential to initiate such biological reactions with high temporal resolution. As light acts as the trigger for cleaving the protecting group, the 'caging' technique provides a number of advantages as it can be intracellular, rapid and controlled in a quantitative manner. We are developing caging strategies to study the catalytic cycle of a number of enzyme systems, such as nitric oxide synthase and ethanolamine ammonia lyase. These include the use of caged substrates, caged electrons and the possibility of caging the enzyme itself. In addition, we are developing a novel freeze-quench instrument to study these reactions, which combines rapid mixing and flashing capabilities. Reaction intermediates will be trapped at low temperatures and will be analysed by using electron paramagnetic resonance (EPR) spectroscopy to identify the involvement of any radical species during catalysis. EPR techniques typically require relatively long measurement times and very often, low temperatures to fully characterise these short-lived species. Therefore, common rapid mixing techniques, such as stopped-flow or quench-flow are not directly suitable. However, the combination of rapid freeze-quench (RFQ) followed by EPR analysis provides the ideal approach to kinetically trap and spectroscopically characterise these transient radical species. In a typical RFQ experiment, two reagent solutions are delivered to the mixer via two syringes driven by a pneumatic actuator or stepper motor. The new mixed solution is then sprayed into a cryogenic liquid or surface, and the frozen sample is then collected and packed into an EPR tube for analysis. The earliest RFQ instrument consisted of a hydraulic ram unit as a drive unit with direct spraying of the sample into a cryogenic liquid (nitrogen, isopentane or petroleum). Improvements to the RFQ technique have arisen from the design of new mixers in order to reduce both the volume and the mixing time. In addition, the cryogenic isopentane bath has been coupled to a filtering system or replaced by spraying the solution onto a surface that is frozen via thermal conductivity with a cryogenic liquid. In our work, we are developing a novel RFQ instrument which combines the freeze-quench technology with flashing capabilities to enable the studies of both thermally-activated and light-activated biological reactions. This instrument also uses a new rotating plate design based on magnetic couplings and removes the need for mechanical motorised rotation, which can otherwise be problematic at cryogenic temperatures.

**Keywords :** caged compounds, freeze-quench apparatus, photolysis, radicals
**Conference Title :** ICSRD 2020 : International Conference on Scientific Research and Development
**Conference Location :** Chicago, United States
**Conference Dates :** December 12-13, 2020