

Making enzyme-responsive PEG-based microparticles

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Responsive polymers are well documented, demonstrating a change in their properties in response to a number of different stimuli (a few examples being temperature, [1] electric field [2] and pH) [3]. Enzyme-responsive materials are particularly interesting due to the properties of enzymes; being highly selective in the reactions they catalyse, working under mild conditions (aqueous, pH 5–8, 37 °C) and playing key roles in healthy and diseased biological pathways [4].

Polyethyleneglycol acrylamide (PEGA) is a polymer hydrogel that is compatible with both aqueous and organic solvents allowing easy chemical modification through solid phase peptide synthesis [5]. Numerous studies have demonstrated that PEGA is also enzyme compatible [6,7]. Its high PEG content prevents non-specific protein absorption and its gel-like matrix facilitates fast diffusion of biomolecules through the polymer. Several workers have shown that PEGA is accessible to small enzymes and that enzyme activity can occur inside the resin [8]. It has also been published that reactions between enzymes and on PEGA give near complete conversion with cleavage occurring at expected sites [9]. Earlier results from our group demonstrated that PEGA can be used as an enzyme-responsive material whereby carefully designed peptides act as the stimuli-responsive elements. [10, 11] PEGA beads that are commercially available are between 100-500 μm in diameter. For future applications in automated screening and in drug delivery much smaller particles are required, giving rise to faster response times and possibilities of automated analysis using a cell sorter.

In this work micron-sized PEGA enzyme particles have been produced by inverse suspension polymerisation. These particles have a mean diameter of 15 μm and 90% of the total volume of polymer is between 10-24 μm in diameter. Using fluorescent labeling and two-photon microscopy we show that these particles have a homogeneous distribution of amine groups for functionalisation. HPLC analysis combined with two-photon microscopy confirmed that the microparticles are compatible with enzymes and enzyme reactions are faster compared to those on conventional macro beads. An enzyme-responsive change in swelling and thus accessibility of the particles has been demonstrated by the incorporation of charged peptides and treatment with an enzyme with the correct specificity. Future work will include the release of an entrapped payload in response to a specific enzyme and the use of microfluidic devices to produce particles with a much more narrow distribution.

References

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