Kinetic characterization of feruloyl-esterase immobilized on nanoparticles. Direct visualisation and characterization of derivatised nanoparticles

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1. Introduction

During recent years, enzymes have attracted the attention of scientists because of their peculiar properties that render them particularly useful in investigating the relationship existing between enzyme stability and flexibility on one hand, and enzyme activity on the other hand. Among these enzymes, the esterases, and particularly the feruloyl esterases, have potential uses over a broad range of applications in the agro-food and pharmaceutical industries.

The importance of Feruloyl esterase also relates to the enzyme product ferulic acid and ferulated oligosaccarides, which have a potential application for food and medicine uses, so that the ferulic acid derivates are used like strong antioxidants and have gel-forming properties.

Furthermore, this effect has been employed to form potential protective agents against skin damages. This enzyme catalyzes the hydrolysis of complex oligosaccharides to produce the ferulic acid. In recent years, the number of microbial feruloyl esterase activities has increased in the growing genome databases. This project is about the feruloyl esterase from a psycrophilic bacterium P.Haloplanktis cloned in E. coli.

The use of enzymes, proteins in applied sciences is broadly expanded due to the fast emerging field of nanoparticle syntheses and characterisation. Immobilisation of biomolecules onto nanoparticles opens for new nanotechnological applications. Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields.

2. Project

This project focuses on the immobilization of the feruloyl esterase on functionalized nanoparticles or on functionalized glass slides and on the kinetic characterization of immobilized enzyme.(fig.1). In order to obtain the enzyme this project includes the expression end purification of the cloned enzyme.

The kinetic characterization on synthetic substrates and natural substrates will be performed using spectroscopic methodologies, like steady state fluorescence and absorption spectroscopy.

Direct visualization and characterization of the derivatised nanoparticles will be done with our new NanoSight instrument.

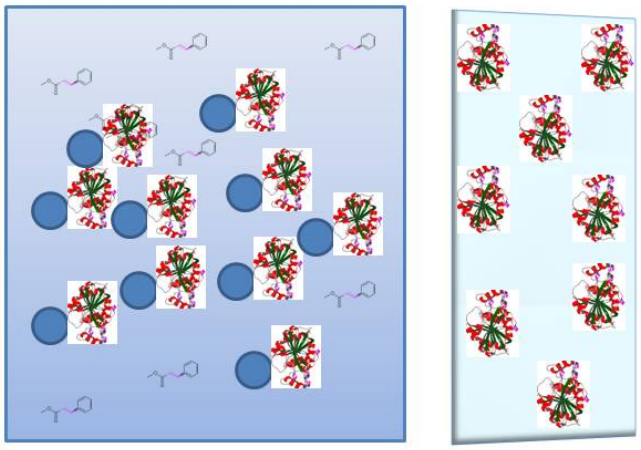

Figure 1: Schematic diagram of feruloyl esterase on nanoparticles in solution and on glass slides.

In order to visualize the nanoparticles you will be using a new technology called Nanoparticle tracking analysis (NTA). It allows nanoscale particles to be individually visualised (but not imaged) in liquids and from which higher resolution particle size distribution profiles can be obtained compared to other light scattering techniques. Sample pre-treatment is minimal requiring only dilution with a suitable solvent to an acceptable concentration range (between 10^5 and 10^{10} per ml depending on sample type). Accurate and reproducible analyses can be obtained from video of only a few seconds duration and the results allow particle number concentration to be recovered. Given the close to real-time nature of the technique, particle-particle interactions are accessible as is information about sample aggregation and dissemination. All particle types can be measured and in any solvent type providing that the particles scatter sufficient light to be visible (i.e. are not indexed matched). The minimum detectable size measurable depends on particle refractive index but can be as low as 9-15nm for high refractive index materials such as colloidal silver.