Trigger enzymes for pectin based bioresponsive polymers

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Introduction

Bioresponsive devices can be obtained by assembling or coating of biomaterials which are partially degradable by enzymes. In general, bioresponsive polymers and capsules can simply react to pH or temperature changes in different environments (Gil et al. 2004 and Nair et al. 2007). A more selective delivery is obtained with devices responding to enzymes.

In this study a selection of indicating and pathogenic microorganisms was screened for different pectinase activities to act as trigger enzymes in bioresponsive devices. Concentrations and time profiles of the enzyme release were determined with different pectin assays. Degradation kinetics by trigger enzymes were studied monitoring the release of oligomers using HPLC.

A semi selective response towards bacteria can be realised with two different layers: a carrier layer and the bioresponsive layer. The schematic assembling is shown in Figure 1. The carrier layer such as polypropylene- and polyamide surface as a scaffold and was coated with a bioresponsive layer consisting of polysaccharide such as pectin. To create a simple screening system the bioresponsive layer was assembled without the carrier layer as beads. The beads were produced by precipitating a pectin in a calcium chloride solution.

One big advantage of pectin is its usage in food industries as a completely harmless additive to improve and optimise the physical conditions of eatables. The second important property is that it can be cleaved by three enzymes: Polygalacturonidase, pectate lyase and pectinesterase are able to transform pectin. The literature shows that a range of other hydrolytic enzymes are able to degrade pectin, but this work focus on these three noted enzymes.

Material and Methods

Enzymatic modification of pectin
Pectin is a polymeric material having carboxylic groups esterified with methanol. As a consequence of the carboxylic groups pectin is an anionic polysaccharide and can be precipitated with divalent ions like calcium. The three representing enzymes were used to monitor the degradation process on the pectin beads. The enzyme activities were measured by different assays (Schnitzhofer et al. 2007). The interactions of all of these three enzymes are shown in Figure 2.

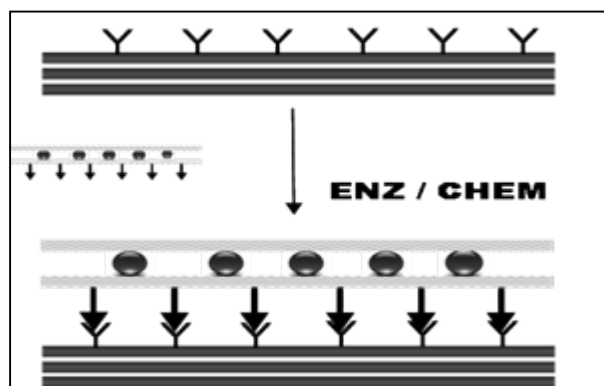

Figure 1 : Assembling of a functionalised Bioresponsive Polymer

Pectinlyase (EC 4.2.2.10)
Pectinlyase is able to cleave eliminative the 1-4-α-D-galacturonan methyl ester bond to give oligosaccharides with 4-deoxy-6-O-methyl-α-D-galact-4-enuronosyl groups.

Pectinesterase (EC 3.1.1.11)
The pectinesterase attacks the ester bond of the galacturonic acid and cleaves the polysaccharide to unestered pectin and methanol. As a difference to the polygalacturonidase as well as the pectinlyase the backbone of the macromolecule is not degraded.

Polygalacturonase (EC 3.2.1.15)
The polygalacturonidase is able to hydrolyse the 1,4-α-D-galactosiduronic linkages of pectate and other polygalacturonans. It is an unspecific enzyme and cleaves the bond in a random way.

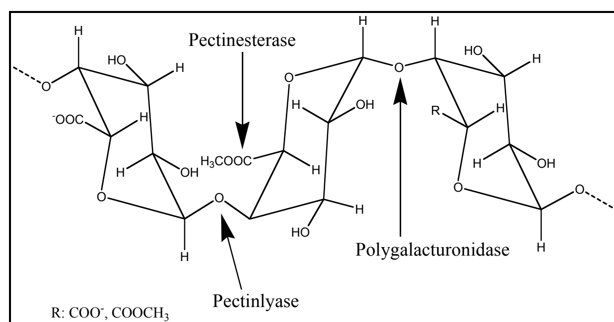

Figure 2 : Pectin cleaving enzymes

Consequently, such enzymes can act as triggers to release active molecules incorporated or coated by pectin as biomaterial.

The structure of pectin with its reactive and "useful" groups like COO^- and $COOCH_3$ is shown in Figure 3. Methylation of these carboxic acid groups forms their methyl ester, which take up a similar space but are much more hydrophobic and the properties of pectin depend on the degree of esterification which is characteristic for the source of the polysaccharide.

The nature of the biomaterial determines the nature of the trigger enzymes required. Degradation of pectins and calcium pectinates by the pectinolytic enzymes of the colonic flora is expected to increase the drug release in the colon (Sinha et al. 2001). Similarly, using specific pectinases, this concept can be adapted for controlled release from medical devices in response to extracellular enzymes from different pathogens.

Screening for trigger enzymes

For the screening process, a selection of different micro organisms, representing indicative and/or pathogenic strains were cultivated in a standard nutrient broth containing pectin. Followed by the investigation of enzymatic activities using different methods.

Polygalacturonidase

To detect Polygalacturonidase activity two different screening methods were used. An example of the spectrometric method is shown in Figure 3.

Spectrometric method
The spectrometric method described by Klug-Santner et al. 2006 consists of two parts. The first step is an incubation of the samples with polygalacturonic acid. After this the generated reducing sugar ends were detected with DNS reagent (3,5-dinitrosalicylic acid, Bernfeld reagent). The measurement itself was performed by absorbance at 540 nm.

Electrophoresis
The second used method was published by Schnitzhofer et al. 2007. It based on a native gel containing polygalacturonic acid as substrate. After a sample preparation with non-denaturating conditions a common polyacrylamide electrophoresis was performed. The gel was incubated in acetate buffer and stained with nil red.

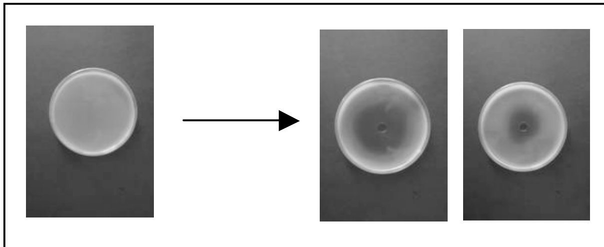

Figure 3 : "Clear zone" of pectin lyase

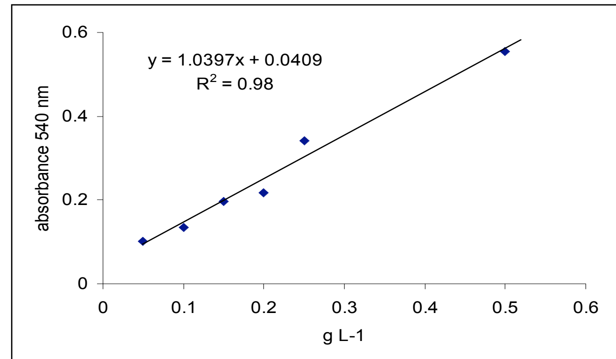

Figure 4: Calibration of the spectrometric method for Polygalacturonidase

Pectin lyase
The technique described by Souza et al. 2003 consists of incorporating pectin in a buffered agar. The mixture was poured in Petri dishes to give 4 mm layer. Cups 5 mm in diameter were cut and filled with 100 µl of the samples. The plates were incubated for 18 h at 37°C, followed by development of zones of hydrolysis with 5 N HCl. An example is shown in Figure 4.

Pectinesterase
Pectin esterase activity was elaborated by a continuous spectrophotometrical method based on the addition of the enzyme pectin indicator solution. The used solutions consitst of a 5% pectin solution added by 10 ml of a 0,017% Bromcresol Green solution as pH indicator. The pH value was adjusted with 1 M NaOH to the point of colour change. The Absorbance was measured at 617 nm and 25°C. The method was described by Ceci et al. 1998. An example of a calibration curve is shown in figure 3.

Preparation of model beads
As mentioned above the used model system is made of pectin and pectin blend micro beads. The beads were prepared using a peristaltic pump and a canula with a defined diameter. By controlling the flow rate different sizes of beads were prepared.

To increase the physical resistance of pectin, test polymer micro beads were prepared with pectin blended with alginate, a biopolymer with similar properties to pectin (Figure 5).

Figure 5 : Model beads

Results and Outlook

A selection of different micro organisms, representing indicative and/or pathogenic strains, was found to release pectin cleaving enzymes (Figure 6). Thus, pectin can be used as biomatrix for semi selective bioresponsive devices. However, it is to say that there is much more work necessary to improve the properties of pectin, e. g. by adding additives.

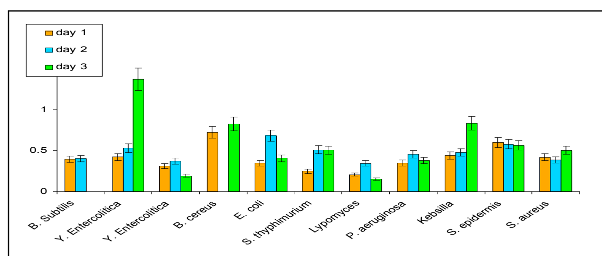

Figure 6 : Bacteria with different pectin-esterase activity

Beside the studies about enzymatic activities further experiments were performed to test the biodegradation of pectin and its blend with alginate. As conclusions it is to say that alginate improves the physical conditions of pectin. The blend becomes a little bit harder and is easier to handle during bacteria degradation processes. As a consequence the biodegradability is lower compared to pure pectin.

As conclusion it is to say that a polymer blend has the ability to optimise the physical properties of pectin to get a better practicability without losing the benefits of a well known non-toxic and cheap biopolymer

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