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Encapsulation of α-amylase into starch-based biomaterials: An enzymatic approach to tailor their degradation rate

Helena S. Azevedo *, Rui L. Reis

3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
IBB – Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Guimarães, Portugal

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Abstract

This paper reports the effect of α-amylase encapsulation on the degradation rate of a starch-based biomaterial. The encapsulation method consisted in mixing a thermostable α-amylase with a blend of corn starch and polycaprolactone (SPCL), which were processed by compression moulding to produce circular disks. The presence of water was avoided to keep the water activity low and consequently to minimize the enzyme activity during the encapsulation process. No degradation of the starch matrix occurred during processing and storage (the encapsulated enzyme remained inactive due to the absence of water), since no significant amount of reducing sugars was detected in solution. After the encapsulation process, the released enzyme activity from the SPCL disks after 28 days was found to be 40% comparatively to the free enzyme (unprocessed). Degradation studies on SPCL disks, with α-amylase encapsulated or free in solution, showed no significant differences on the degradation behaviour between both conditions. This indicates that α-amylase enzyme was successfully encapsulated with almost full retention of its enzymatic activity and the encapsulation of α-amylase clearly accelerates the degradation rate of the SPCL disks, when compared with the enzyme-free disks. The results obtained in this work show that degradation kinetics of the starch polymer can be controlled by the amount of encapsulated α-amylase into the matrix.

Keywords: Starch-based biomaterials; Controlled degradation rate; Thermostable α-amylase; Encapsulation; Enzyme activity

1. Introduction

The demands for biomaterials with controlled, predictable degradation kinetics includes a wide range of biomedical applications, such as resorbable surgical sutures, matrices for the controlled release of drugs and scaffolds for tissue engineering [1–4]. In fact, the performance of many biomaterials depends largely on their degradation behaviour since the degradation process may affect a range of events, such as cell growth, tissue regeneration, drug release, host response and material function.

Biodegradable polymers are materials with the ability of functioning for a temporary period and subsequently degrade in physiological conditions, under a controlled mechanism, into products easily eliminated in the body's metabolic pathways.

Several strategies have been developed to obtain biomaterials with a controlled degradation rate. Those have been based on molecular design principles, such as the introduction of hydrolysable bonds into polymer backbones [5], copolymerization and blending techniques [6], crosslinking [7,8] and surface modification methods [9,10], and inclusion of certain additives [11–13] into polymeric matrices (e.g. excipients, drugs, salts, etc.).

An interesting approach has been the development of polymeric systems with a self-regulated degradation

* Corresponding author. Address: 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal. Tel.: +351 253 510907; fax: +351 253 510909.
E-mail address: hazevedo@dep.uminho.pt (H.S. Azevedo).

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mechanism. In these systems, the degradation process is initiated and/or controlled under certain environment conditions or in response to cell activities [3,14,15]. For instance, Hubbell and co-workers [16–18] developed a novel class of hydrogels that are sensitive to the activity of cell-associated proteases. This cell-mediated degradation approach consists in the inclusion of enzymatic recognition sites into polymer backbones to make the material sensitive to the feedback provided by the cells involved in the healing response. In this way, the materials will degrade in response to cellular activities and the tissues may determine the degradation rate of the materials rather than the calendar.

Materials to be used in some applications, such as hard-tissue replacement, must combine adequate mechanical properties with controlled biodegradability. The material should degrade while maintaining a specified minimum mechanical strength to support the formation of new tissue. It may be difficult to achieve the desired combination of degradation and physical properties in a single material. In this context, enzyme encapsulation technology can be used to incorporate hydrolytic enzymes into the polymeric matrices and then provide systems with controlled degradation. Controlled degradation by enzymatic means presents several advantages considering the high specificity of enzymes for their substrates and also because enzyme activity can be regulated by environmental conditions (e.g. pH, temperature, the presence of certain substances, like metal ions) [19]. In addition, the degradation kinetics can be adjusted by the amount of encapsulated enzyme into the matrix. For instance, Goldbart et al. [14] developed an enzymatically controlled responsive drug delivery system consisting of a starch-based tablet incorporating a non-active α-amylase and a protein. The enzyme reactivation was made by the presence of calcium ions (which is known to be essential for enzyme's tertiary structure and catalytic activity) from the medium, which causes the tablet degradation and the concomitant release of the protein.

A basic requirement in enzyme encapsulation technology is that the integrity of the enzyme structure must be maintained during the encapsulation process. This is often a difficult challenge as most proteins are dependent on a three-dimensional conformation for their bioactivity and that conformation can be easily compromised. For instance, most of the polymers that are used in biomedical applications are not soluble in water and consequently the protein is exposed to an organic solvent during the encapsulation step. Examples of other stress conditions associated with the manufacture of medical devices, and that may compromise protein integrity, are the high shear forces used during extrusion processes or to form droplets of the polymer solution in a continuous phase, exposure to polymer reactions, high temperature and adverse pH values [20].

Starch is a fully biodegradable material that is readily available and can easily be modified, therefore constituting a good material to produce encapsulation matrices [21–24]. Several starch-based formulations have been prepared containing encapsulated α-amylase to develop enzymatically controlled drug delivery systems [14,21,24,25]. The methods used for the preparation of the tablets have been based mainly on mechanical compression [14,24]. Physical compression does not allow good adhesion to be obtained between components, leading to the formation of tablets without enough mechanical integrity. Starch has thus been blended with synthetic polymers (polycaprolactone, poly(lactic acid), poly(butylene succinate adipate, poly(ethylene-*co*-vinyl alcohol), etc.) to improve its weakness and obtain better mechanical properties [26–31]. Starch-based polymers have been studied and proposed as potential materials to be used in several biomedical applications [32–38], namely as carriers for drug delivery [36,37], hydrogels and partially degradable bone cements [37,39], and porous structures to be used as scaffolds in bone tissue engineering [33,35]. The susceptibility of these starch polymeric blends to enzymatic degradation has also been reported [40,41].

The processing conditions of thermoplastics, however, does not allow the incorporation of biomolecules like proteins or enzymes during processing, since the high temperatures used during melt-based processes can cause irreversible changes to the protein structure, leading to its denaturation. The use of enzymes with high heat stability can overcome this obstacle and constitute a good alternative that can efficiently encapsulate enzymes by melting techniques.

In this work, a thermostable α-amylase was encapsulated in a starch matrix (processed by compression moulding) with the aim of tailoring its degradation rate.

2. Materials and methods

2.1. Materials

The material used in this work was a commercially available thermoplastic starch-based polymer (Z grade MATER-BI®, ZI01U), supplied by Novamont S.P.A. (Novara, Italy) in granular form, containing starch, poly(ϵ-caprolactone) (PCL) and plasticizers (glycerol) [42]. This material is described in several patents [31,42–45], and is based on native maize starch and PCL with a molecular weight of 118,000 [42]. The properties and characteristics of the starch/PCL blend (SPCL) can be found in previous publications [46,47]. Soluble potato starch for measuring α-amylase activity was obtained from Sigma–Aldrich Inc. (St. Louis, MO, USA).

The encapsulant enzyme was a thermostable α-amylase supplied in a liquid formulation (SPEZYME® FRED, Genencor International Inc., Rochester, NY, USA) produced by a genetically modified strain of *Bacillus licheniformis*.

2.2. Preparation of starch-based matrices: the encapsulation method

Prior to use, the enzyme was first lyophilized to obtain the enzyme in powder form. Then the enzyme was mixed with the polymer powder (previously milled in a high speed milling equipment) at different weight percentages (0.5% and

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