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Dodecenyl succinylated alginate as a novel material for encapsulation and hyperactivation of lipases

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ABSTRACT

Alginate was modified with dodecenyl succinic anhydride (SAC12) in an aqueous reaction medium at neutral pH. The highest degree of succinylation (33.9 ± 3.5%) was obtained after 4 h at 30 °C, using four mole SAC12 per mol alginate monomer. Alginate was modified with succinic anhydride (SAC0) for comparison, and the structures and thermal properties of alg-SAC0 and alg-SAC12 were evaluated using FTIR, ^{1}H NMR, and DSC. Calcium-hydrogel beads were formed from native and modified alginates, in which lipases were encapsulated with a load of averagely 76 μg lipase per mg alginate, irrespective of the type of alginate. Lipases with a "lid", which usually are dependent on interfacial activation, showed a 3-fold increase in specific activity toward water-soluble substrates when encapsulated in alg-SAC12, compared to the free lipase. Such hyperactivation was not observed for lipases independent of interfacial activation, or for lipases encapsulated in native alginate or alg-SAC0 hydrogels.

1. Introduction

Alginate is a naturally occurring polysaccharide composed of α-L-guluronate and β-D-mannuronate arranged as linear homopolymeric and heteropolymeric blocks (Pawar & Edgar, 2012). Alginate is widely used for encapsulation purposes, commonly in the form of a hydrogel, which forms through coordination of divalent cations to the carboxylate groups in the guluronate moieties (Lee & Mooney, 2012). Calcium(II) is commonly used to form alginate hydrogels, and calcium–alginate hydrogels have been used to encapsulate probiotics (Dong et al., 2013), live cells for use in biomedicine (Lim & Sun, 1980), as well as enzymes for various technical applications (Won, Kim, Kima, Park, & Moon, 2005; Zhang Shang et al., 2014).

Numerous derivatizations of alginate have been performed in order to tailor alginate toward specific applications (Pawar & Edgar, 2012; Yang, Xie, & He, 2011). Generally, the alginate polymer can be modified at the secondary hydroxyl groups at C2 and C3, and/or at the carboxyl groups. In this study, alginate was modified with succinic anhydrides (SAC0) and dodecenyl succinic anhydrides (SAC12), forming succinylated alginates (alg-SAC0 and alg-SAC12), as illustrated in Fig. 1.

Lipases (E.C. 3.1.1.3) are biocatalyst used in various industrial applications (Reis, Holmberg, Watzke, Leser, & Miller, 2009). Encapsulation of lipases can aid their reusability and protect the biocatalyst from external stresses. Lipases have successfully been encapsulated in alginate hydrogels by mixing the lipase with an aqueous solution of alginate, followed by drop-wise addition of the alginate–enzyme solution into an aqueous solution of calcium chloride (Toscano, Montero, Stoytcheva, Cervantes, & Gochev, 2014; Won et al., 2005; Zhang Shang et al., 2014). Lipases encapsulated in alginate hydrogels show good stability and good reusability, but generally suffer from lower activity compared to the free lipase, primarily due to mass transfer limitations (Toscano et al., 2014; Won et al., 2005).

Most lipases have elements of secondary structure covering their active site (generally termed the "lid"), which opens in the presence of interfaces such as an oil–water interface or the surface of a micelle (Reis et al., 2009). The lipase is fully active only with the "lid" open, and lipases with a "lid", such as the lipase from *Thermomyces lanuginosus* (TLL), show only low activity against water-soluble substrates. Studies have shown that encapsulating such lipases using hydrophobic supports can lead to hyperactivation of the lipase, as the hydrophobicity of the support enables the lipase to be encapsulated in an active "open" form (Bastida et al., 1998; Rodrigues, Ortiz, Berenguer-Murcia, Torres,

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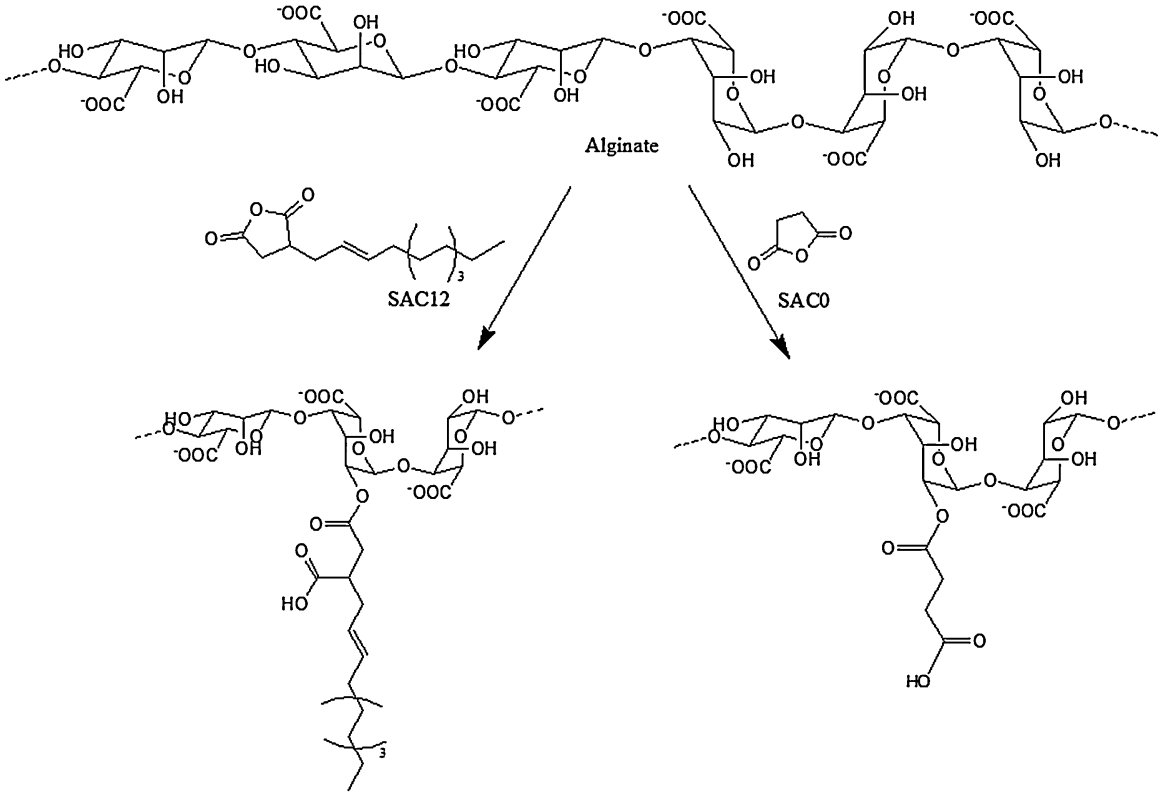

Fig. 1. Reaction scheme of succinylation of alginate with SAC12 (left) or SAC0 (right).

& Fernandez-Lafuente, 2013; Wilson et al., 2006). For example, Bastida et al. (1998) showed that lipases from *Rubus niveus*, *Rhizomucor miehei*, and *Humicola lanuginosa* were 6-, 7-, and 20-fold more active toward water-soluble substrates when encapsulated in octyl-agarose gels, compared to the corresponding free lipases. Such hyperactivation of lipases towards water-soluble substrates is particularly advantageous in medical applications, where lipase-catalyzed resolution of drugs (water soluble or partially soluble), e.g., menthol (Bai, Guo, Liu, & Sun, 2006), is an important area of lipase application.

The present work is based on our recent findings with alginate-based oligomers/polymers, obtained upon depolymerization or modification, which demonstrated interesting properties as ingredients in lipid encapsulation and formulation (Falkeborg & Guo, 2015; Falkeborg et al., 2014). We hypothesize that modification of alginate with hydrophilic SAC0 and hydrophobic SAC12 may not only change the chemical structure, but also alter the polymer packing and chemical properties of alginate, and create possible interactions with proteins/enzymes. Hence, the properties and activities of lipases encapsulated in these modified alginates were examined in this study. Two lipases, *Candida antartica* lipase B (CALB) and *T. lanuginosus* lipase (TLL), were selected as respective representatives of non-interfacial- and interfacial active lipases. This work was aimed to exploit the application potentials of alg-SAC12 as a novel biomimicking material for use in biotechnological areas.

2. Materials and methods

2.1. Materials

Sodium alginate (Grindsted® Alginate FD 170) was provided by DuPont, Brabrand, Denmark. This alginate originated from brown algae, and had an average molecular weight of 100 kDa. The ratio of α-L-guluronate units to β-D-mannuronate units was 40–60. Lipase B from *C. antartica* (CALB) and lipase from *T. lanuginosus* (TLL) were obtained from Novozymes Denmark in liquid formulations containing 25% propylene glycol. The formulations contained 30 mg lipase/mL as determined from the bicinchoninic acid (BCA) protein assay (Section 2.8), and were used as received. Succinic anhydride (SAC0), 2-dodecen-1-yl succinic anhydride (SAC12), tris(hydroxymethyl) aminomethane (Tris; ≥99.8%), *p*-nitrophenylbutyrate (*p*-NPB; 98%), cupric acetate–pyridine reagent, and calcium chloride ($CaCl_2$), were from Sigma–Aldrich Co., Ltd, Denmark.

2.2. Alginate succinylation

Alginate was succinylated with SAC0 and SAC12 with inspiration from previously reported methods (Le-Tien, Millette, Mateescu, & Lacroix, 2004; Rao, Prakasham, Rao, & Yadav, 2008). Small-scale alginate succinylations for process optimization were performed in 20 mL capped vials, thermostated using circulating water and stirred with magnetic bars at 300 rpm. Sodium alginate (100 mg, corresponding to 0.51 mmol monomeric units) was dissolved in 10 mL pure water, followed by addition of 2.02 mmol SAC0 or SAC12. The pH was adjusted to 7.0 ± 0.3 using 1% sodium hydroxide (NaOH) and an automated pH-meter (inoLab pH 7110, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). After 4 h, the succinylated products (alg-SAC0 and alg-SAC12) were precipitated using 35 mL ethanol and isolated by centrifugation at 4000 rpm for 10 min at 20 °C. Excess SAC0 and SAC12, and water, were removed by washing four times with 50 mL acetone. The reaction velocities were followed by determining the degree of succinylation (DS) after varying reaction times (1–8 h). Optimization toward a high DS was performed with SAC12 by varying the reaction temperature (25, 30, 40, and 50 °C) and the mole