Journal of Biotechnology 167 (2013) 16–23

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Lipases efficiently stearate and cutinases acetylate the surface of arabinoxylan films

A.M. Stepan[a,b,*], G.E. Anasontzis[a,c], T. Matama[d], A. Cavaco-Paulo[d], L. Olsson[a,c], P. Gatenholm[a,b]

[a] WWSC, Wallenberg Wood Science Center, Chalmers University of Technology, Kemivägen 10, SE-412 96 Göteborg, Sweden
[b] Biopolymer Technology, Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-412 96 Göteborg, Sweden
[c] Industrial Biotechnology, Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-412 96 Göteborg, Sweden
[d] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

ARTICLE INFO

Article history:
Received 17 December 2012
Received in revised form 30 May 2013
Accepted 6 June 2013
Available online 14 June 2013

Keywords:
Hemicellulose
Arabinoxylan
Film
Surface esterification
Enzymatic modification

ABSTRACT

This is the first report on successful enzyme catalyzed surface esterification of hemicellulose films. Enzyme catalyzed surface acetylation with vinyl acetate and stearation with vinyl stearate were studied on rye arabinoxylan (AX) films. Different surface analytical techniques (FT-IR, TOF-SIMS, ESCA, CA) show that lipases from *Mucor javanicus*, *Rhizopus oryzae* and *Candida rugosa* successfully surface stearate AX films and that a cutinase from *Fusarium solani pisi* surface acetylates these films. The specificities of cutinase and lipases were also compared, and higher activity was observed for lipases utilizing long alkyl chain substrates while higher activity was observed for cutinase utilizing shorter alkyl chain substrates. The contact angle analysis showed films with increased initial hydrophobicity on the surfaces.

1. Introduction

Current plastic production is chiefly based on non-renewable resources, and the carbon dioxide emissions resulting from this process and the accumulating non-biodegradable waste are a serious challenge to the ecosystem's balance. Hemicelluloses, being the second most abundant biopolymer on Earth, can make a major contribution toward a sustainable bio-based economy. Besides their potential conversion to biofuels and biochemicals, hemicelluloses and some of their derivatives can be utilized as novel value added bio-based and biodegradable materials such as gels, packaging material, matrices for composites and binders (Ebringerova and Heinze, 2000).

Hemicelluloses are a group of polysaccharides consisting of diverse monosaccharides building up a complex composition and structure (Timell, 1967). In contrast to cellulose, hemicelluloses are nonlinear heteropolymers with a lower molecular weight, which gives them significantly different chemical and material properties compared to cellulose (Tombs and Harding, 1997). For example, arabinoxylan forms films with good oxygen barrier properties, which show a potential as novel packaging materials (Escalante et al., 2011; Grondahl et al., 2004; Hoije et al., 2008). Furthermore, the acetylated arabinoxylans have improved water resistance and attractive mechanical and thermal properties compared to other commercially available bio-based packaging materials (Stepan et al., 2012).

Due to the more complex structure of hemicellulose as compared with cellulose, targeted hemicellulose derivatization is still a challenge. Most of the hemicellulose conversions to date proceed through chemical reactions involving in most cases significant excess of several organic solvents for significant esterification levels (Sun et al., 1999, 2004; Timell, 1967). Surface-only modification, such as the surface octanoylation of starch films, has been developed to decrease the amount of chemicals needed for the conversion (Bengtsson et al., 2003). Enzymes offer an alternative option to carry out surface esterification. Such biocatalytic systems can decrease the amount of chemicals required, many of which can be toxic and dangerous, while enzymes, when immobilized, can be reused, reducing the cost of the enzyme production. Chemical surface modifications may also have high energy demands, with

* Corresponding author at: Biopolymer Technology, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden. Tel.: +46 317723409; fax: +46 317723418.
E-mail addresses: agnes.stepan@chalmers.se, stepan@chalmers.se (A.M. Stepan).

0168-1656/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2013.06.004

reactions taking place at high temperatures, such as 80 °C in the case of the octanoylation, while most enzymes used in this study have their temperature optimum at around 40 °C. Further advantage of enzymatic surface modifications is that it is less intrusive for the treated material, as they depend on its porosity to access the potential active groups. Enzymes can also provide high level of reaction specificity, which is important for the development of custom-made biomaterials and not always controllable in chemical synthesis systems. Nevertheless, the push toward environmental processes is a main trigger to turn toward enzyme applications for hemicellulose derivatization. The literature on enzyme catalyzed chemical modifications of carbohydrates is continuously expanding. Numerous studies have been done on the activity, stability and specificity of lipases and cutinases in hydrolyzing different ester bonds or transesterification reactions, which have inspired researchers to use lipases and cutinases in a reverse reaction for esterification of hemicelluloses (Kontkanen et al., 2004; Pio and Macedo, 2009; Poulsen et al., 2005; Sakai et al., 2008; Utsugi et al., 2009). It has been shown that some lipases, esterases and cutinases that show activity on esterified substances can also have monosaccharide, oligosaccharide and polysaccharide derivatives as a substrate (Guebitz and Cavaco-Paulo, 2009; Woudenberg-van et al., 1996). The work of (Sakai et al., 2008) and (Utsugi et al., 2009) provides a good overview of some lipases. Their research helped in choosing the lipases from *Mucor javanicus*, *Rhizopus oryzae* and *Candida rugosa* and a cutinase from *Fusarium solani pisi* in our study. Even though they were selected for their attractive properties, the enzymes used in the presented study have to the best of our knowledge not been applied for esterification of arabinoxylans. Klibanov and co-workers were pioneers in the field of using enzymes in non-aqueous media (Ikeda and Klibanov, 1993; Klibanov, 1989; Patel et al., 1996; Zaks and Klibanov, 1988). While the presence of water is essential in the hydrolysis reaction, esterification on various substrates has been demonstrated using esterases and lipases in non-aqueous media (Chen et al., 2006; Gremos et al., 2011; Kirk et al., 1995; Ljunger et al., 1994; Micaelo et al., 2005; Patel et al., 1996). Enzymatic acylation of polysaccharide derivatives in organic media was also an important landmark in the field, showing activity on not only monomer or oligomer units (Sereti et al., 1998, 2001). Native or unmodified polysaccharides remained beyond the scope of enzymatic functionalization until the last few years (Gremos et al., 2011; Matama et al., 2009). A significant breakthrough was achieved when Gremos et al., 2011 succeeded in performing acylation on cellulose with lipases and a cutinase with different esterifying reagents.

This study is the first report on enzymatic surface esterification of hemicellulose films. The work had two major goals. One of the aims of the project was to substitute the surface of rye arabinoxylan (AX) films in a heterogeneous reaction with a short acyl chain (acetyl) and a long acyl chain (stearate) and to characterize both with respect to chemical modification and possible changes in material properties. The second goal was to investigate and compare the activity of different lipases and a cutinase toward different alkyl chain lengths in esterification reactions of hemicelluloses. By modifying only the surface in a heterogeneous reaction, the reported good oxygen barrier properties of the bulk can be maintained while increasing the hydrophobicity of the film surface (Groendahl et al., 2006; Grondahl et al., 2003; Hoije et al., 2008; Stepan et al., 2012). Electron spectroscopy for chemical analysis (ESCA)/X-ray photoelectron spectroscopy (XPS) provided information about surface acetylation and stearation levels, after which samples (taken after three days of enzymatic surface treatment) were chosen for further analysis. To observe changes on a thinner outer layer of the surfaces, time of flight secondary ion spectroscopy (TOF-SIMS) and Fourier transform infrared (FT-IR) spectroscopy were used. Static contact angle measurements provided information on the hydrophilic and hydrophobic properties of the film surfaces.

2. Materials and methods

2.1. Materials

Rye arabinoxylan (AX) was purchased from Megazyme (Ireland) (~95% purity, LOT 20601a). Deionized water was used to dissolve and cast the AX films to be treated. The reagents for the enzymatic surface modifications were purchased from Sigma-Aldrich: vinyl-stearate (95% purity, 43362-08) and vinyl-acetate (V1503). The lipases were also purchased from Sigma–Aldrich: lipase from *C. rugosa* (L1754), lipase from *R. oryzae* (86012), lipase from *Mucor miehei* (L9031) and lipase from *M. javanicus* (L8906). Cutinase from *F. solani pisi* was produced as described earlier (Araújo et al., 2007). Hexane (208752) and methanol (32213N) were used to wash the samples after the enzyme treatments.

2.2. Methods

2.2.1. Film preparation

1 g of rye AX was dissolved in deionized water (40 ml). The solution was used to cast films on polystyrene petri dishes. The films were dried in room temperature in a fume hood. The films were freed from the petri dish and cut into 1 cm-wide strips, of which a 2 cm-long portion was hung in the treatment solution. Thus each strip yielded a treated film surface of 4 cm^2 for each test specimen.

2.2.2. Enzymatic surface acylation

The enzyme loading was as follows: 10 mg of dry enzyme (cutinase or lipase) was suspended in 10 ml of reagent (vinyl acetate or vinyl stearate). A strip of (~80 μm thick) film with a total surface of 4 cm^2 (see above) was kept suspended in the reaction mixture under continuous stirring at 300 rpm and 40 °C in closed systems to avoid evaporation of the reagents. Separate batches were run for 3 h, 6 h, 12 h, 24 h, 72 h, 96 h and 168 h. The samples were then washed several times with methanol and hexane, left to dry in fume hood for 5 min and stored, covered until surface analysis. Blank samples were treated in pure vinyl acetate (VAc blank) or vinyl stearate (VSt blank) without enzymes for 3 and 7 days. Untreated AX film is also discussed as untreated blank (AX blank), to serve as double blank for both treated blank studies.

2.2.3. Time-of-flight secondary ion mass spectrometry (TOF-SIMS)

ToF.SIMS5 from ION-TOF was used and controlled by the Surfacelab 6.1 software for analysis of the outermost one to two monolayers of the modified films. The primary ions used were Bi^{3+} ions, and the distance to the sample surface was 2 mm with a primary beam angle of 45° with respect to the surface. The area analyzed was selected manually (~100 μm × 100 μm), and the spectra were recorded for 50 s in high current bunched mode. A flood gun was used for charge compensation. Measurements were made in both positive and negative modes.

2.2.4. Fourier transform infrared spectroscopy (FT-IR)

The surface of the AX films was scraped off with a scalpel, and this powder was mixed with KBr to press tablets. The samples were analyzed with a System 2000 FT-IR from Perkin Elmer controlled by Spectrum software version 5.3 in transmission mode using a triglycine sulphate (TGS) detector. Twenty scans were taken with a resolution of 4 cm^{-1}. Samples were also run in reflection absorption spectra (RAS) mode on a Spectratech FT-80 with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. In this mode, 200 scans were taken with a resolution of 4 cm^{-1}. The